

New Alternatives to Chemical Pesticides: Deciphering the Action Mechanisms of Lipid based Plant Elicitors via Complementary Biophysical and Biological Approaches

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An **elicitor** may be defined as any molecule produced by pathogenic or non-pathogenic microorganisms or by host plants (synthesizing or accumulating the molecule) capable of **inducing** or **promoting** the **natural resistance** of plants to one or more pathogens, which resistance may be **local** or **systemic**.



Priming: defense responses are not activated directly by the priming agent, but are accelerated following perception of biotic or abiotic stress signals, resulting in an enhanced level of resistance.

Pieterse & Dicke., Trends Plant Sci., 2007, 12, 564.



Primed plant

Corné & Dicke, Trends Plant Sci., 2007, 12, 564



Pathogen



www.123rf.com

Dogma = elicitor recognition mechanism is based on the involvement of membrane protein receptors

Our hypothesis : the lipid fraction of plant plasma membrane has a key role during the immune related defense response by some specific lipidic elicitor

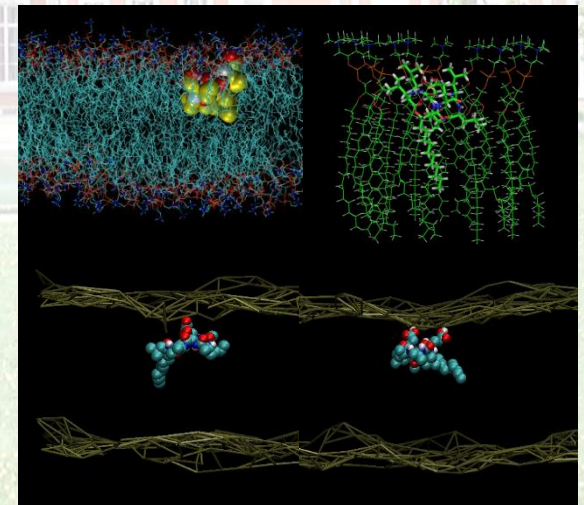
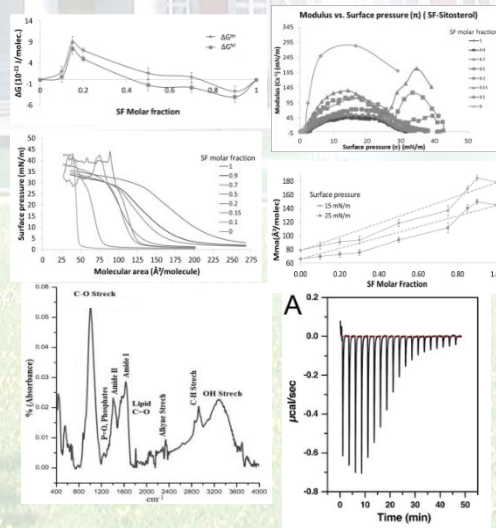
Our strategy :

in silico modelling

Tests *in planta*

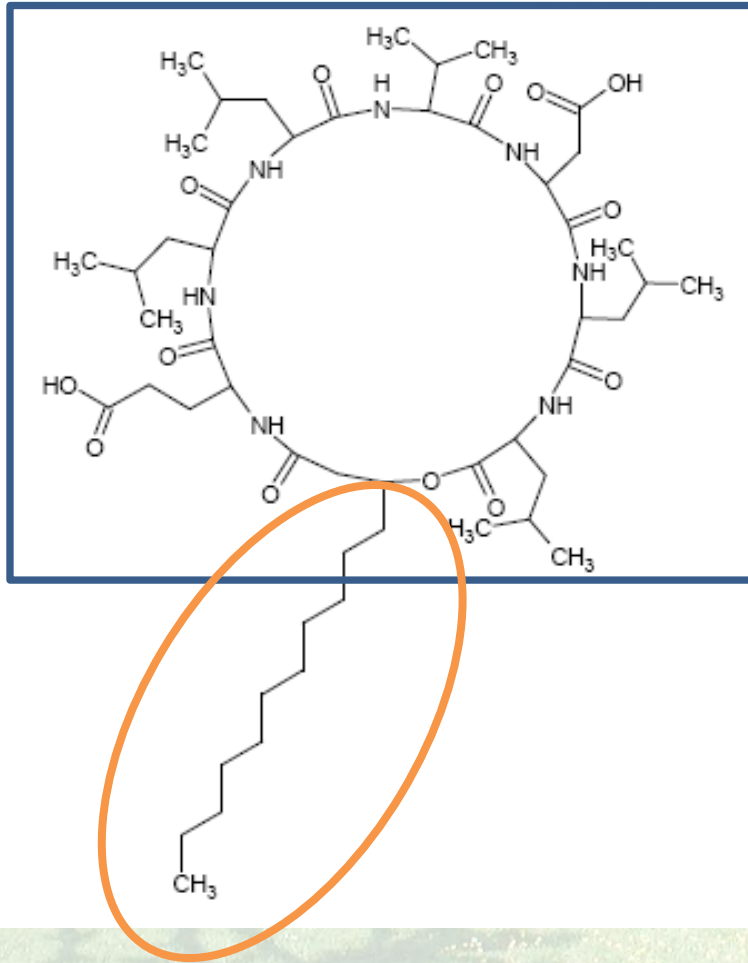


Biophysics



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First example : surfactin

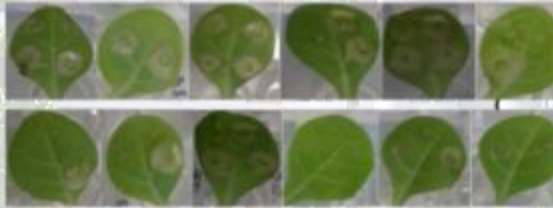


- Produced by *Bacillus* species
- Seven amino acids
- β -hydroxy fatty acid chain (variable length)

Surfactin : biological assays

Eliciting activity demonstrated on many species (rice, cucumber, tomato, tobacco, bean, *Arabidopsis*, ...)

In tobacco – 2/10 μM



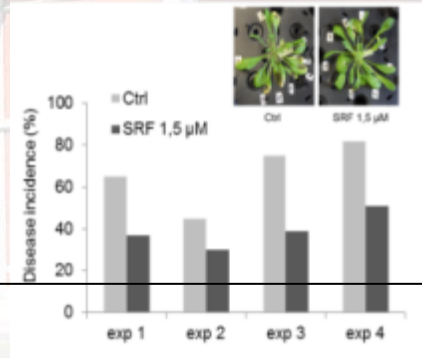
In tomato – 5/10 μM



Control

Treated

In *Arabidopsis* – 1/10 μM



- Stimulates early defense events at micromolar levels
- Does not cause fitness cost (No growth inhibition)
- Only slight induction of defense gene expression if no infection
- Still active after treating plant cells with protease
- Still active after a first elicitation (no saturation)
- Only active with fatty acid chain > C13

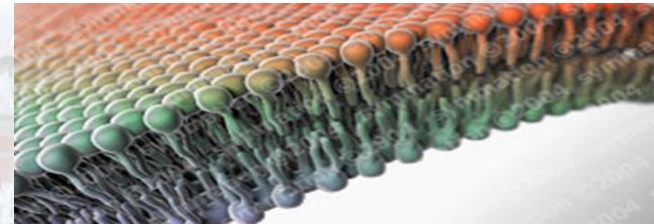
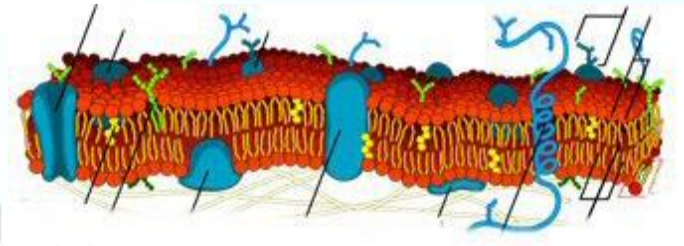
Surfactin : biophysics tools

PPM is a complex edifice.

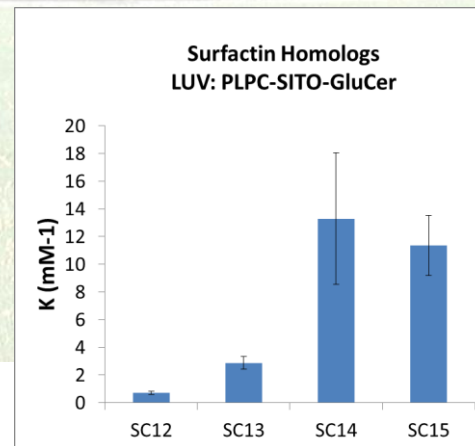
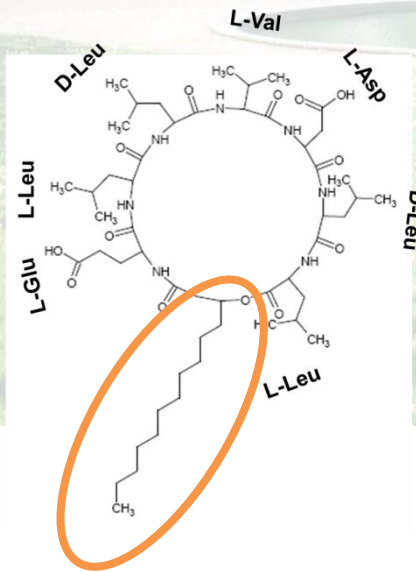
Reconstitution of lipid membranes in biomimetic systems

Isothermal Titration Calorimetry

- To quantify the interactions
- To determine the binding coefficient



Surfactin binds to membranes containing PLPC, sitosterol and GLuCer.



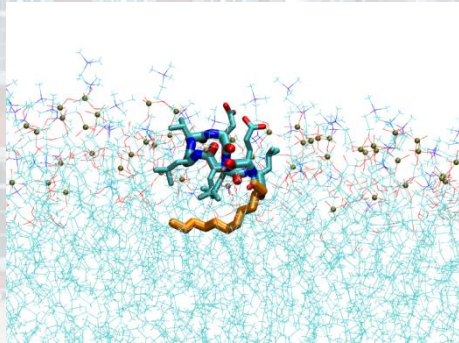
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Surfactin : bio-informatics

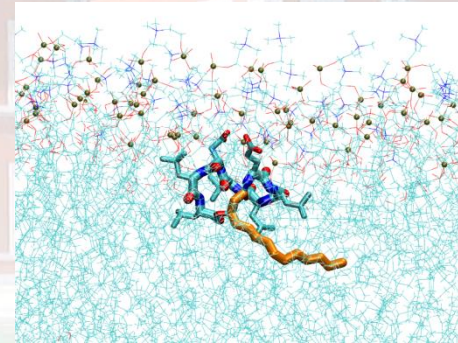
Molecular dynamics

Position and orientation of surfactin homologues within a PLPC bilayers

SC12



SC14



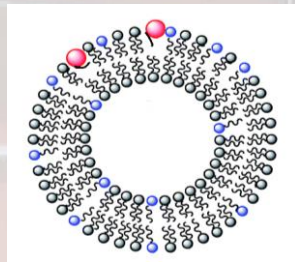
SC14 is more deeply inserted in the bilayer than SC12
SC14 has less freedom of motion

film

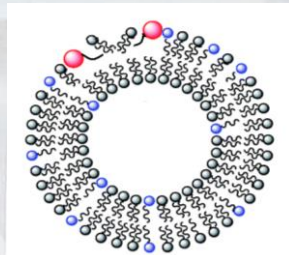
Hypothesis on surfactin mechanism

Differential effect of surfactin homologues

SC12



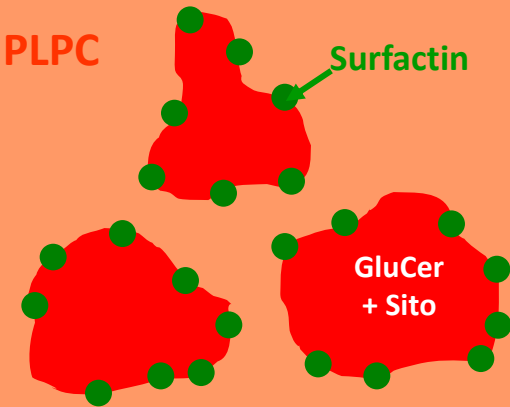
SC14



Destabilizing effect = key parameter

Lipid specificity

PLPC



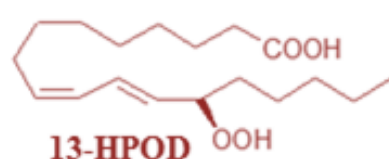
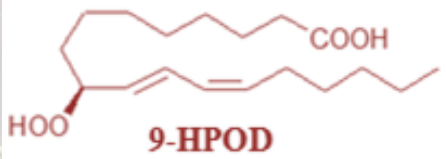
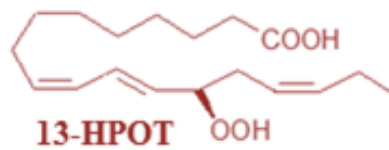
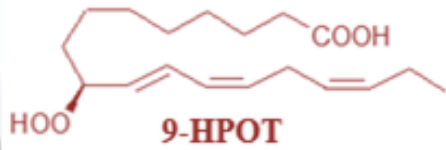
Surfactin elicitation does not require some specific proteic (co)receptor

Modification of the lipid bilayer organization by long fatty acid homologues



« In/out » domain trafficking and subsequent activation of key proteins involved in signalling/defense

Example 2 : HPOD/T



HPOD: in vitro antibacterial effect
 HPOT : antifungal effect
 (Proost et al. 2005)

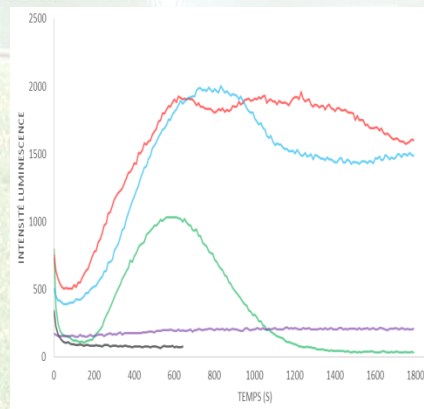
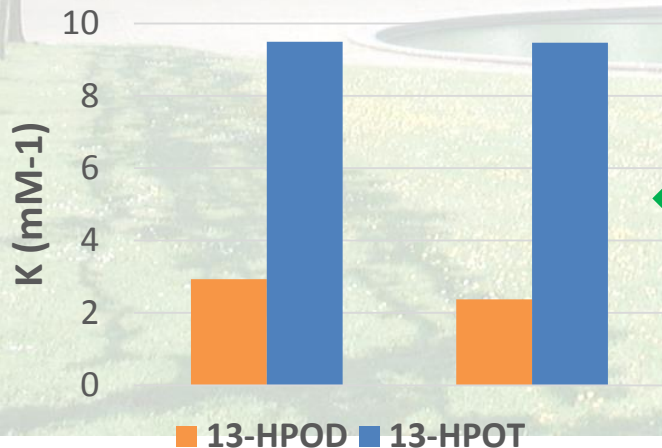


Elicitor effect ?

Role of pathogen PM in
 the mechanism ?

PLPC

PLPC/Gluco



Biomimetic membranes with
 representative pathogen
 plasma membrane lipids

General conclusions : powerful tools to investigate complex mechanisms

_ development of new lipidic elicitors and comprehension of the mechanisms of action

- Prediction of the most active structure based on biophysics → total chemical synthesis, extraction from natural source

- Comprehension of antimicrobial activity and prediction of action spectrum

The FIELD project : Finding Interesting Elicitor Lipids

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Biophysics:

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Biological assays

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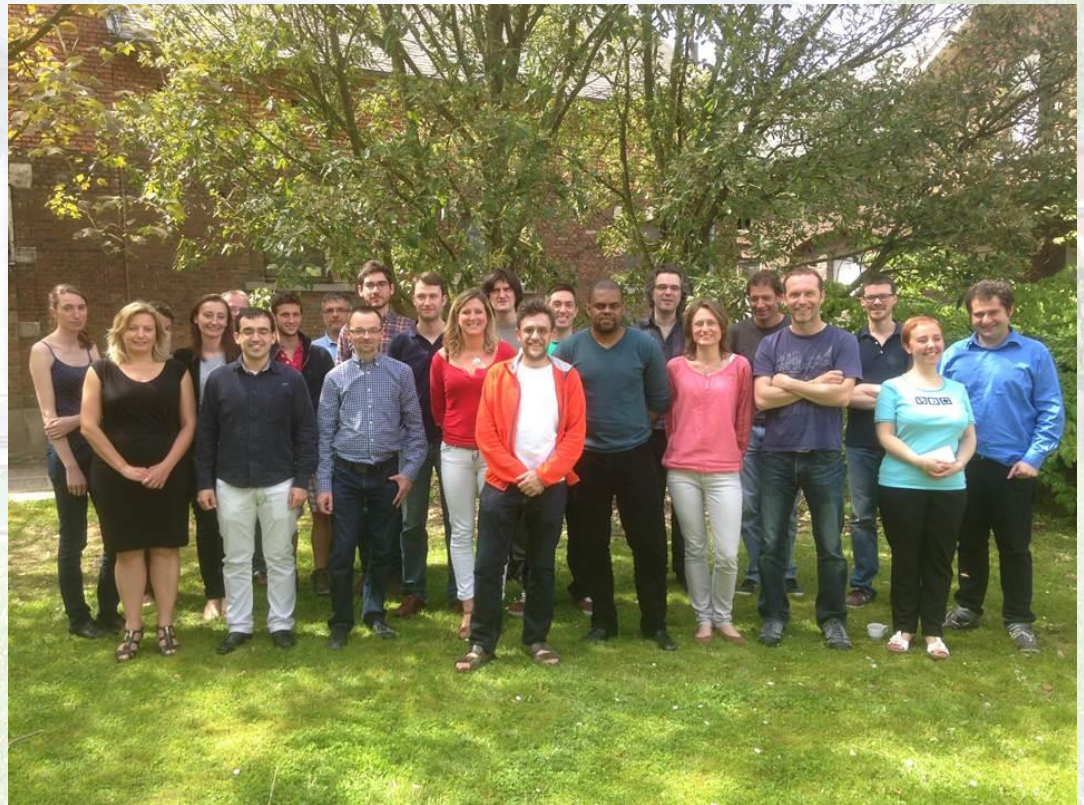
C. Sarazin, Amiens/FR

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Lipid characterization

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Total chemical synthesis : **F. Obounou Akong Gembloux/BE**



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