

WORKSHOP 1

ON REACTIVE OXYGEN SPECIES

**By
Florence
&
Lionel**

Sète 2019

BPMP

Brainstorming

A ROS/RNS OVERVIEW

- Reactive oxygen and other oxide species, localization, detection

4 BPMP RESEARCH STORIES WITH ROS

- An overview of some research projects dealing with ROS at BPMP

EXPECTATION FROM THE AUDIENCE

- Questions, suggestions, exchange of experience, ...

HOW IMPROVING A ROS COMMUNITY

- All ideas that could improve development of tools and expertise

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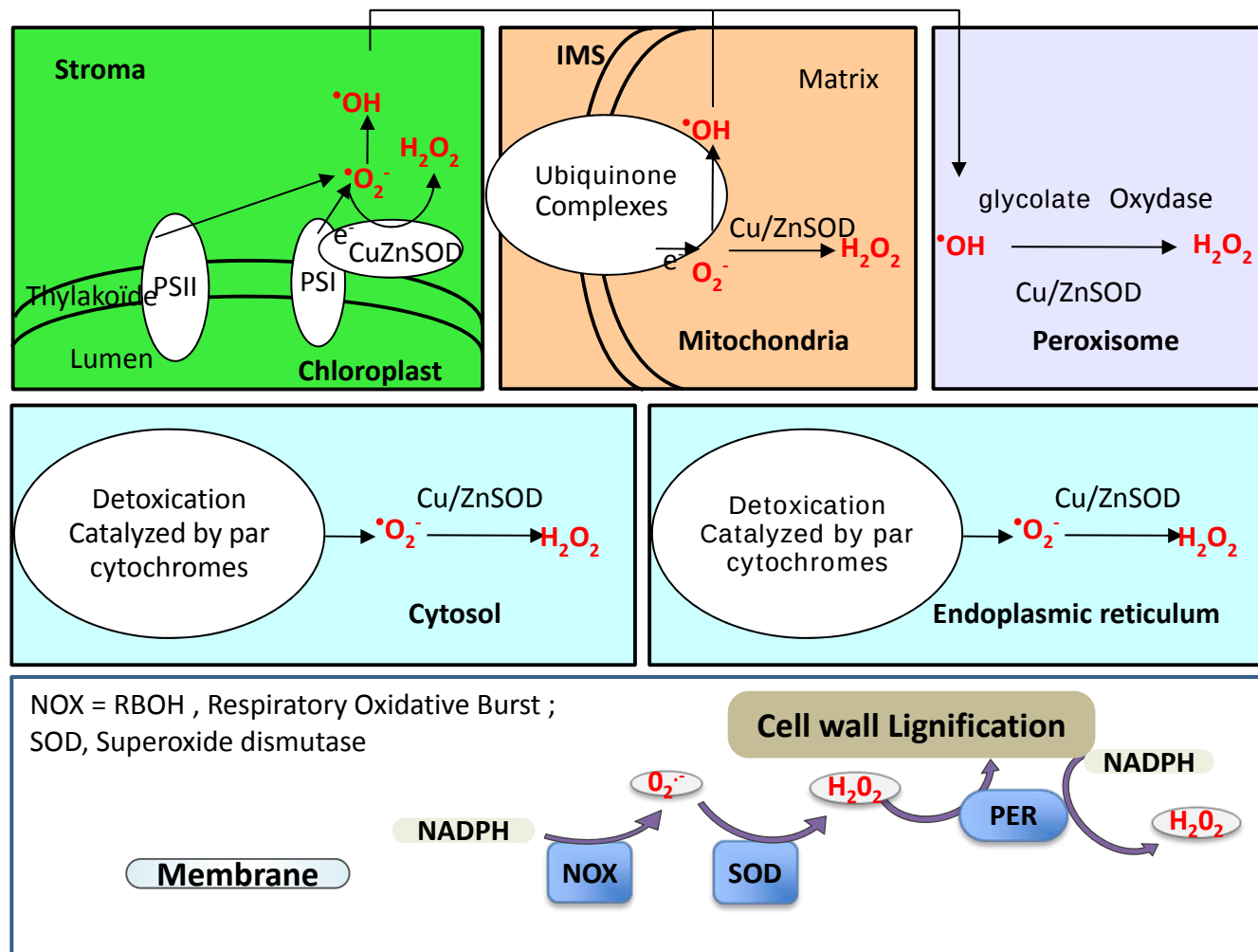
ON

REACTIVE OXYGEN SPECIES

A ROS/RNS OVERVIEW

➤ ROS/RNS are produced by the basal metabolism of the cell

Endogenous generation of ROS/RNS is essential for correct adaptation and signaling in normal cellular functioning including stress conditions



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A ROS/RNS OVERVIEW

➤ ROS/RNS include a panel of molecules with or without radicals... a lot of them being undetectable

Reactive oxygen species (detectable)	
Radicals Superoxide $O_2^{\bullet-}$ Hydroxyl radical $OH^{\bullet}$ Peroxyl $RO_2^{\bullet}$ Alcoxyl $RO^{\bullet}$ Hydroperoxyl $HO_2^{\bullet}$	Non radicaux Hydrogen peroxide H_2O_2 Acide hypochloreux $HOCl$ Ozone O_3 Oxygène singulet O_2 Acide hypobromeux $HOBr$
Reactive nitric oxide species (detectable)	
Radicals Nitric oxide $NO^{\bullet}$ Nitrogen dioxide $NO_2^{\bullet}$	Non radicaux Nitrous acid HNO_2 Cation nitrosylé NO^+ Anion nitrosylé NO^- Tétroxyde de dinitrogène N_2O_4 Trioxyde de dinitrogène N_2O_3 Peroxynitrite $ONOO^{\bullet}$ Cation nitronium NO_2^+ Chlorure de nitryle NO_2Cl Alkyl peroxyxynitrate $ROONO^{\bullet}$ Anion nitroxyle $NO^{\bullet}$

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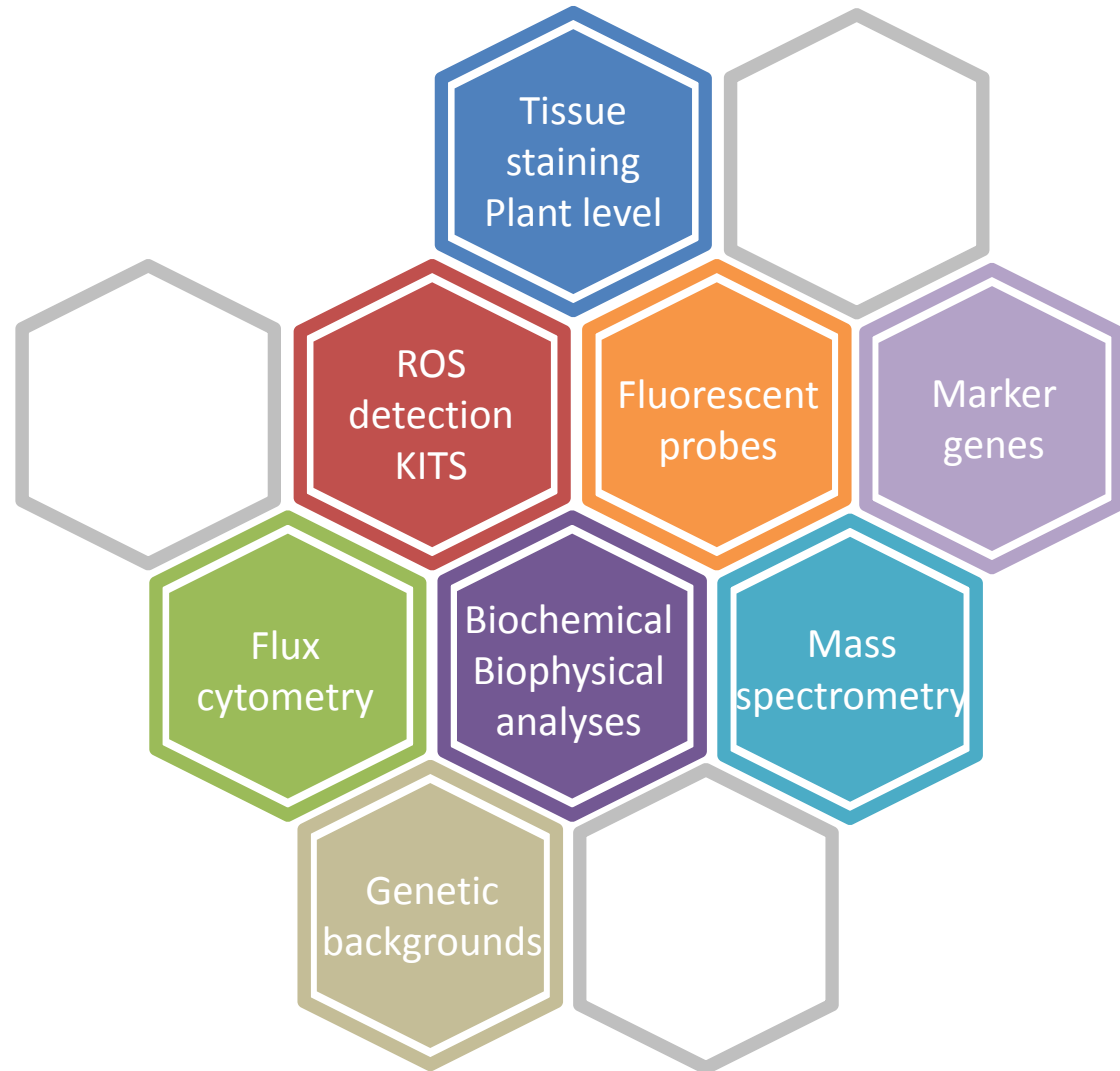
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A ROS/RNS OVERVIEW

➤ **How to detect oxygen species, in cells or in acellular systems**



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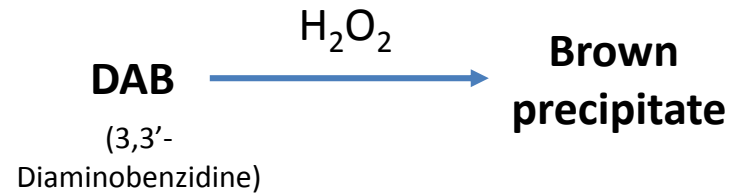
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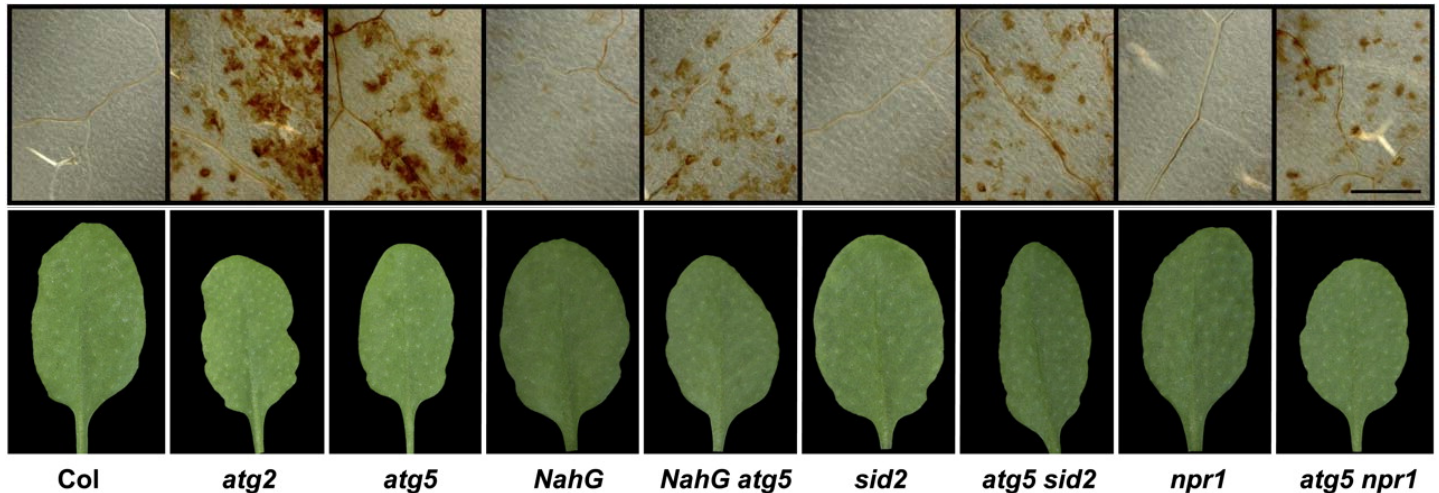
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A ROS/RNS OVERVIEW

➤ How to detect oxygen species, in cells or in acellular systems



Diffuses into tissues where it precipitates into a brownish compound upon oxidation



Reacts mostly with H_2O_2
May diffuse to surrounding tissues

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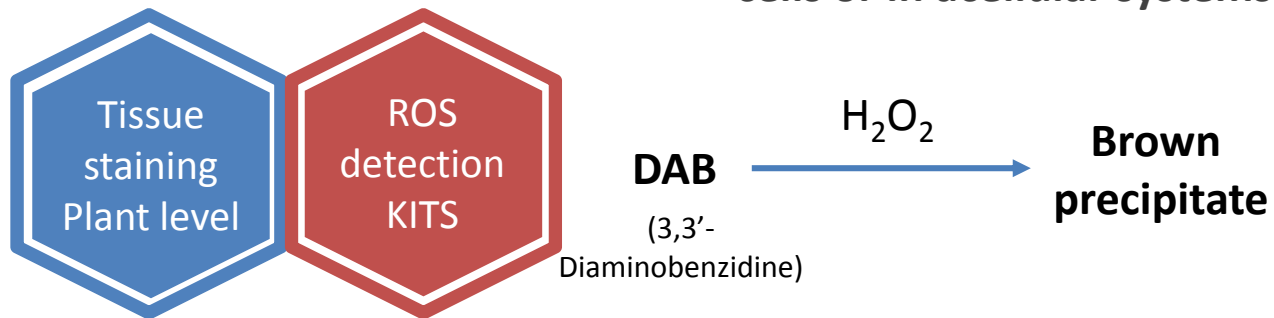
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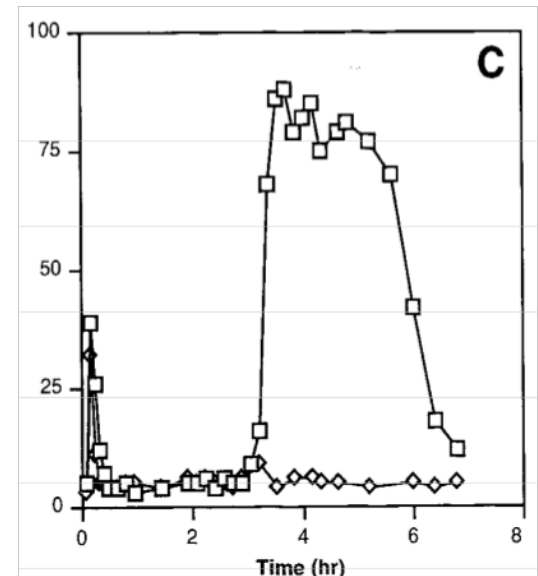
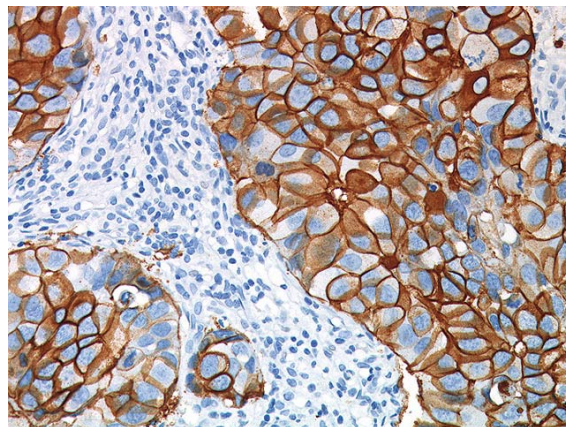
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A ROS/RNS OVERVIEW

➤ How to detect oxygen species, in cells or in acellular systems



Can be combined with histochemistry on peroxidase-treated tissues for recording peroxidase activity



May diffuse to surrounding tissues

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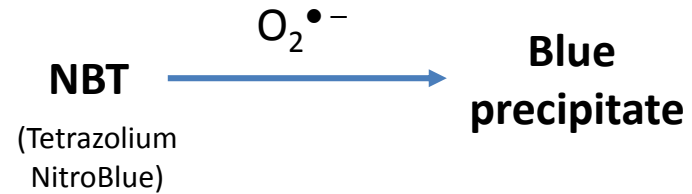
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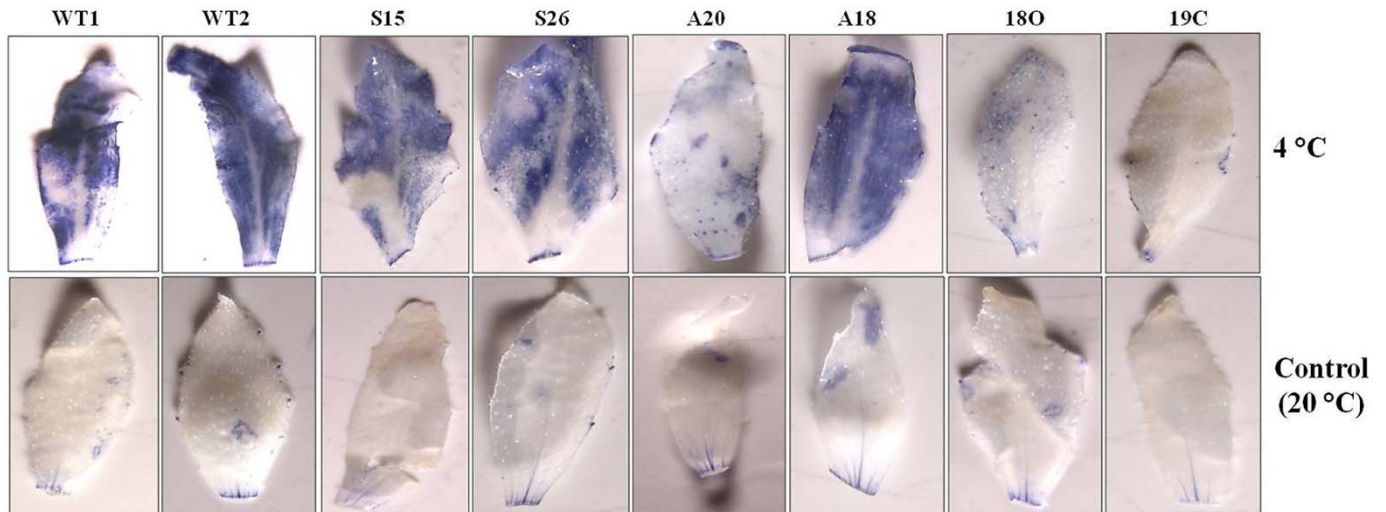
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A ROS/RNS OVERVIEW

➤ How to detect oxygen species, in cells or in acellular systems



Diffuses into tissues where it precipitates into a blue compound in the presence of superoxide anion



May diffuse to surrounding tissues

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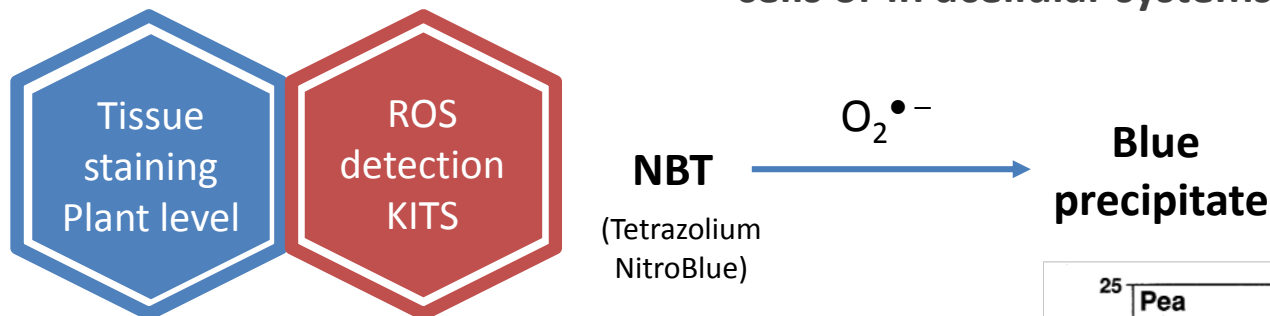
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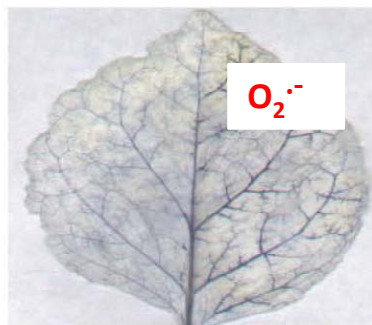
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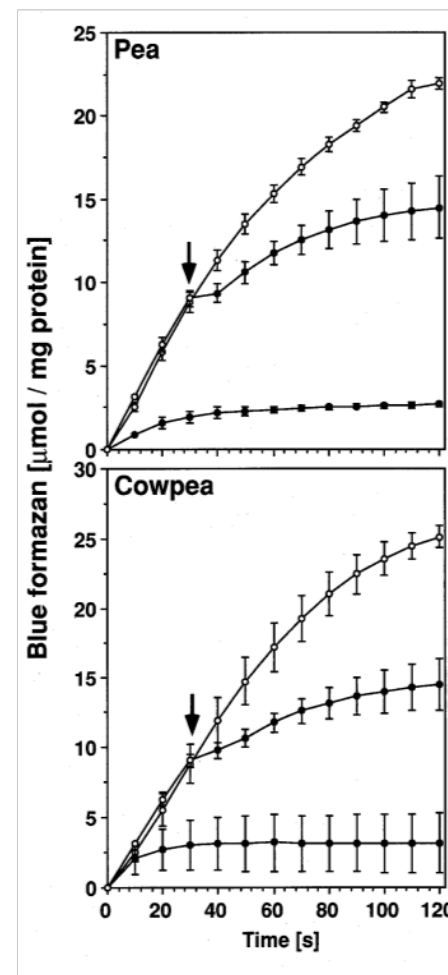
➤ How to detect oxygen species, in cells or in acellular systems



Can be used combined with kits



May diffuse to surrounding tissues



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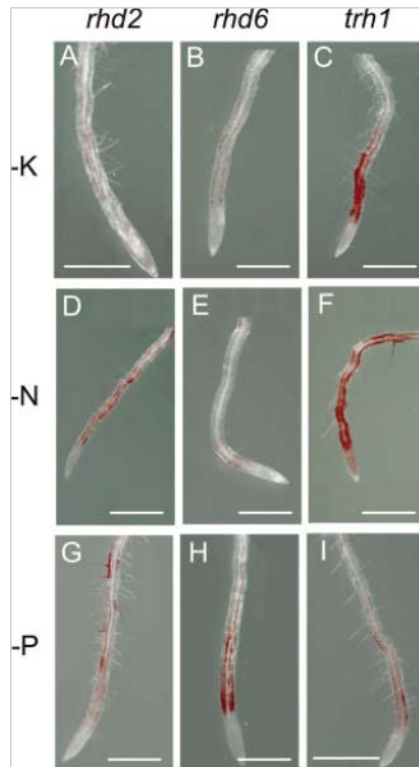
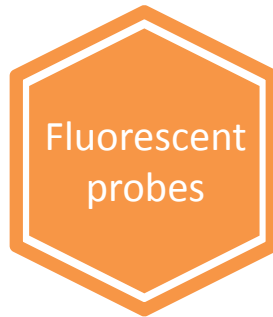
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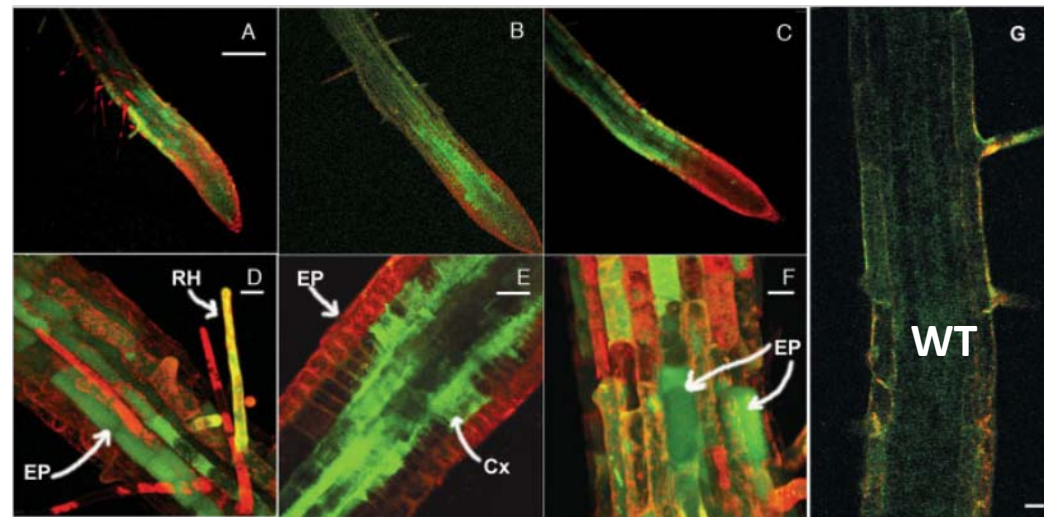
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A ROS/RNS OVERVIEW

➤ How to detect oxygen species, in cells or in acellular systems



Confocal projection of nutrient deprived Arabidopsis roots after incubation in CM-H2DCFDA (pseudo Green color) and with MitoTracker (red)



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Nikon SMZ1500 dissecting microscope (460–500 nm bandpass excitation. 510–560 nm bandpass emission)

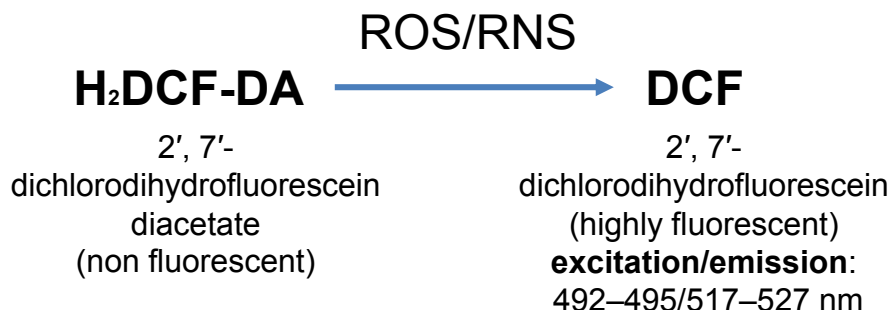
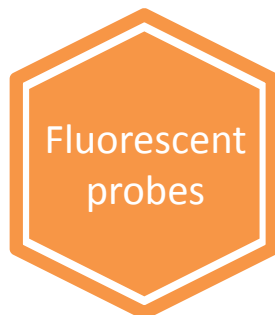
Shin et al. Plant Cell Physiol (2005)

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A ROS/RNS OVERVIEW

➤ How to detect oxygen species, in cells or in acellular systems



Diffuses into cells and is retained in the intracellular level after cleavage by intracellular esterases.



The chloromethyl derivative (CM-H₂DCFDA) provides even much better retention in live cells than H₂DCFDA.



Best used in combination with Propidium iodide (labels cell walls but penetrates also in dead cells that may produce ROS)



React with several ROS including hydroxyl radicals and peroxynitrite (HO[•], ROO[•], ONOO[•]) but also with H₂O₂

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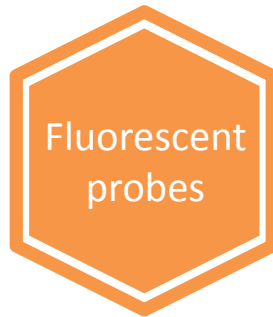
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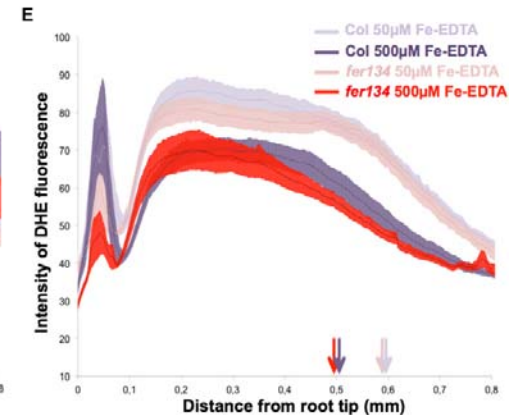
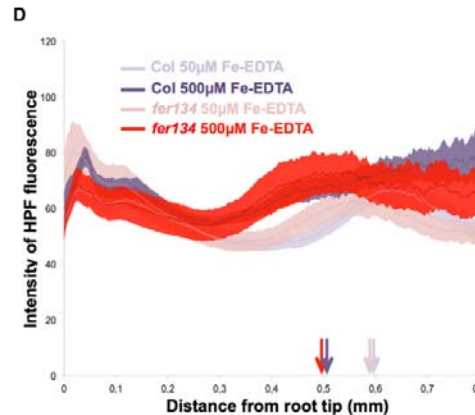
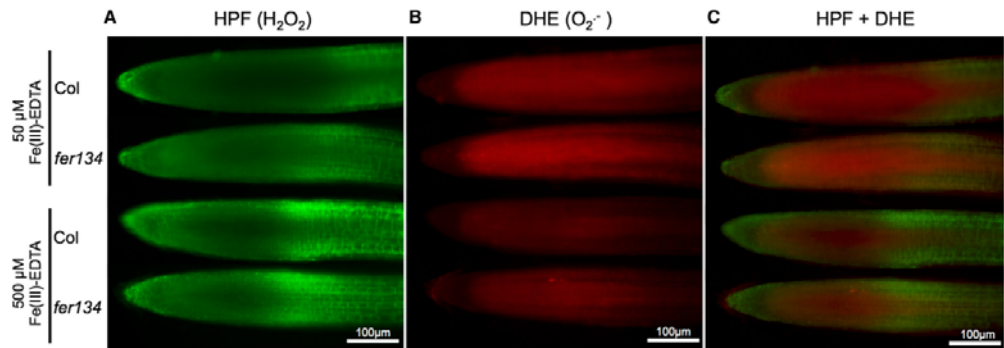
A ROS/RNS OVERVIEW

➤ How to detect oxygen species, in cells or in acellular systems



DHE (Dihydroethidium) $\xrightarrow{\text{Superoxide}}$ **2-hydroxy Ethidium**

HPF
(30-(p-hydroxyphenyl) fluorescein) $\xrightarrow{\text{H}_2\text{O}_2}$ **2-hydroxy Ethidium**



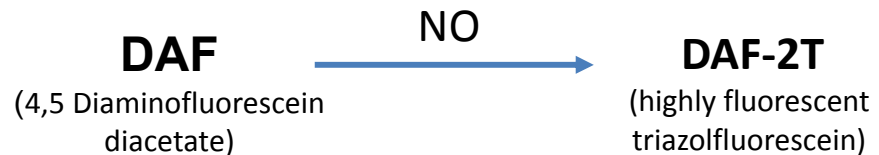
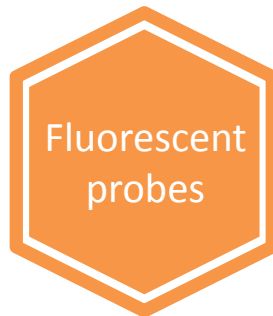
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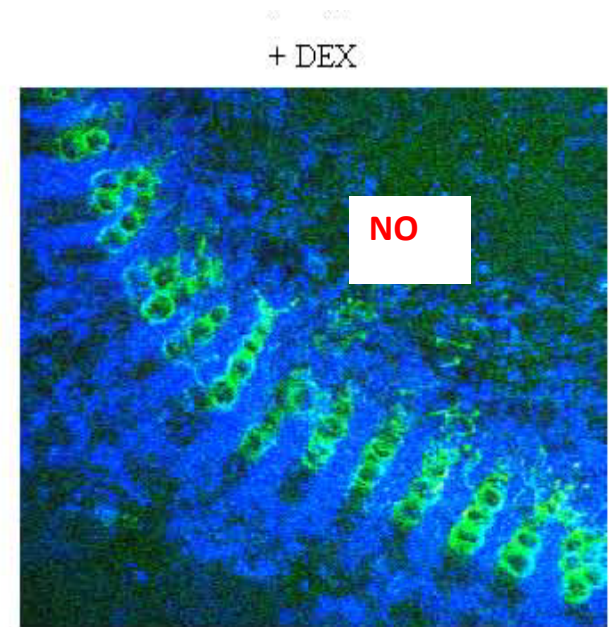
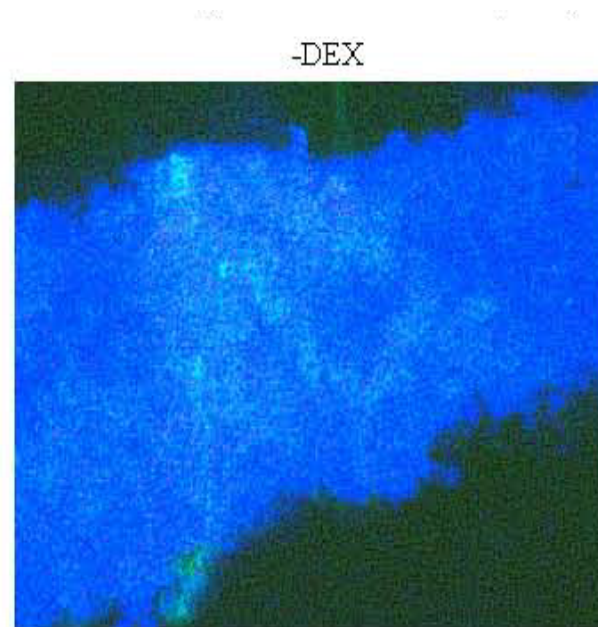
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A ROS/RNS OVERVIEW

➤ How to detect oxygen species, in cells or in acellular systems



Main constraint of nitric oxide:
half life < 5s ; [NO] within cells is very low (detection limit for NO is 2-5 nM)



DEX inducible::AvrPto (overexpression in N Benthamiana)

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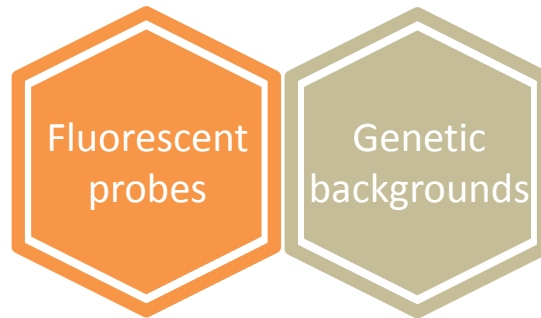
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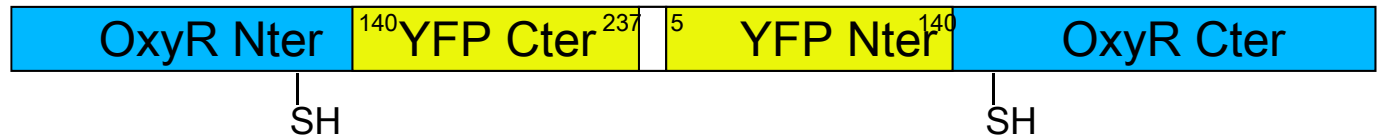
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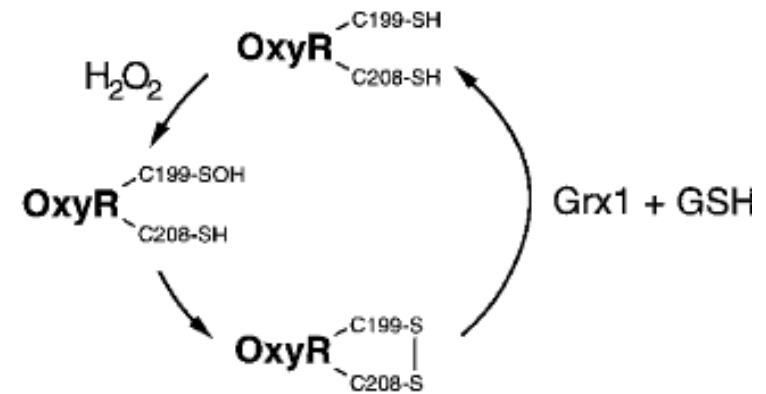
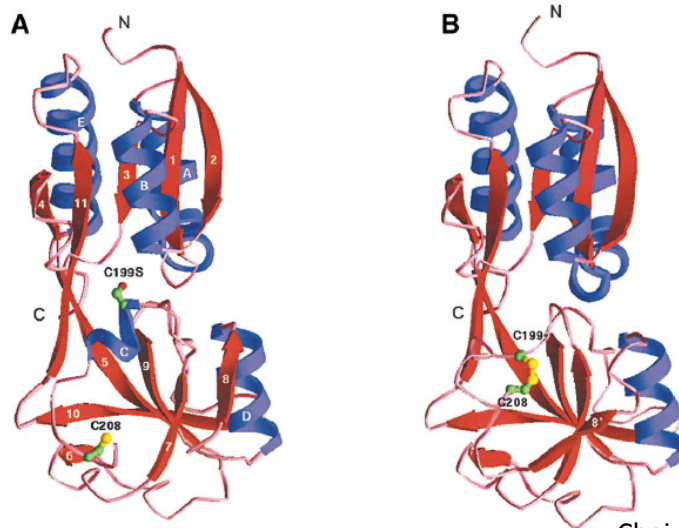
➤ How to detect oxygen species, in cells or in acellular systems



HyPer: a genetic probe for specific detection of cytoplasmic H_2O_2



The circularly permuted YFP chromophore can be refolded upon disulfide bridge formation of the two Cys residues of OxyR.



Choi *et al.*, Cell (2001)

Zheng *et al.*, Science (1998)

Figure 1. Structure of the OxyR Regulatory Domain

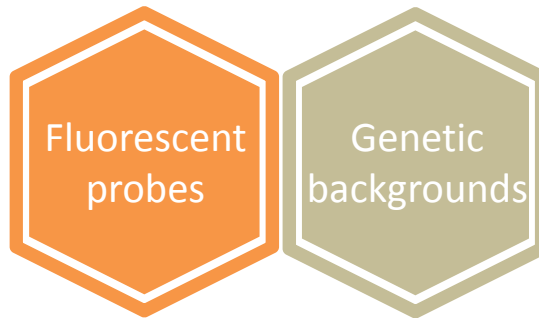
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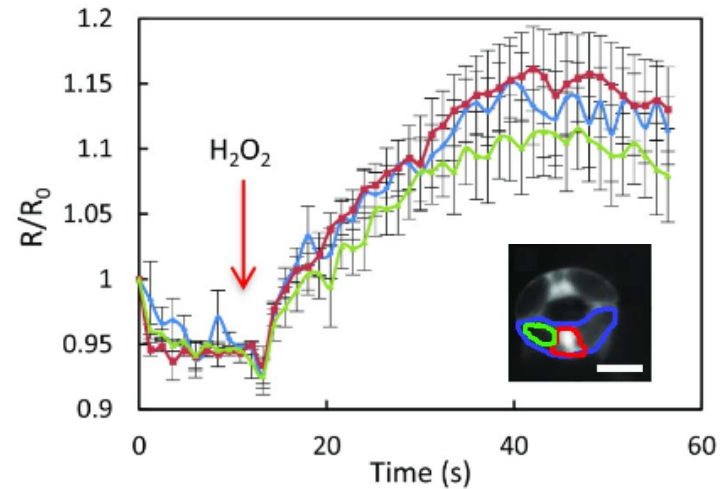
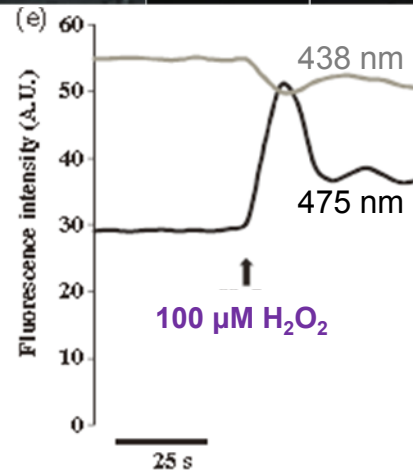
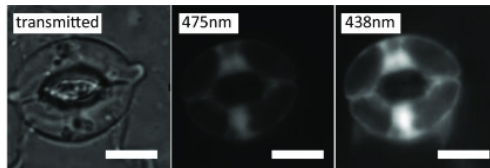
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HyPer: a genetic probe for specific detection of cytoplasmic H_2O_2



Rodrigues *et al.* (2017)

The fluorescence ratio change, here presented as $\Delta R/R_0$, is proportional to the amount of exogenously applied H_2O_2

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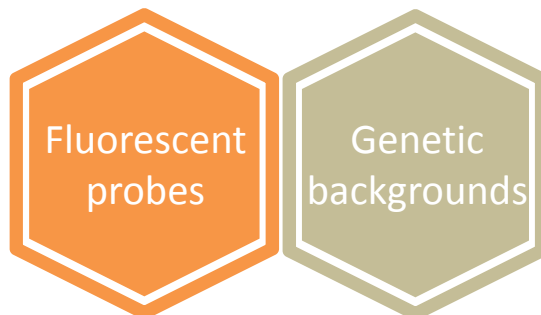
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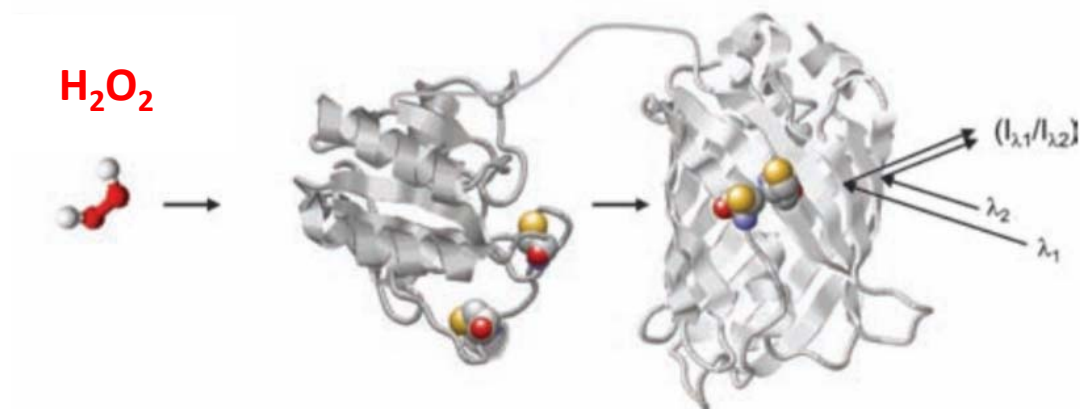
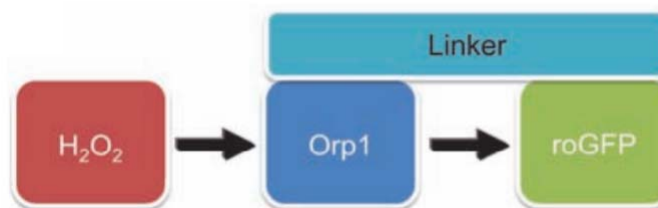
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Orp1-roGFP2:
 H_2O_2 -specific genetic probe



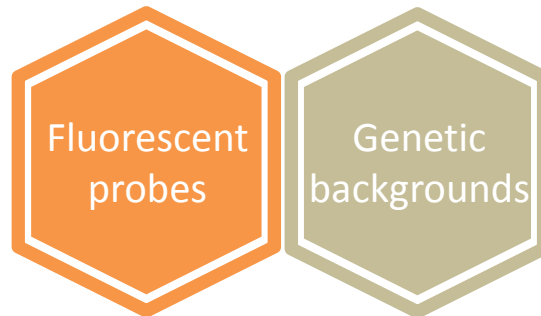
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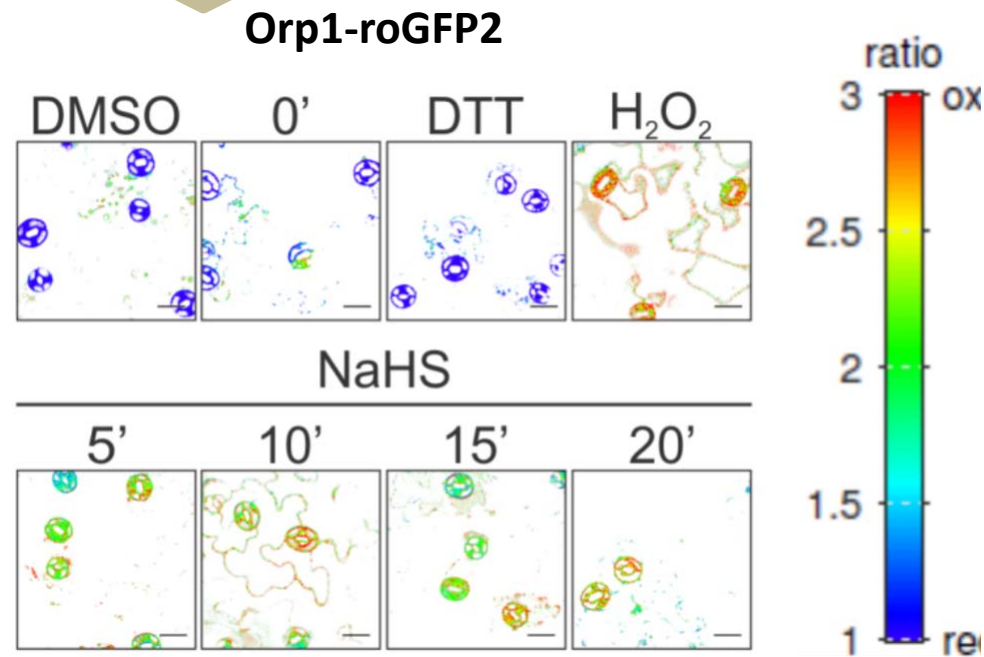
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Orp1-roGFP2:
 H_2O_2 -specific genetic probe



Scuffi *et al.* (2018)

Here again, the fluorescence ratio change is proportional to cytosolic H_2O_2 concentration

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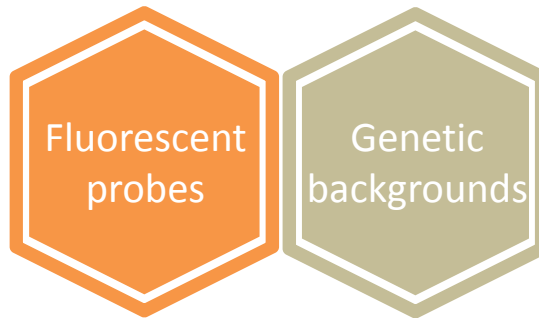
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Orp1-roGFP2:
H₂O₂-specific genetic probe



Highly H₂O₂-specific probes



Allows ratiometric measurements of H₂O₂ concentrations



Generation of biological samples takes more time than using chemical probes



HyPer is a pH-sensitive fluorescent probe

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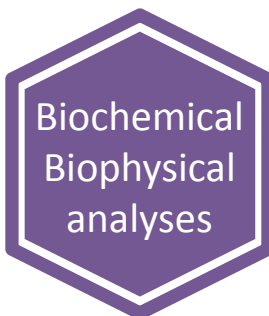
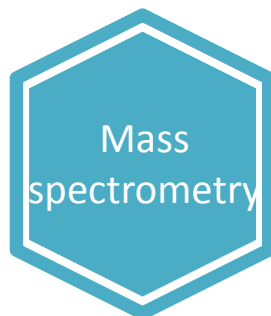
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A ROS/RNS OVERVIEW ➤ Redox-based modifications of proteins



Redox-based PTMs :

Dynamic and versatile means to rapidly alter the activity or functional structure of proteins, including key regulators of other PTMs, such as phosphorylation, acetylation, ubiquitination, etc...

TABLE 1 | Common ROS/RNS induced modifications.

Common ROS/RNS modification	Δ Mass	Selective reduction	Probes/Antibody
Disulfide bond formation (S-S-)	2	Thioredoxin system	Directly by MS
Glutathionylation (S-S-G)	305.3	Glutaredoxin system	BioGEE, Anti-PSSG
Nitrosylation (SNO)	28.99	Cu/Ascorbate	Anti-SNO
Sulfenylation (SOH)	15.99	Sodium Arsenite	Dimedone based
Sulfinic acid (SO ₂ H)	31.99	Sulfiredoxin*	NO-Bio
Sulfonic acid (SO ₃ H)	47.99	–	Directly by MS
3-Nitrotyrosine	44.98	Sodium dithionite	Anti-3NT
Carbonylation (C=O)	**		Hydrazide chemistry

Adapted from McDonagh, July 2017, Frontiers in Physiology

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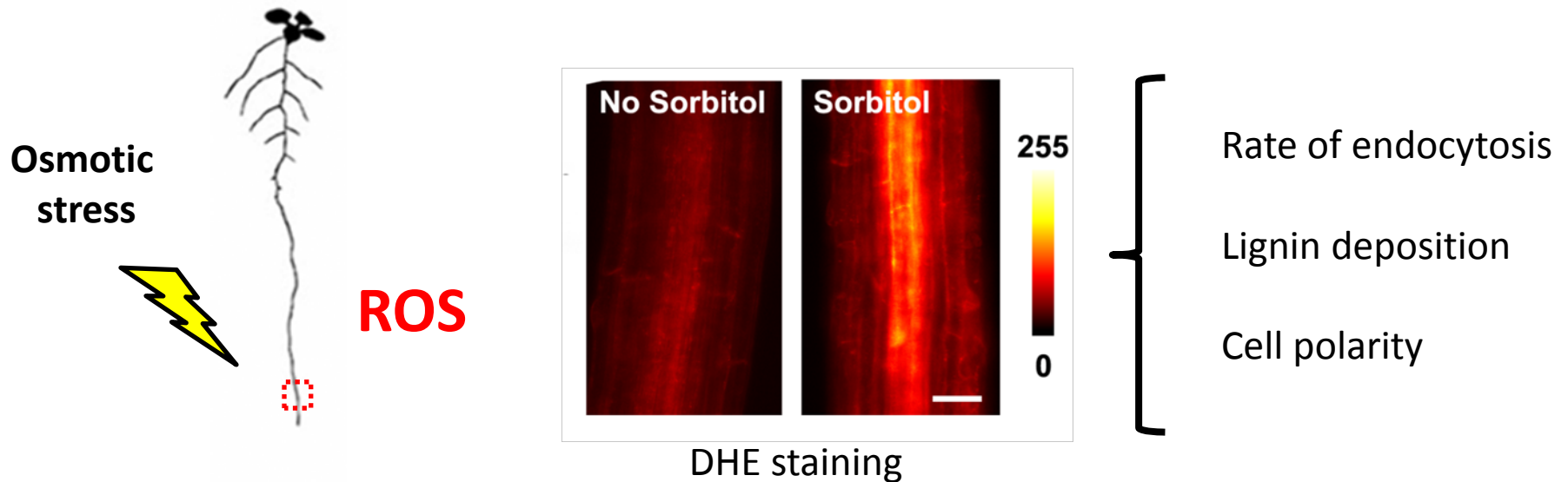
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A contribution of Alexandre Martinière



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Identification of new regulators of ROS accumulation

- Candidate genes
- GWAS

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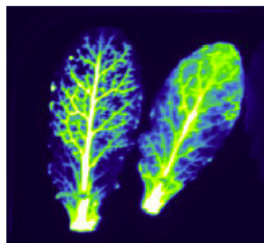
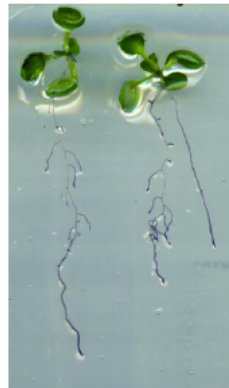
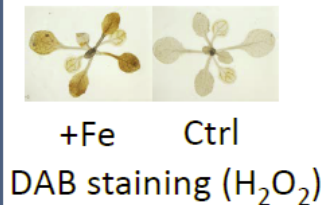
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A contribution of Tou Cheu Xiong

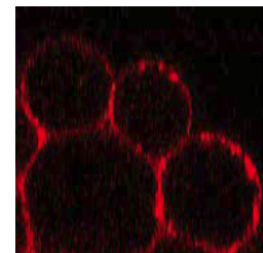
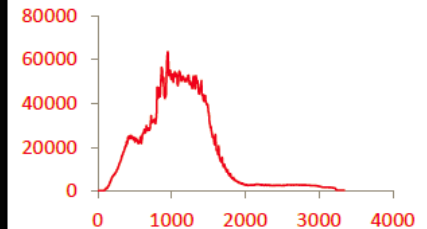
Long distance signalling /ROS

rbohF

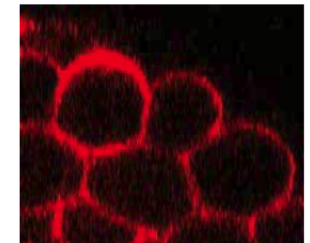


➤ An overview of some research projects dealing with ROS at BPMP

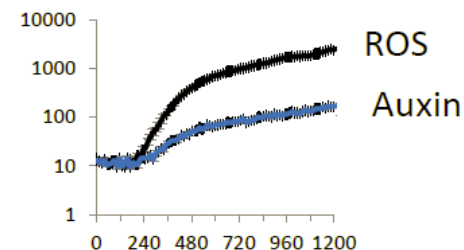
ROS/Fe deficiency/root



-Fe



ROS/Seed germination



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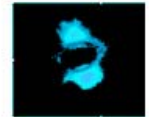
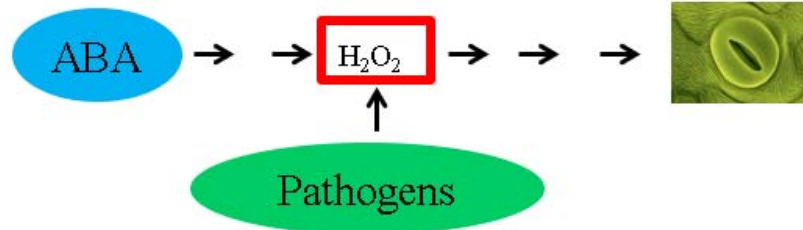
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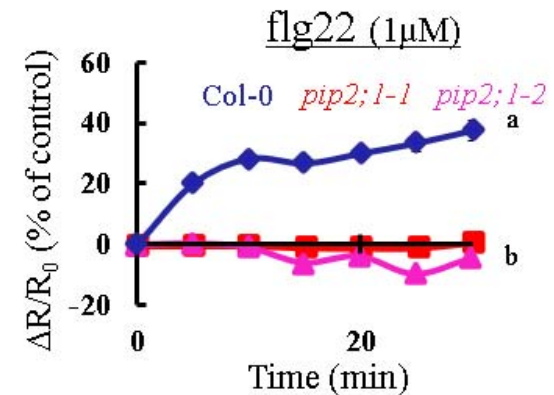
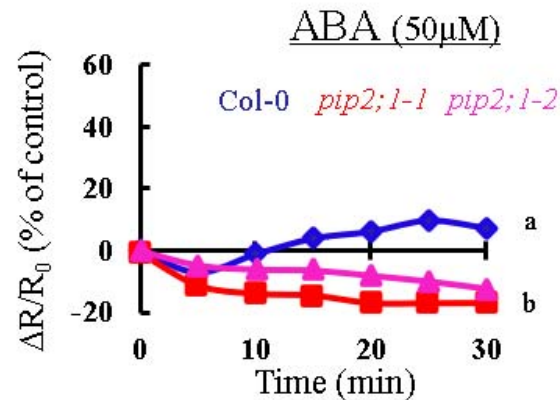
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A contribution of Lionel Verdoucq



HyPer imaging



PIP2;1 contributes to the accumulation of H₂O₂ induced by ABA and flg22 in guard cells

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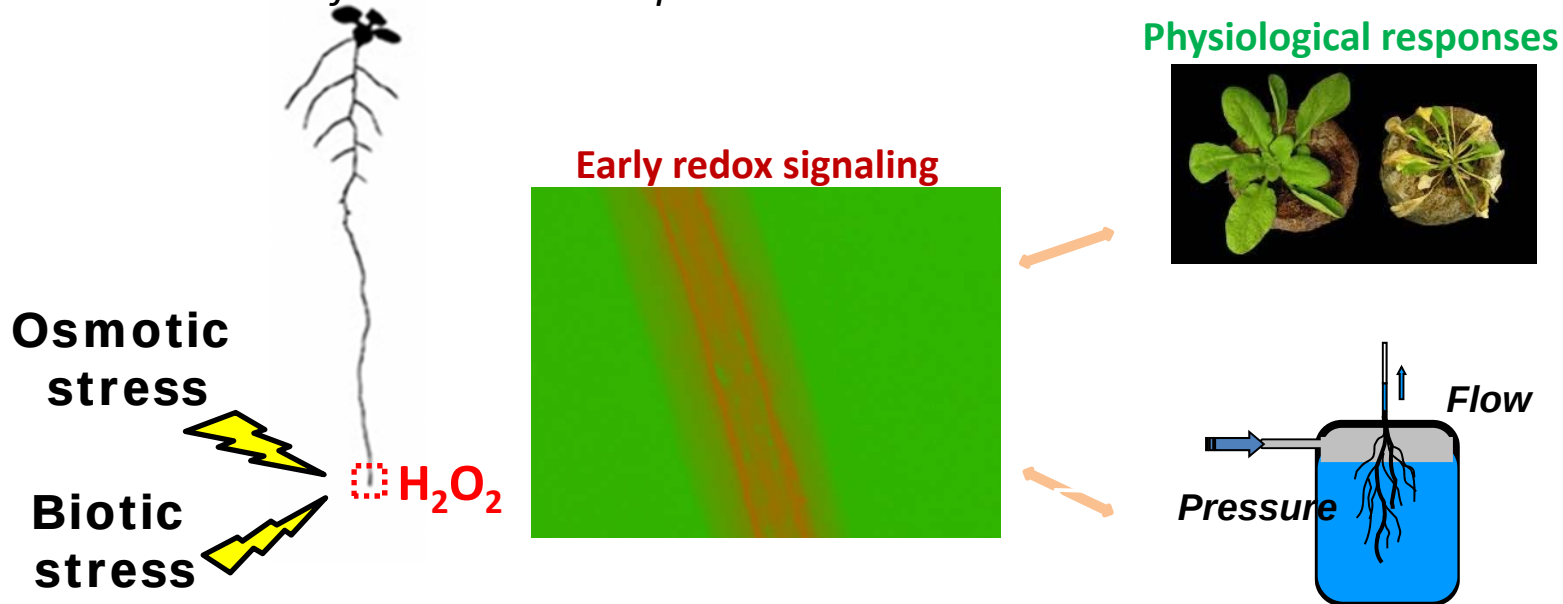
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A contribution of Lionel Verdoucq



Importance of H_2O_2 signature in combined stresses

- ✓ Kinetics of H_2O_2 accumulation in altered genetic backgrounds (AQP, redox homeostasis) using biosensors
- ✓ Phenotypal characterization (pathogenicity, hydraulic conductivity)

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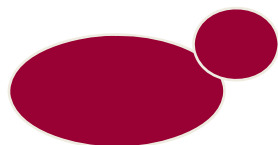
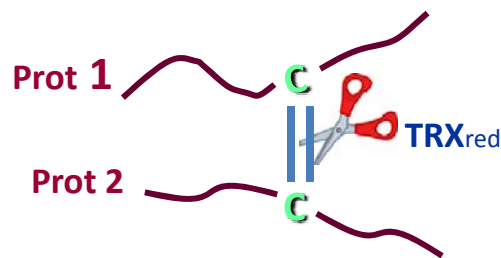
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A contribution of Florence Vignols

A Yeast Two-hybrid Strain To Reveal Labile Protein-protein Interactions Mediated By Disulfide Bonds

Proteins exhibiting active CXXC redox centers with reducing activity within cells (TRX, GRX) are **major constraints for disulfide bond-mediated PPIs**:

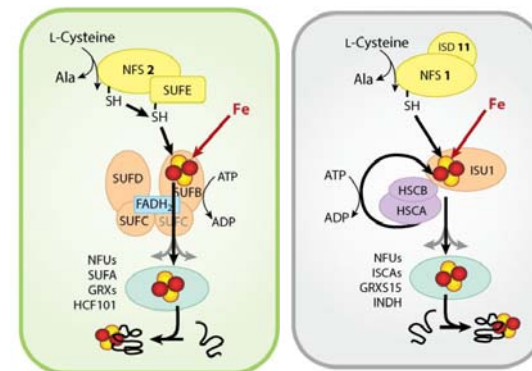


Gal4 reporter, HIS3, *trx1Δ*, *trx2Δ*

CY306, a Gal4-reporter system based yeast strain, depleted of ScTRX1 and ScTRX2

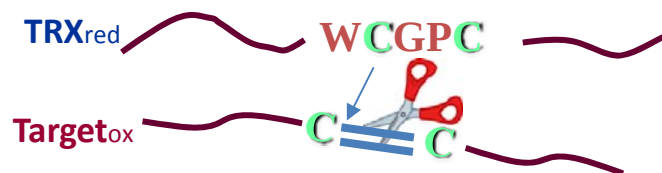
➤ No more cytosolic thioredoxins

Iron-sulfur assembly machineries



➤ Extensively used for PPI studies involving FeS- or Zn-binding proteins

➤ Search for redoxin targets



Sète 2019

BPMP

Brainstorming

WORKSHOP 1

ON REACTIVE OXYGEN SPECIES

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Brainstorming

A ROS/RNS OVERVIEW

- Reactive oxygen and other oxide species, localization, detection

4 BPMP RESEARCH STORIES WITH ROS

- An overview of some research projects dealing with ROS at BPMP

EXPECTATION FROM THE AUDIENCE

- Questions, suggestions, exchange of experience, ...

HOW IMPROVING A ROS COMMUNITY

- All ideas that could improve development of tools and expertise

WORKSHOP 1
ON
REACTIVE
OXYGEN
SPECIES

**HOW IMPROVING A
ROS COMMUNITY**

➤ All ideas that could improve
development of tools and expertise

B&PMP seminar on
ROS SIGNALLING in PLANTS
Thursday 12th of April 2018
cancelled for strike reasons, To recall in 2019 ?

Andreas J. MEYER, Chemical Signalling Lab, INES Institute, Bonn, Germany
Jaakko J. KANGASJÄRVI, Viikki Plant Science Center, Univ. of Helsinki, Finland

A local B&PMP “Wine/Beer-accompanied
workshop ?
How many people interested in ?
Once a year? Twice a year? More?...

A Mistral-like (even
shorter) training on
ROS utilization &
detection ?

What else?...

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WORKSHOP 1 ON REACTIVE OXYGEN SPECIES

4 BPMP RESEARCH STORIES WITH ROS

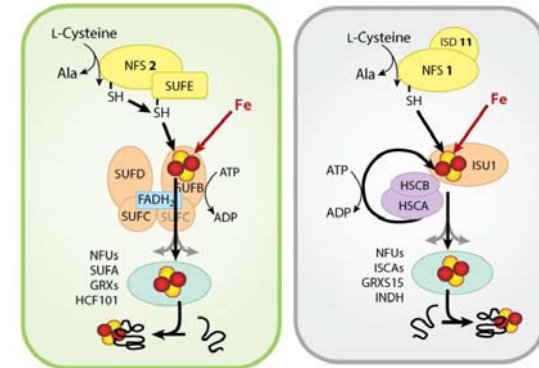
➤ How BPMP “ROS” projects connect to national & international research

➤ Redox regulations of key components in FeS assembly, FeS protein interactomes

ANR FIRES 2011-2013 & Fe-S TRAFFIC 2014-2016, still on-going

National Coll°: INRA Nancy, CEA Cadarache, LGDP Perpignan, CBS Montpellier, CBS Montpellier

International Coll°: University Texas (US), John Innes Institute (UK), Georg-August-University (D)



➤ [OsmoPathOx](#) : OSMOtIC and PATHogen crosstalks in roots: role of redOX signaling (Projet Lionel / JP Reichheld / Fabienne Vailleau)

AAP ANR 2019, BPMP, LGDP Perpignan, LIPM Toulouse

InterLabEx TULIP/Agro, on-going

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