Complementary biophysical tools to investigate the membrane activities of essential oils

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Fields of use of essential oils

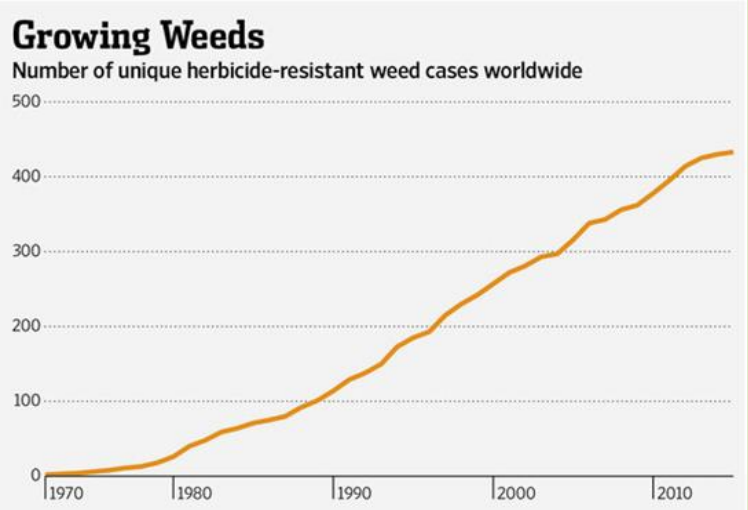
- « Historical applications »
  - Flavoring / preservative agent in food
  - Fragrance in cosmetics
  - Aromatherapy / massage
  - Human and animal health
  - …
- Recent trends in agronomy: bio-based pesticide or biopesticide
  - Antimicrobial agent
  - Antifungal agent
  - Insecticide
  - **Herbicide**

Essential oils as bio-herbicides

Conventional herbicides
- resistance
- impact on environment
- impact of human health

➔ high demand for bio-based herbicides

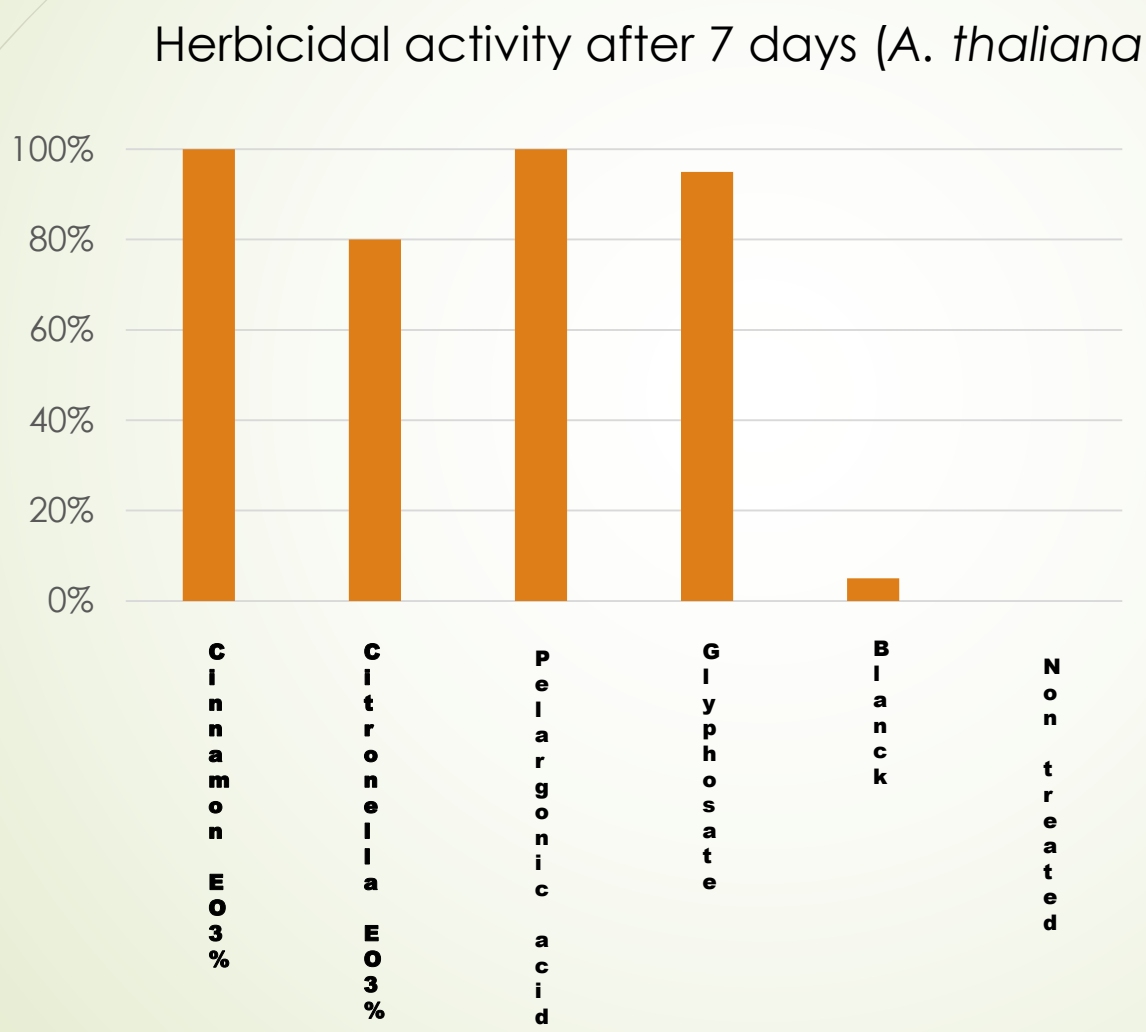
➔ Development of a bio-herbicide based on essential oils

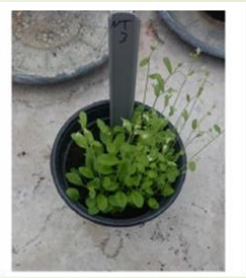


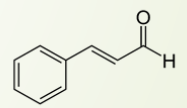
Cinnamon EO

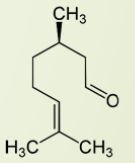
Java Citronella EO

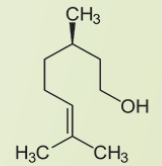
Bio-herbicide based on essential oils











Cinnamaldehyde Citronellal Citronellol

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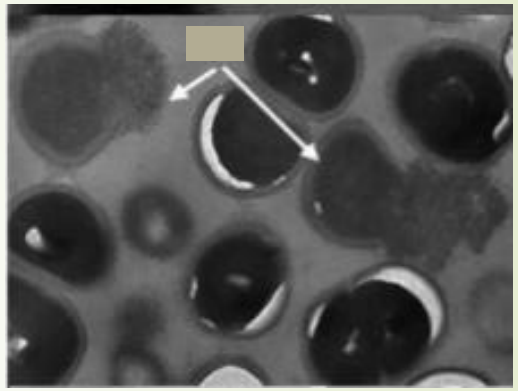
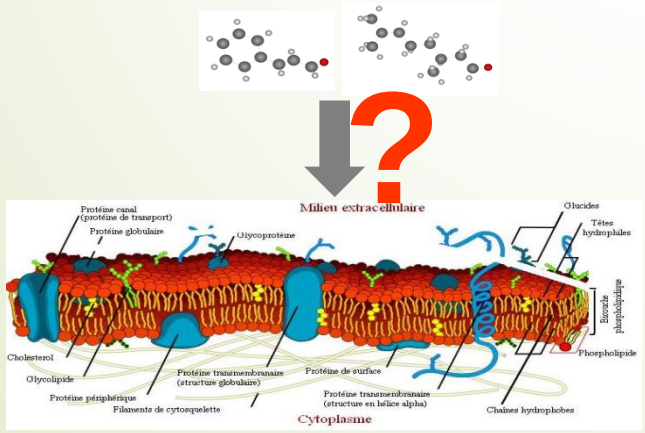
Bio-herbicide based on essential oils

- Action modes of Eos as herbicide in the literature:
  - waxy cuticular layer removal
  - disruption of microtubule polymerization
  - cellular respiration decrease
  - mitosis inhibition
  - ion leakage and membrane depolarization
  - oxidative damages
  - chlorophyll content decrease

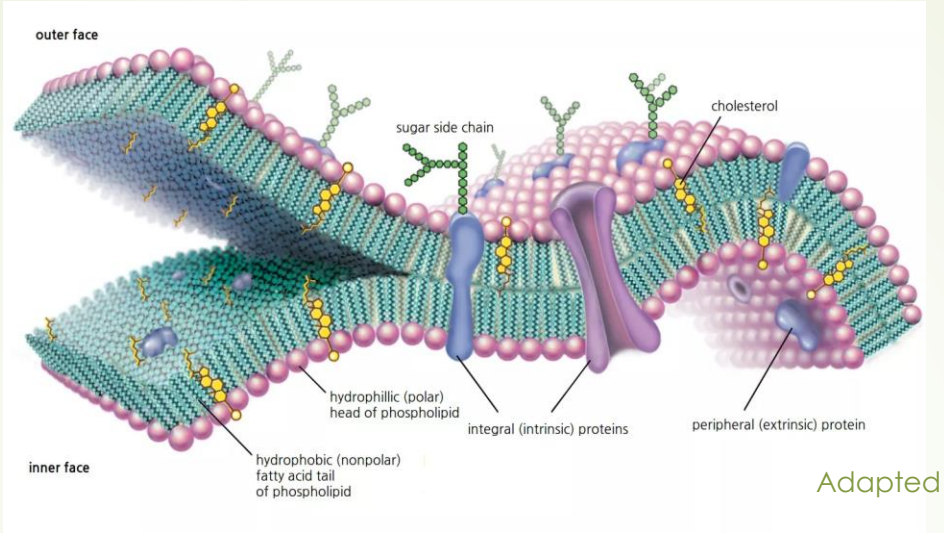


Membrane damage to *E. Coli* and *S. aureus* by *cinnamaldehyde* *(Shen et al, Food control,2015)*



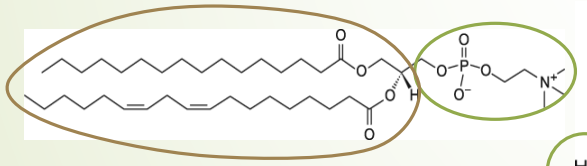
- detailed molecular mechanisms ??????
  - small amphiphilic molecules
  - Could interact with the plant plasma membrane?
  - Demonstrated in fungicide and bactericide activities

Plant plasma membrane



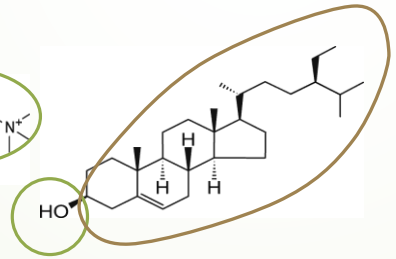
Adapted from *Encyclopædia Britannica*, Inc

Glycerolipids



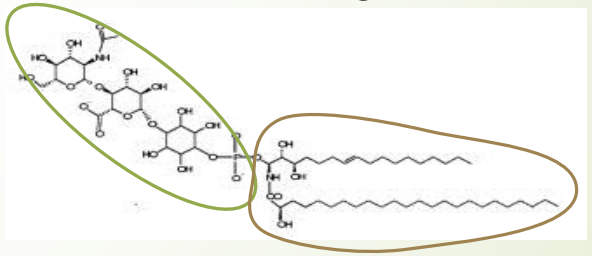
Palmitoyl linoleoyl Phosphatidylcholine (PLPC)

Sterols



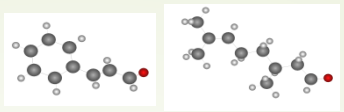
β-Sitosterol

Sphingolipids



Glycosyl inositol phosphoceramides (GIPC)

Model membranes PLPC/sito 80/20 with 10% CitO, CitA or CIN



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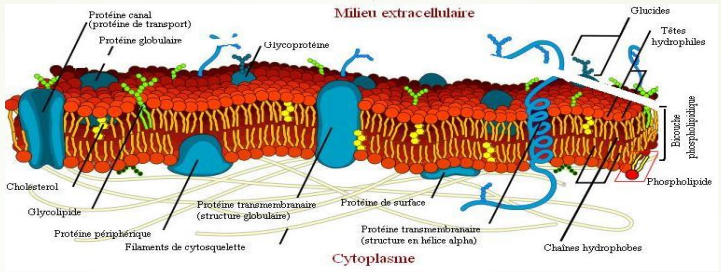
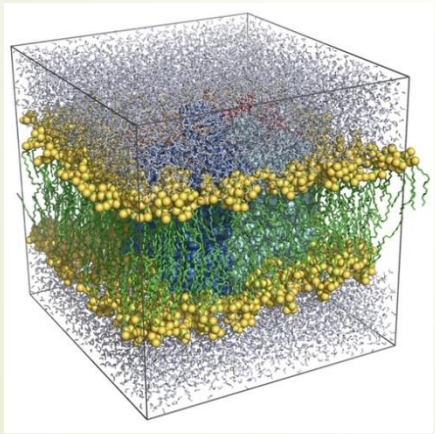
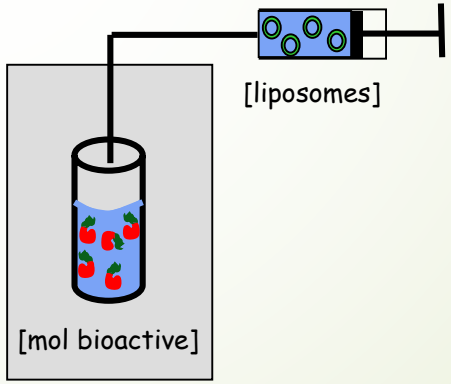


In silico approaches

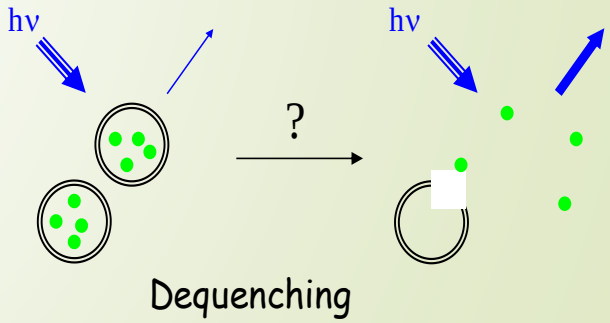
In vitro biophysical tools



Molecular dynamics simulations



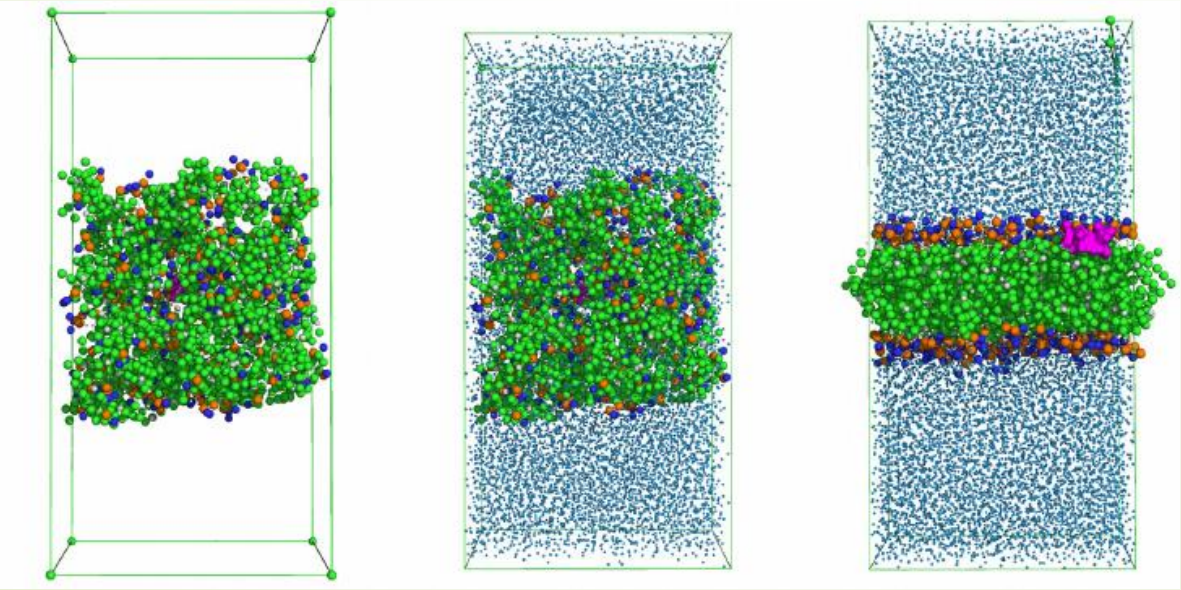
Isothermal titration calorimetry (ITC)



Lipid Permeability

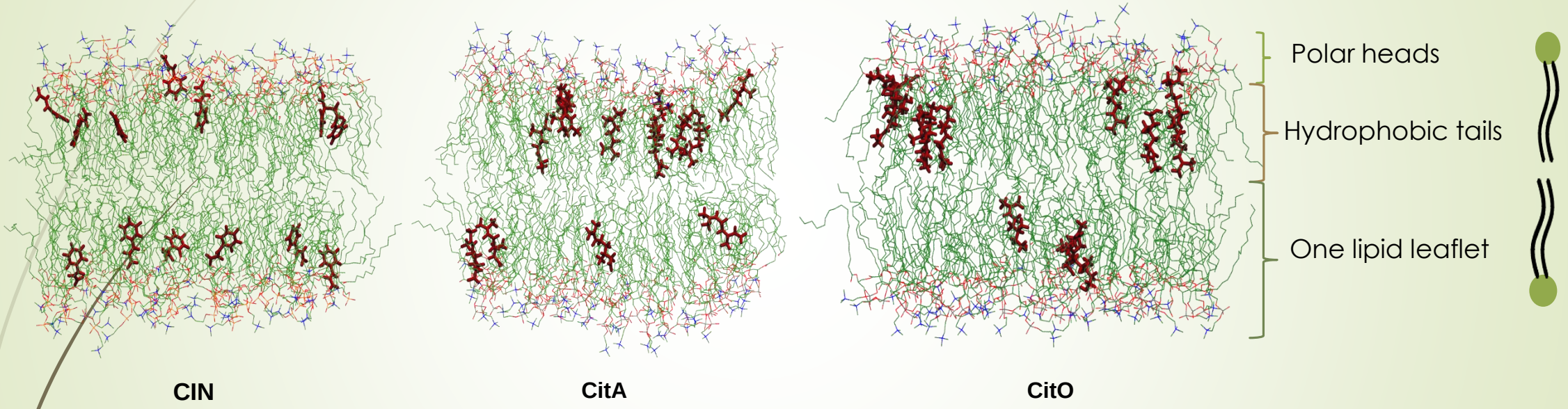
MD simulations

- Evolution of a molecular system with time
- Based on Newton $\vec{F} = m\vec{a}$
- Give insight into the inter- and intramolecular interactions

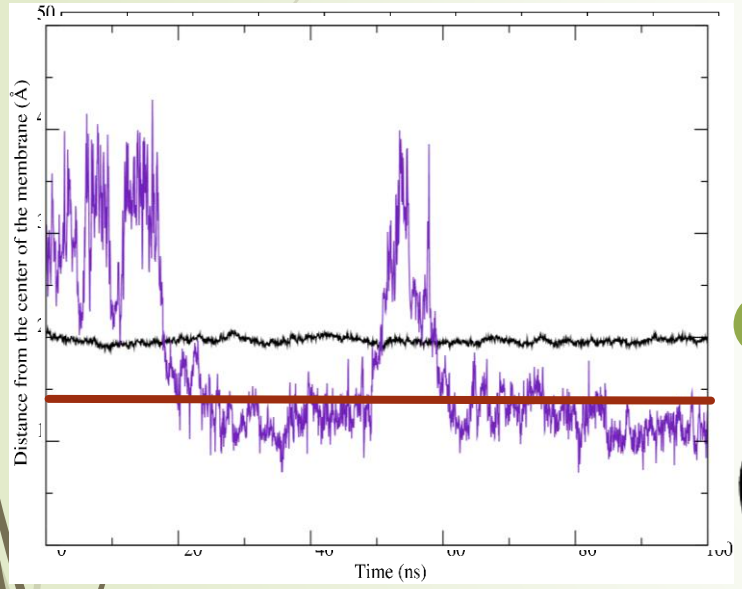


Adapted from Crowet *et al.* (2012)

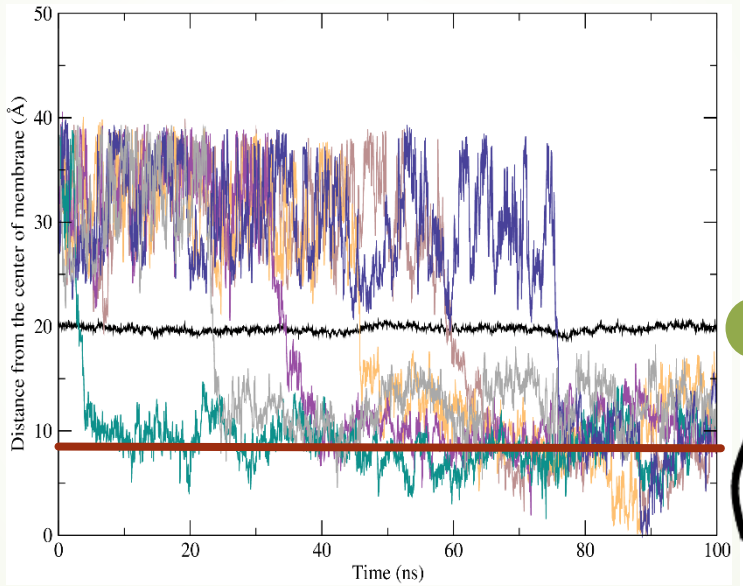
The EO molecules can insert into a model PPM



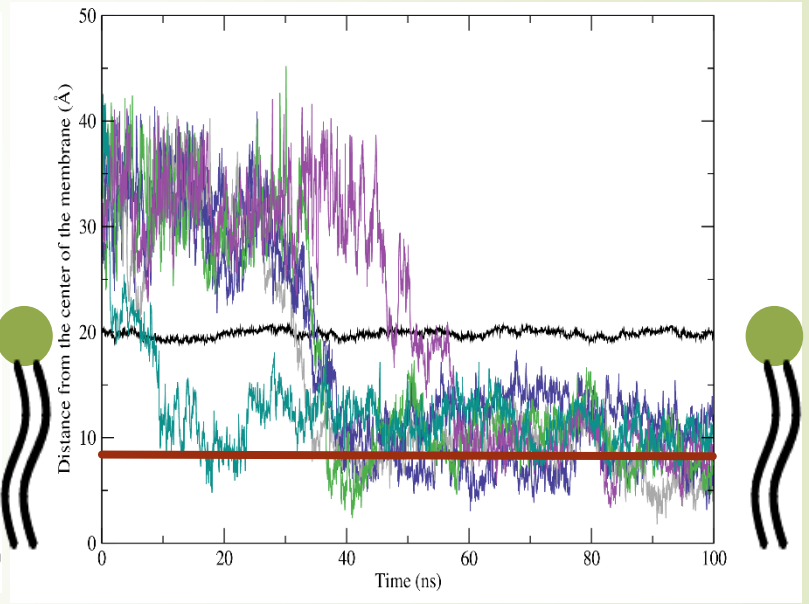
CIN, CitA et CitO have a different behavior



CIN



CitA

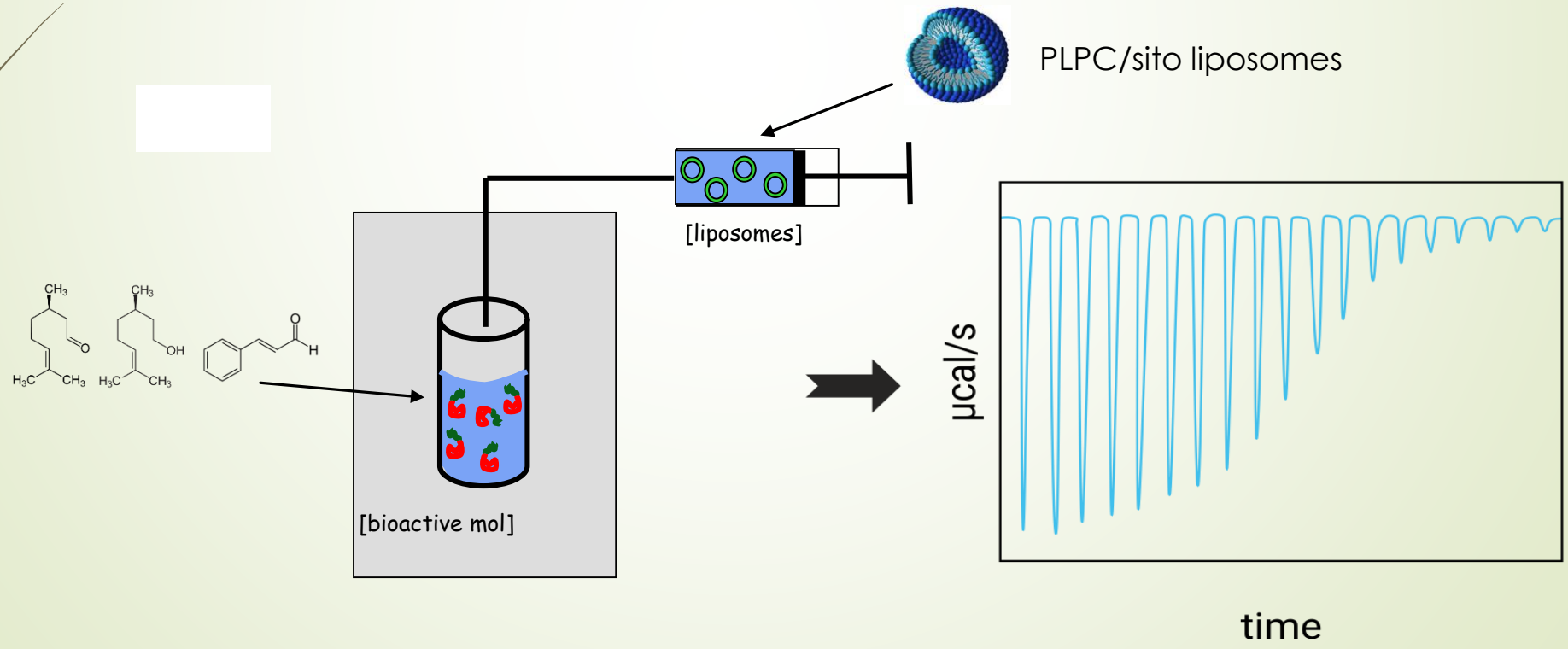


CitO

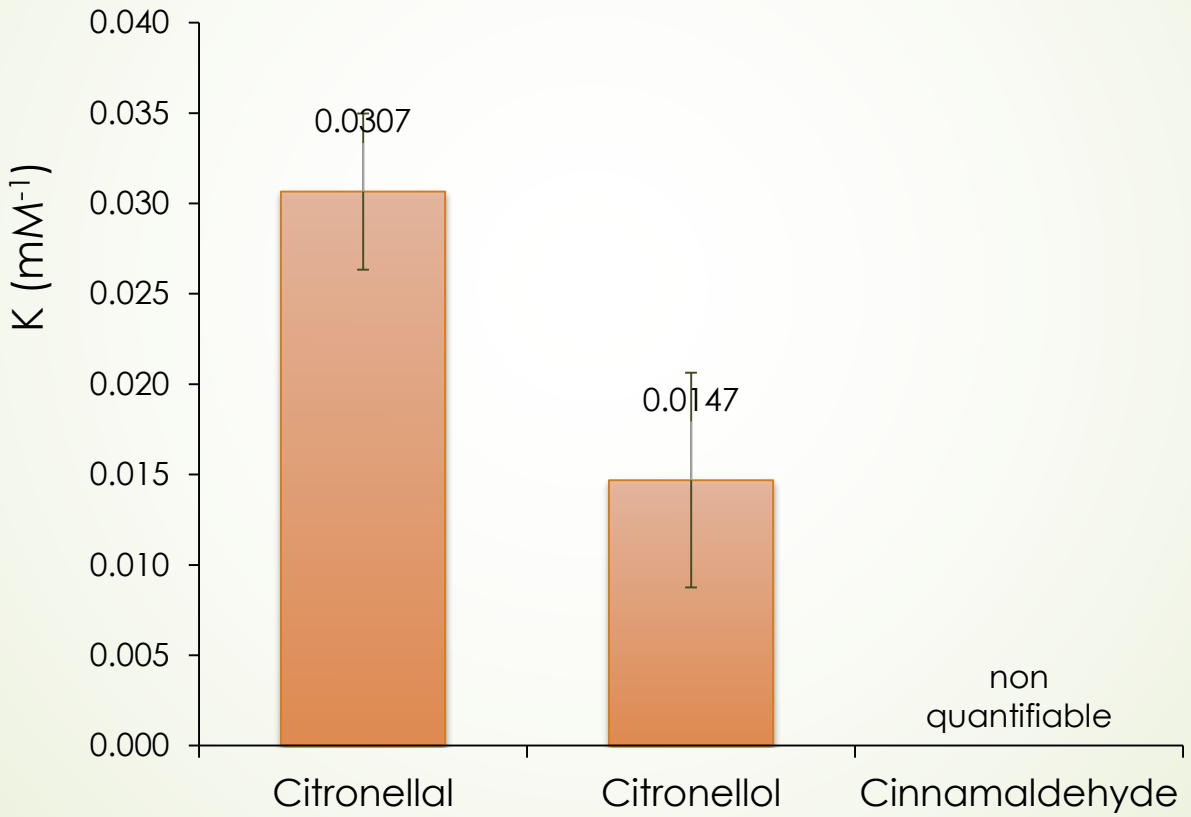
- Insertion CIN less deeper than CitO/CitA
- CIN can get out of the membrane

Isothermal Titration Calorimetry (ITC)

- Full thermodynamical characterization of the interaction (ΔG, ΔH, ΔS, K_D)



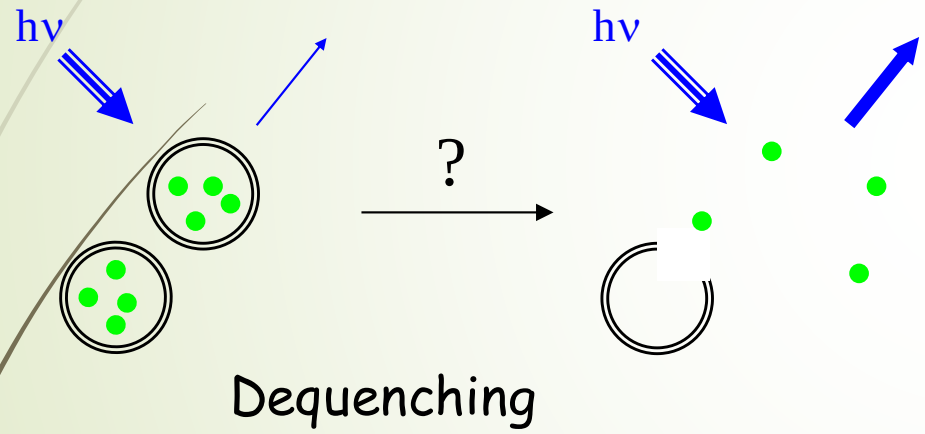
- CitA et CitO interact with the membrane but not CIN

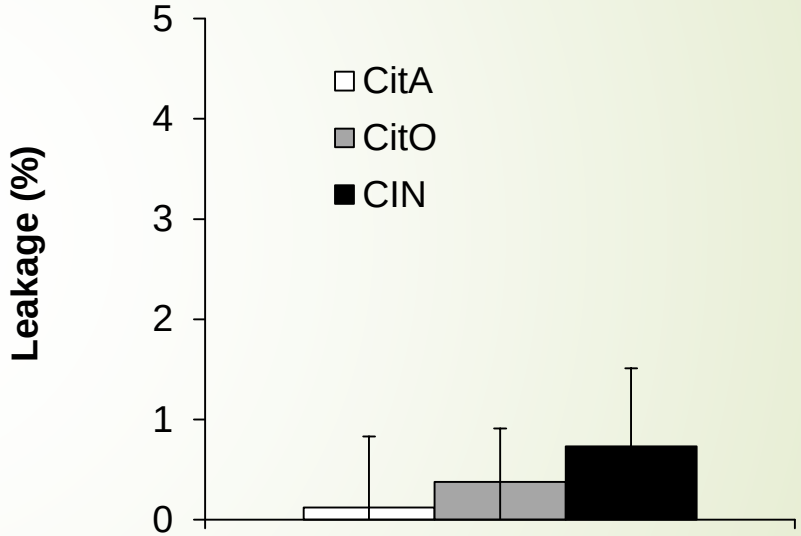


compound	K (mM^{-1})	$\Delta H_D^{W\rightarrow D}$ ($kj.mol^{-1}$)	$T\Delta S_D^{W\rightarrow D}$ ($kj.mol^{-1}$)	$\Delta G_D^{W\rightarrow D}$ ($kj.mol^{-1}$)
Citronellal	0.0307 ±0.004	5.13 ±0.64	21.62 ±0.34	-16.53 ±0.93
Citronellol	0.0147 ±0.006	5.05 ±0.52	23.28 ±0.50	-18.47 ±0.46

- K for PLPC/sito liposomes similar for CitA and CitO
- Interaction is entropy driven (hydrophobic)

Fluorescence assays



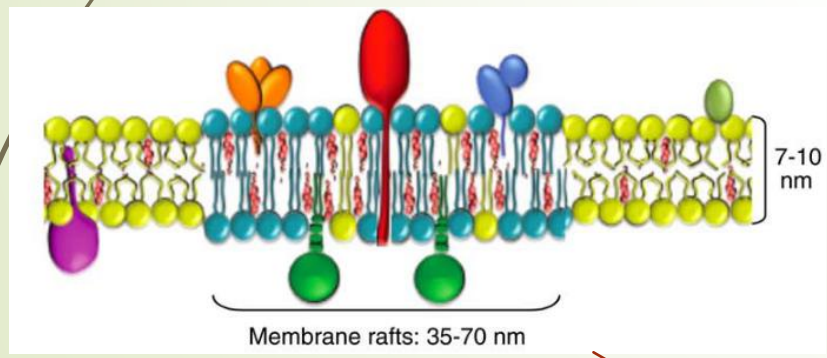


- No permeabilization of the membrane

How is the membrane activity of EO components related to their herbicidal effects ?

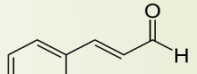
CitO/CitA

no membrane permeabilization/effects of sterol: Metabolism perturbation via interaction with lipid domains (signalling platform) ?

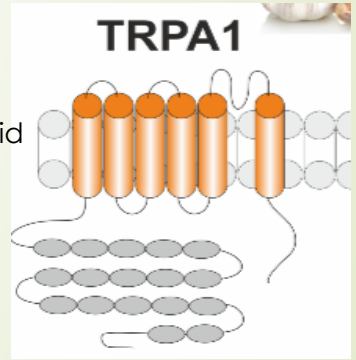


CIN

no direct interaction with the lipid membrane: Interaction with membrane receptors ?



GCPR (phenylpropanoid receptors)



Signalling pathways modified

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Conclusions

- Promising herbicide activities
- More than one action mode
- Many work to be done …
  - More in depth action mode studies: biophysics, molecular biology (proteomics, metabolomics,…)
  - Formulation
  - Environment effects

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Thank you for your attention

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