

# Modulation of gastric lipase adsorption onto mixed galactolipid-DPPC films by the addition of phytosterols

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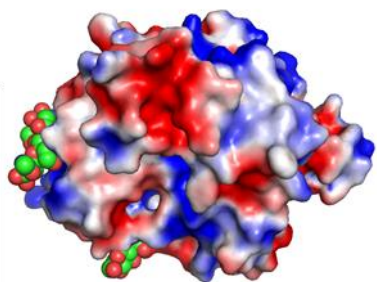
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## Human gastric lipase (HGL)



Globular glycosylated protein

379 aa, 47 KDa, pHi 5.8

Belong to  $\alpha/\beta$  hydrolases

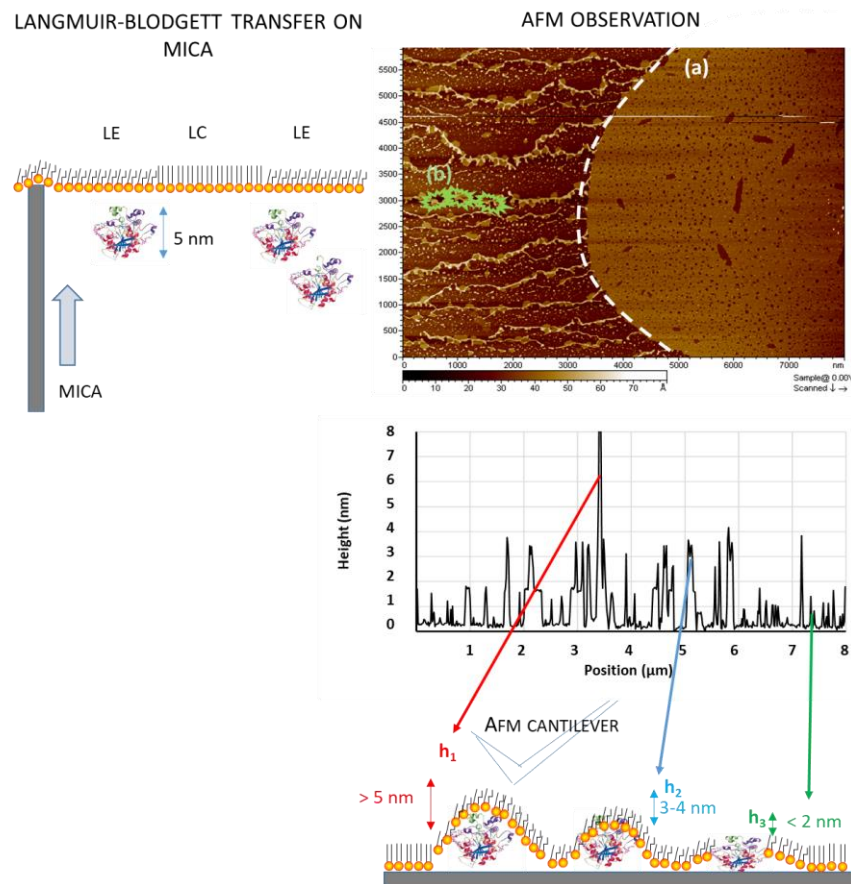
3D-Structure obtained in 1999  
(3.0-Å)

Gastric enzyme, initiates  
intraluminal lipolysis (10-25 %  
triglycerides) in humans

Highly active in the acidic  
environment of the stomach (pH  
4-5.4)

Key player in neonatal digestion

### Adsorption of gastric lipase onto multicomponent model lipid monolayers with phase separation, Bourlieu et al, 2016



### rDGL adsorption on milk fat membrane (heterogenous system)

It has been shown in animal model membrane presenting **phase coexistences** (Liquid condensed – liquid expanded) that gastric lipase gets **very rapidly adsorbed**:

- **into fluid phase**
- **at phase boundaries as patches of enzymes at different levels of insertion, suggesting a molecular cooperation.**

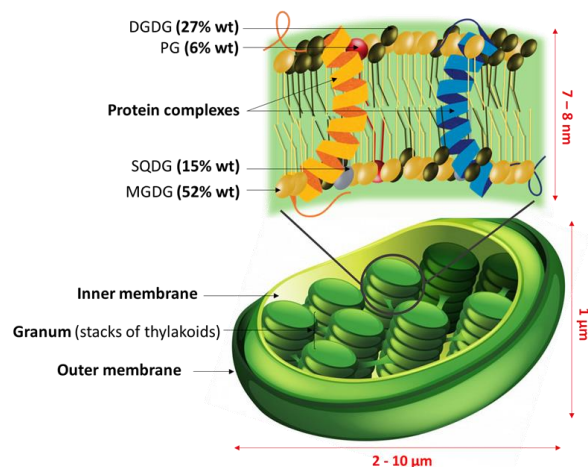
\*recombinant Dog gastric lipase 86% aa homology with HGL

**What about rDGL adsorption on vegetal model membrane ?**

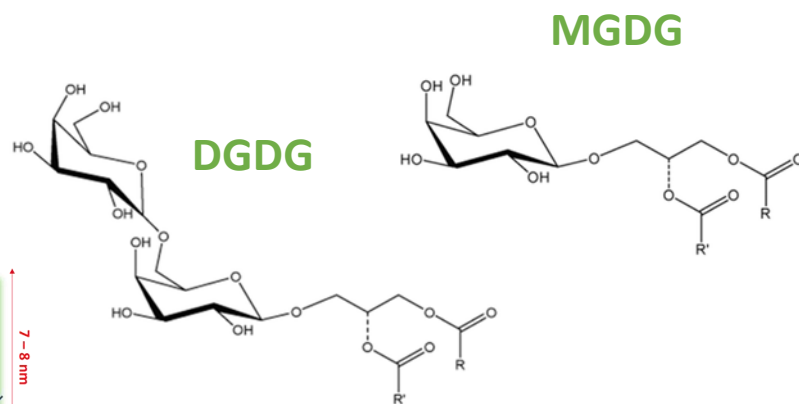
→ Gastric lipase adsorption into photosynthetic plant membranes has not been yet characterized



Rich in phospholipids, galactolipids and micronutrients (phytosterols...)



Rich in PUFA (C16:3, C18:3)



→ Polar lipids are not hydrolyzed in the upper gastrointestinal tract, but gastric lipase has been shown to have a strong ability to insert in PL monolayers thanks to its **high tensioactive capacity** (Bénarouche et al., 2013; de La Fournière et al., 1994).

Sahaka et al, 2020

gPLRP2

Essential  $\omega$ 3  
(2,2 g/jour, ANSES)

→ What about galactolipid monolayer?

\*MGDG: monogalactosyldiacylglycerol  
\*\*DGDG: digalactosyldiacylglycerol



What is the physico-chemical behavior of plant membrane assemblies?  
What is the interfacial behavior of human gastric lipase on plant polar lipids?

# Materials and Methods

## Substrates studied

### Model systems

(3) GL = MGDG\*/DGDG\*\* (60:40 mol/mol) (Pribil et al., 2014)

(4) GL/DPPC (50:50 mol/mol) → *To add condensed domains*

(5) GL/DPPC/pS\*\*\* (45:45:10 mol/mol/mol) → *To mimic photosynthetic membrane*

\*MGDG: monogalactosyldiacylglycerol

\*\*DGDG: digalactosyldiacylglycerol

\*\*\*pS=brassicasterol/campesterol/ $\beta$ -sitosterol (10:40:50 mol:mol:mol)

## Enzyme studied

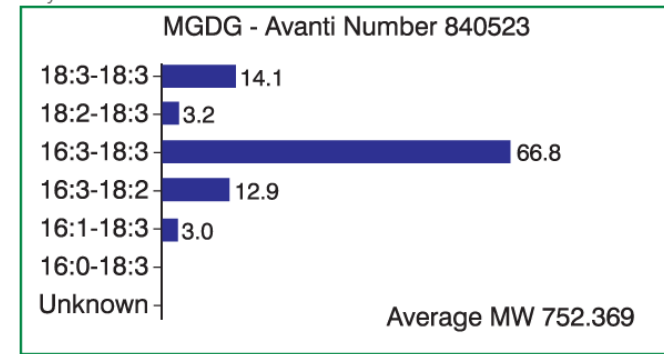
GL = gastric lipase – pH 5, 40 nM → rDGL\*\*\*\* (86% amino acid sequence identity with HGL)

How does lipase adsorb and insert into lipid monolayers at the air/water interface?

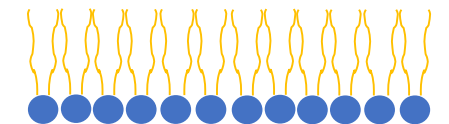
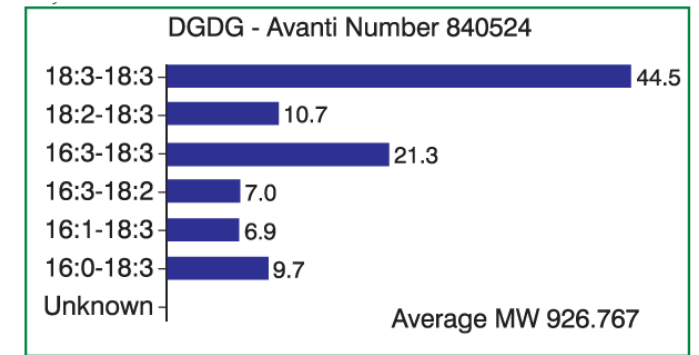
What is the impact of the lipid packing and the interfacial composition?

What is the impact of the addition of phytosterols in the monolayer on lipase adsorption?

GL = galactolipids  
PL=phospholipids



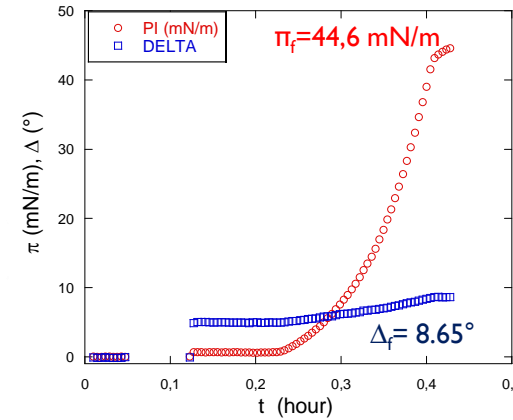
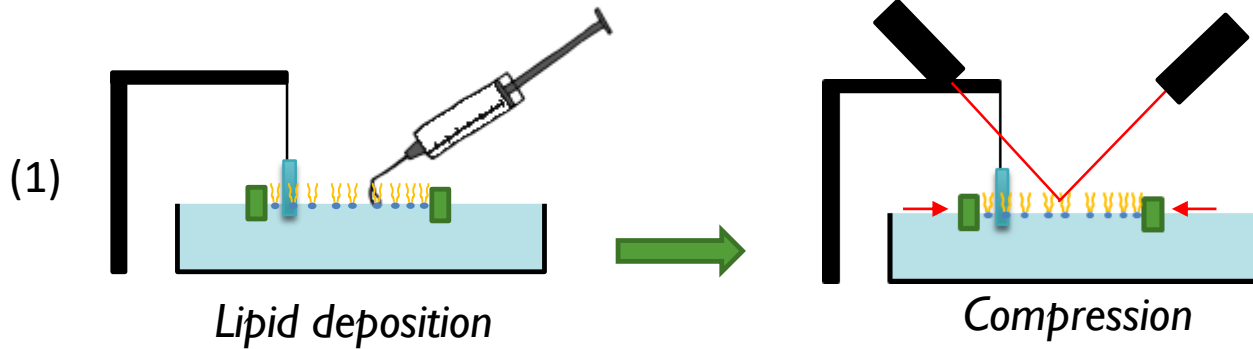
(Nitenberg, 2018)



\*\*\*\*rDGL: recombinant dog gastric lipase

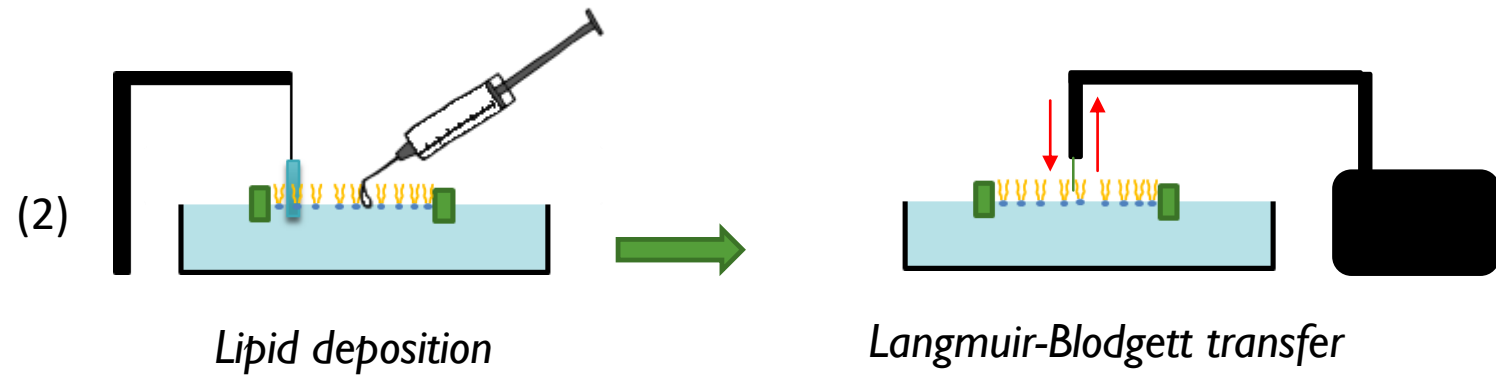
# Materials and Methods

## Ellipsometry/tensiometry

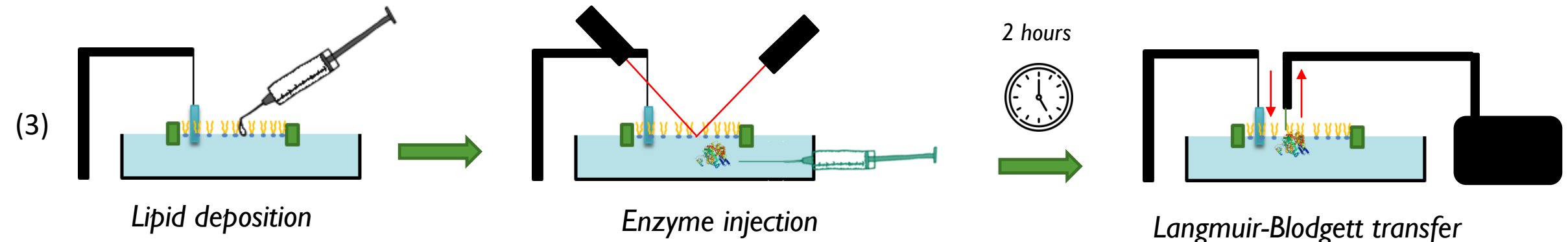
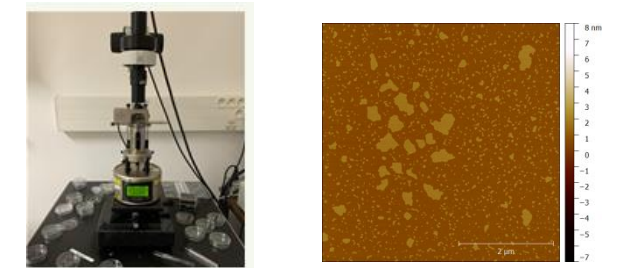


$\pi$  (surface pressure) is indicative of molecule interactions

$\Delta$  (ellipsometric angle) is related to the amount of matter at the interface



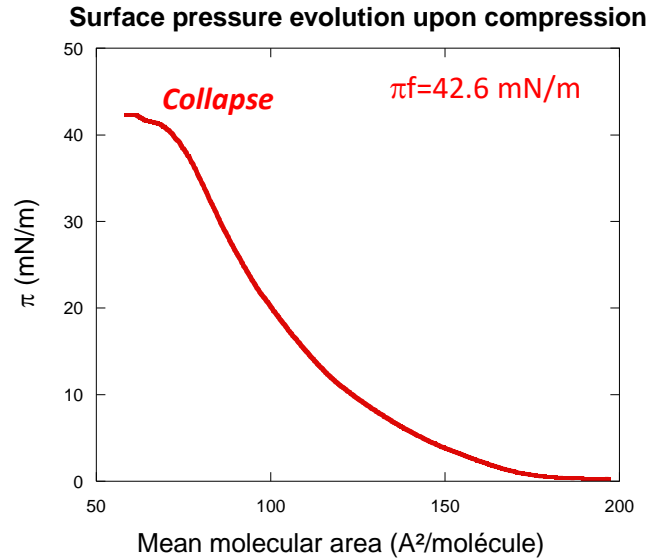
## Atomic Force Microscopy



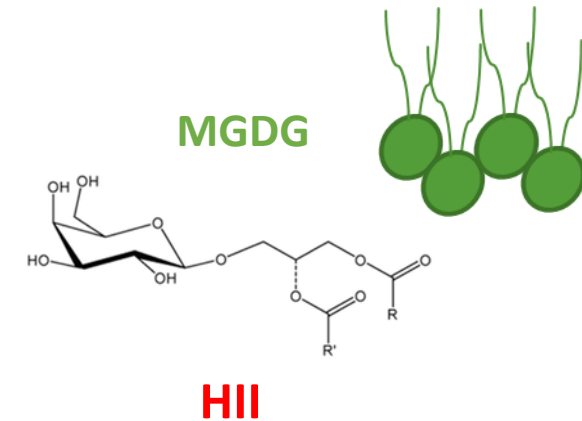
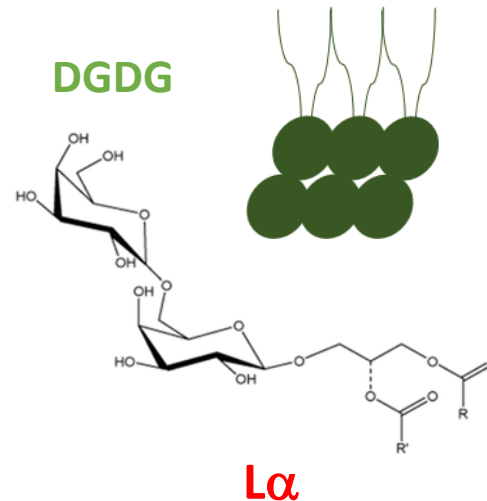
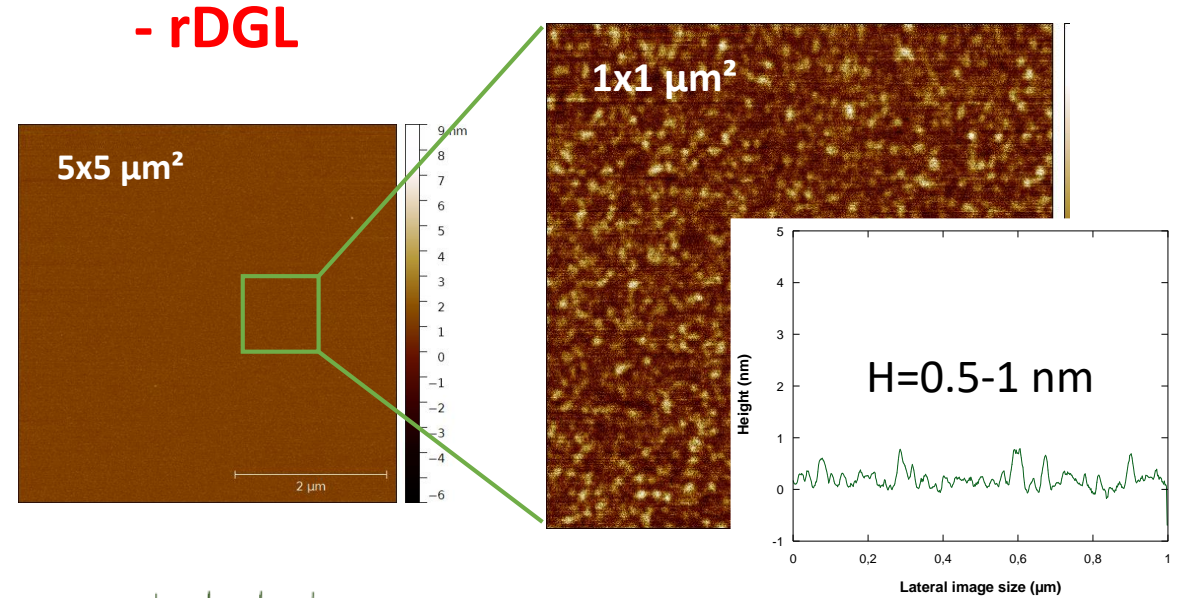
# Results and discussion

## Model systems

GL (MGDG/DGDG, 60:40 mol/mol)



$\pi = 20 \pm 0 \text{ mN/m}$   
 $\Delta = 7.5 \pm 0.1^\circ$

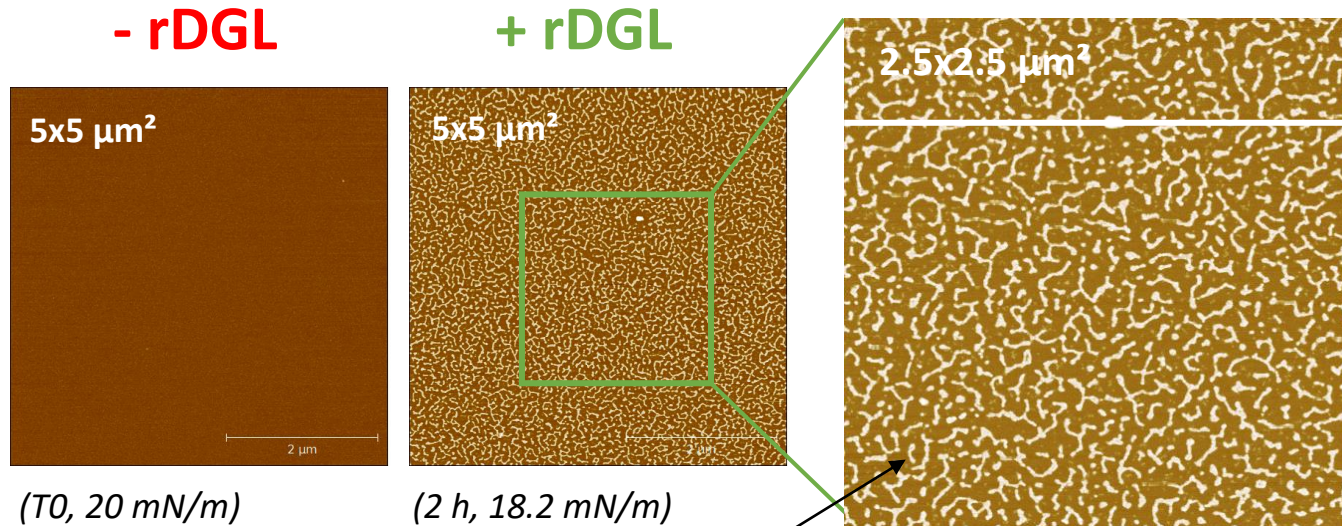


- Fluid phase (expanded liquid)
  - Roughness induced by the intercalation of MGDG sugar polar heads
- (Bottier et al, 2007)

# Results and discussion

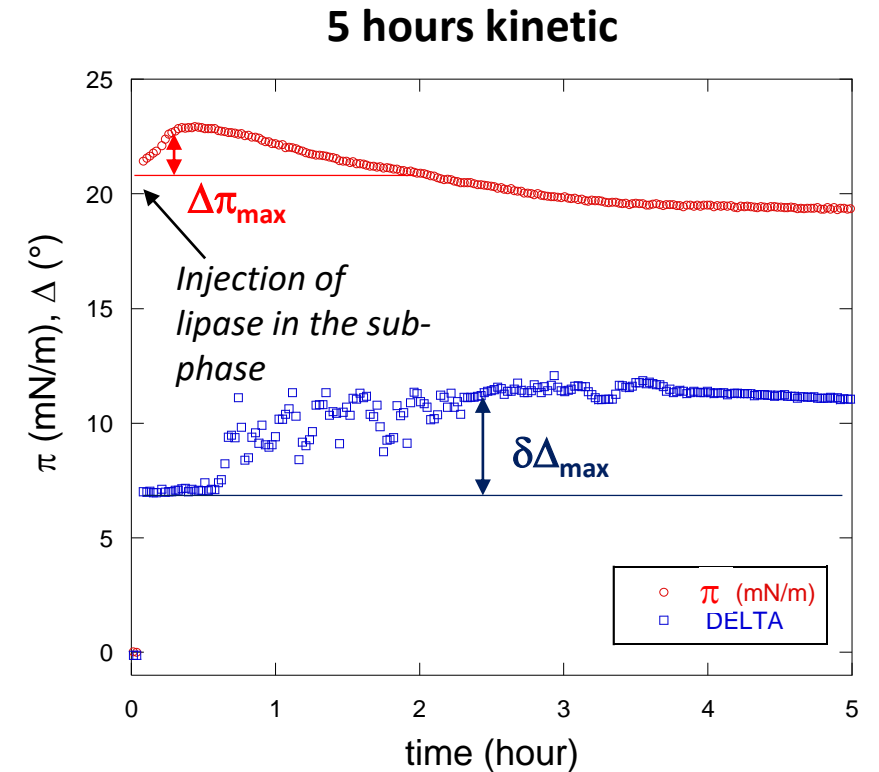
## Model systems

GL (MGDG/DGDG, 60:40 mol/mol)



Formation of a lipase network **onto** the fluid phase

Loss of roughness induced by MGDG polar heads  
→ Modification of interactions between GL and rDGL



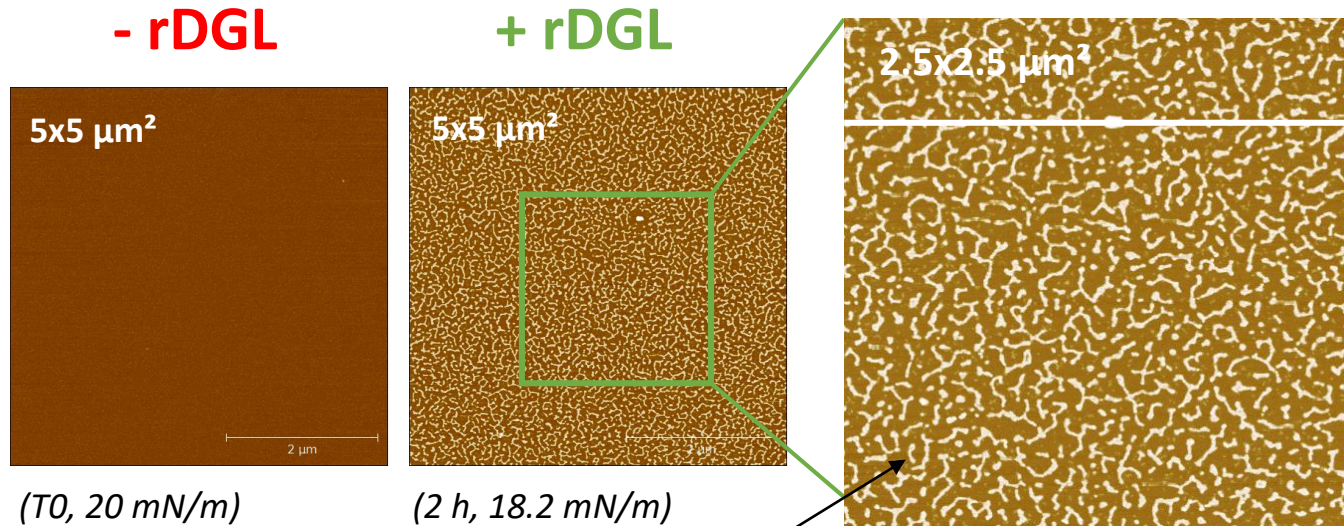
→ Increased pressure due to the adsorption of lipases onto the monolayer shortly after the injection

( $\pi$  (surface pressure) is indicative of molecule interactions)  
( $\Delta$  (ellipsometric angle) is related to the amount of matter at the interface)

# Results and discussion

## Model systems

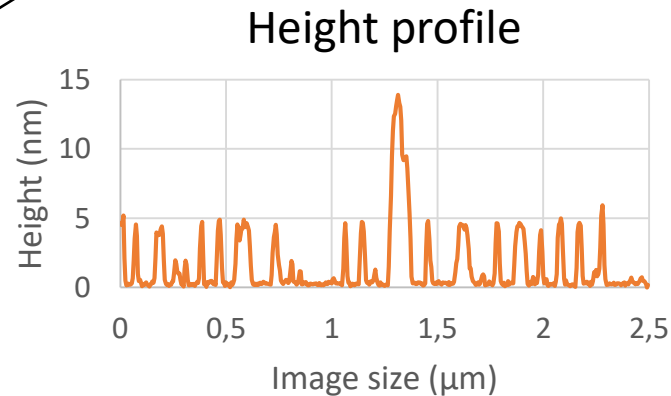
GL (MGDG/DGDG, 60:40 mol/mol)



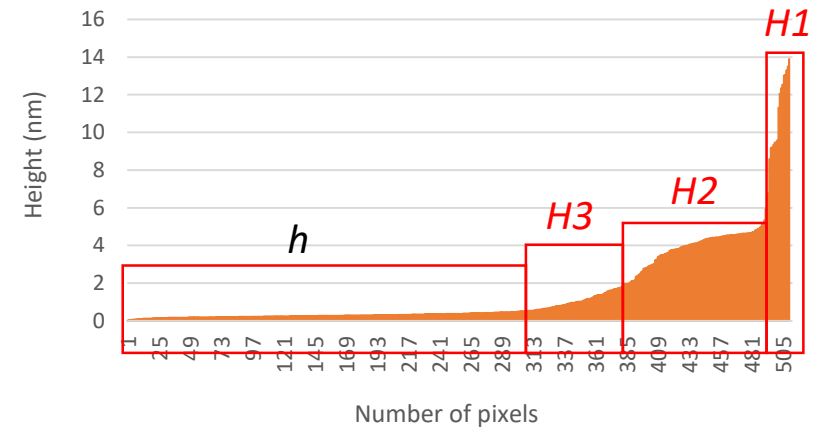
(T0, 20 mN/m)

(2 h, 18.2 mN/m)

Formation of a lipase network **onto** the fluid phase



## Identification of 3 major height profiles



- (H1 → > 5.4 nm, aggregates)
- H2 → 2.0 – 5.4 nm
- H3 → 0.5 – 2.0 nm
- h → 0 – 0.5 nm

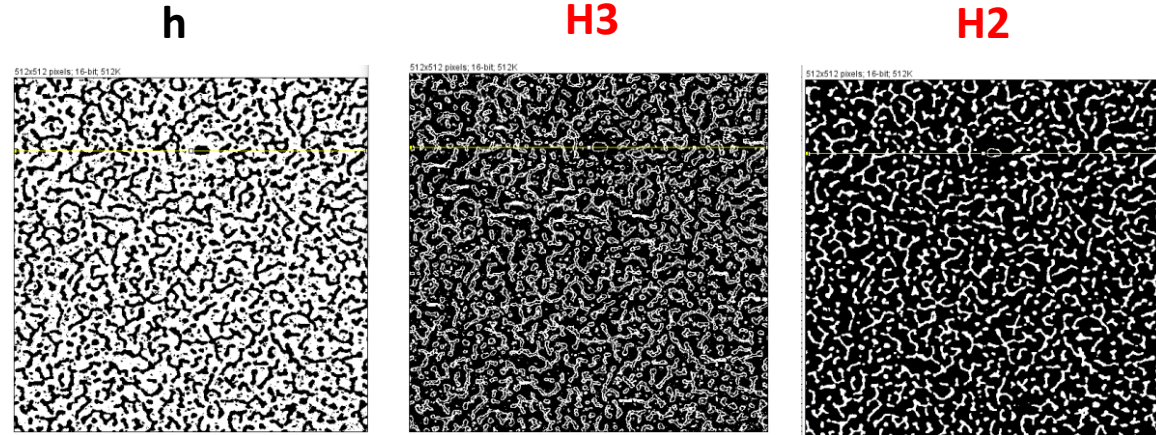
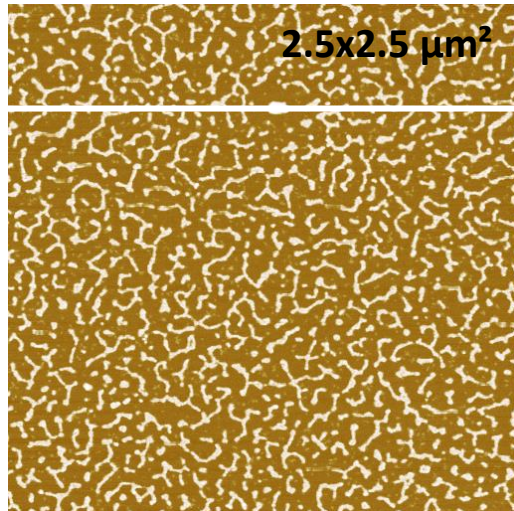


# Results and discussion

## Model heterogenous systems

GL (MGDG/DGDG, 60:40 mol/mol)

+ rDGL



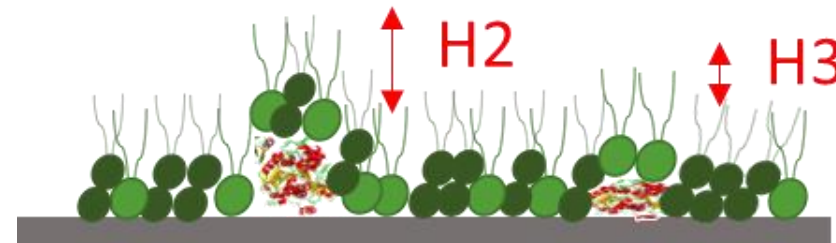
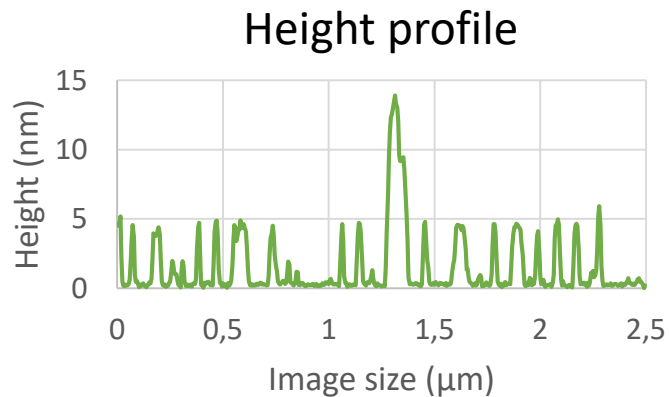
ImageJ

Threshold

0 – 0.48 nm  
Fluid lipidic  
background

0.5 – 2.0 nm  
Unfolded lipases  
(sacrificing agents)

2.0 – 5.4 nm  
Insert lipase on  
1-2 nm depth,  
intermediate  
level



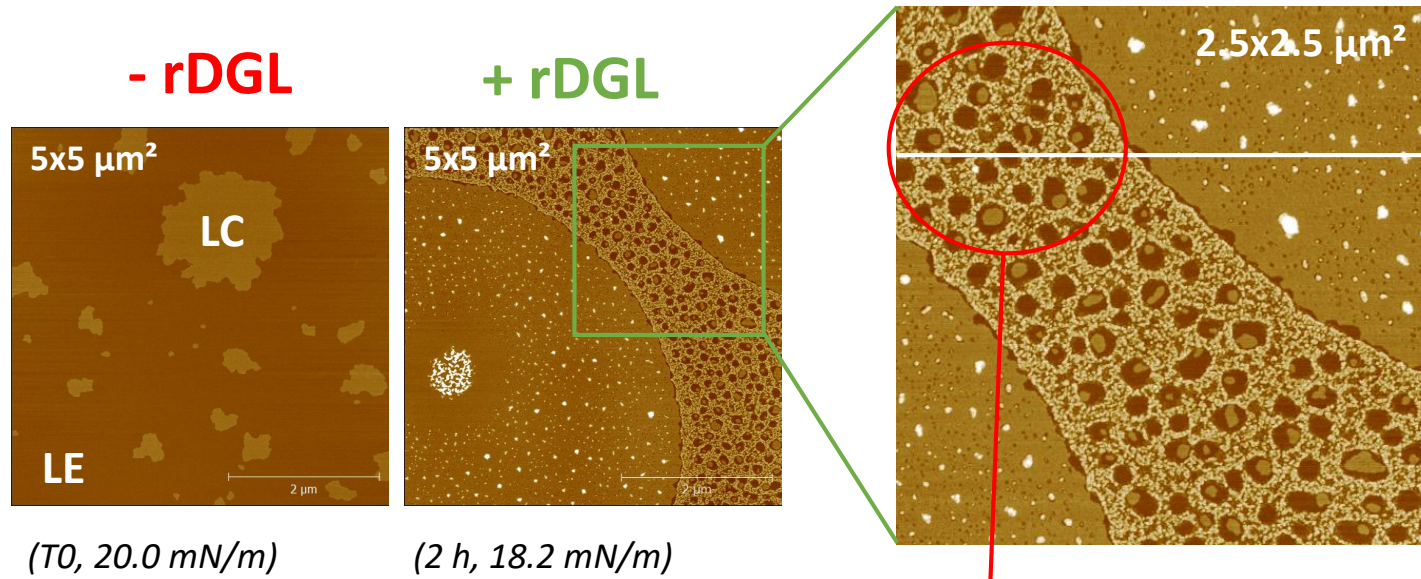
# Results and discussion

## Model heterogenous systems

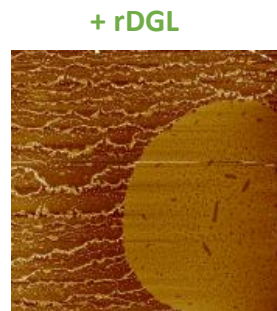
GL-DPPC (50:50 mol/mol)

Addition of saturated phospholipids (dipalmitoylphosphatidylcholine)

Identification of 5 major height profiles



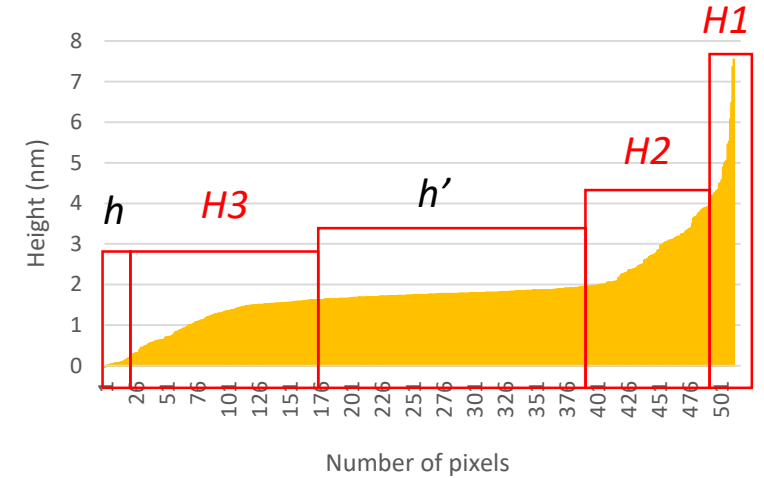
Visualization of condensed domains (LC)  
Loss of roughness



Milk bovine membrane



Protein network in fluid phase



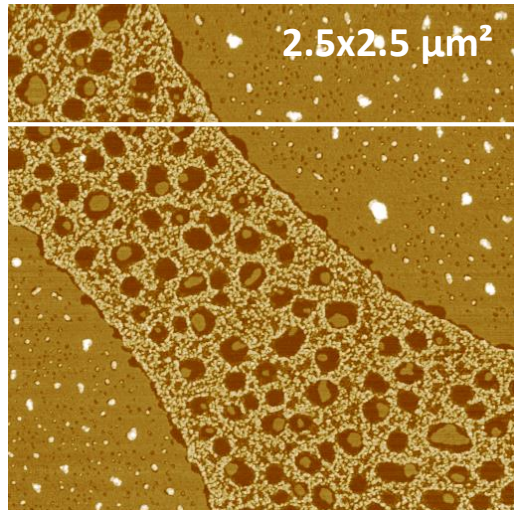
- **H1** → > 4.8 nm (aggregates)
- **H2** → 2.3 – 4.8 nm
- **h'** → 1.7 – 2.3 nm
- **H3** → 0.2 – 1.7 nm
- **h** → 0 – 0.2 nm

Based on ImageJ analyses of 5 plot profile

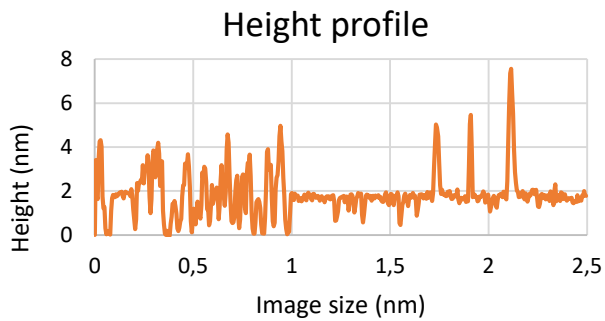
# Results and discussion

## Model heterogenous systems

GL-DPPC (50:50 mol/mol)



ImageJ  
Threshold

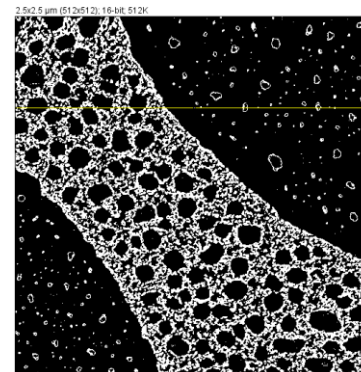


**h**  
0 – 0.2 nm  
Fluid lipidic  
background

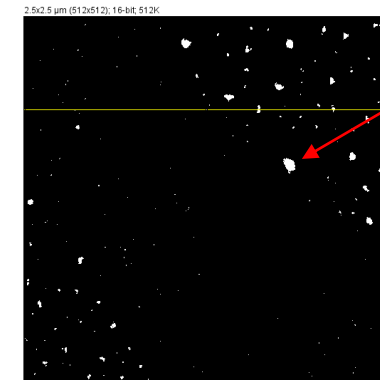
**H3**  
0.48 – 1.95 nm  
Unfolded  
inserted lipases

**h'**  
1.7 – 2.3 nm  
Condensed lipid  
domains

**H2**  
2.3 – 4.8 nm  
Intermediate  
adsorbed  
lipases in the  
fluid phase



**H1**  
> 4.8 nm  
Aggregated  
proteins



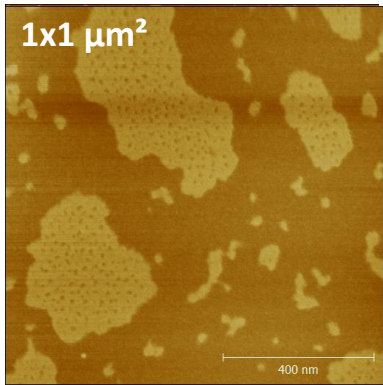
protuberances

# Results and discussion

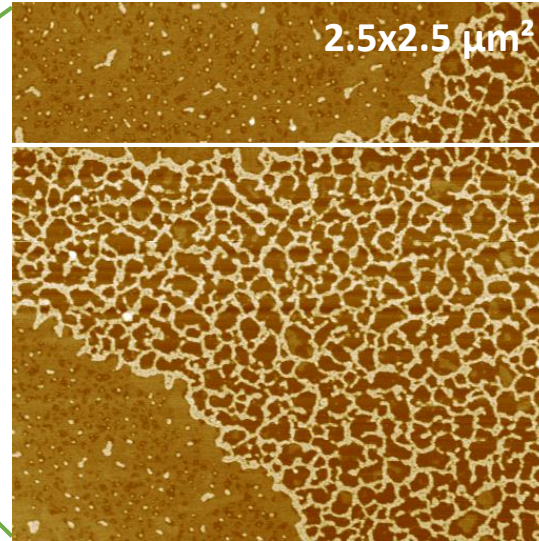
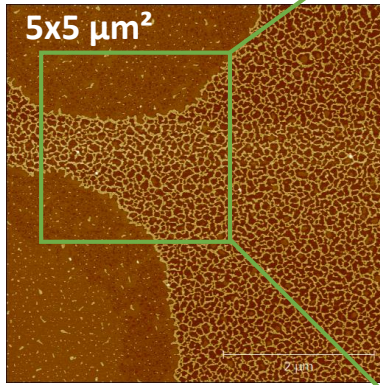
GL-DPPC-pS (45:45:10 mol/mol/mol)

*Addition of phytosterols*

**- rDGL**



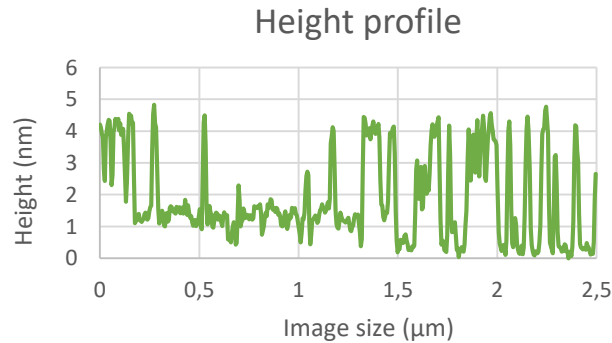
**+ rDGL**



*Are phytosterol-induced defects likely to enhance lipase adsorption onto condensed domains?*

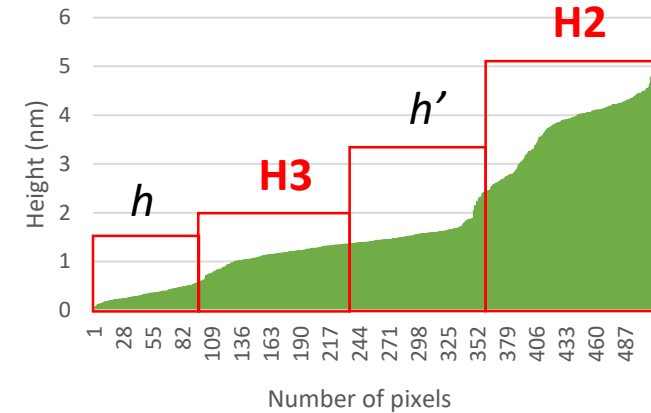


→ Phytosterols seem to induce a better adsorption of rDGL on the condensed phases



## Model heterogenous systems + pS

### Identification of 4 major height profiles



- **H2** → 2.3 – 5 nm – **Inserted lipases (intermediate level, active conformation?)**
- **h'** → 1.5 – 2.3 nm - Condensed lipid domains
- **H3** → 0.6 – 1.5 nm - **Unfolded inserted lipases**
- **h** → 0 – 0.6 nm – Fluid lipidic background

# Conclusions

## The addition of DPPC and/or phytosterols has induced a modification of the phase behavior of GL

→ The addition of DPPC to the mixture led to the loss of the roughness induced by the GL polar heads. The addition of pS has caused the formation of defects in the condensed phases

## The adsorption of rDGL was modulated by the presence of phytosterols in the mixture

→ The rDGL has adsorbed and created a more or less dense network in the fluid phase, and was excluded from the condensed phase. The addition of pS in the mixture has improved the adsorption of the enzyme in the condensed phase

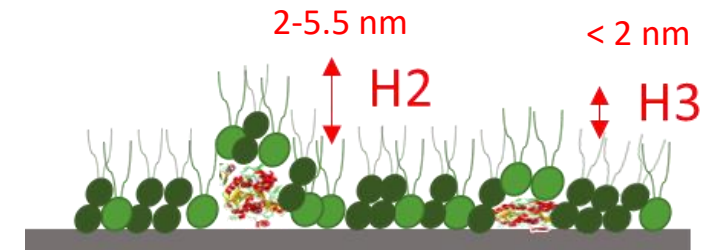
## Different levels of adsorption/insertion heights of the enzyme have been identified

→ The insertion of denatured "sacrificial" lipase modified the lateral pressure of the lipids and facilitated the adsorption of the enzyme in a second step.

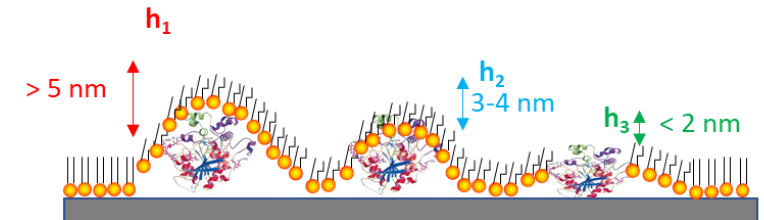
## Similar behaviors were identified for rDGL adsorption at milk and vegetal fat model membranes

→ However, it is difficult to compare the two systems due to their different composition. Indeed, the reorientation of the polar heads of galactolipids can induce a minimization of height variations. In the case of the plant membrane, there is little insertion of lipase but a more homogeneous protein network

## Galactolipid model membrane



## Milk fat model membrane



What is the precise organization of lipase with respect to the GL polar heads and what reorganization does it induce?

Will the same type of behavior be found with another globular surfactant protein?

What happens at the intestinal level (PLRP2, PTL, PLA2)?

Thank you for your attention!

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Agropolymer engineering  
& emerging technologies