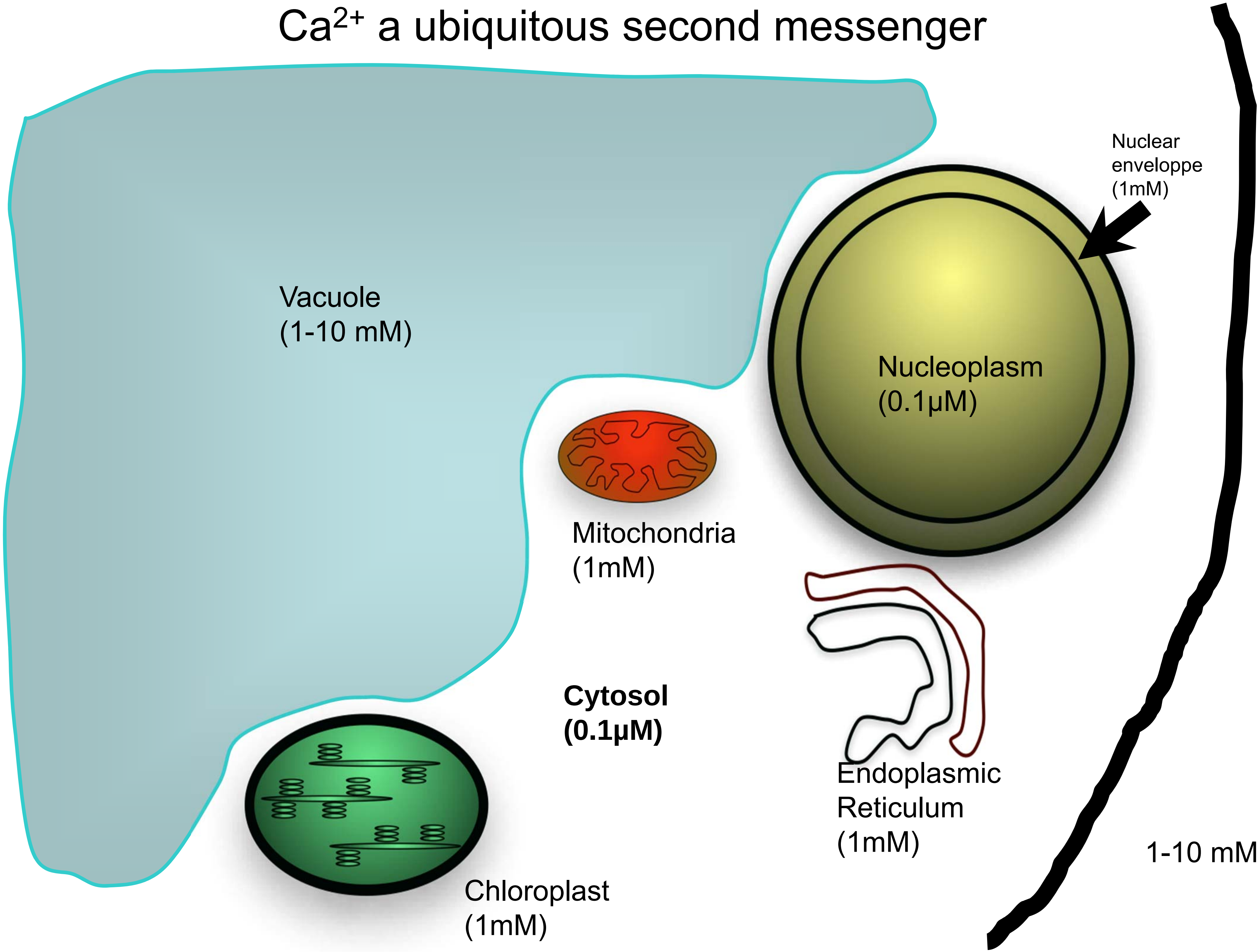
Ca^{2+} a ubiquitous second messenger



Calcium Transport and Calcium-Binding Protein Homologs of Human, Rice and Arabidopsis

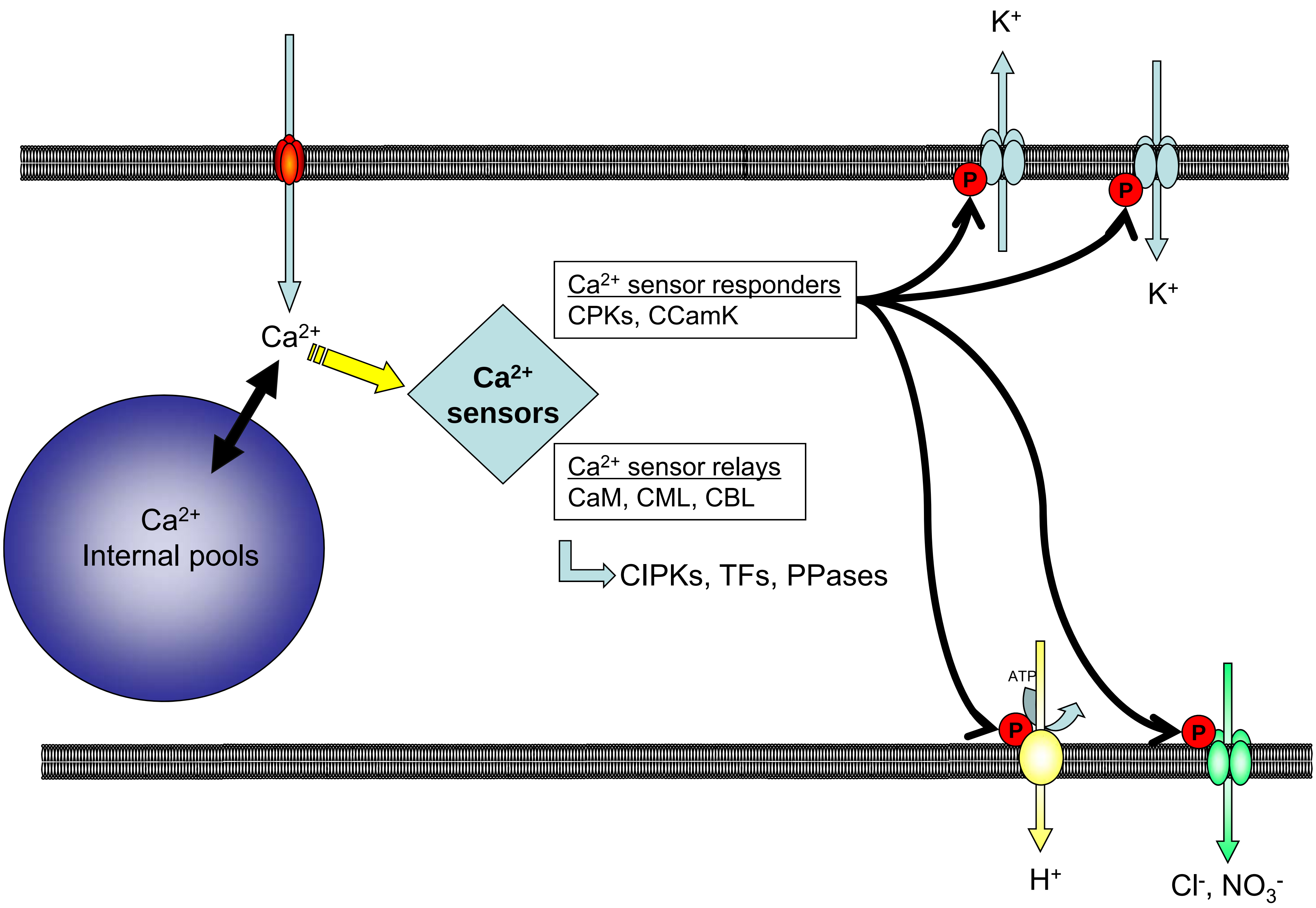
					Type	*H. sapiens*	*O. sativa*	*A.thaliana*
Ⅰ.Transporter protein								
	channel	**1.VDCC**	**α- I**	a) LVA	T	3	0	0
				b) HVA	L	6+1(TPC1)	2(TPC1)	1(TPC1)
					N	1	0	0
					P/Q	1	0	0
					R	1	0	0
			α II			4	0	0
			β			4	0	0
			γ			8	0	0
			δ			1	0	0
		2.ROCC		a) CNGC		4	8	20
				b) Glu R	AMPA	5	8	20
					NMPA	0	0	0
					KININ	0	0	0
				e) Ryanodine receptor		8	0	0
				f) IP3 receptor		3	0	0
				g) LCT		0	1	1
	pump			c) P-type Ca^{2+} ATPase	II A	8	5	3
					II B	11	10	8
	transporter			d) Ca^{2+} exchanger	Ca^{2+},H^{+}	0	5	8
					Ca2+,Na+	3	0	0
					Ca^{2+},Na^{+},(K^{+})	5	3	5

NOTE.—VDCC: voltage-dependent calcium channel; ROCC: receptor-opened calcium channel; LVA: low voltage-activated calcium channels; HVA: high voltage-activated calcium channels; Human: *Homo sapiens*; ory: *Oryza sativa*; Ara: *Arabidopsis thaliana*.

Nagata et al. 2004

				Type	H. sapiens	O. sativa	A.thaliana
II.Ca^{2+} binding protein							
	1.EF-hand protein		**①mono type**				
		A. internal cell	a) calbindin		3	0	0
			b) calcineurin B		5	7	10
			c) calmodulin		30	23	54
			d) caltractin(centrin)		5	4	2
			e) myosin regulatory light chain kinase2		1	0	0
			f) parvalbumin		1	0	0
			g) recoverin		1	0	0
			h) Penta-EF-hand		2	2	2
			i) S100		18	0	0
			j) troponin C		2	0	0
		B. ER ・golgi	a) ERC55		1	0	0
			b) calumenin		2	1	1
			②complex type				
		A. outside of cell	a) BM-40		1	0	0
			b) thrombospondin(1,4)		2	0	0
			c) NADPH oxidase		4	8	12
		B. internal cell	a) actinin		4	0	0
			b) fimbrin		5	1	2
			c) calpain	large subunit	11	2	1
				small subunit	1	1	1
			d) diacylglycerol kinaseα		1	0	0
			e) EGF receptor substrate15		1	0	0
			f) EH-domain		4	3	3
			h) CCaMK		6	3	2
			i) CaMK		4	3	3
			j) CDPK		0	20	34
			k) CRK		0	4	4

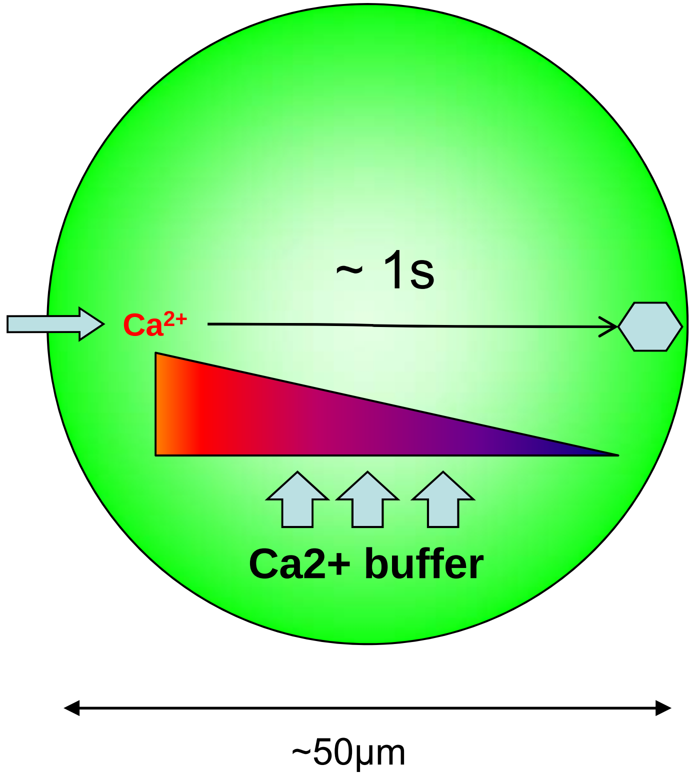
Nagata et al. 2004

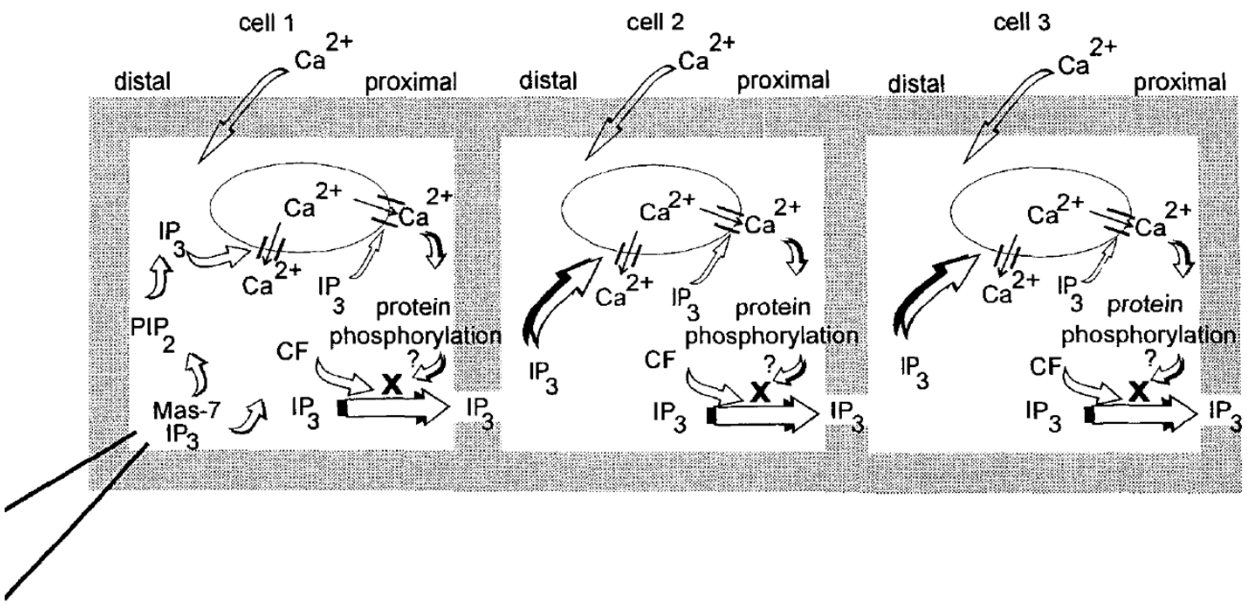

K+
K+
Ca2+ sensor responders
CPKs, CCamK
Ca2+
Ca2+ sensors
Ca2+ sensor relays
CaM, CML, CBL
Ca2+ Internal pools
CIPKs, TFs, PPases
P
ATP
H+
Cl-, NO3-

Ca^{2+} waves in plant tissue ?

***In vitro* diffusion coefficient (*D*)**

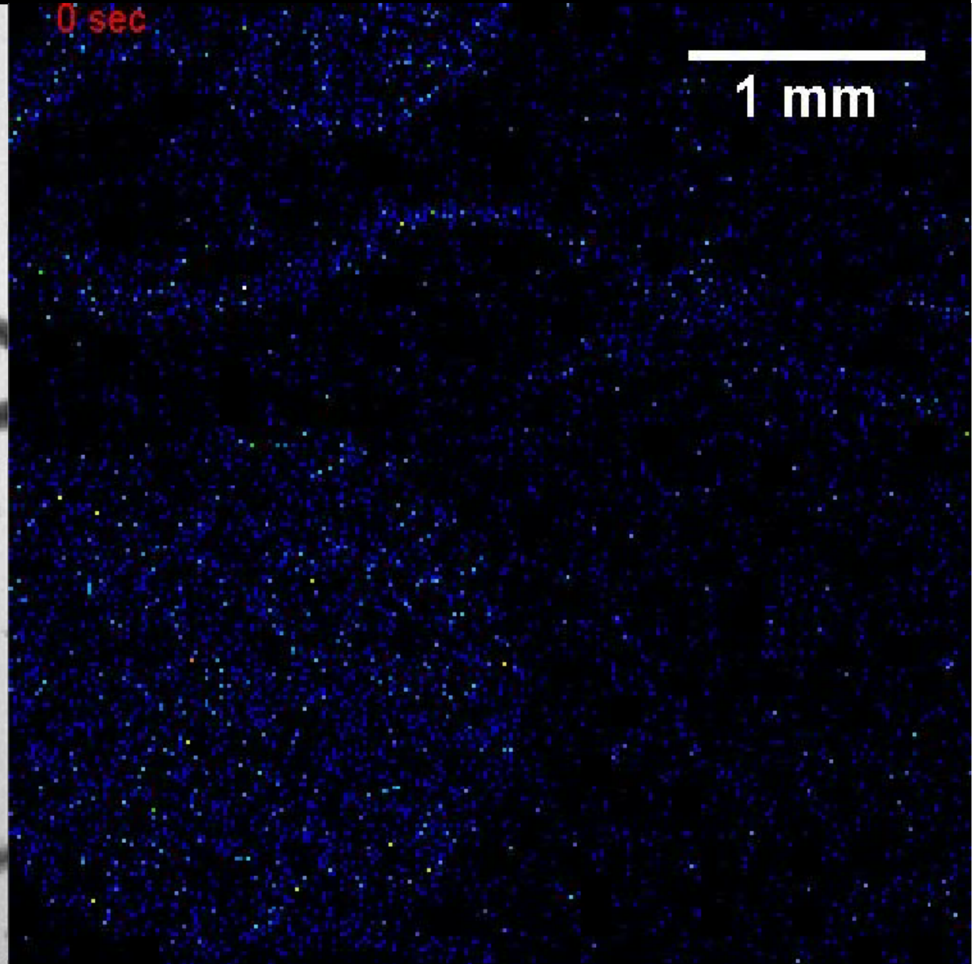
Proteins	*D* ~ 1-10 µm^2 s^{-1}	(Swaminathan *et al*., 1997; Bray, 1998; Dayel *et al*., 1999)
Ca^{2+}	*D* ~ 13-65 µm^2 .s^{-1}	(Allbritton *et al*.,1992)
NAADP	*D* ~ 100 µm^2 .s^{-1}	(Churchill & Galione, 2000)
$InsP_3$	*D* ~ 283 µm^2 .s^{-1}	(Allbritton *et al*., 1992)
cAMP	*D* ~ 270 µm^2 .s^{-1}	(Chen *et al*., 1999)
H_2O_2	*D* ~ 1000 µm^2 .s^{-1}	(Vestergaard *et al*., 2012)
NO	*D* ~ 3300 µm^2 .s^{-1}	(Malinski *et al*., 1993)



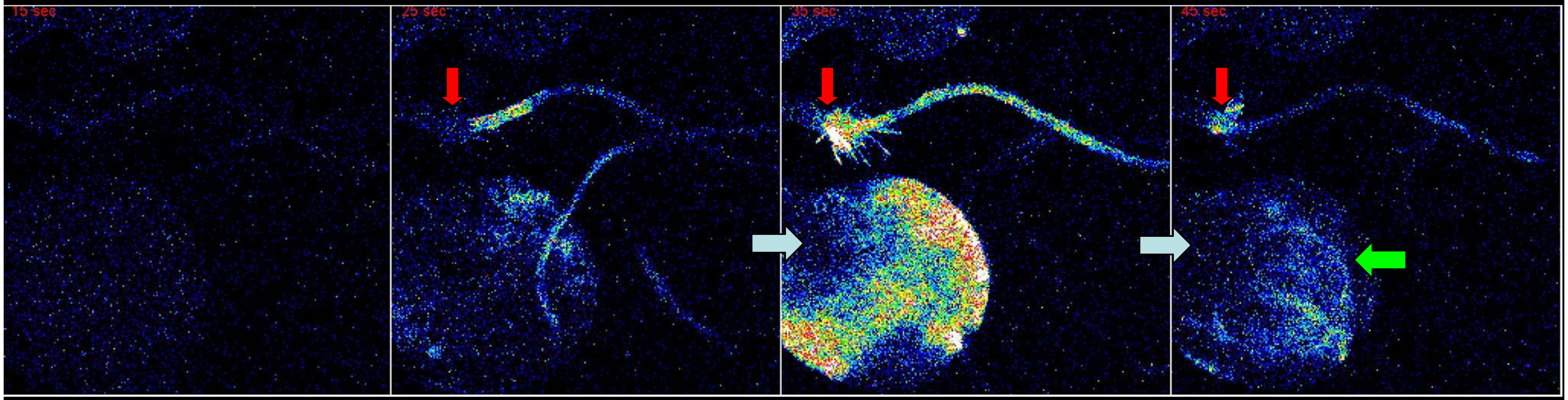


Arabidopsis seedling harboring bioluminescent Ca2+ reporter

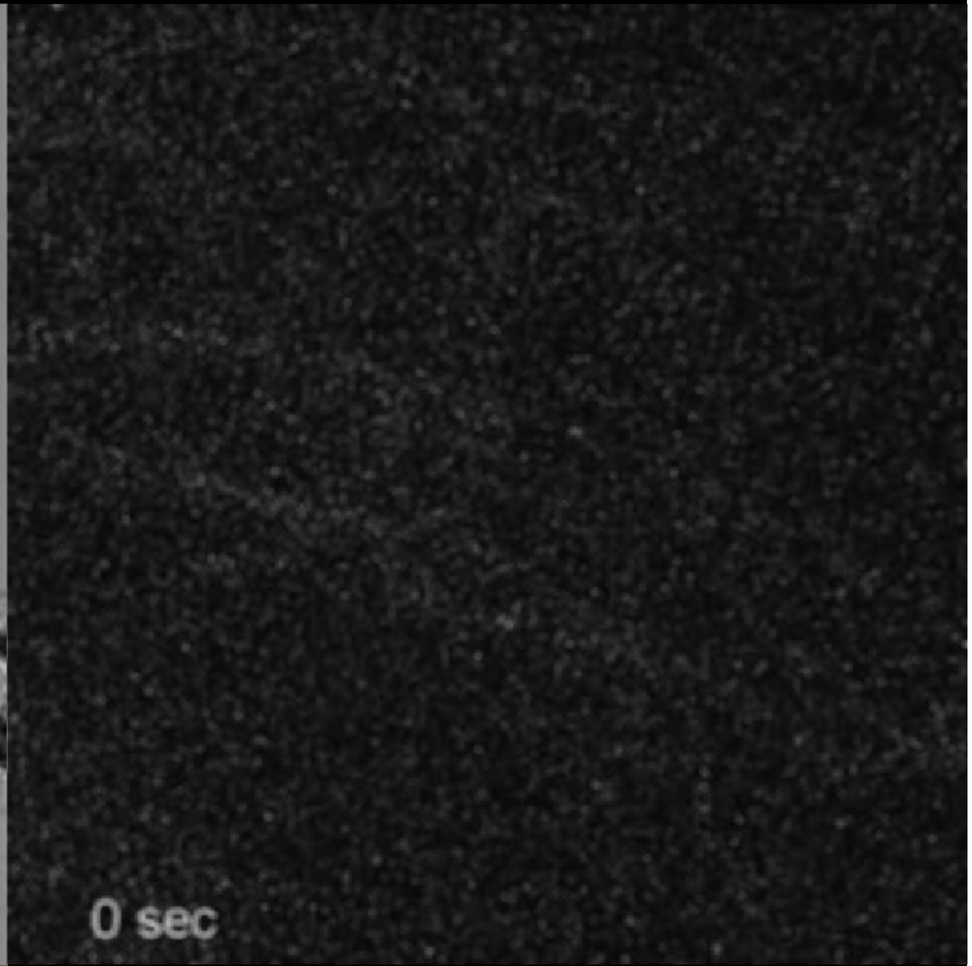
Bright field



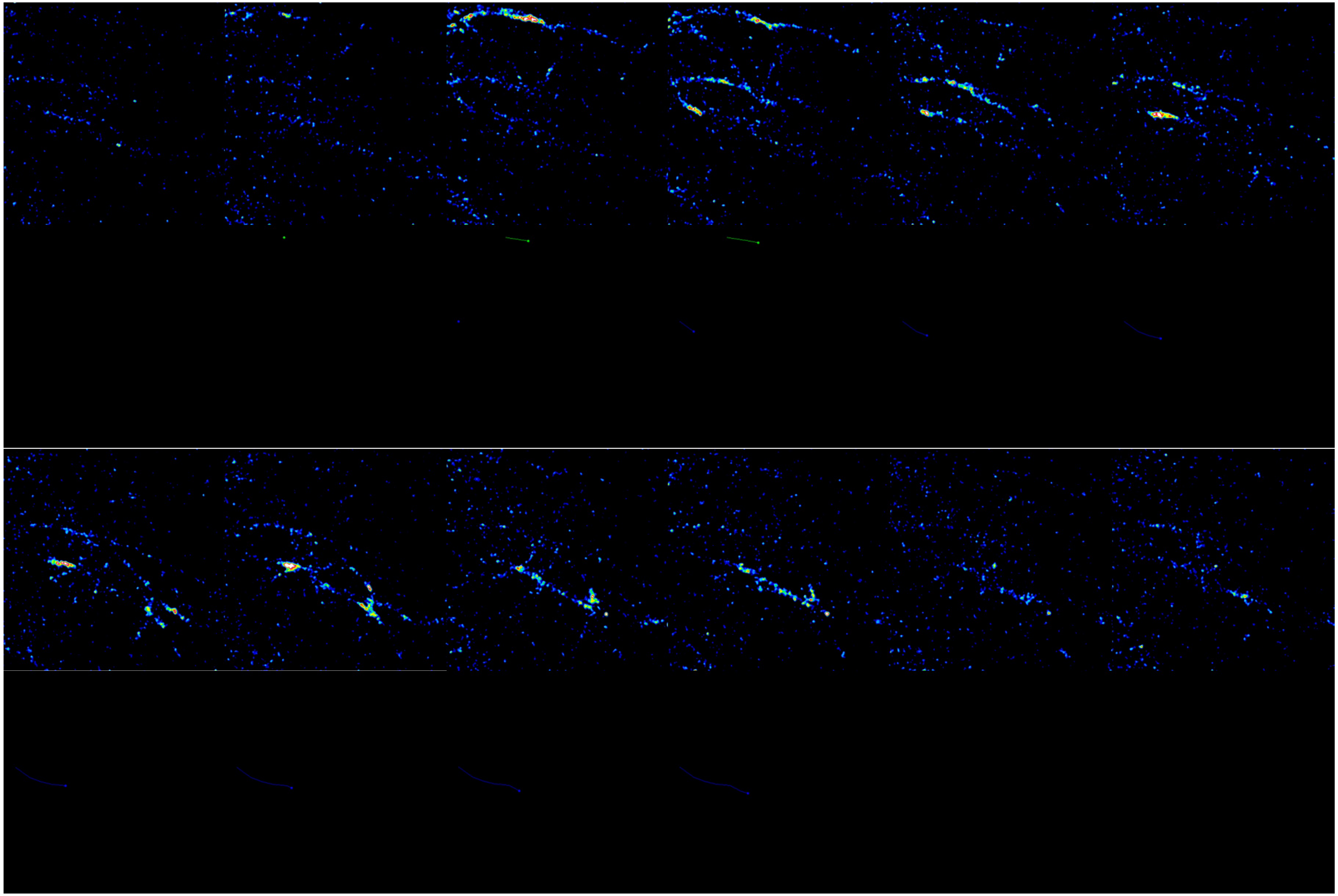
Bioluminescent


15 sec
25 sec
35 sec
45 sec

Bright field

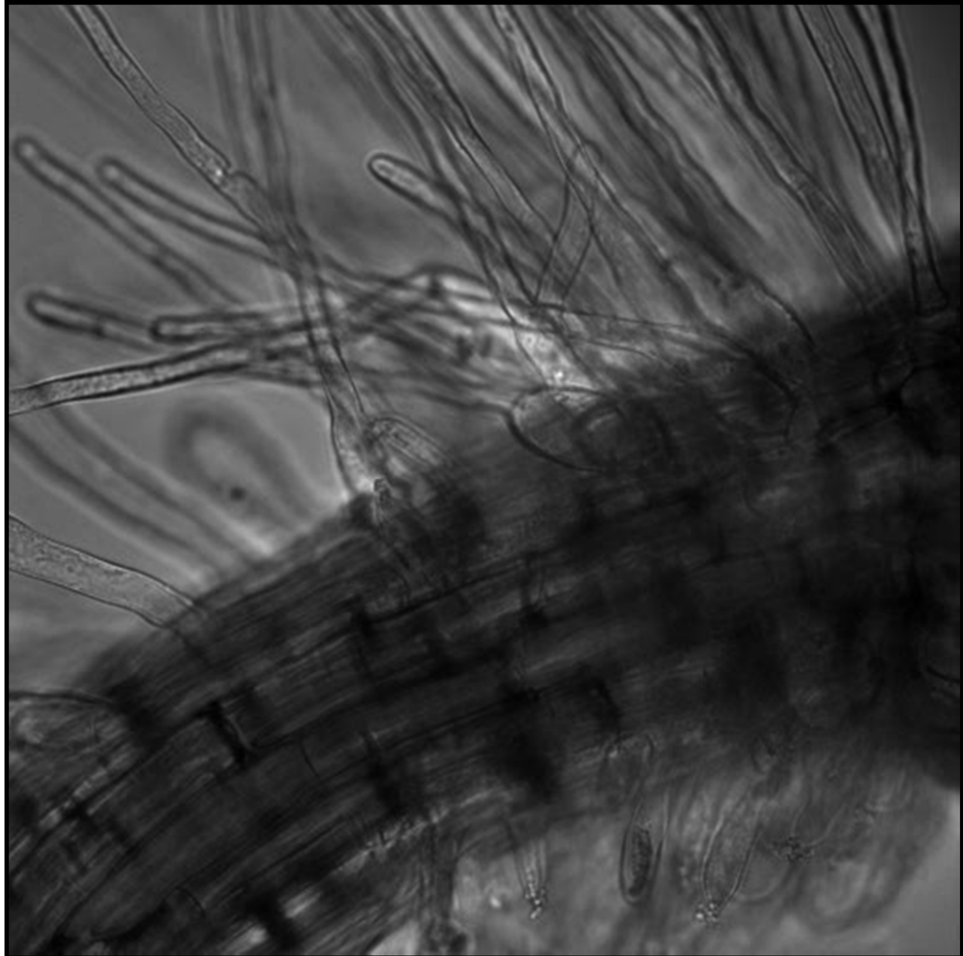


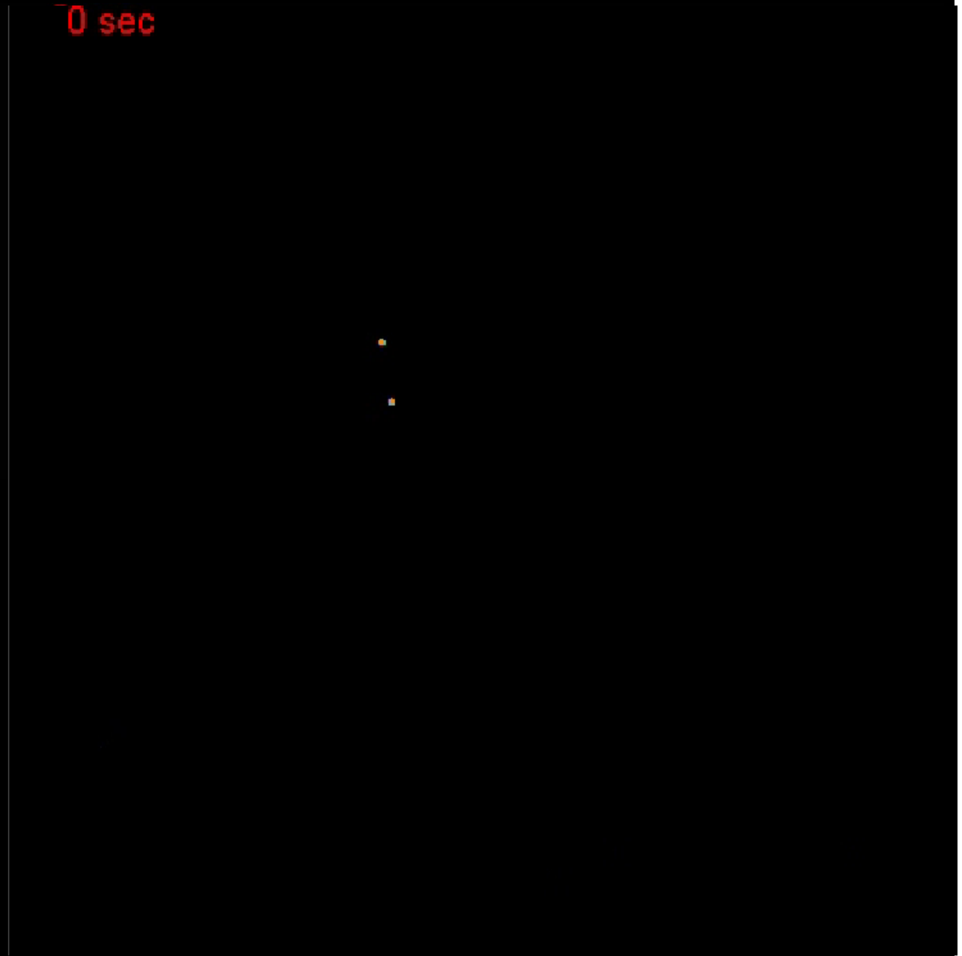
Bioluminescent

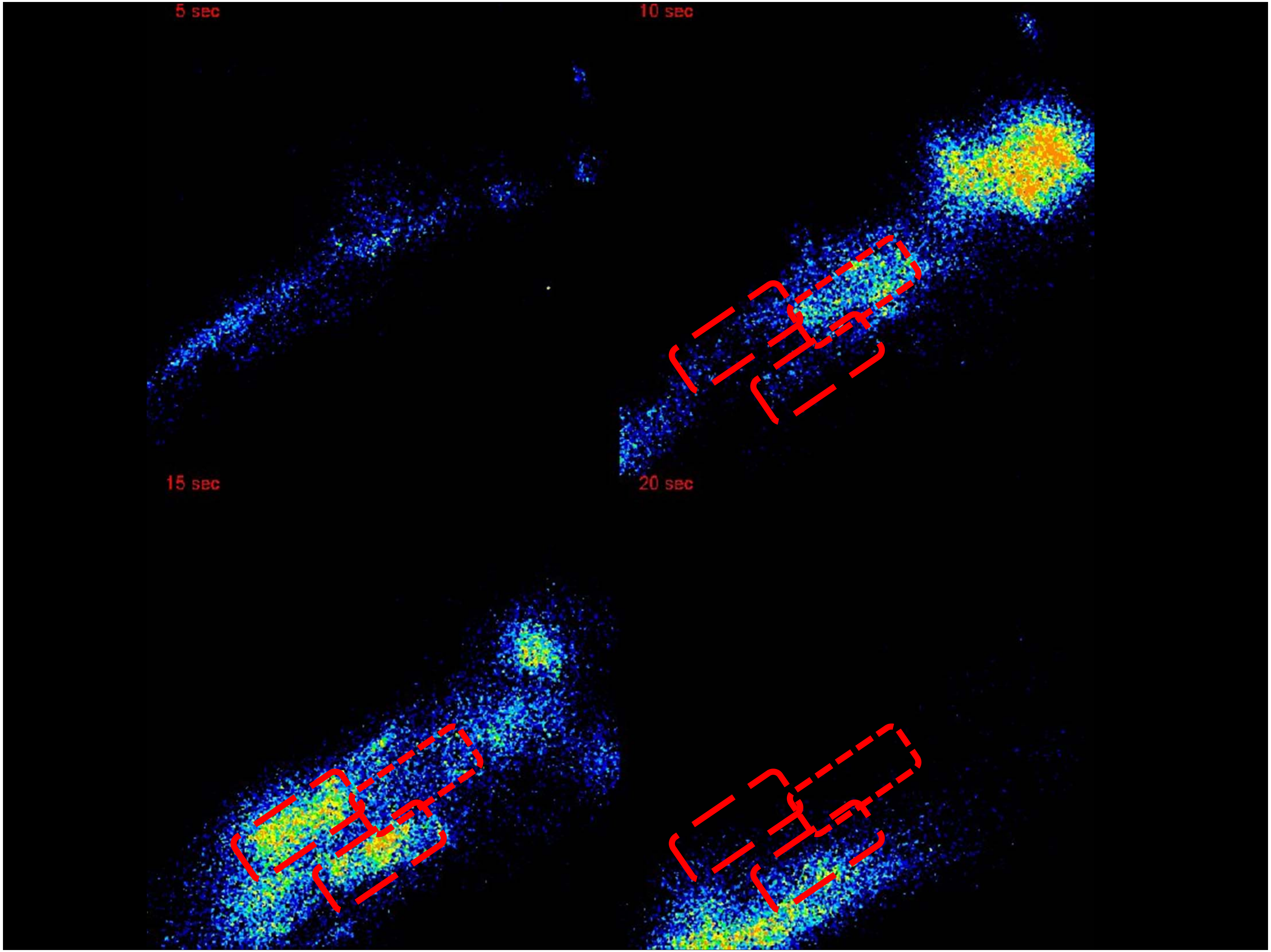


Speed~43μm.s^{-1}

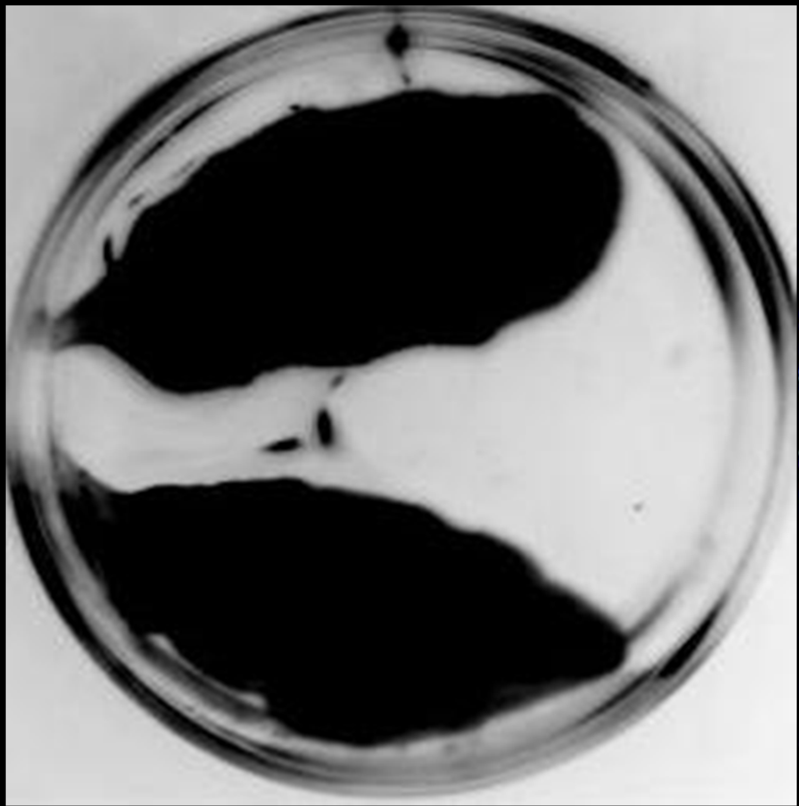
Cell to Cell propagation



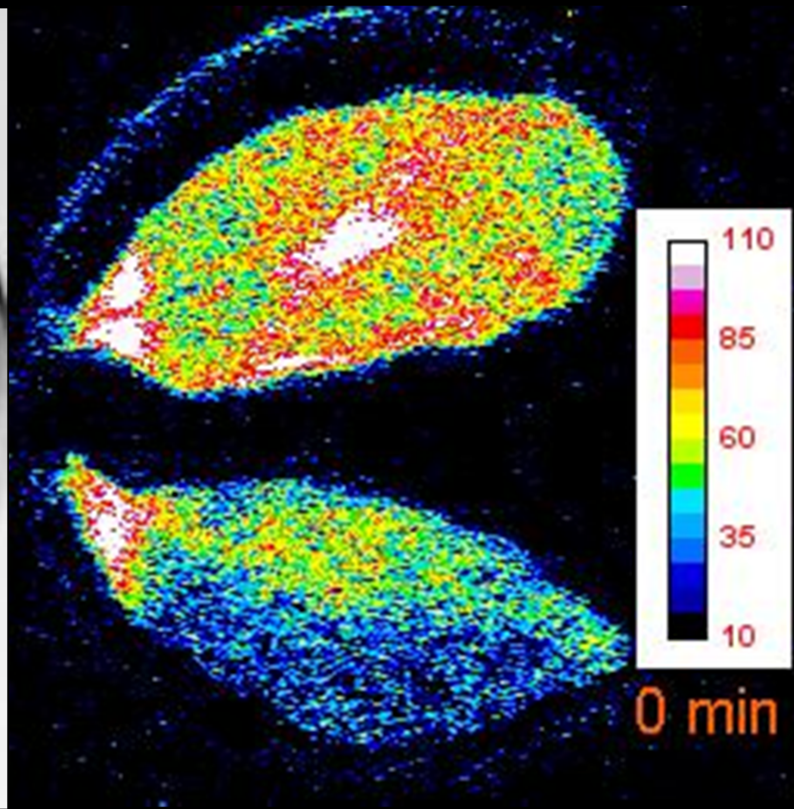



5 sec
10 sec
15 sec
20 sec

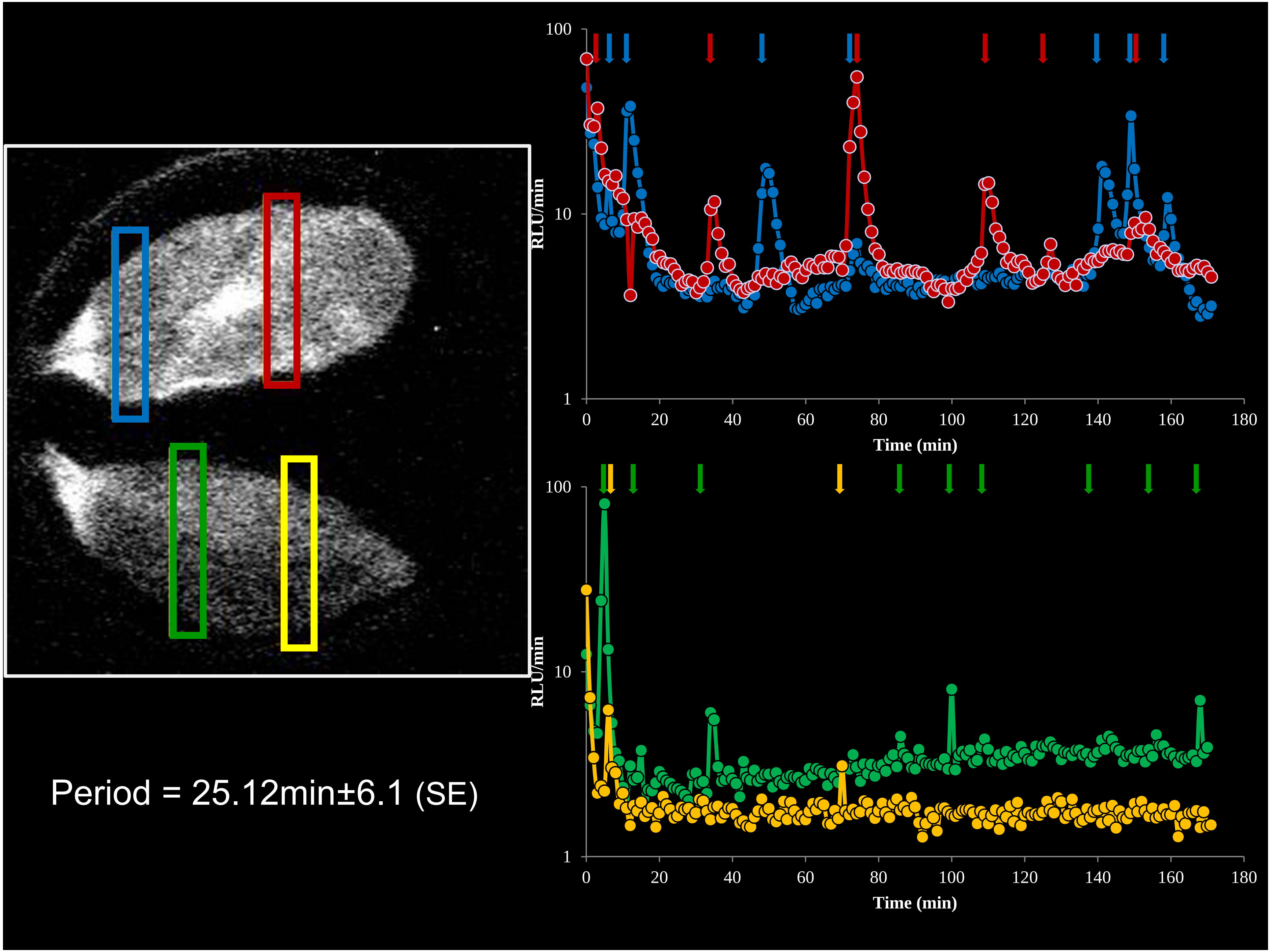
Dark induces calcium waves in leaves



Bright Field

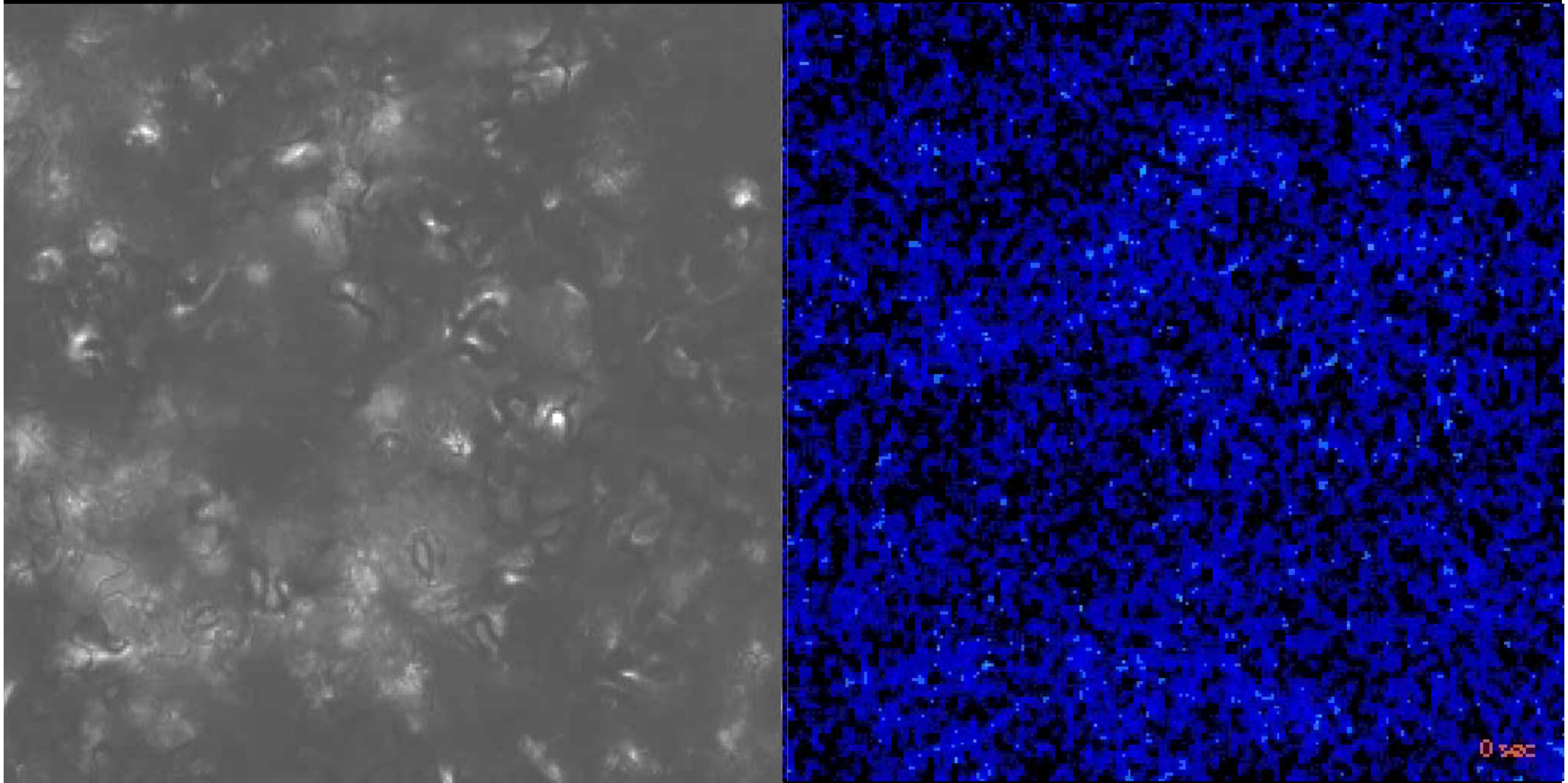


Bioluminescent

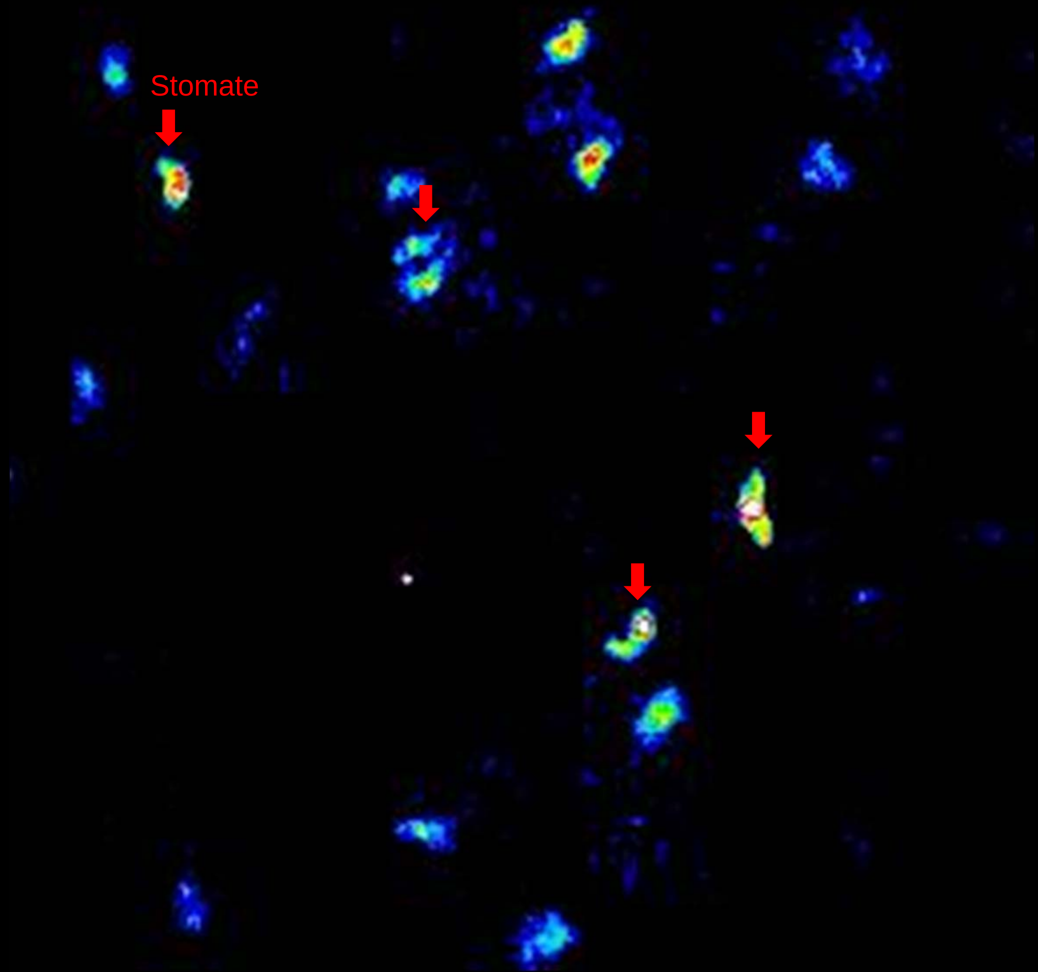


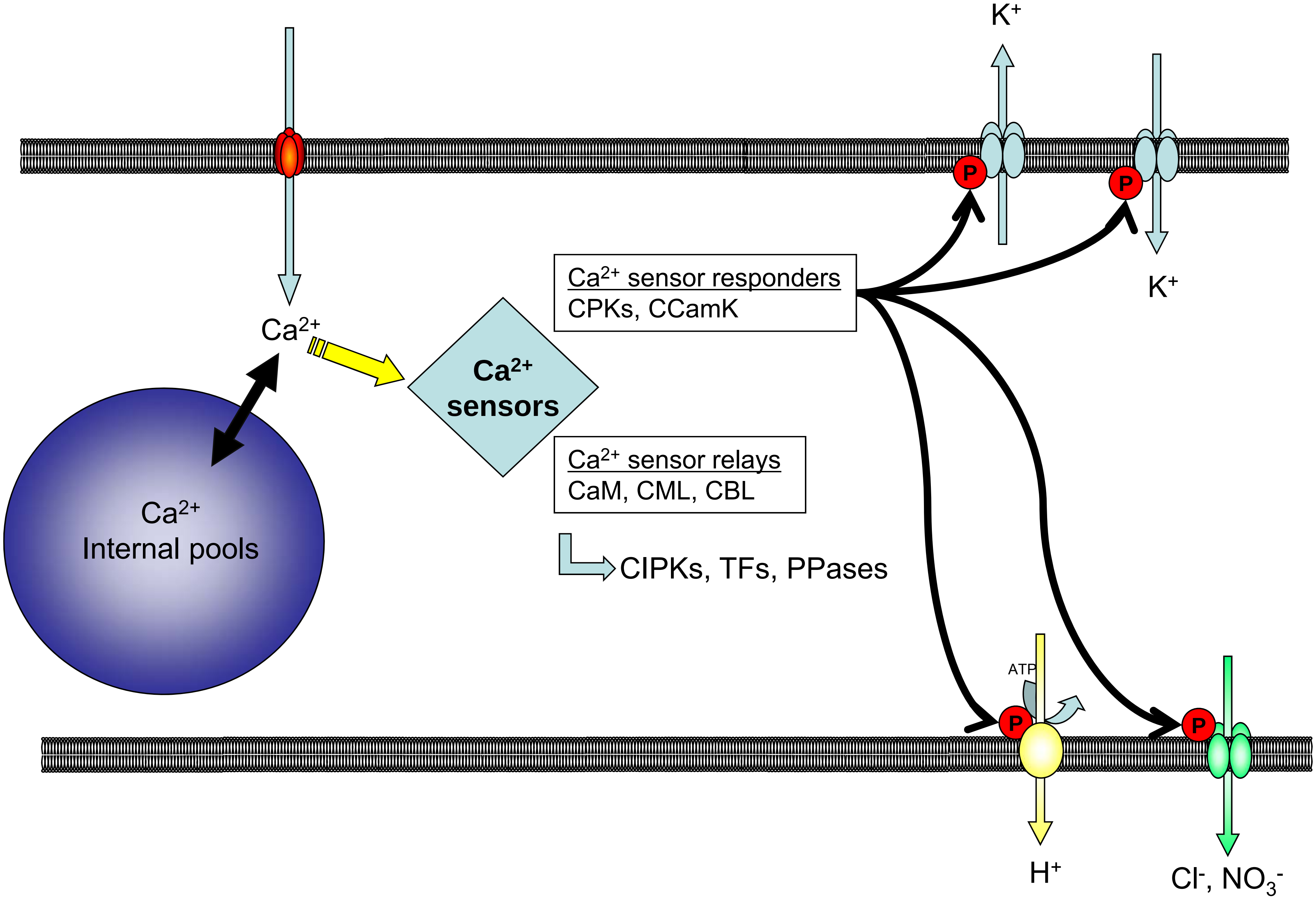
Period = 25.12min±6.1 (SE)
RLU/min
100
10
1
0
20
40
60
80
100
120
140
160
180
Time (min)

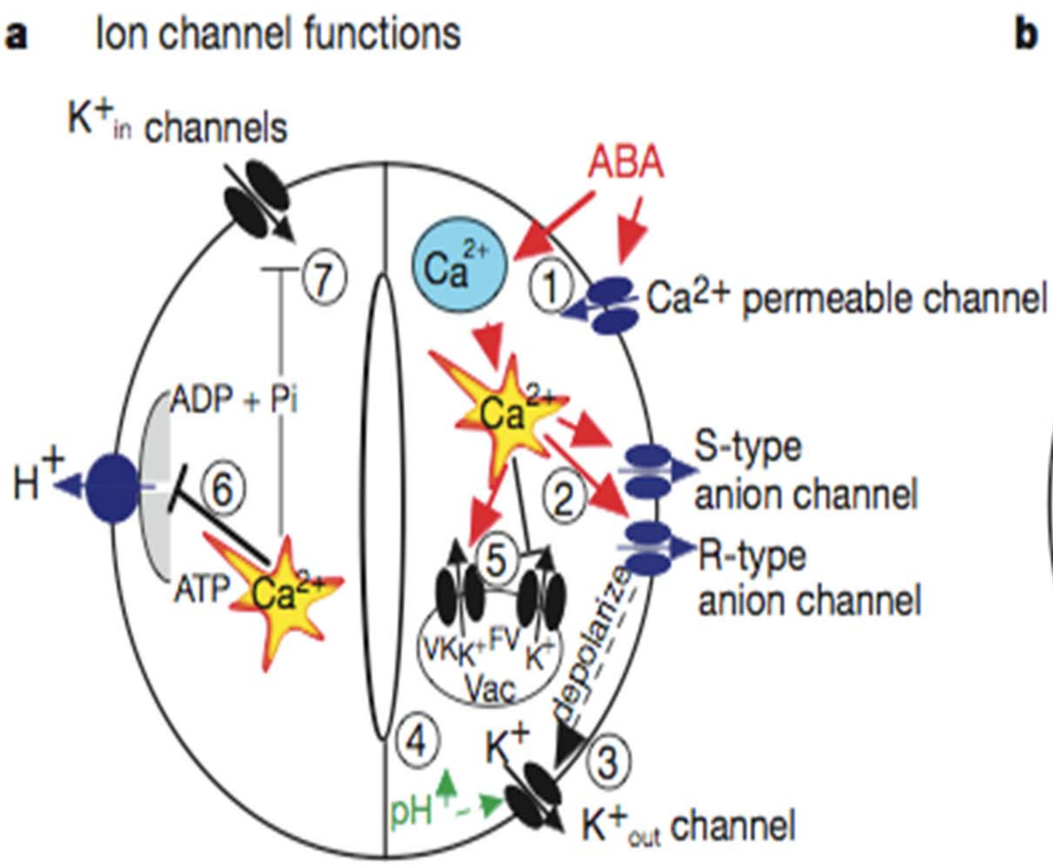
Aequorin Arabidopsis leaf



Z-Stack



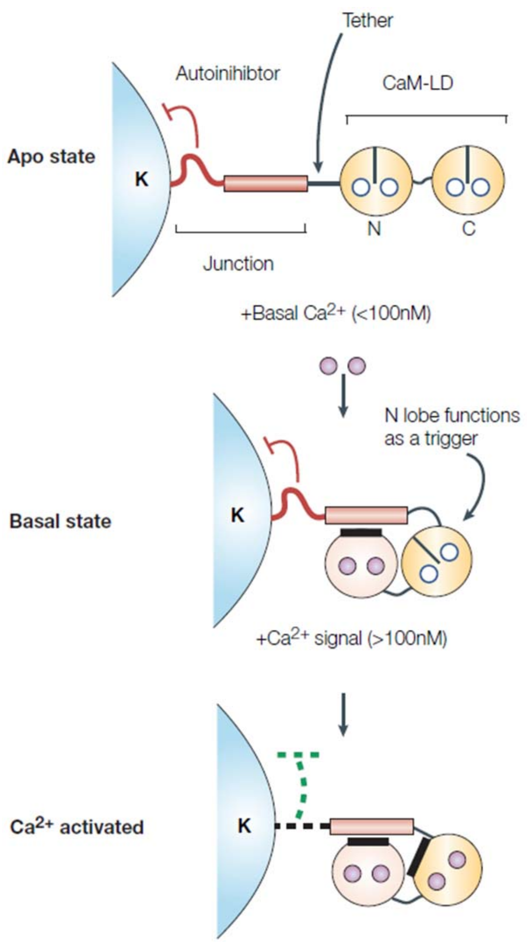

K+
K+
Ca2+
Ca2+ sensors
Ca2+ sensor responders
CPKs, CCamK
Ca2+ sensor relays
CaM, CML, CBL
CIPKs, TFs, PPases
Ca2+
Internal pools
P
P
ATP
P
P
H+
Cl-, NO3-



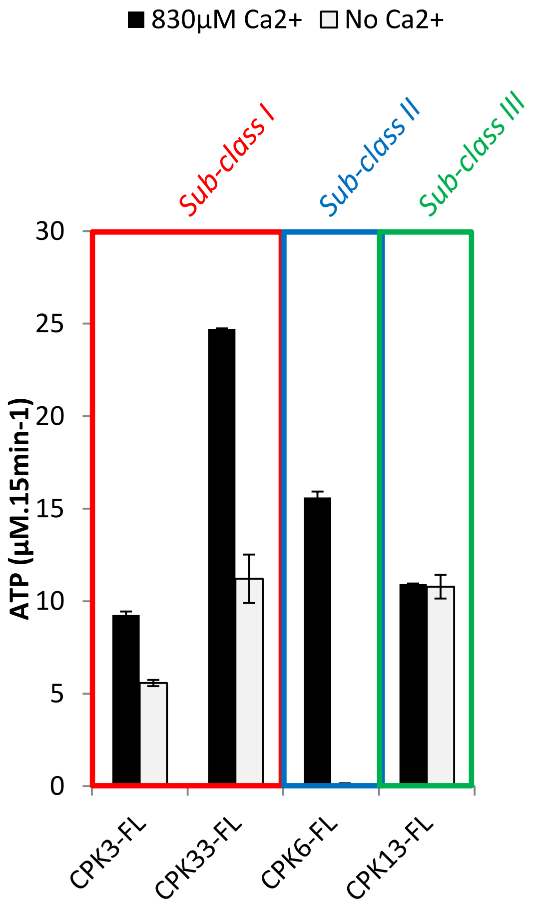
Schroeder et al. 2001

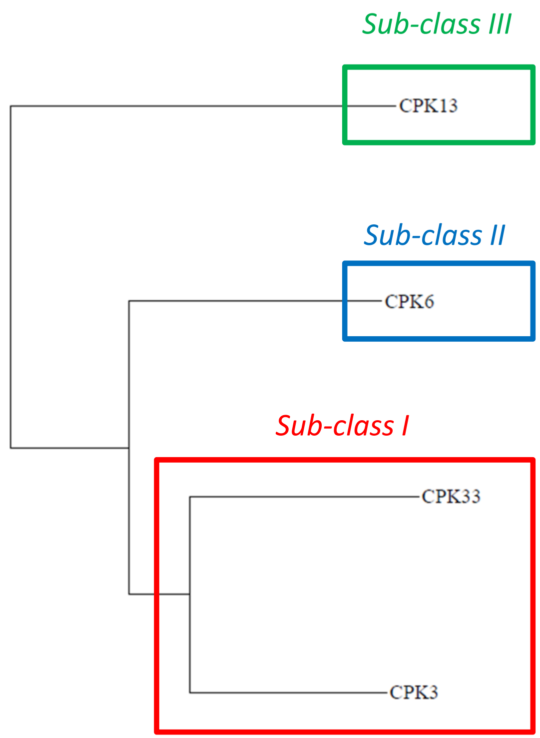
Calcium dependent Protein Kinase (CPK)

In vitro CPKs characterisation



Harper et al. 2011

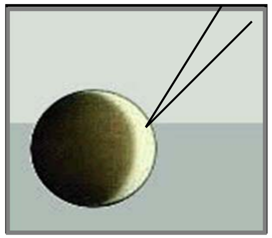


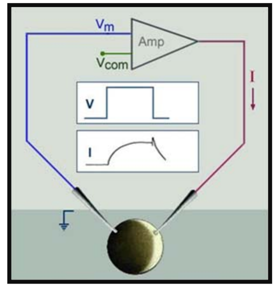


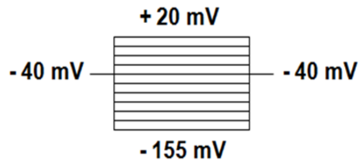
Functional screening in oocyte: CPKs regulate Shaker channel

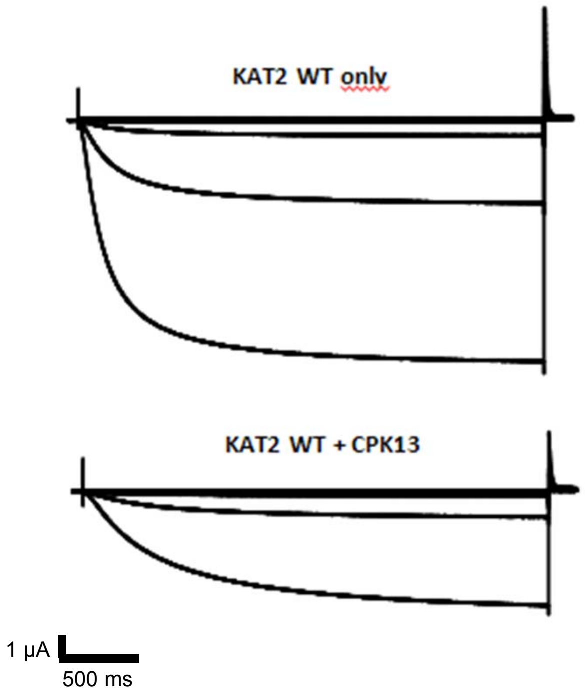
Xenopus laevis

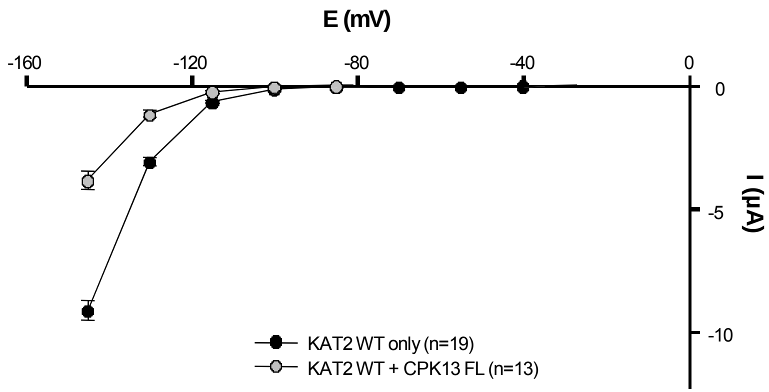
cRNA injection in oocytes







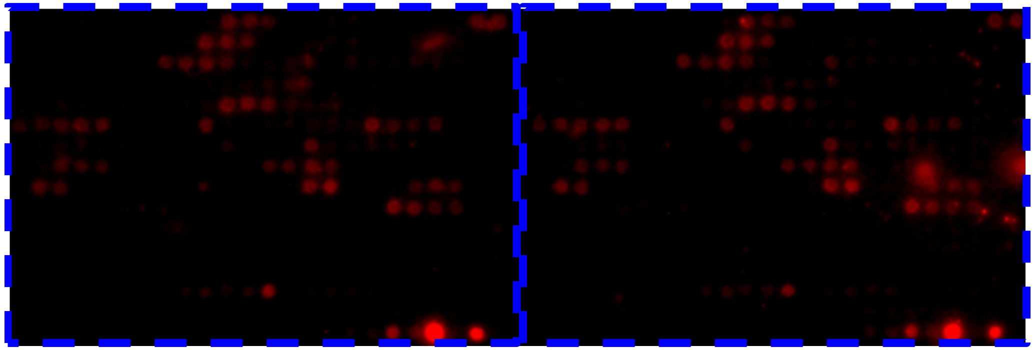


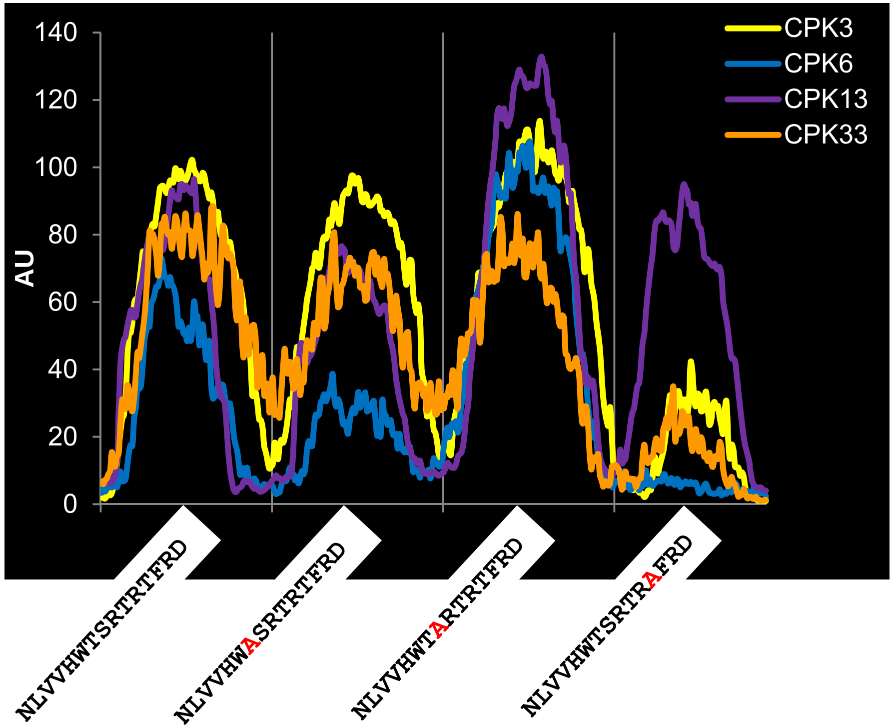


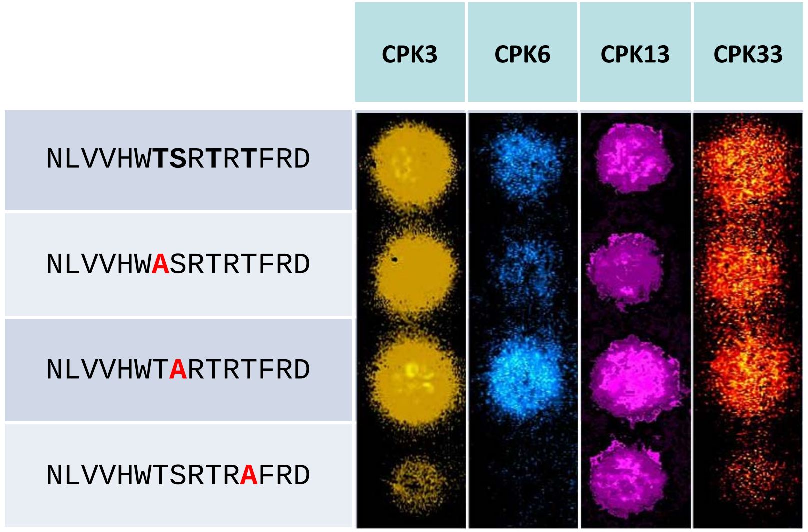
KAT2 peptides are phosphorylated by CPKs

In vitro phosphorylation

CPK + ATP [γ^{32}P] + Ca^{2+}







Mastermind game analysis

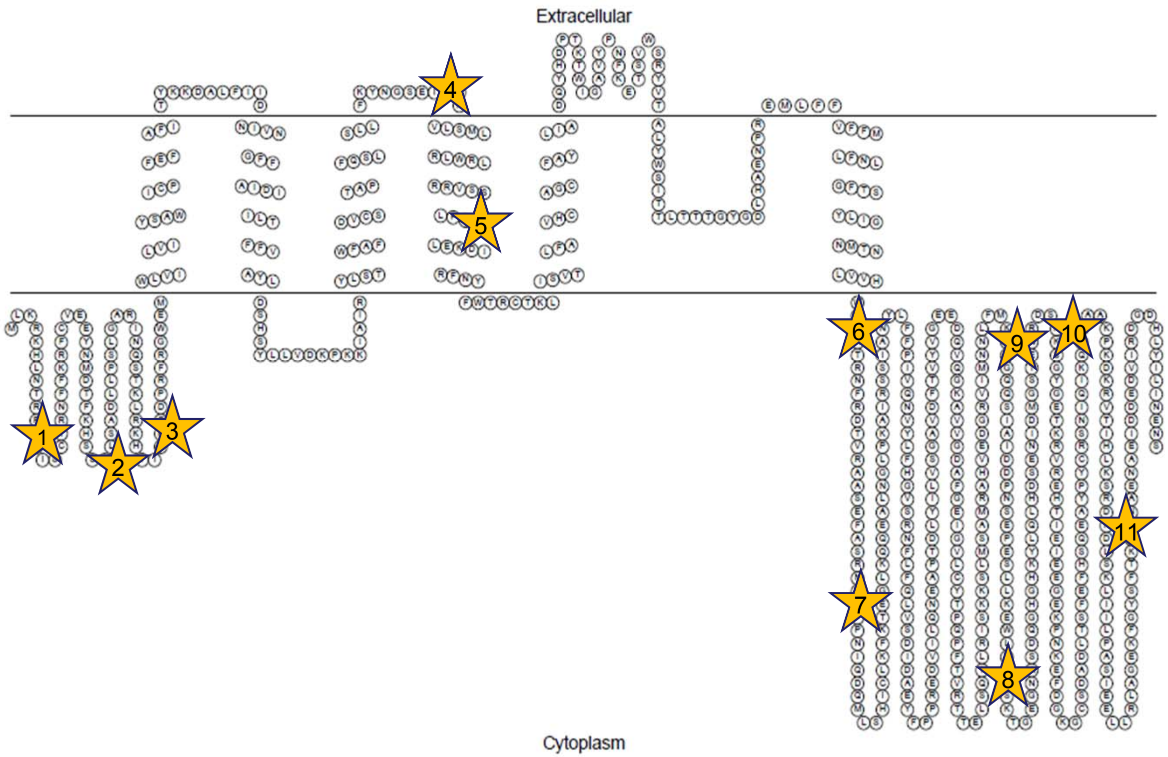
CPK13

NLVVHW**T**S**R**T**R**TFRD

CPK6

CPK3
CPK6
CPK33

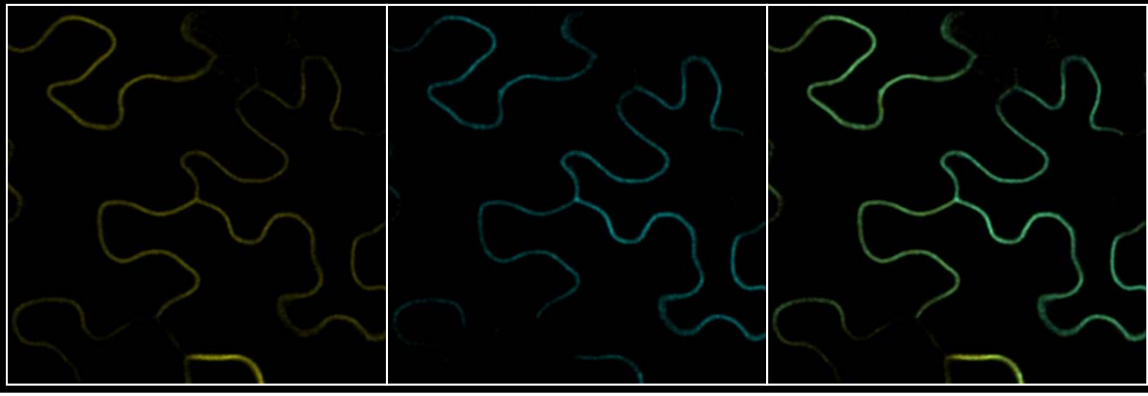
KAT2 phosphorylation sites



Phosphorylation residues

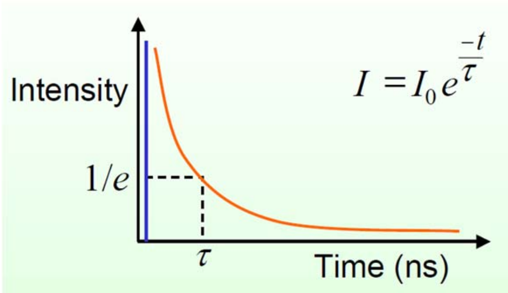
55 Ser 40 Thr	18 Ser 7 Thr

CPK13 and KAT2 are co-localised *in planta*

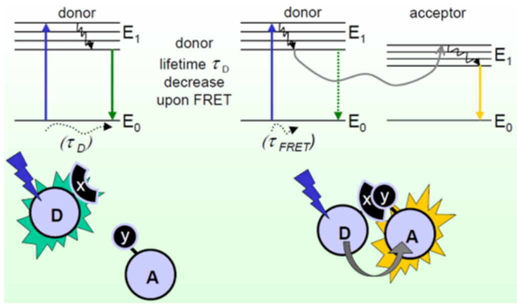


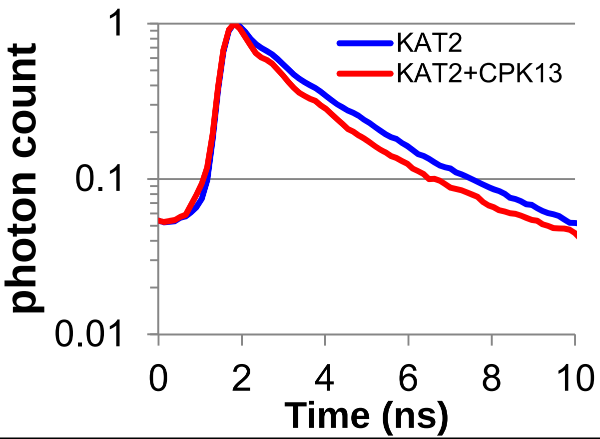
CPK13 interacts with KAT2 *in planta*

FLIM



FRET-FLIM

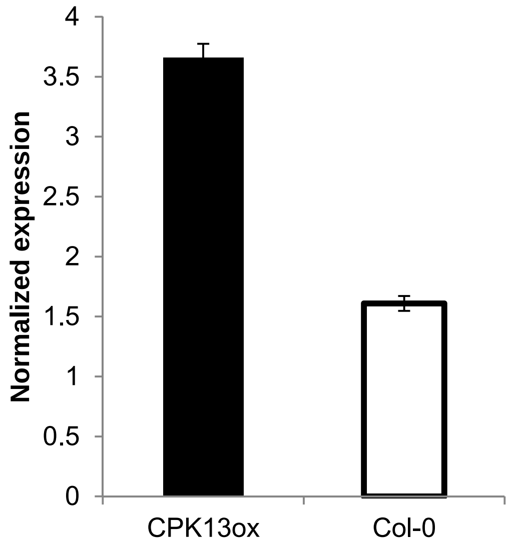


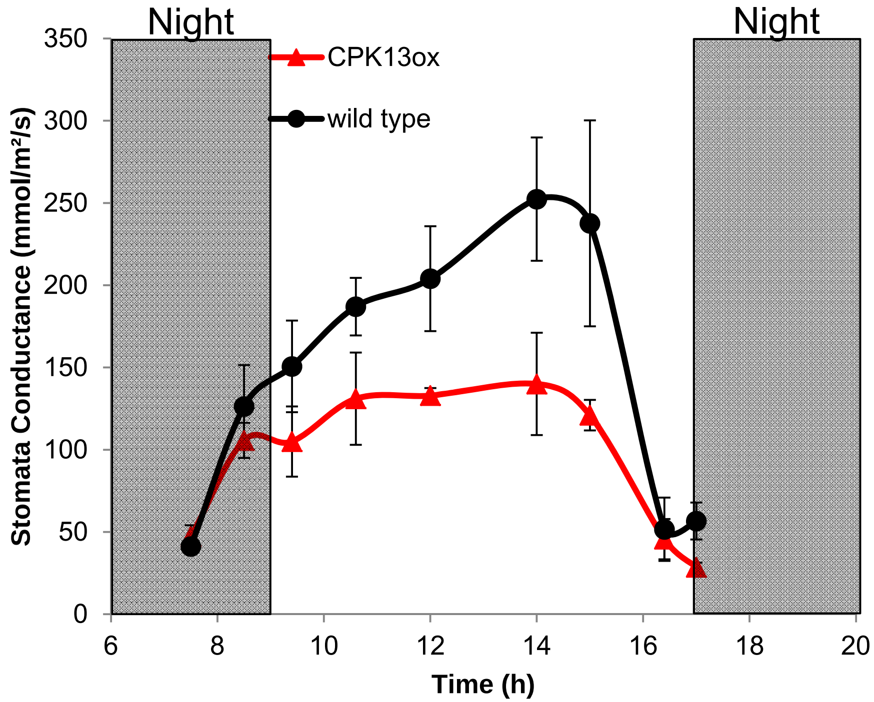


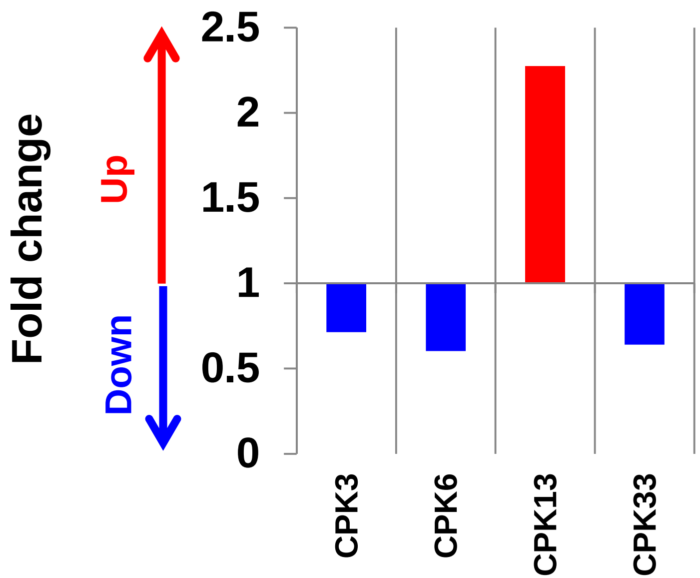
	KAT2::CFP	KAT2::CFP + CPK13::YFP
τ (ns) (Mean ±SE)	2.233 ±0.017	1.861 ±0.031
FRET efficiency (%)		16.7

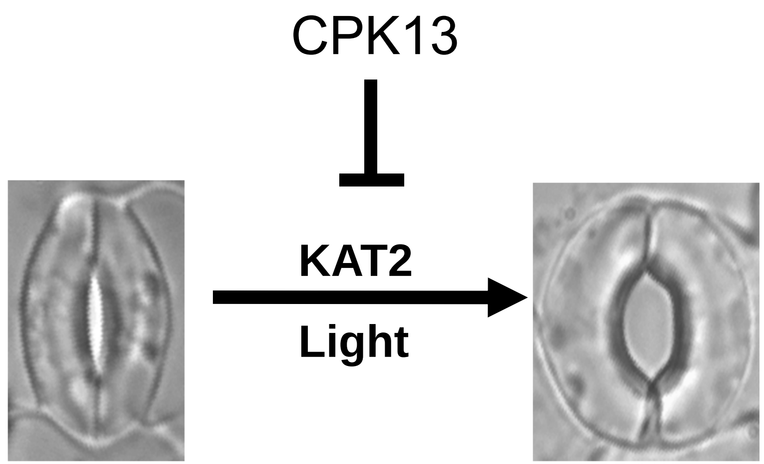
Collaboration
C. Brière, Toulouse

Overexpression of CPK13 affects stomata movement



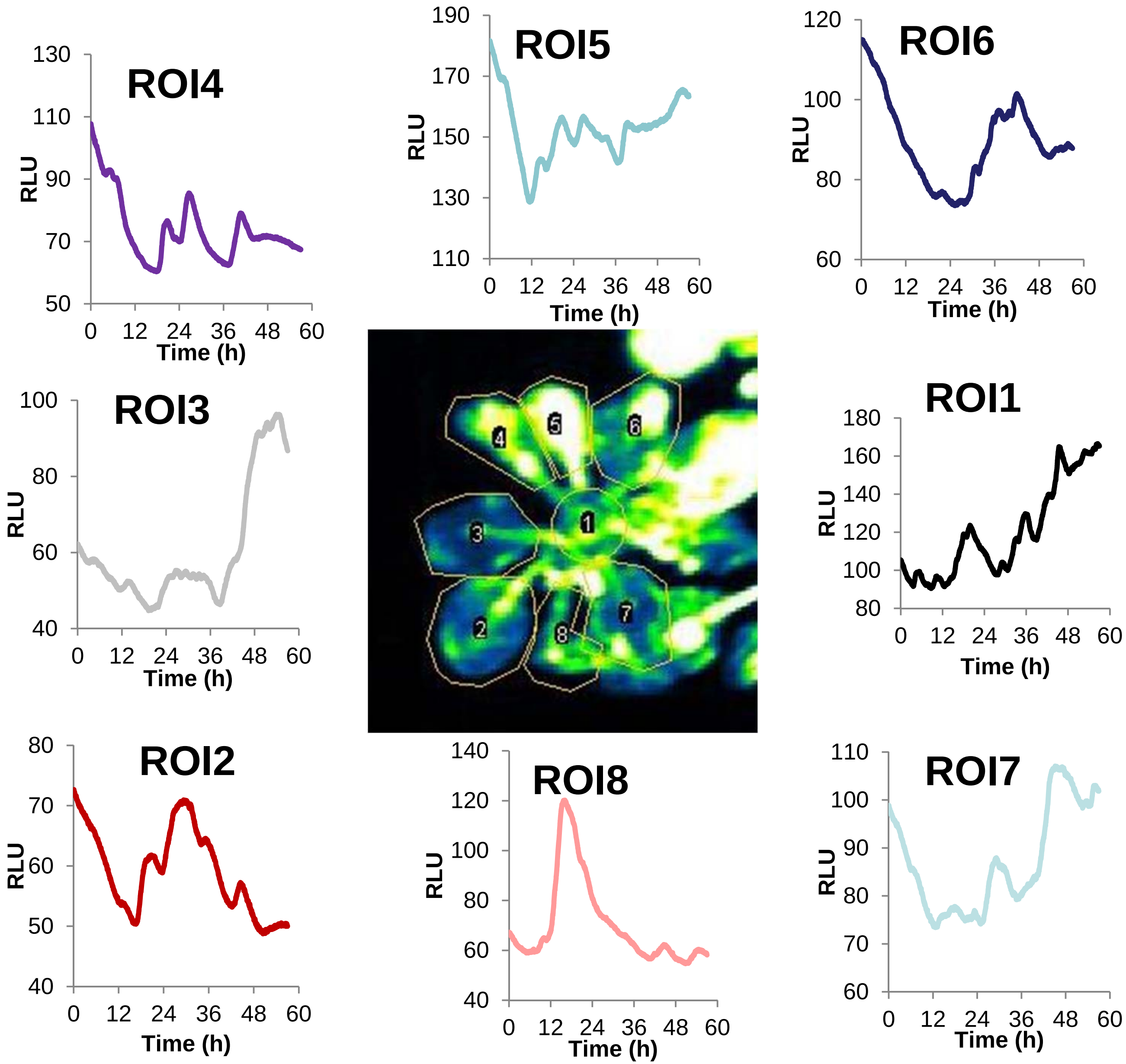




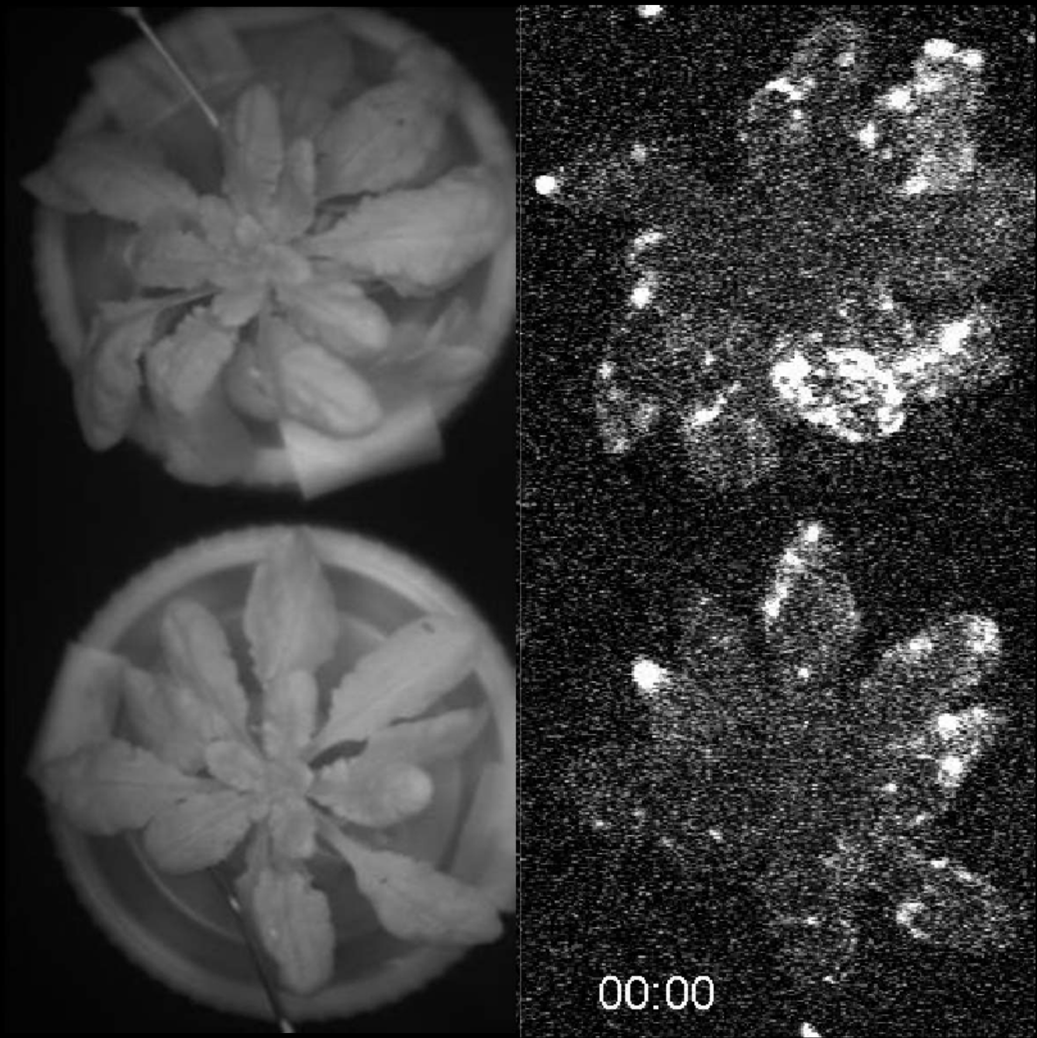


Spatial and temporal waves of CPK gene expression

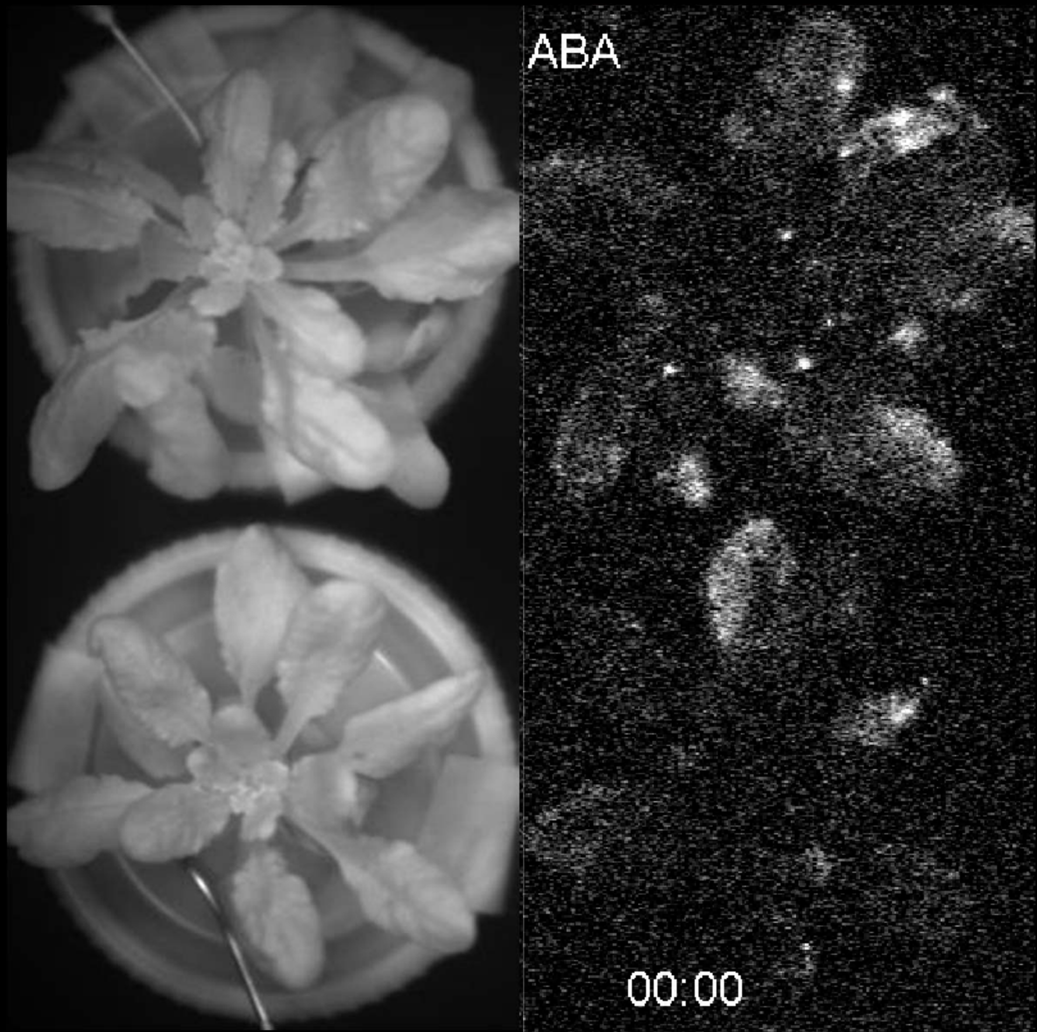
pCPK::Luciferase



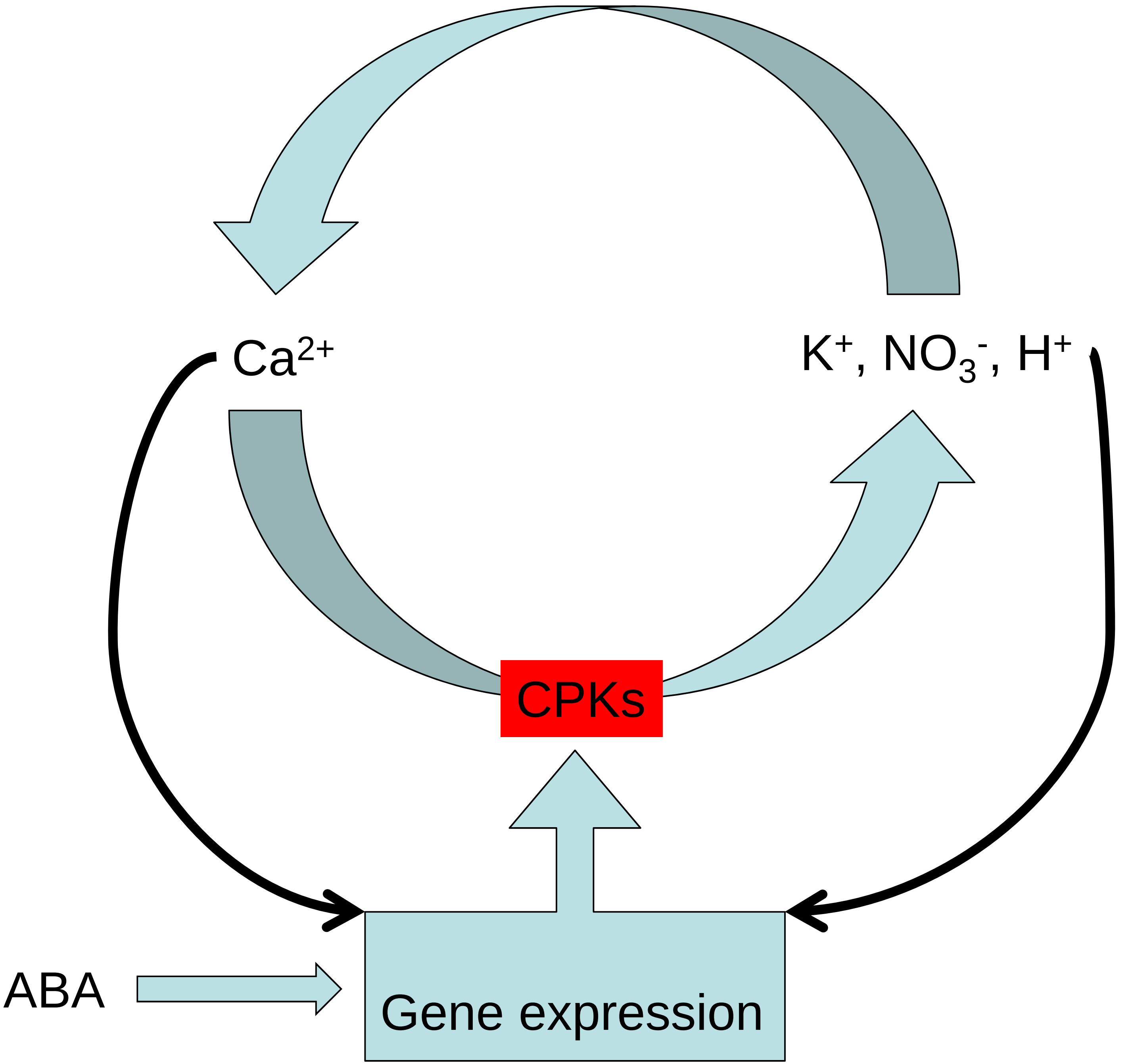
Dark induces Ca^{2+} spark in intact plant leaves



ABA application on roots stimulates Ca^{2+} spark in intact plant leaves



Current model



Perspectives

Identification of active phosphorylation site

Ca2+ signals/waves in *cpk* mutant (Ox and KO)

Ca2+ homeostasis modeling

Identification of CPKs' substrates (H^+-ATPase, etc.)