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## **Galactolipids from microalgae as a new source of omega 3 and naturally structured surfactants for human nutrition**

Jeanne Kergomard, Nathalie Barouh, Frédéric Carrière, Pierre Villeneuve, Claire Vigor, Thierry Durand, Pierre Emmanuel Millet, Maeva Subileau, Véronique Vié, Claire Bourlieu-Lacanal

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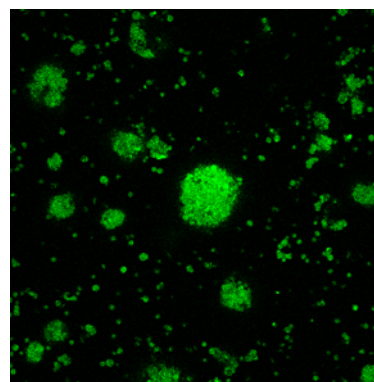
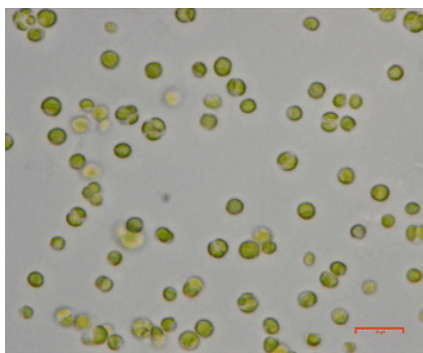
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# Galactolipids from microalgae as a new source of omega 3 and naturally structured surfactants for human nutrition



Jeanne Kergomard, Nathalie Barouh, Frédéric Carrière, Pierre Villeneuve, Claire Vigor, Thierry Durand, Pierre Emmanuel Millet, Maeva Subileau\*#, Véronique Vié\*# and Claire Bourlieu\*#

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*#authors contributed equally to the work*

# CONTEXT

- ✓ Global spread of obesity, overweight and diet-related chronic diseases with its burden of comorbidities. How can we turn the tide off ?



<https://www.fda.gov/news-events/fda-voices/improving-nutrition-turn-tide-diet-related-chronic-disease>

↘ cardiovascular diseases (17.9 million deaths globally/y), cancers (9 million deaths/y), and diabetes (1.6 million deaths/y) (WHO, 2019)

- ✓ Global picture but lipid nutrition matters (35-40 % TEI)

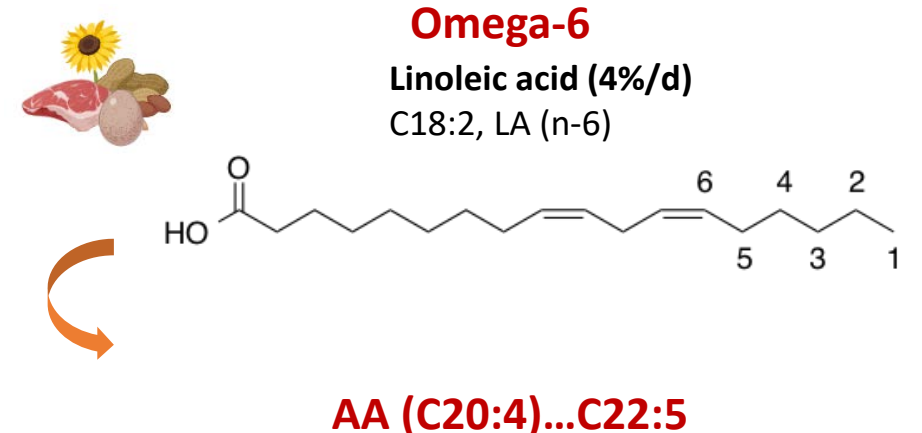
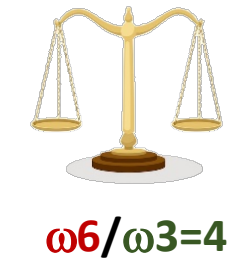
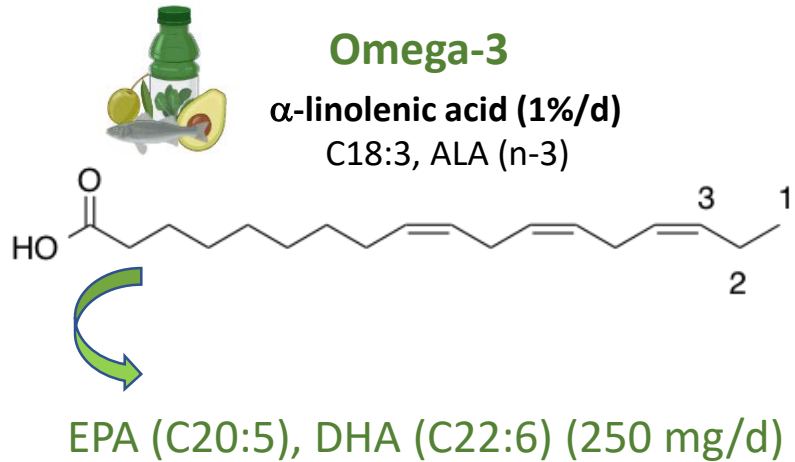


Fatty acids	RDA* (% TEI)		% TFA*
Essential	LA (C18:2, ω6)	4 %	10-12 %
	ALA (C18:3, ω3)	1 %	2.5-3 %
	Docosahexaenoic acid, DHA (C22:6 ω3)	250 mg	
Non essential	Eicosapentaenoic acid, EPA (C20:5 ω3)	250 mg	
	Lauric + myristic + palmitic FA	≤ 8 %	20-23 %
	<b>Total saturated FA</b>	<b>≤12 %</b>	<b>30-34 %</b>
	Oleic FA (C18:1, ω9)	15-20 %	38-50 %

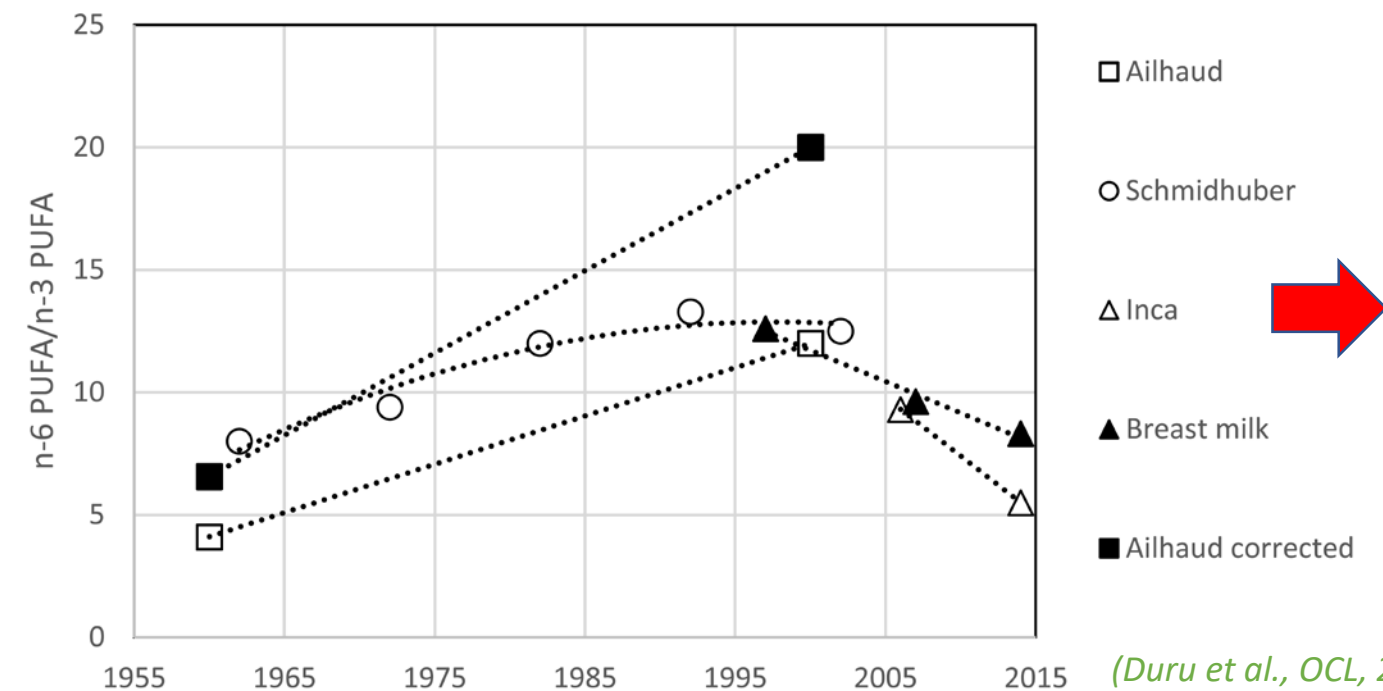
\*RDA = Recommended Dietary allowances, TEI=Total energy intake, TFA=total fatty acids of lipid source

(ANSES 2010 recommendations)

# CONTEXT



(ANSES 2010 recommandations)



(Duru et al., OCL, 2019)

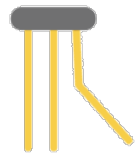
- ✓ Balancing our lipid intake by regulating our intake of omega-6 fatty acids and increasing our intake of omega-3 fatty acids = strong nutritional recommendation from the WHO
- ✓ Unbalanced diets (excess omega-6) promote the onset of chronic inflammatory diseases
- ✓ High imbalance in  $\omega 6/\omega 3$  ratio, still very strong in infants and adolescents (INCA3; Chuy et al., Chevreul Congress 2023; Simopoulos, Biomed. Pharmacol., 2002)

# CONTEXT

✓ To rebalance intakes what are the sources that can be used in nutritional strategies ?



Seeds and nuts rich  $\omega 3$  precursors – main molecular form TAG >> PL

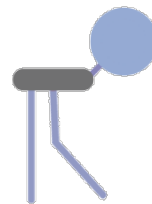


Triacylglycerols (TAG)

80 g/d



Fish and marine resources rich  $\omega 3$  VLCPUFA – main molecular form TAG >> PL

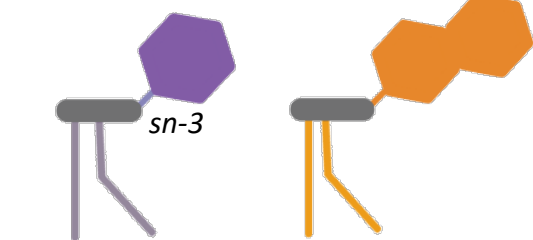


Phospholipids (PL)

2-10 g/d



Photosynthetic vegetals from marine or terrestrial sources

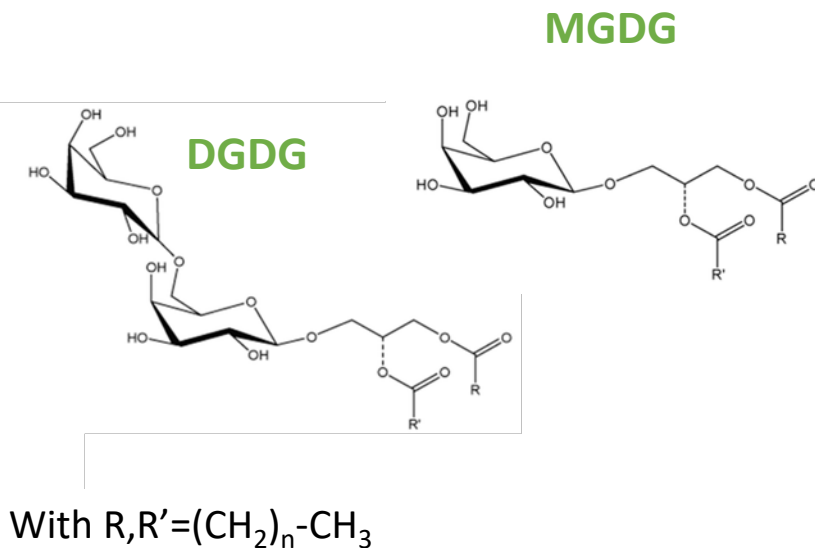
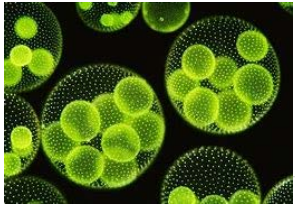


Galactolipids (PL)

0.2 g/d

# CONTEXT

- ✓ In some microalgae, fatty acids present under specific molecular forms: glycolipids



Digested by specific enzymes (pancreatic lipase related type 2 (PLRP2) or cholesterol ester hydrolase (CEH) and not by human pancreatic lipase (HPL)

When structured under form of thylakoid membrane  
=> inactivation of pancreatic lipase and weight loss

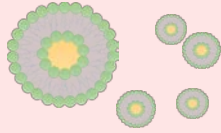
*(Köhnke et al., Scand. J. Gastrol., 2009; Köhnke et al., Phytotherapy Res., 2009; Tabrizi & Farhangi, FRI, 2021)*



## Gastric compartment

*Aggregated but stable*

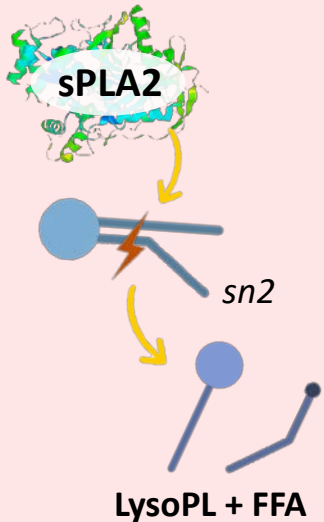
Membrane fragments  
(Thylakoïds)



## Intestinal compartment

*Polar lipid hydrolysis*

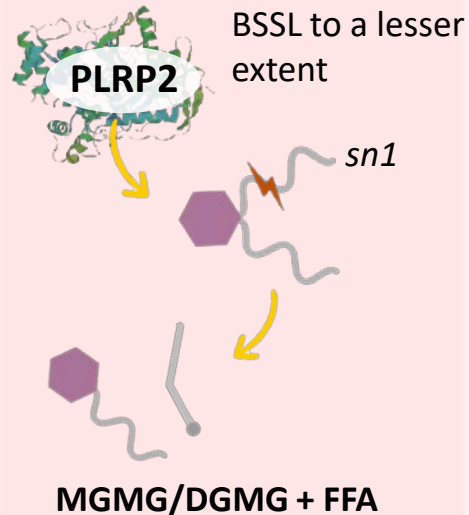
Phospholipids



Latency in HPL  
lipolysis

*(Beisson et al., BBA, 2001)*

Galactolipids



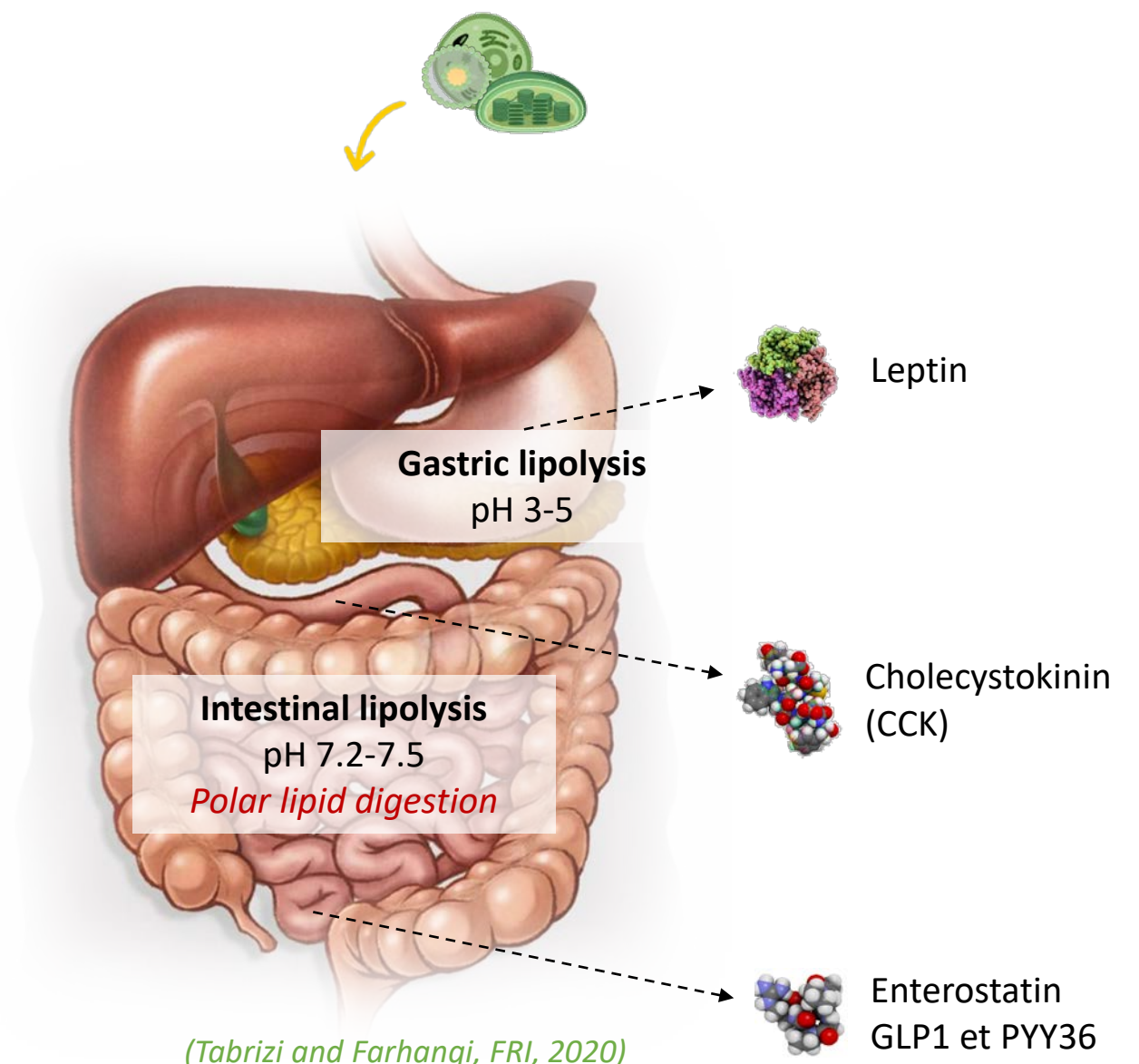
MGDG > DGDG  
*(Amara et al., BBA, 2010)*  
**Reduction of digestion  
and TAG absorption**

*(Stenkula et al., Nutr Metab, 2017)*



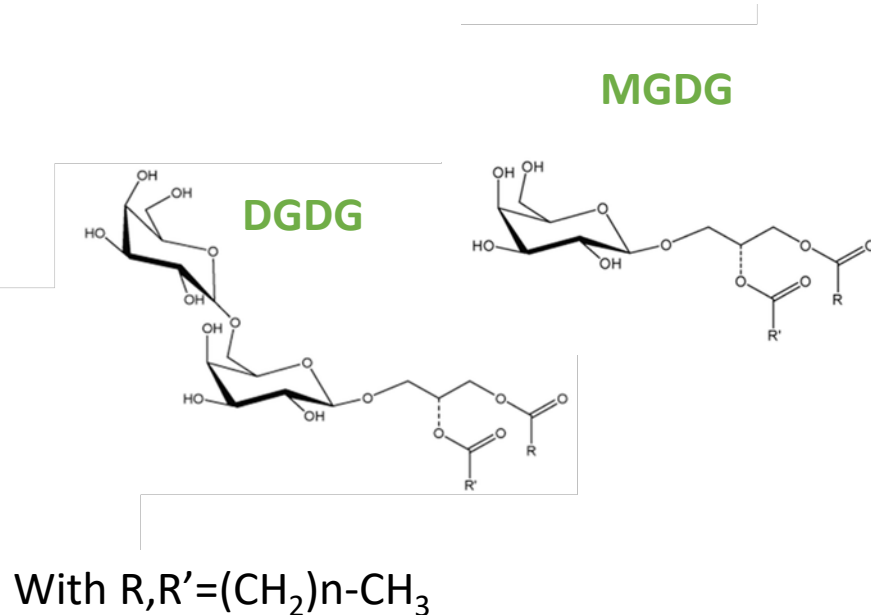
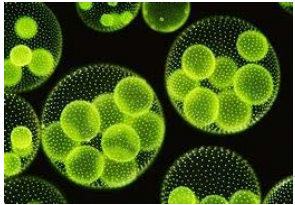
↑ **satiety hormones**

*Ab: Pancreatic phospholipase A2 (sPLA2), Colipase-dependent pancreatic lipase (HPL), Pancreatic lipase related protein 2 (PLRP2), BSSL : bile salt stimulated lipase, DGDG: digalactosyldiacylglycerol, DGMG: digalactosylmonoacylglycerol, FFA: free fatty acids, MGDG: monogalactosyldiacylglycerol, MGMG: monogalactosylmonoacylglycerol, lysoPL: lysophospholipids*



# CONTEXT

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Digested by specific enzymes (pancreatic lipase related type 2 (PLRP2) or cholesterol ester hydrolase (CEH) and not by human pancreatic lipase (HPL)

When structured under form of thylakoid membrane => inactivation of pancreatic lipase and weight loss

*(Köhnke et al., Scand. J. Gastrol., 2009; Köhnke et al., Phytotherapy Res., 2009; Fard Tabrizi & Abbasalizad Farhangi, FRI, 2021)*

Antioxidant – DGDG > MGDG > TAG

*(Yamaguchi et al., J. Oleo Sci., 2012; Hazahari, et al., Food Nutr. Sci., 2018)*

Natural surfactants

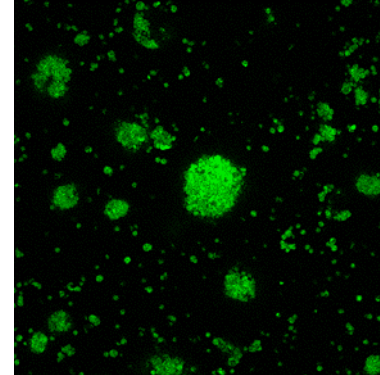


# OUTLINE OF THE PRESENTATION

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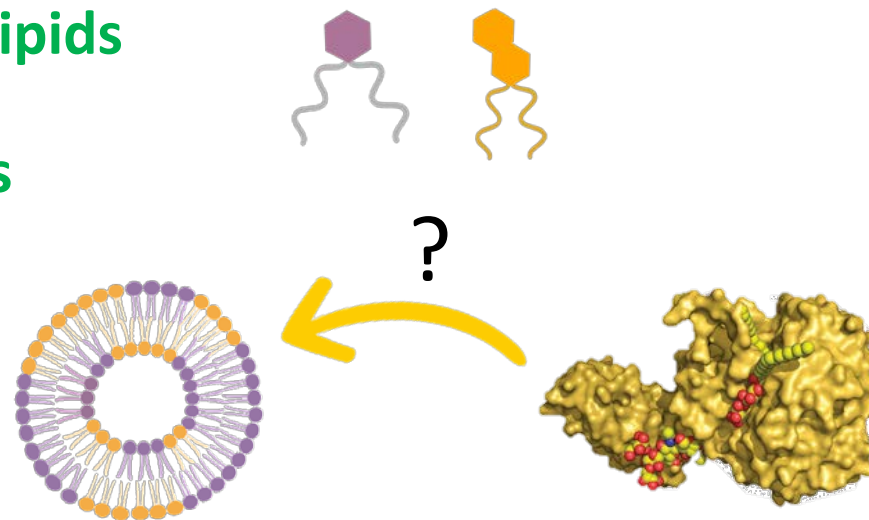
## 1) Nutritional interest of microalgae lipids

- content in omega 3 and its plasticity
- content in galactolipids
- potential presence of oxylipins

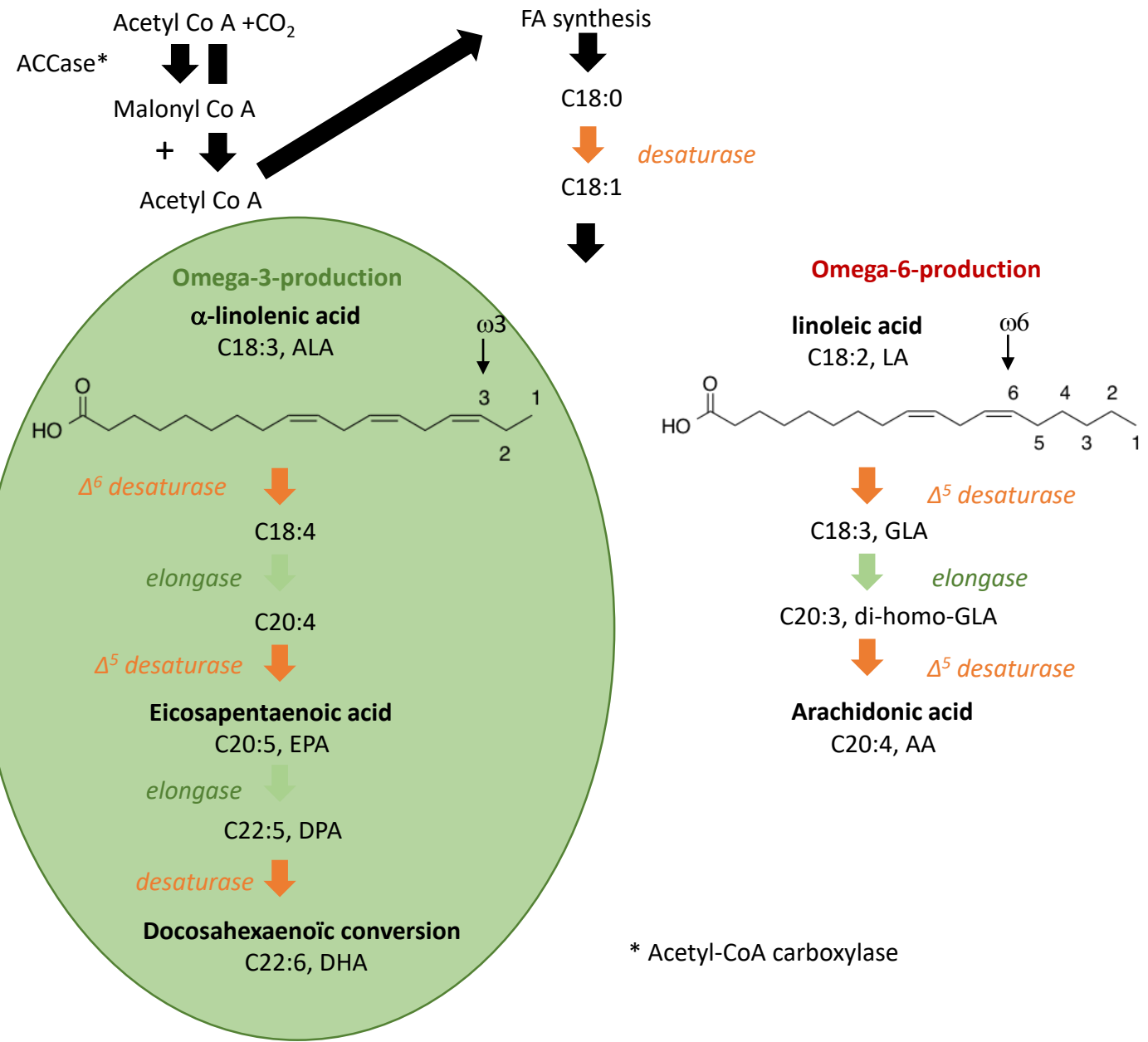


## 2) Interfacial behaviour of microalgae lipids

## 3) Digestion of microalgae galactolipids



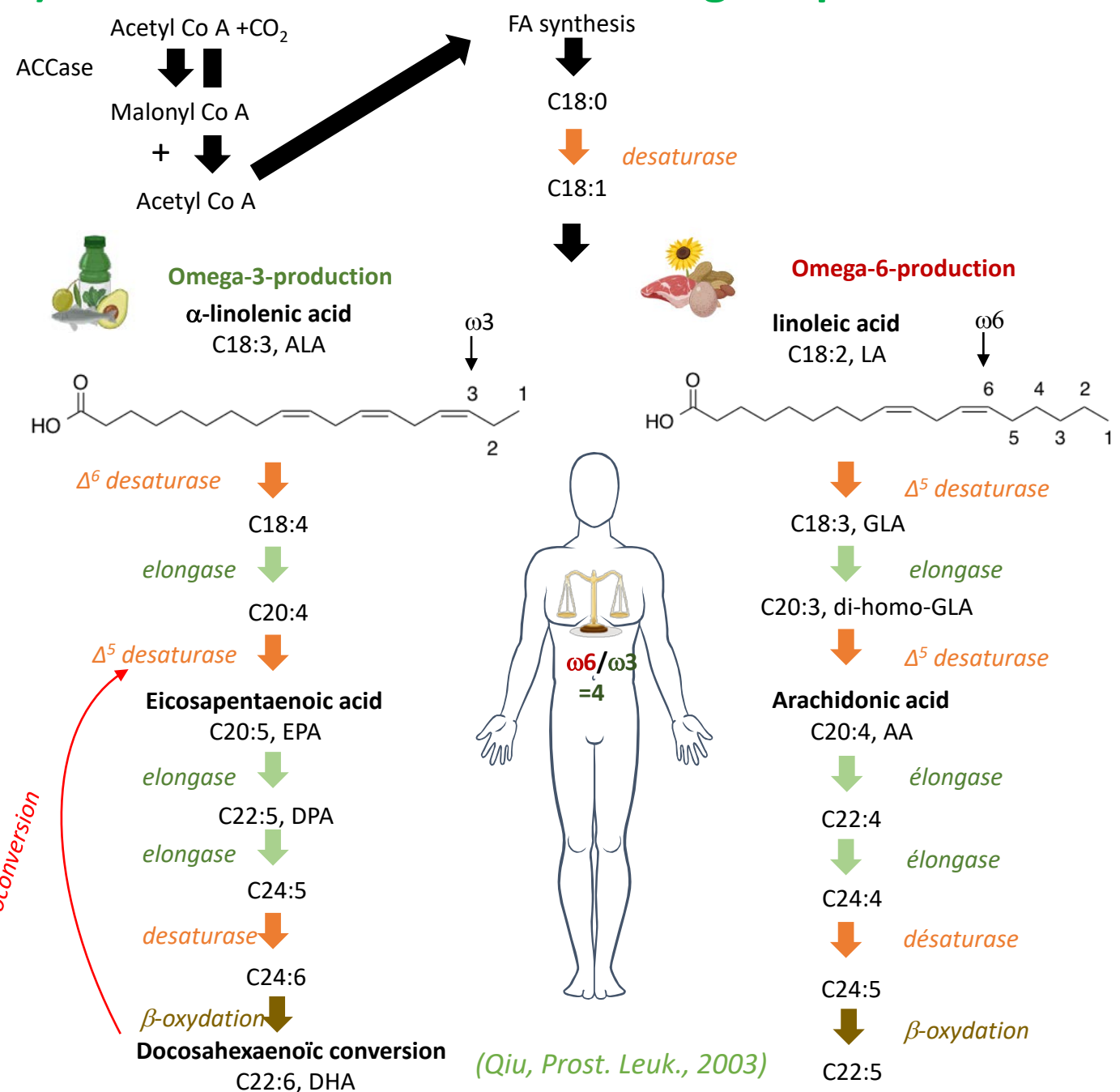
# 1) Nutritional interest of microalgae lipids



	PUFA (% wof total FA)			
	AA	ALA	EPA	DHA
<i>Isochrysis galbana</i>	0.7	1.2	22.6	8.4
<i>Phaeodactylum tricornutum</i>	3.4	0.6	29.8	0.8
<i>Porphyridium cruentum</i>	23	1.0	23.9	0.2
<i>Cryptocodinium chnii</i>	-	-	-	51.12
<i>Schizochytrium sp.</i>	--	-	-	25-50
<i>Aurantiochytrium sp.</i>	-	-	-	23-64
<i>Nitzschia sp.</i>	-	-	16-23	-
Cod liver oil	2.7	0.8	12.5	9.2
Tuna oil	0.92	-	7.81	24.56
Shark liver oil	0.05	-	0.05	0.28
Soft-shell turtle oil	0.64	-	0.19	0.42
Lemuru oil	2.00	-	14.36	4.60
Flaxseed, whole, (1 tbsp)	2.35 g/serving	-	-	-
English walnuts (1oz)	2.57 g/serving	-	-	-

(Katiyar et Arora, Algal Res., 2020)

# 1) Nutritional interest of microalgae lipids

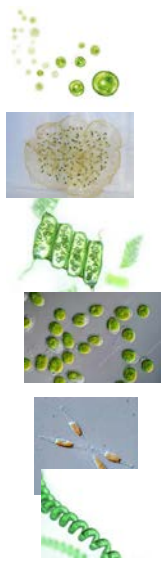
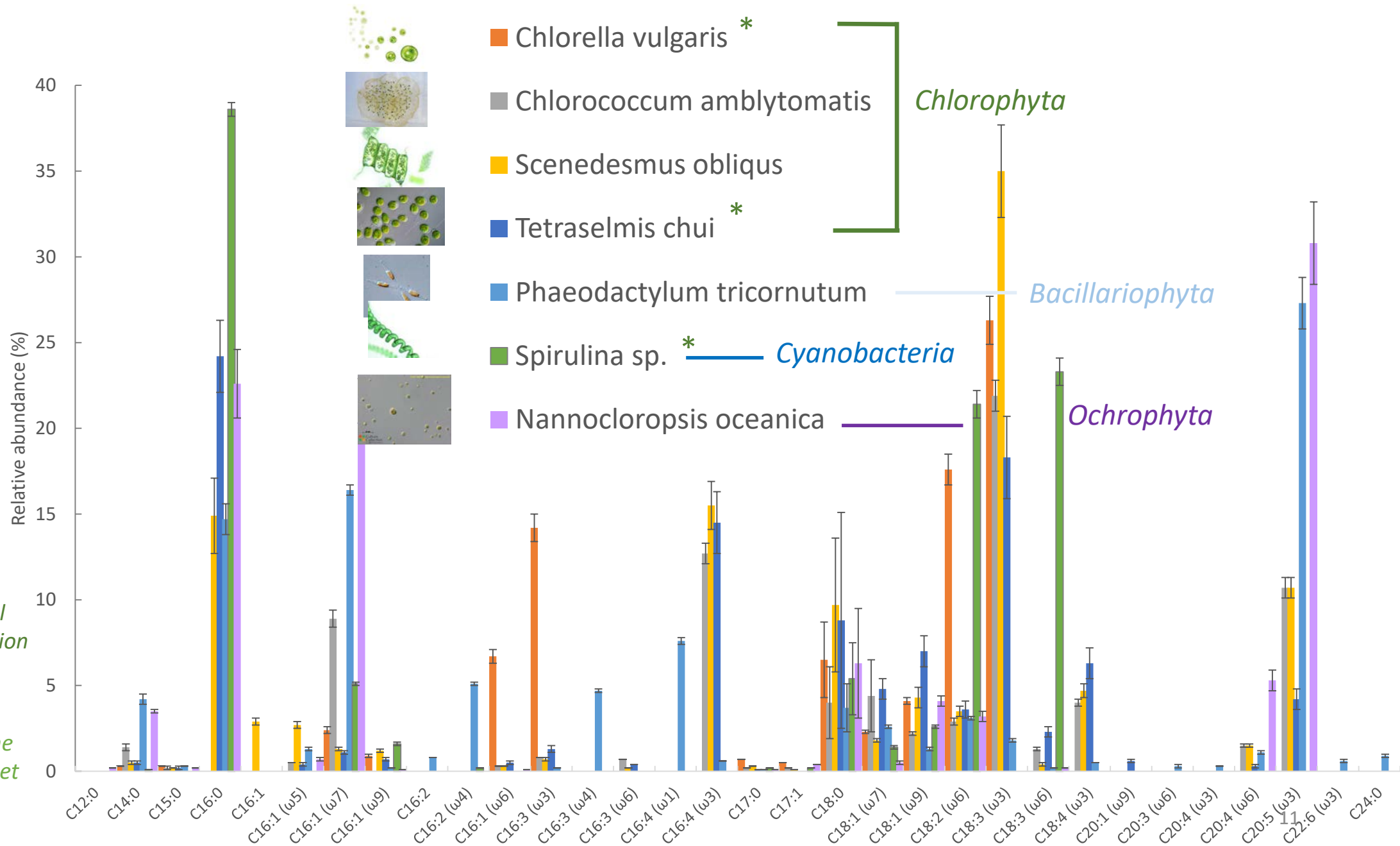


(Qiu, Prost. Leuk., 2003)

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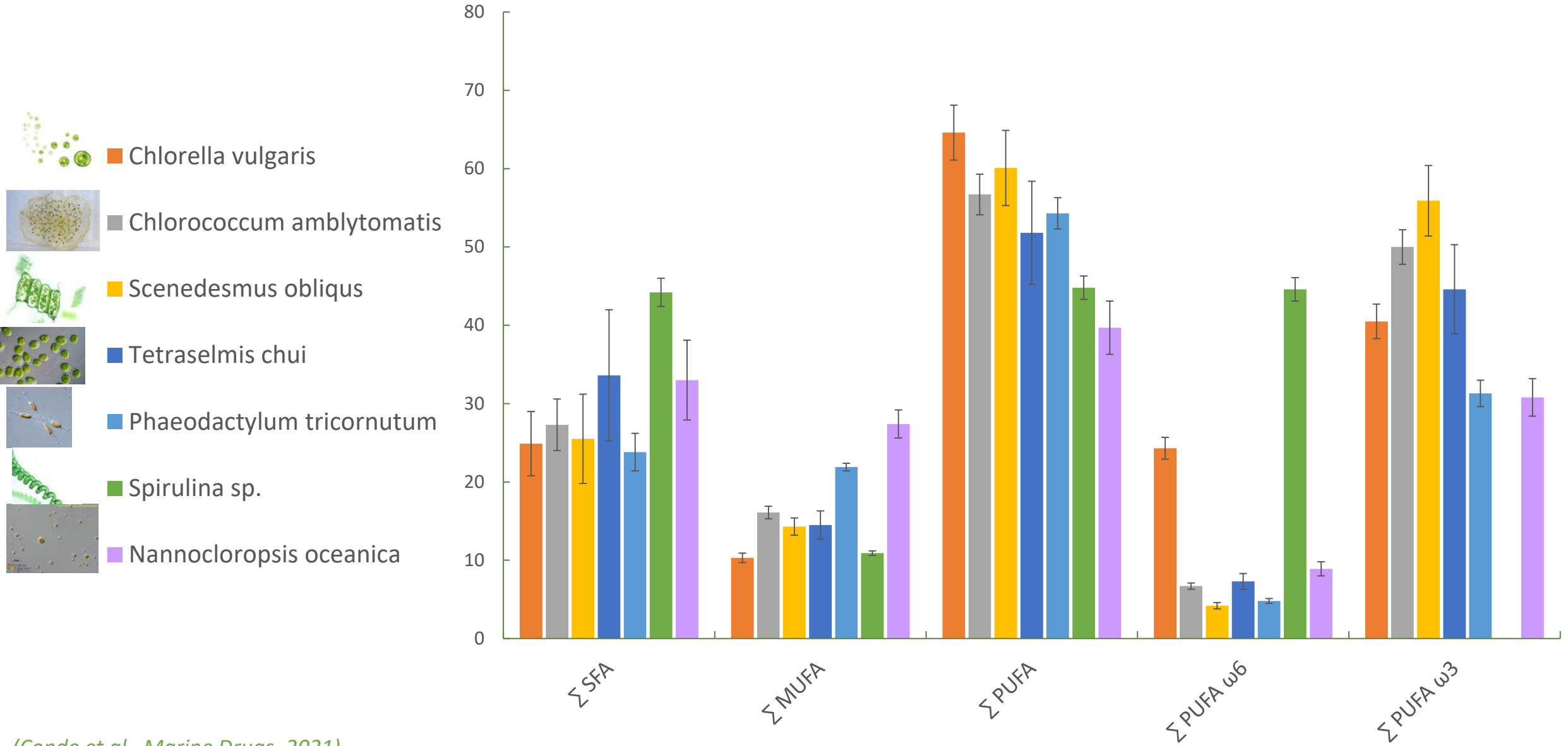
# 1) Nutritional interest of microalgae lipids



\*food grade approval for human consumption

(Conde et al., Marine Drugs, 2021; Jeong et al., BMC Plant Biol., 2011)

# 1) Nutritional interest of microalgae lipids



(Conde et al., Marine Drugs, 2021)

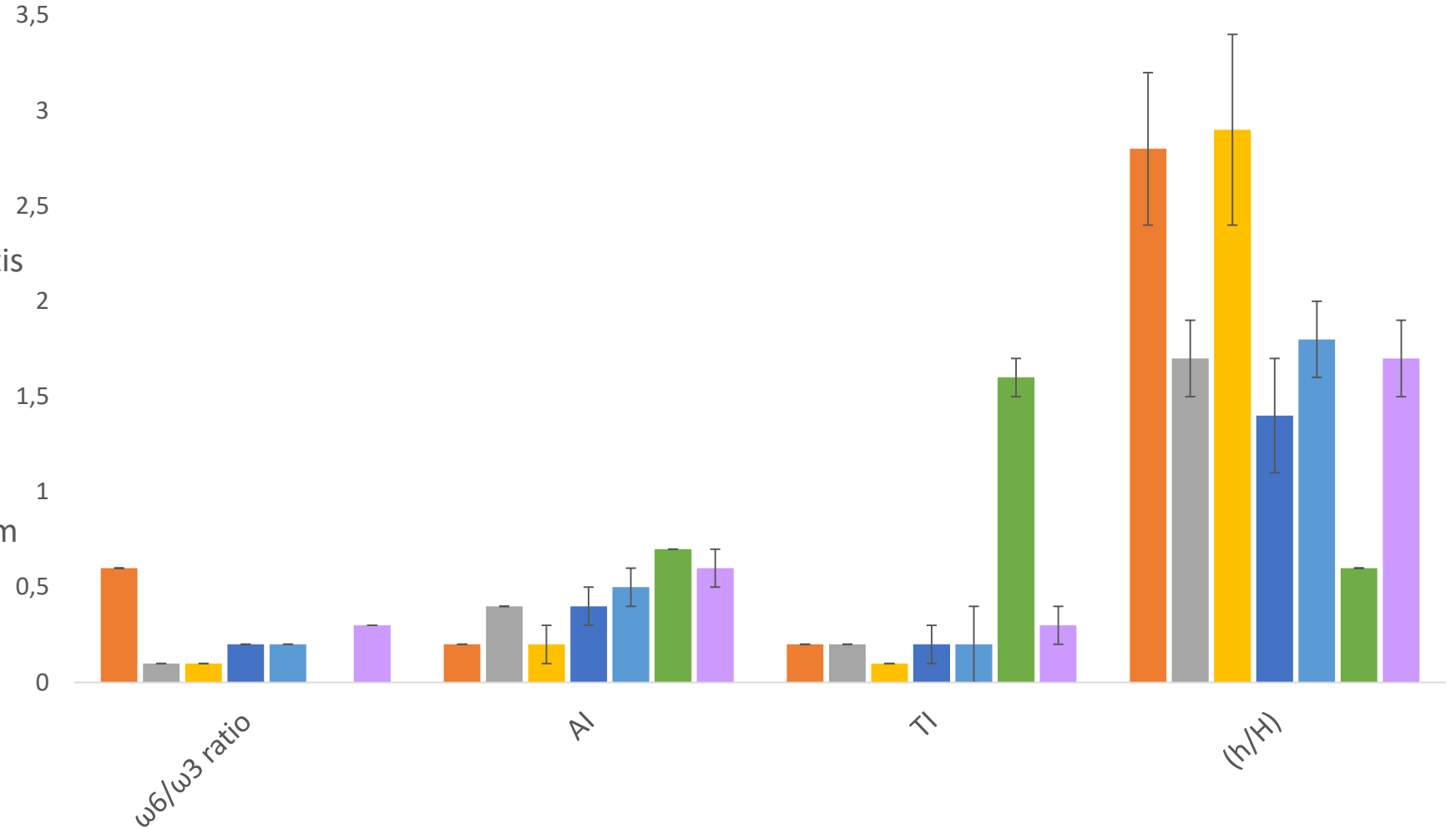


# 1) Nutritional interest of microalgae lipids

4



- Chlorella vulgaris
- Chlorococcum amblytomatis
- Scenedesmus obliquus
- Tetraselmis chui
- Phaeodactylum tricornutum
- Spirulina sp.
- Nannochloropsis oceanica



(Conde et al., Marine Drugs, 2021)

Ab: AI=atherogenic index, TI= thrombotic index, h/H= hypo/hypercholesterolemic index (Ulbricht and Southgate, Lancet, 1991)

$$AI = \frac{[C12:0 + 4] \times [(C14:0) + C16:0]}{[\sum MUFA + \sum(n-6) + \sum(n-3)]}$$
 Coconut oil AI=14, TI=6, Olive oil AI=0.14, TI=0.32, sunflower oil AI=0.07, TI=0.28, butter AI=2.03, TI=2.07

$$TI = \frac{[C14:0 + C16:0 + C18:0]}{[0.5 \times \sum MUFA + 0.5 \times \sum(n-6) + 3 \times \sum(n-3) + (\frac{\sum(n-6)}{\sum(n-3)})]}$$

$$(h/H) = \frac{[C18:1(\omega-9) + 18:2(\omega-6) + 18:3(\omega-3) + C20:4(\omega-6) + C20:5(\omega-3)]}{[C14:0 + C16:0]}$$

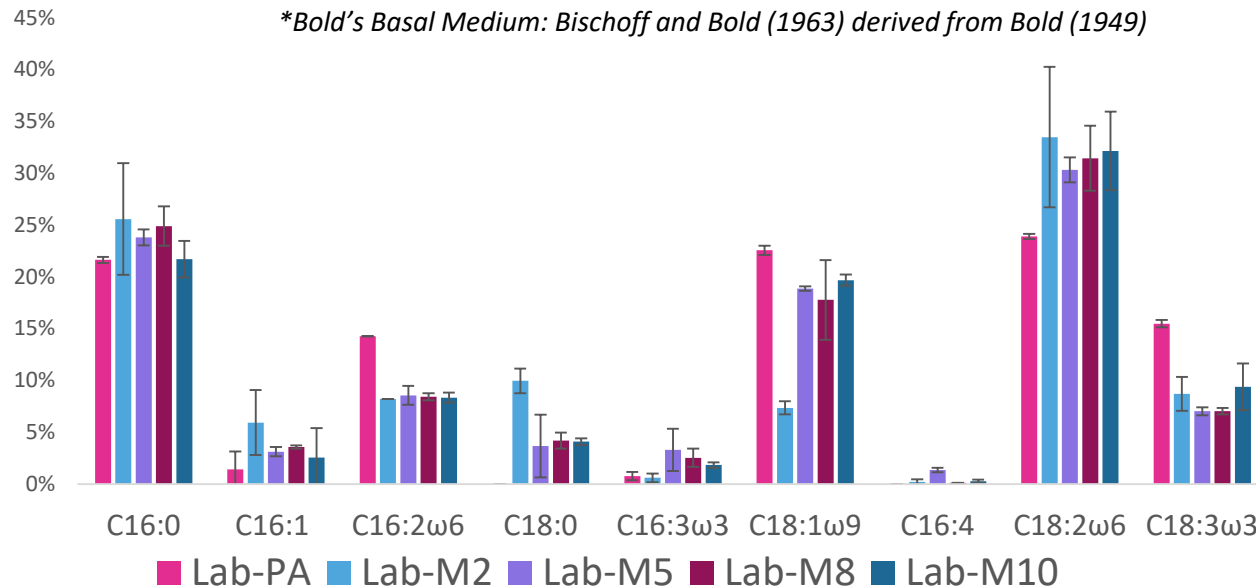
# 1) Nutritional interest of microalgae lipids

✓ Plasticity of FA profiles and molecules on which these FA are esterified

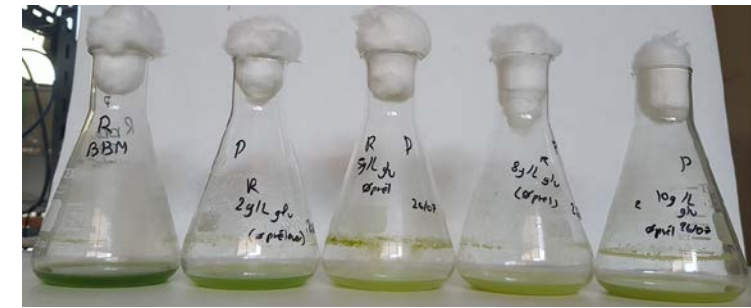


Trophic mode	[Glucose] (g/L added to BBM*)	% lipids in the dry biomass	Abbreviation	$\omega 6/\omega 3$
Photo-autotrophy	0	22	Lab-PA	2.4
Mixotrophy	2	20	Lab-M2	4.5
Mixotrophy	5	13	Lab-M5	3.8
Mixotrophy	8	13	Lab-M8	4.2
Mixotrophy	10	16	Lab-M10	3.6

\*Bold's Basal Medium: Bischoff and Bold (1963) derived from Bold (1949)

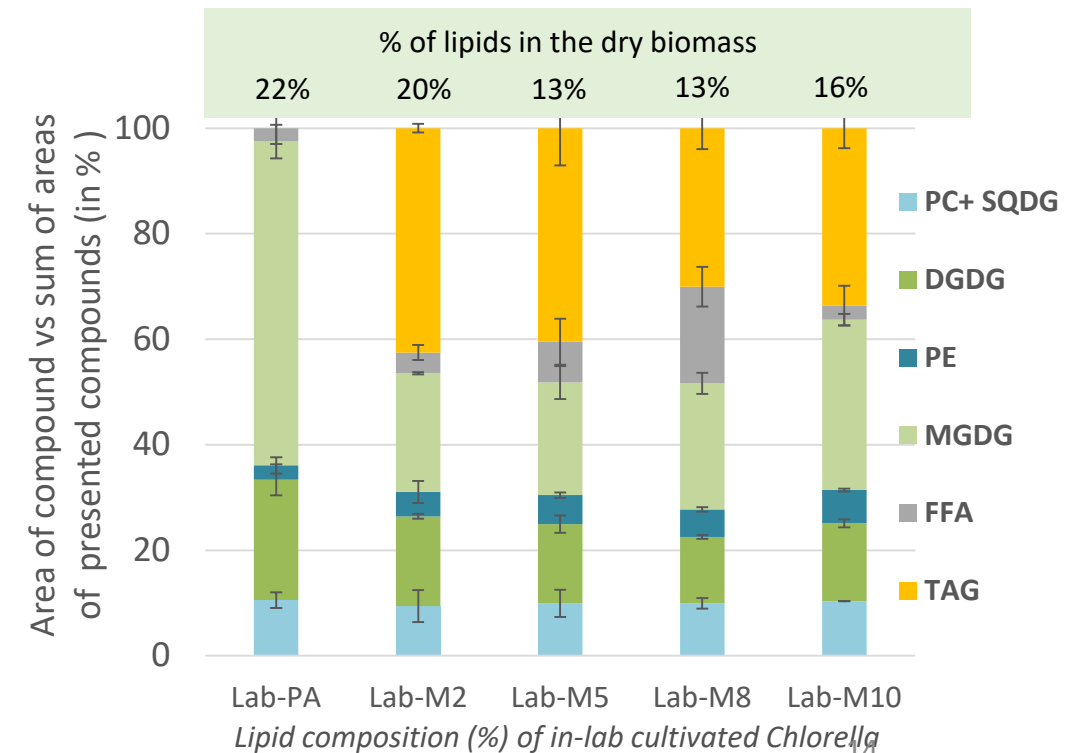


Fatty acid profiles of in-lab cultivated Chlorella



Lab-PA Lab-M2 Lab-M4 Lab-M8 Lab-M10

Visual aspect of in-lab cultivated Chlorella Sorokiniana

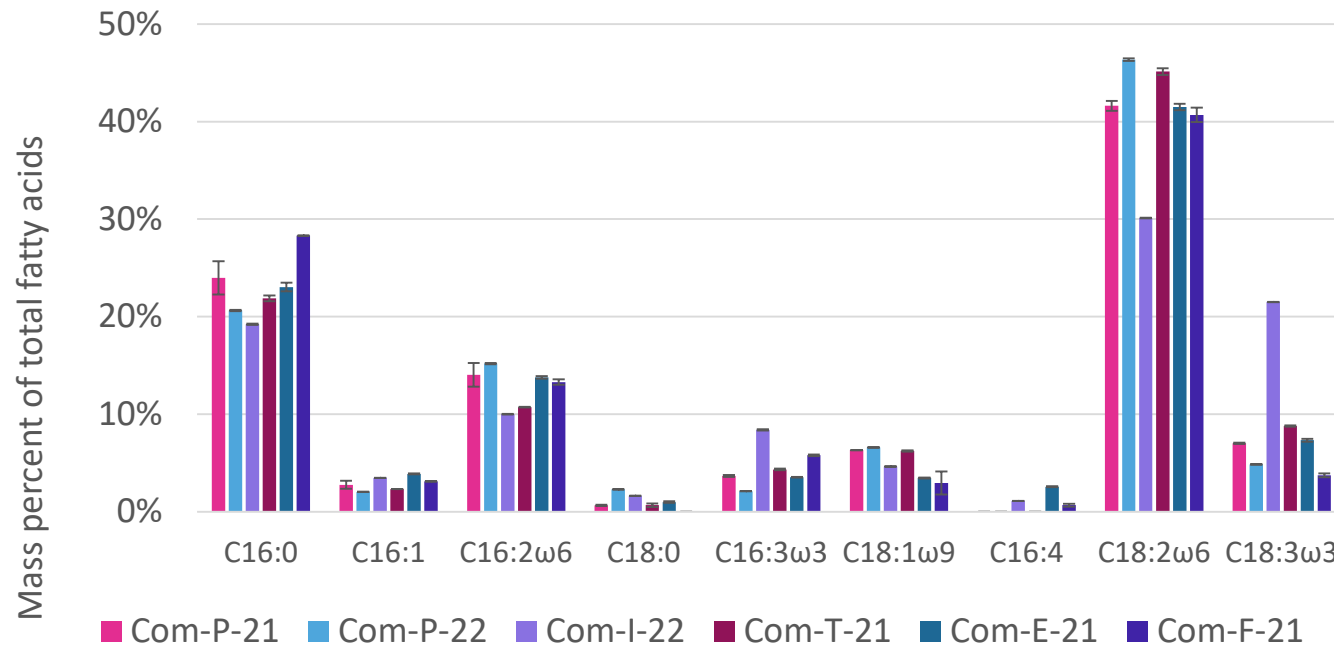


# 1) Nutritional interest of microalgae lipids

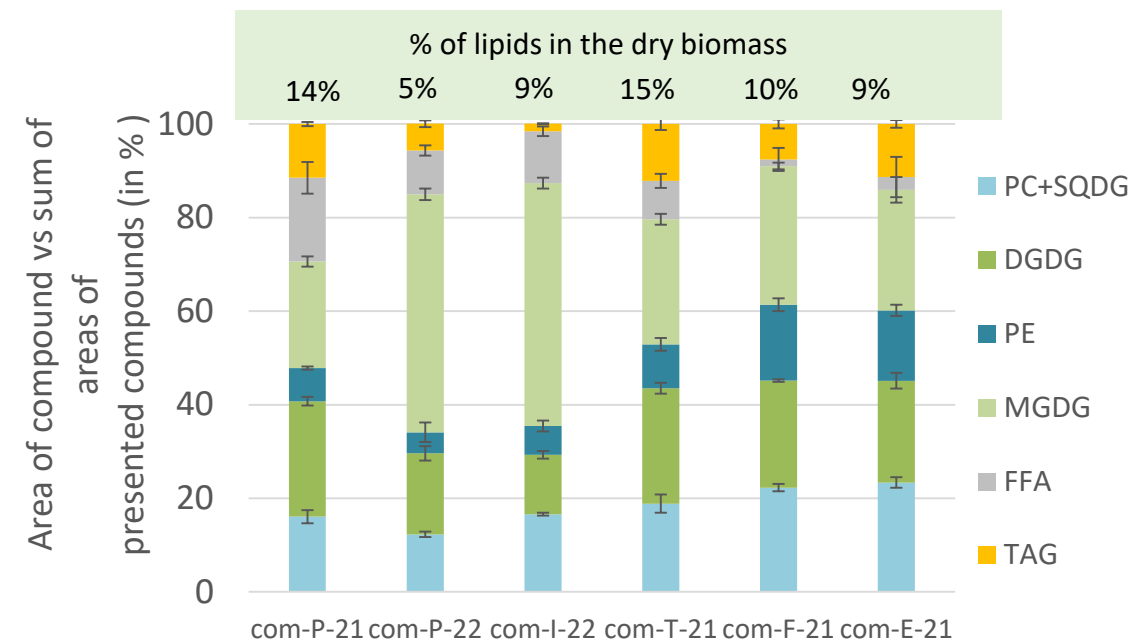
✓ Plasticity of FA profiles and molecules on which these FA are esterified



Origin	Species	Year of production	Specifications	Abbreviation	$\omega 6/\omega 3$
Mongolia or Hainan island	<i>C. vulgaris</i>	2021	Broken cell wall	Com-P-21	5.2
	<i>C. vulgaris</i>	2022	Broken cell wall	Com-P-22	8.9
France	<i>C. vulgaris</i>	2021	Whole cells	Com-T-21	4.3
France	<i>C. vulgaris</i>	2021	Whole cells	Com-F-21	5.7
France	<i>C. pyrenoidosa</i>	2022	Broken cell wall	Com-I-22	1.3
Netherlands	<i>C. sorokinana</i>	2021	Broken cell wall - "fermented"	Com-E-21	5.1



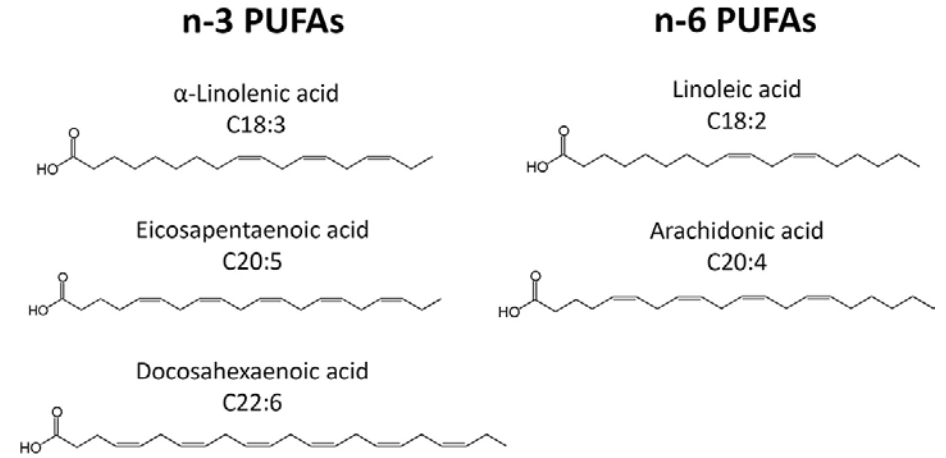
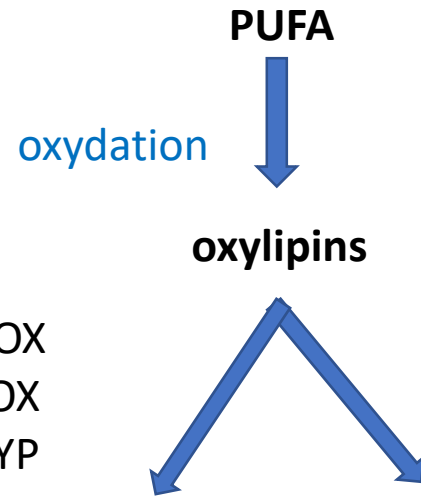
Fatty acid profiles of commercial *Chlorella*



Lipid composition (%) of commercial *Chlorella* 15  
(Wind, Master 2, 2021; Barouh et al., Chevreul Congress 2023, Paris)

# 1) Nutritional interest of microalgae lipids

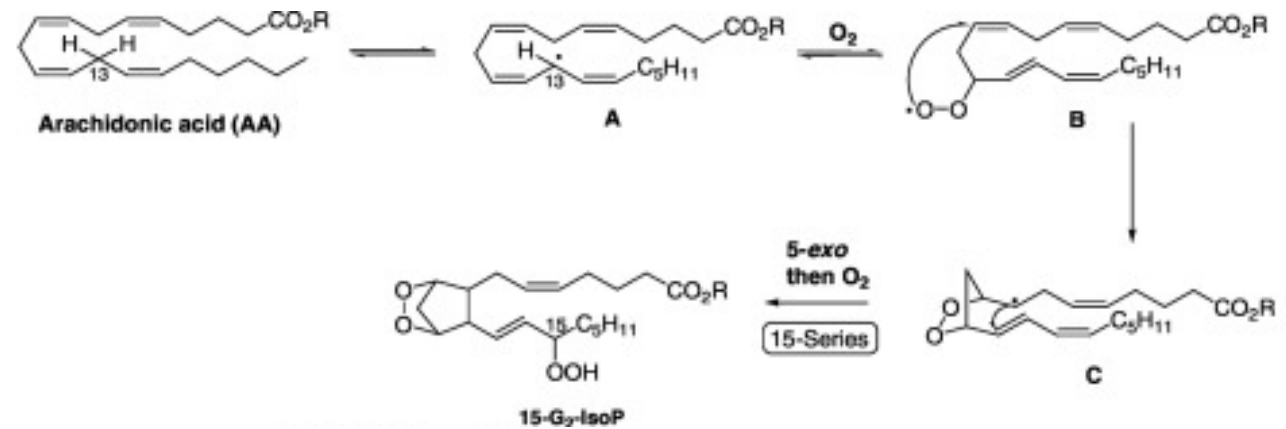
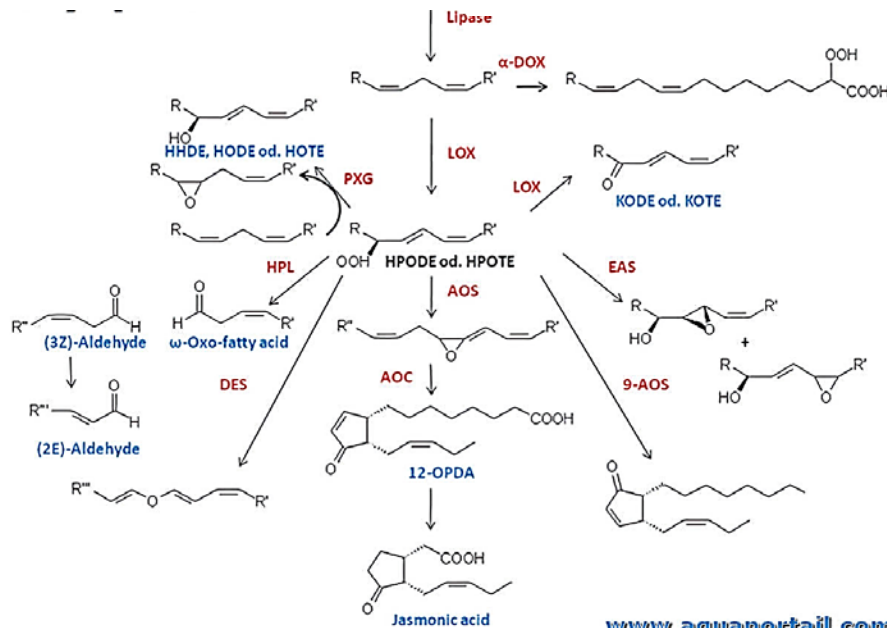
✓ Presence of oxylipins ?



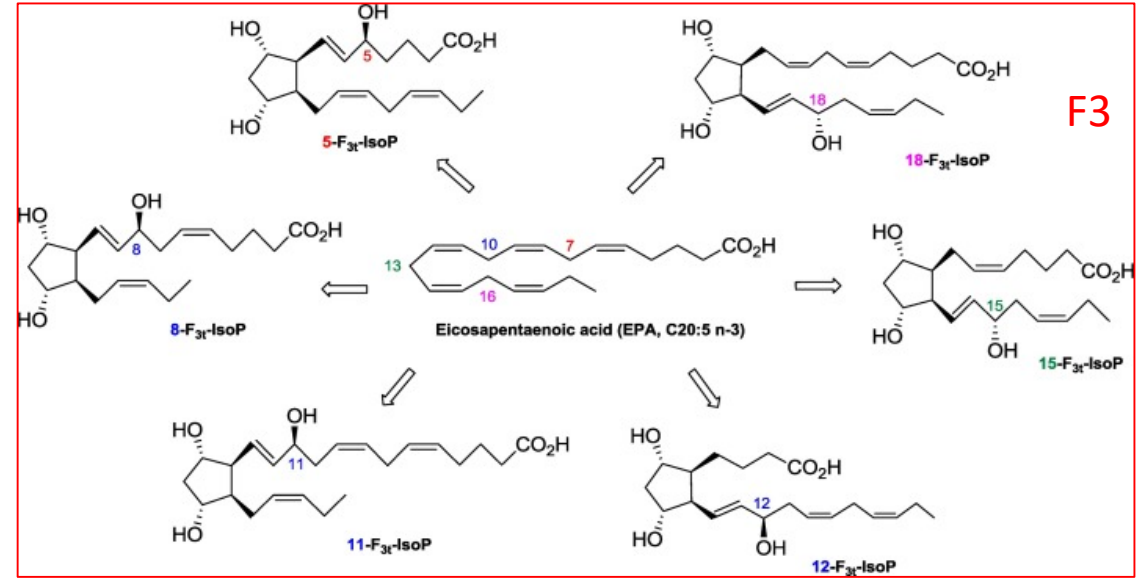
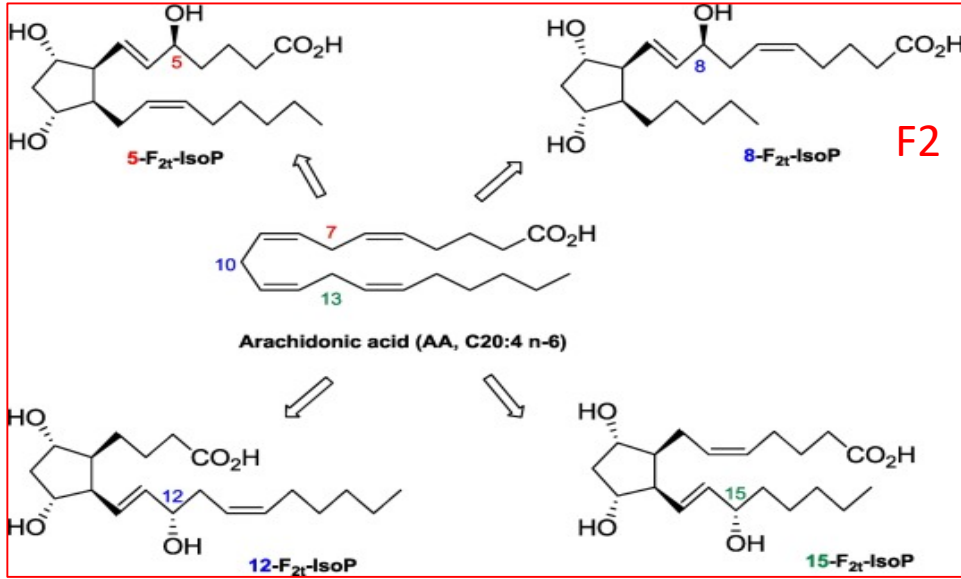
Enzymatic

Non – enzymatic

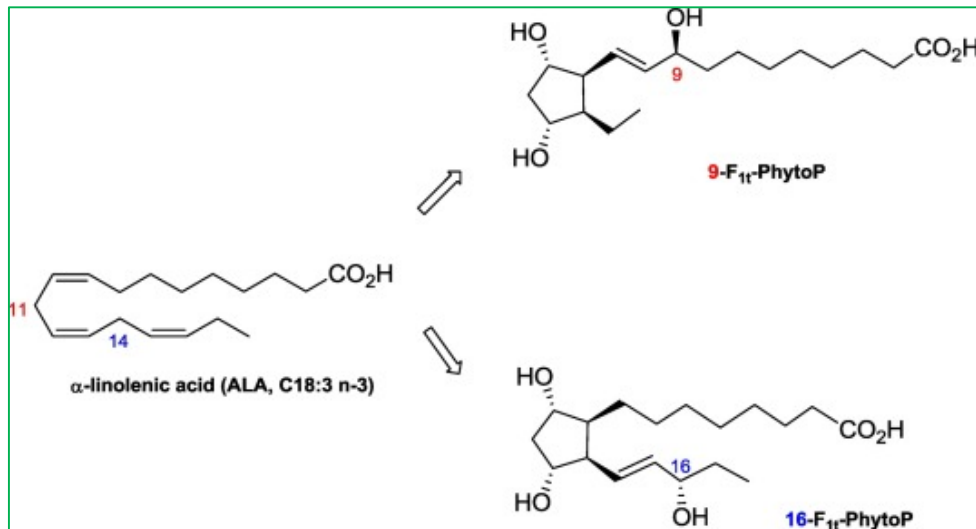
= created by ROS / radical reaction



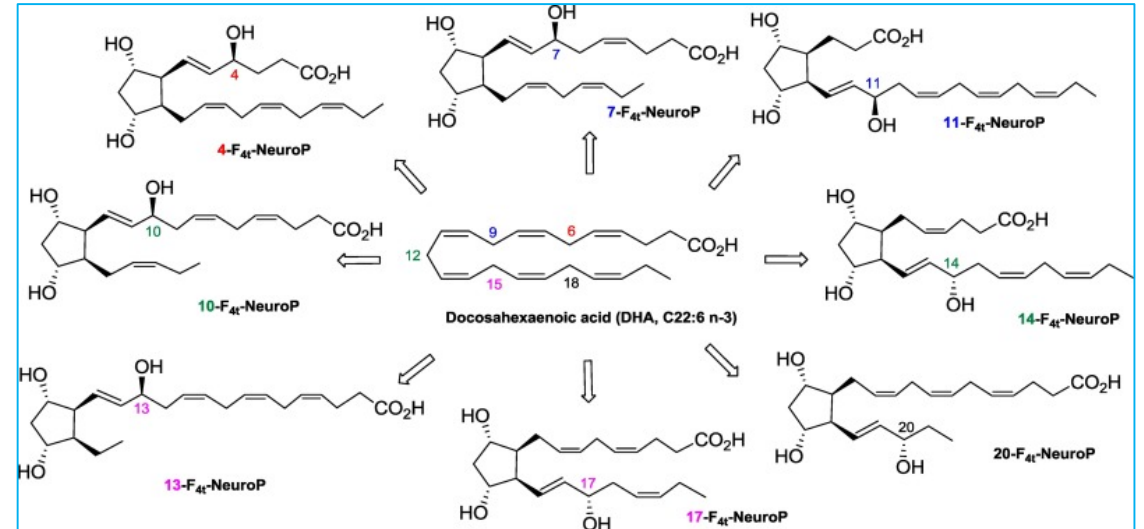
# Main classes of NE oxylipins



Arachidonic acid (AA, C<sub>20</sub>:4 n-6) et eicosapentaenoic acid (EPA, C<sub>20</sub>:5 n-3) → **Isoprostanes (IsoPs)**



Alpha-linolenic acid (ALA, C<sub>18</sub>:3 n-3) → **Phytoprostanes (PhytoPs)**



Docosahexaenoic acid (DHA) → **Neuroprostanes (NeuroPs)**



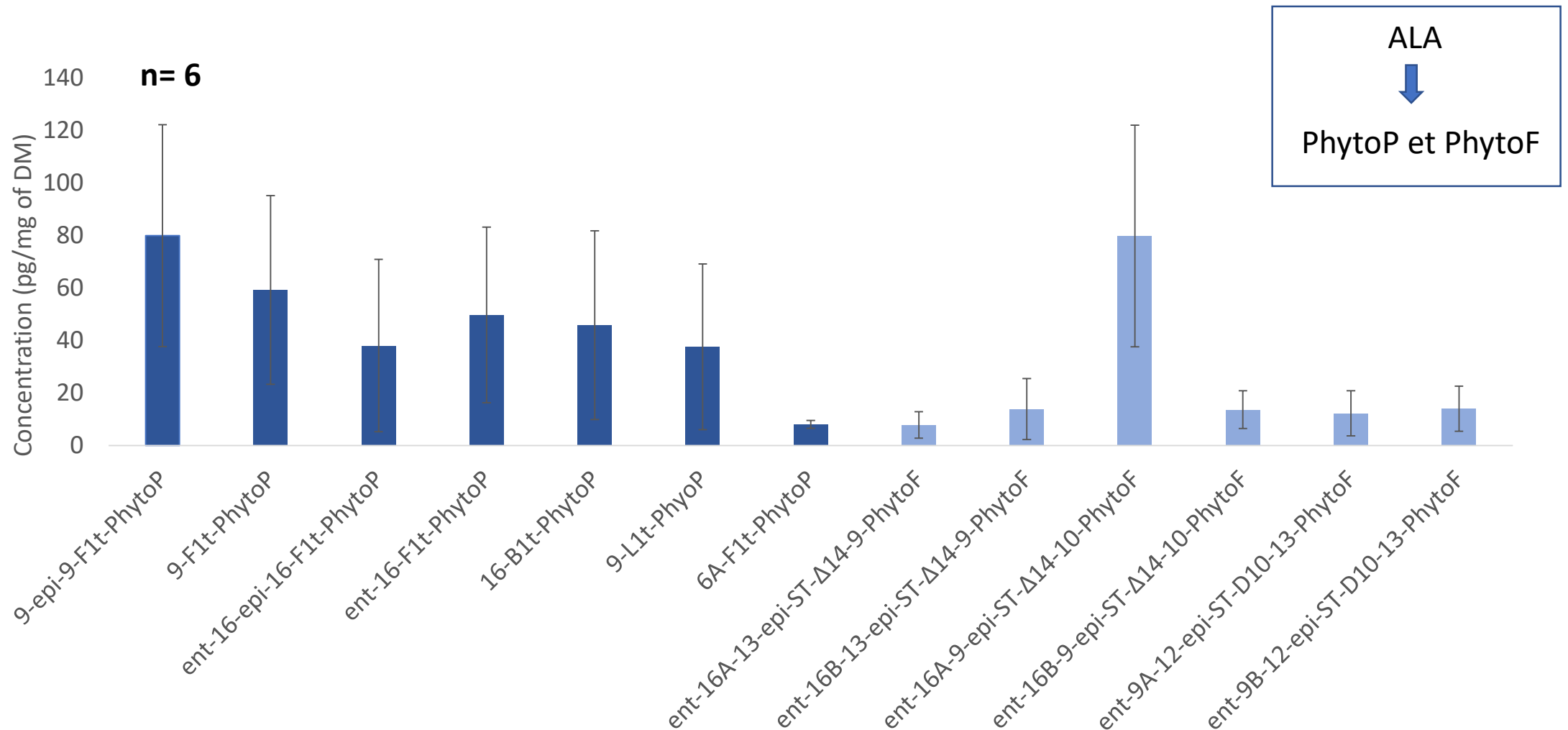
# 1) Nutritional interest of microalgae lipids

✓ Presence of oxylipins ?

Oxylipins	Activity
Ent-16B1t-PhytoP	Neuroprotectivity
Ent-9L1t-PhytoP	Inflammatory
8-F3-IsoP	Pro-arythmia
10-F4t-NeuroP	Artherosclerosis prevention Anti VIDD (Ventilator Induce Diaphragm Disfunction)
4+F4t-NeuroP	Anti-inflammatory Artherosclerosis prevention Anti VIDD (Ventilator Induce Diaphragm Disfunction) Anti-arythmia Neuroprotectivity Sperm capacitation
14-A4t-NeuroP	Anti-inflammatory

# 1) Nutritional interest of microalgae lipids

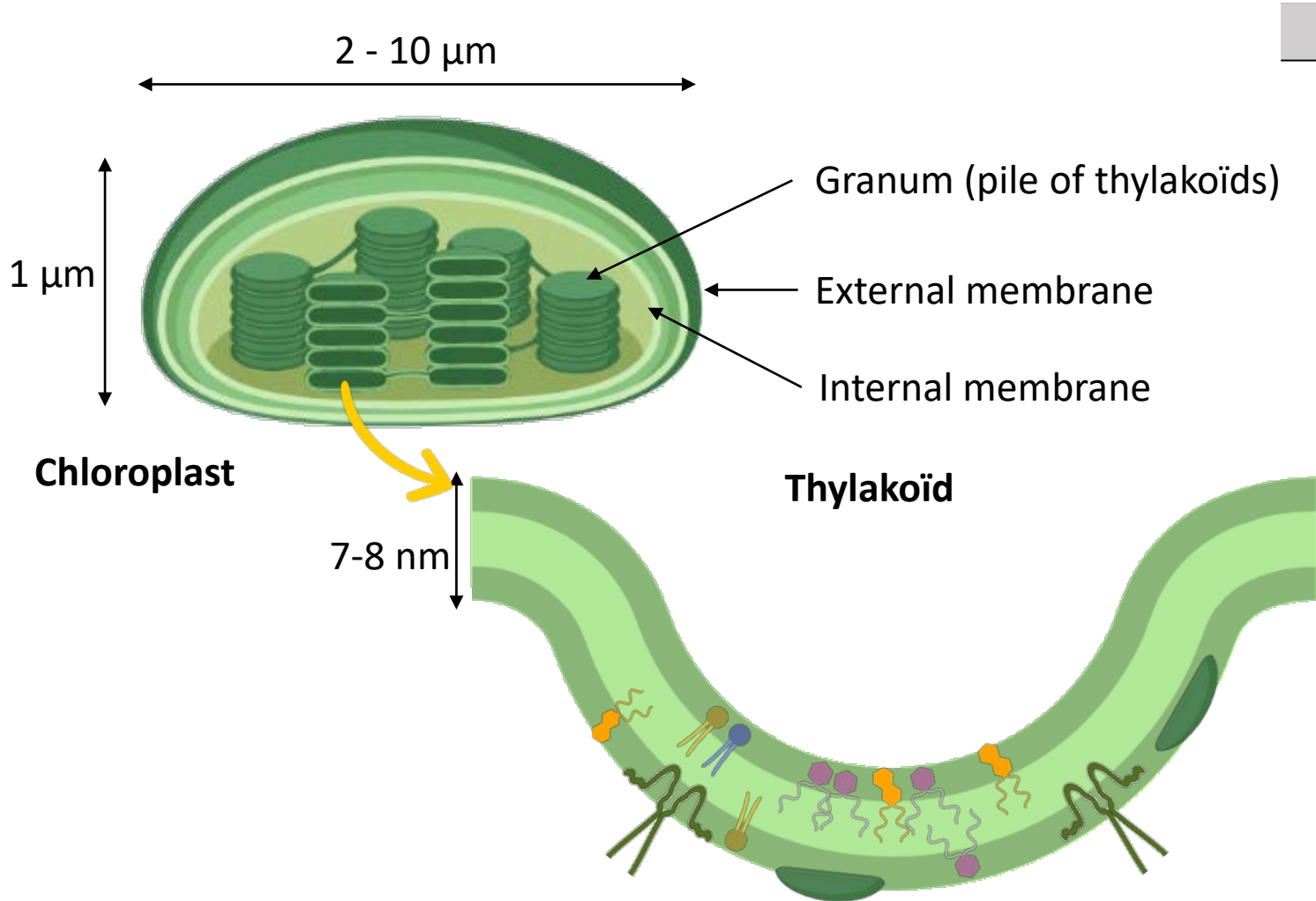
✓ Presence of oxylipins ?



**Profile in non enzymatic oxylipins detected in lipid extract of *C. sorokiniana* cultivated in photoautotrophy**

(Avila-Roman et al., Pharmacol Res., 2018; Los Reyes et al., Phytochem, 2014; Conde, et al. Int. J. Mol. Sci. 2021)

## 2) Interfacial behaviour of microalgae lipids



Protein complexes  
(60-70% wt.)



DGDG (27% wt.)



SQDG (15% wt.)



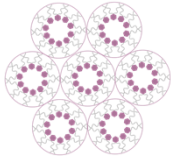

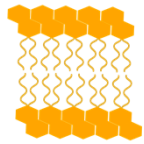
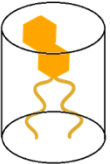
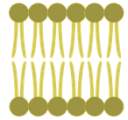
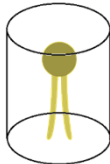
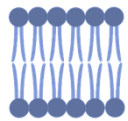
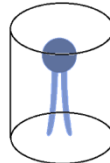
Chlorophyll



MGDG (52% wt.)



PG (6% wt.)

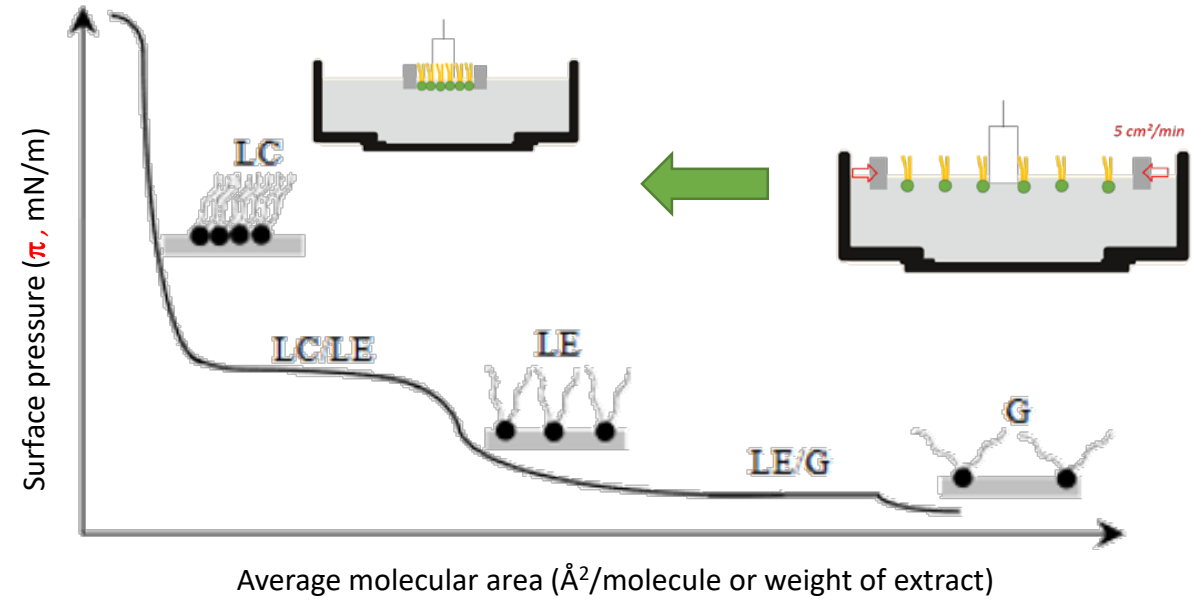
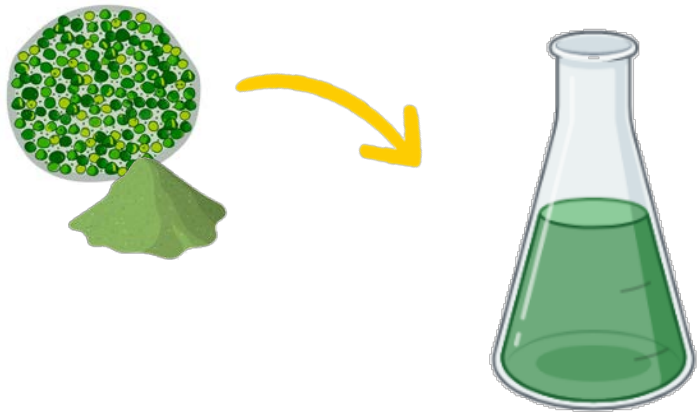
Lipid	Phase	Molecular arrangement
MGDG	Hexagonal inverse (HII)	  Conic
DGDG	Lamellar ( $L\alpha$ )	  Cylindrical
SQDG	Lamellar ( $L\alpha$ )	  Cylindrical
PG	Lamellar ( $L\alpha$ )	  Cylindrical

Ab: DGDG: digalactosyldiacylglycerol, DGMG: digalactosylmonoacylglycerol, MGDG: monogalactosyldiacylglycerol, SQDG: sulfoquinovosyldiacylglycerol, PG: Phosphatidylglycerol

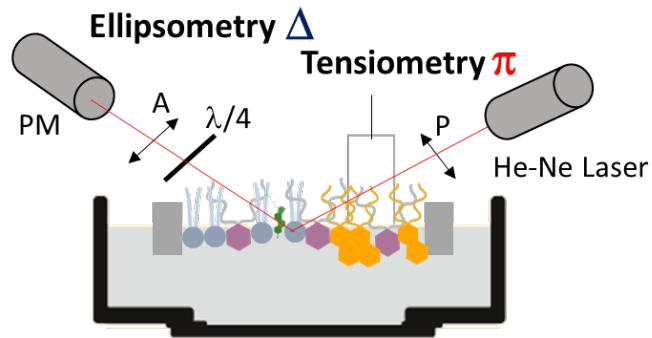
(Gurevich et al., Eur. J. Int. Med., 1997; Jouet et al., Front. Plant Sci., 2013; Kergomard et al., CRFSN, 2021; Luesse et al. Plant Physiology, 2006)

## 2) Interfacial behaviour of microalgae lipids

Folch extraction of total lipids on commercial or in-lab produced *C. sorokiniana* extracts



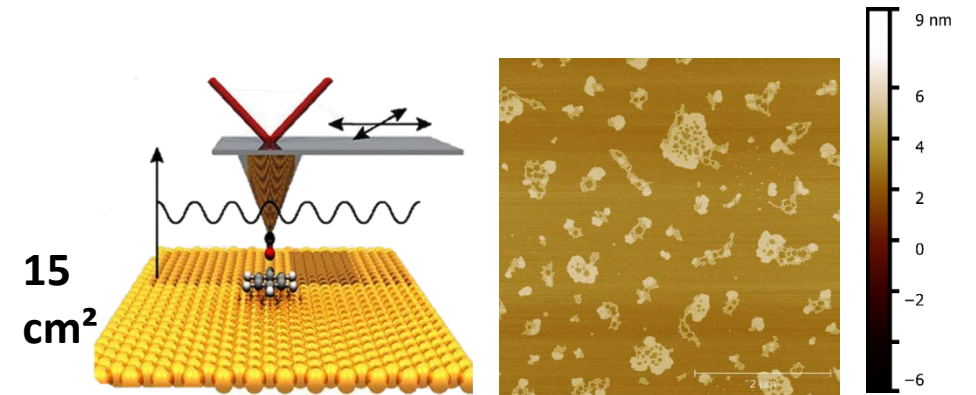
### Ellipsometry/Tensiometry



Langmuir Blodgett transfer



### Atomic Force Microscopy (AFM)

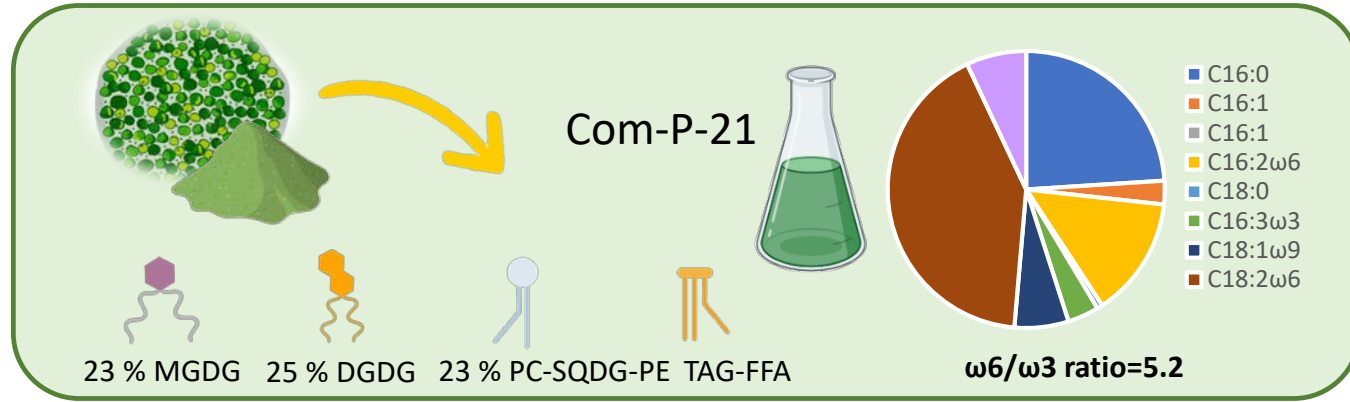


$\pi$  (surface pressure)  $\rightarrow$  molecular interfacial interactions

$\Delta$  (ellipsometric angle)  $\rightarrow$  amount of matter at the a/w interface

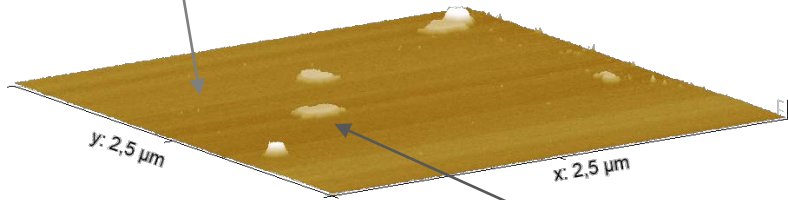
Topographic visualisation at the nanometric scale

## 2) Interfacial behaviour of microalgae lipids

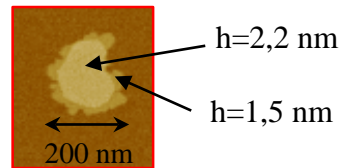


✓ In monolayers,  $\pi=19.7\pm 0.5$  mN/m,  $\Delta= 8.1\pm 0.5$  °

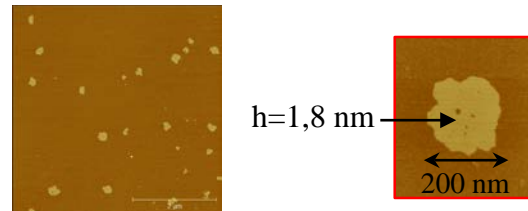
Fluid main organization  
(Liquid Expanded, 75 % unsaturated FA)



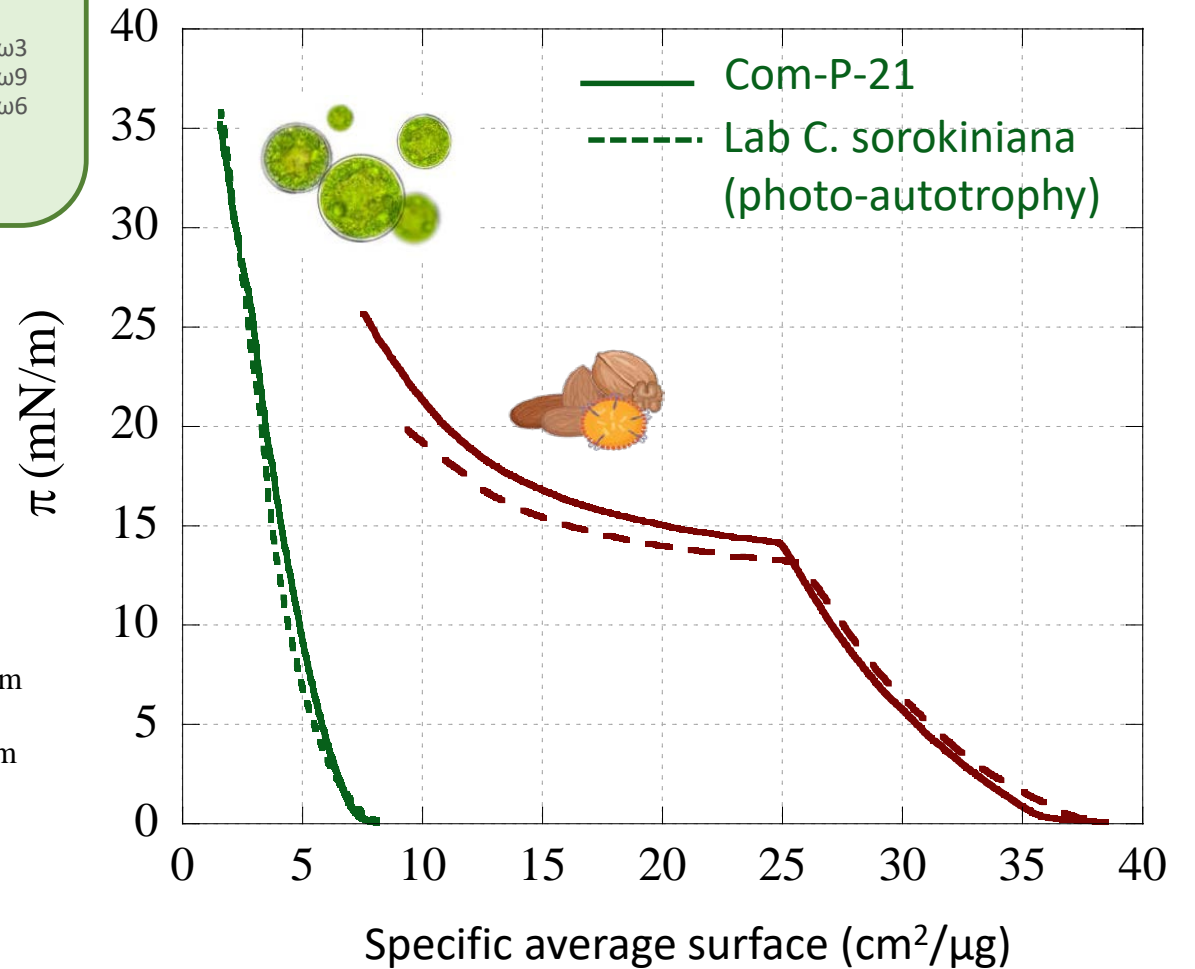
Presence of small liquid condensed domains  
(high affinity between saturated FA)  
with 2 different heights



✓ Similar behaviour in lab biomass despite variations in chemical composition



✓ High compressibility specially in comparison to glycerophospholipids dominated sources





### 3) Digestion of microalgae galactolipids

## Enzymes at work in Humans



No lipolytic enzyme acting on polar lipids is produced in the gastric compartment

*But the stability of emulsions resulting from gastric digestion influences the kinetics of intestinal digestion.*

*(Infantes-Garcia et al., JAFC, 2021)*

**Gastric lipase (HGL) – 47 kDa**

*Responsible for the digestion of 10 to 30% of TAG*

**Gastric lipolysis**  
pH 3 -5

**Pancreatic phospholipase A2 (sPLA2) - 14 kDa**

*Phospholipase activity in sn-2 position*

**Intestinal lipolysis**  
pH 6 -7.5

***Polar lipid lipolysis***

**Colipase-dependent pancreatic lipase (HPL) – 48 kDa**

*Responsible for the digestion of 56% of TAG*

**Pancreatic lipase related protein 2 (PLRP2) – 50 kDa**

*(N'Goma et al. 2012)*

Responsible for the digestion of galactolipids

# 3) Digestion of microalgae galactolipids

## Model systems

1 GL

MGDG/DGDG 60:40 mol%

Homogeneous

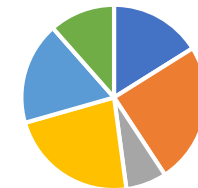
2 MGDG/DGDG/SQDG/PG

MGDG/DGDG/SQDG/PG 56:24:10:10 mol%

Biomimetic

3

Lipid monolayer from *Chlorella vulgaris* (Commercial, Com-P-21)



- PC+SQDG
- DGDG
- PE
- MGDG

## Molecular scale (nm)

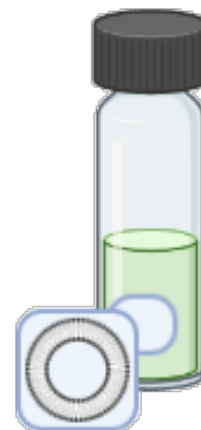
Interfacial characterization in Langmuir trough



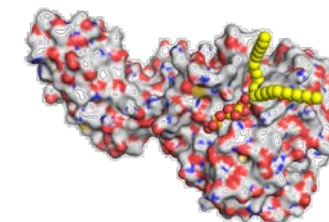
Lipid monolayers

## Assembly scale (µm)

Static *in vitro* model (mL) - Bulk



Giant liposomes



50 kDa

**Guinea pig Pancreatic lipase protein-related 2 PLRP2**

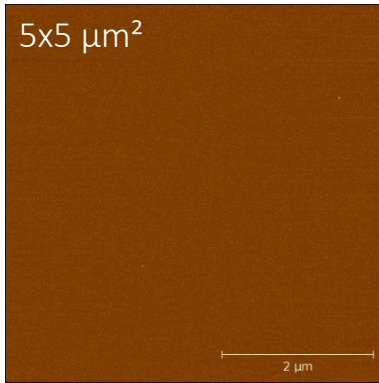
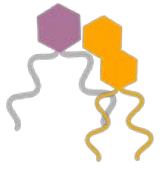
Provided by F. Carrière

Low activity toward TAG and phospholipids  
 High galactolipase activity on galactolipid films and mixed micelles (best substrate)  
 Specificity for the hydrolysis of ester bonds at the sn-1 position

### 3) Digestion of microalgae galactolipids

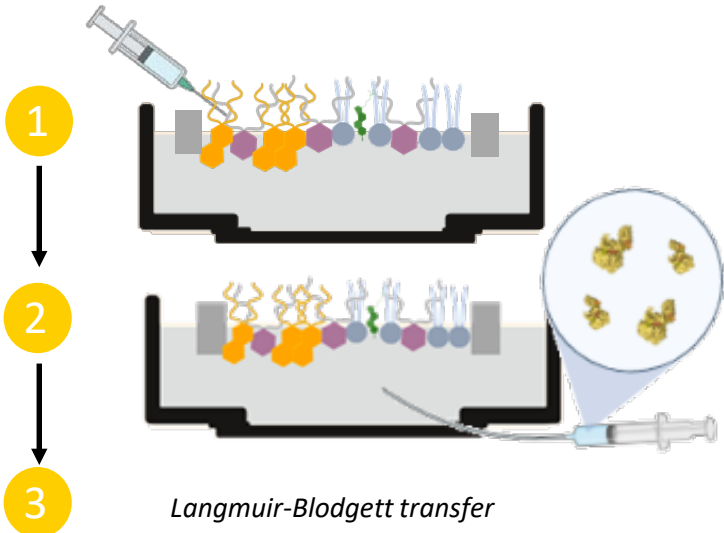
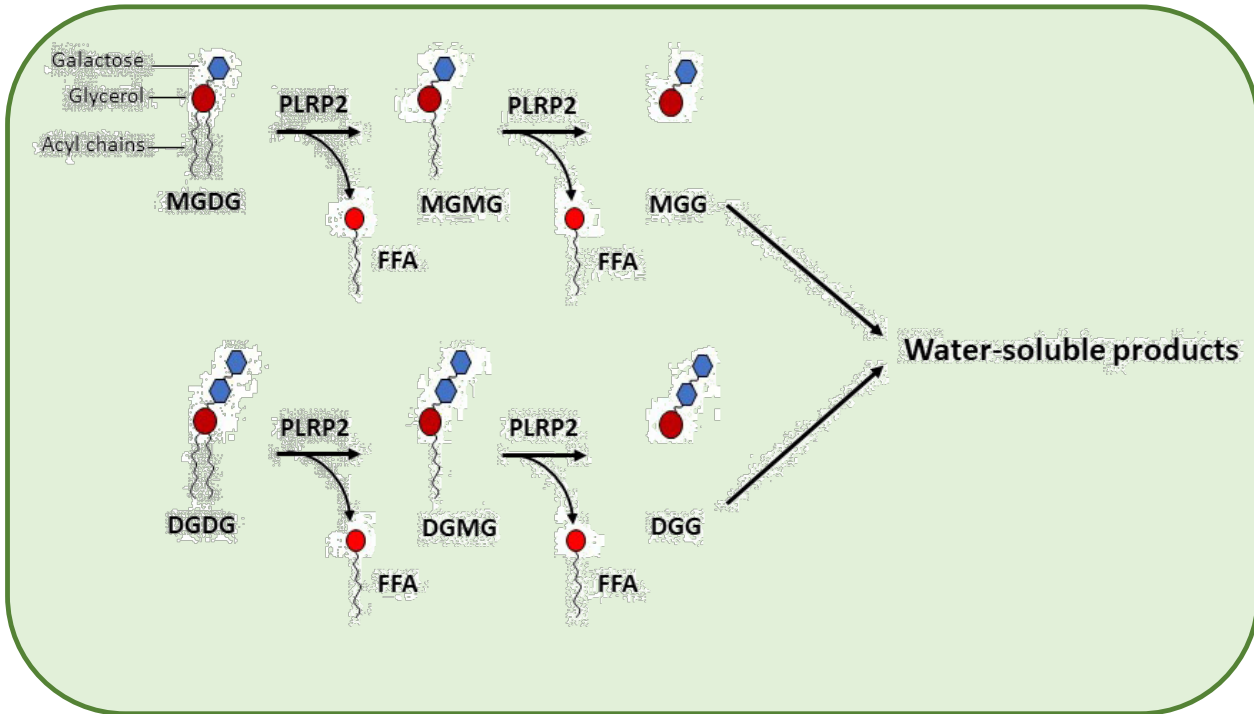
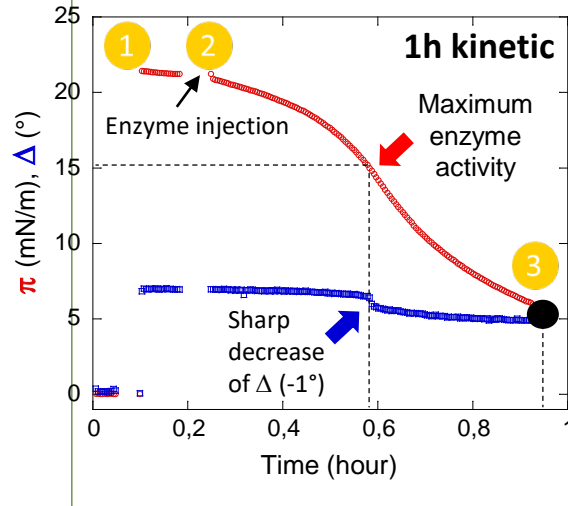
✓ gPLRP2 adsorption and lipolytic activity onto model monolayers at the air/water interface

System 1 – GL



$\pi=20.0$  mN/m  
 $\Delta=7.5^\circ$

+ gPLRP2 (0.128 mg/L)



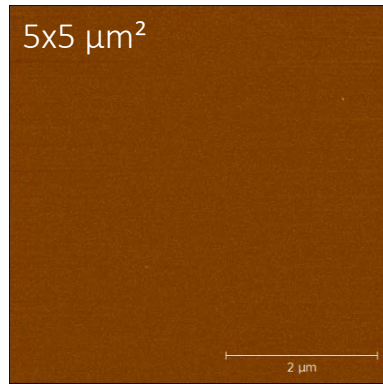
Langmuir-Blodgett transfer

(Kergomard et al, 2022 submitted Biochimie)

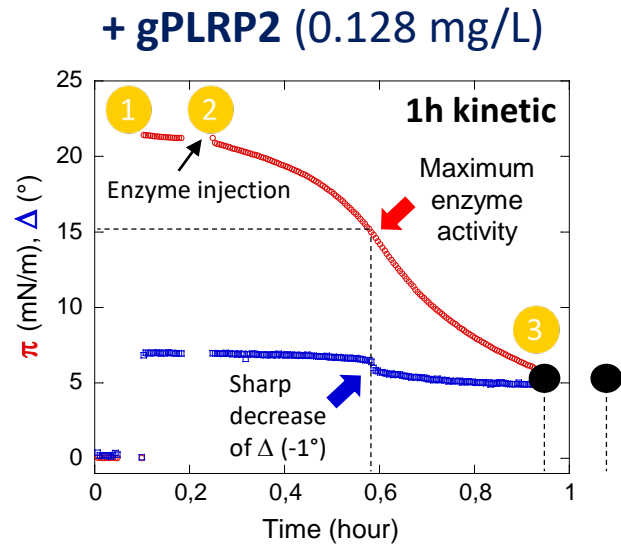
### 3) Digestion of microalgae galactolipids

✓ gPLRP2 adsorption and lipolytic activity onto model monolayers at the air/water interface

#### System 1 – GL



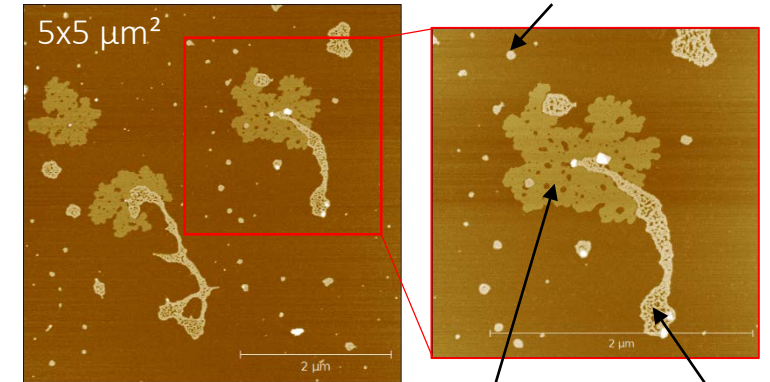
$\pi=20.0$  mN/m  
 $\Delta=7.5^\circ$



Langmuir-Blodgett transfer

$\pi=6.0$  mN/m  
 $\Delta=4.9^\circ$

#### AFM



Adsorbed enzyme?  $h=4-5$  nm

$h=2.5-3$  nm FFA?  $h=3.5$  nm Adsorbed enzyme?

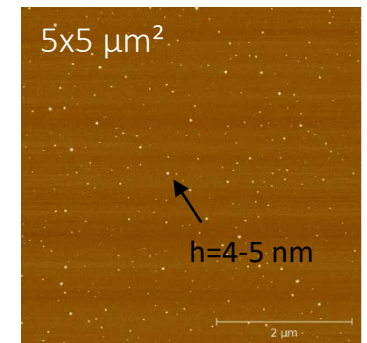
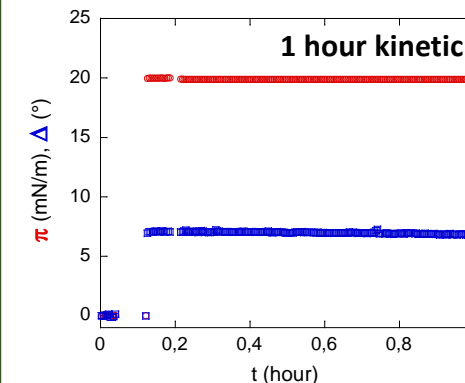
1h kinetic

- **Enzymatic activity of gPLRP2 on GL monolayer**  
Optimal activity at 15 mN/m, decrease of  $\pi$ , marked reduction in thickness ( $\Delta$ ).

- **Modification of the interfacial organization**  
Appearance of domains related to the generation of lipolysis products, adsorption of PLRP2 at the interface

- **No lipolysis activity for the PLRP2 inactive variant**  
No evolution of  $\pi$  and  $\Delta$ , preservation of the PLRP2 structure (central domain 50 Å)

#### GL + gPLRP2 inactive variant

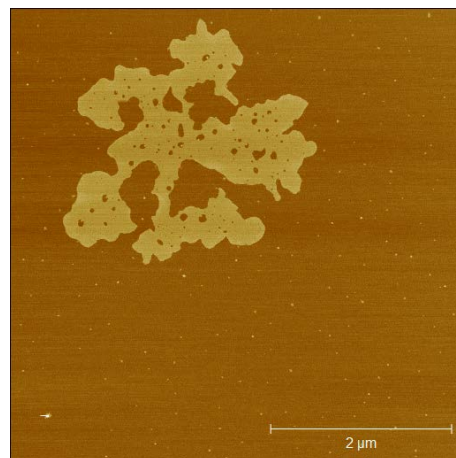
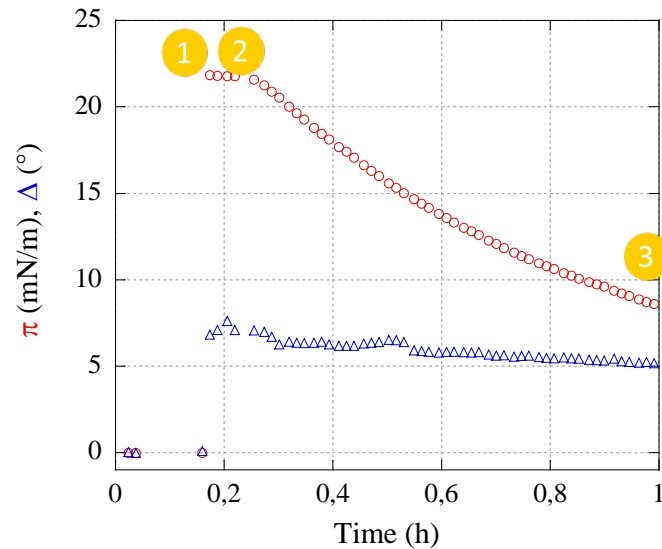




### 3) Digestion of microalgae galactolipids

Comparison of gPLRP2 adsorption and lipolytic activity onto biomimetic system (2) and **Lipid monolayer from *Chlorella vulgaris*** (System 3 Commercial, Com-P-21)

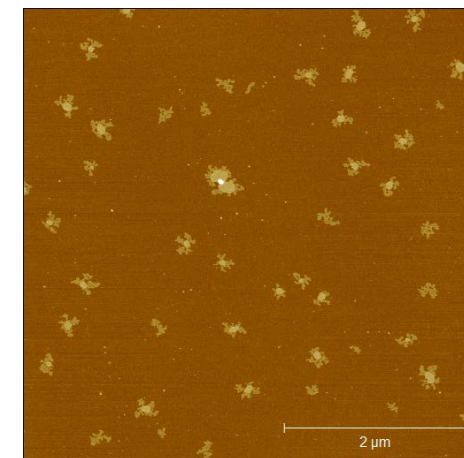
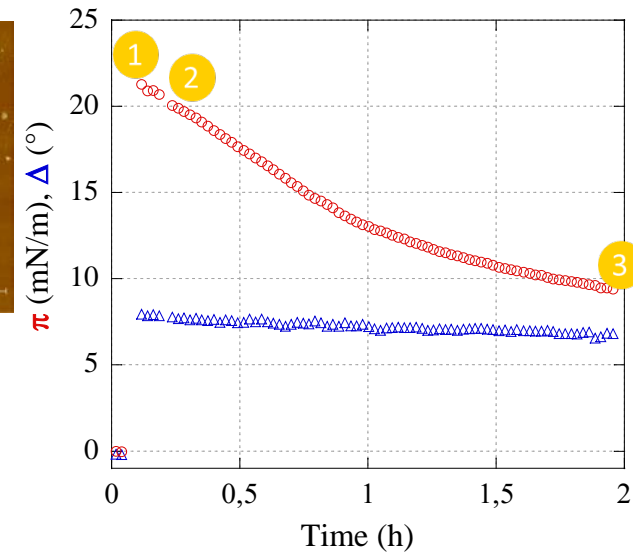
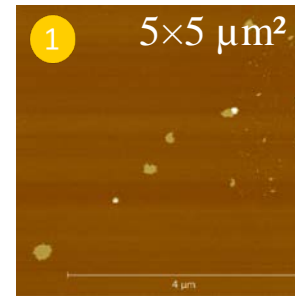
**System 2 – GL/SQDG/PG**



**+ gPLRP2**

1h,  $\pi=8.5$  mN/m,  $\Delta=5.2^\circ$

**System 3 – Commercial, Com-P-21**



**+ gPLRP2**

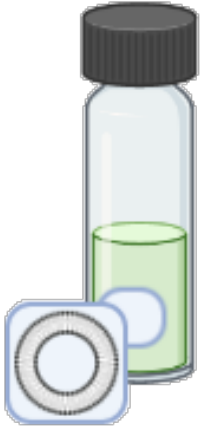
2h,  $\pi=9.4$  mN/m,  $\Delta=6.9^\circ$

(Kergomard et al, 2022  
PHD unsubmitted work)

### 3) Digestion of microalgae galactolipids

Assembly scale ( $\mu\text{m}$ )

Static *in vitro* model  
(mL) - Bulk



Giant liposomes

1  $\mu\text{m}$  liposomes  
stabilized by model lipid  
extract (0.2% wt.)

(pH 7, Tris HCl buffer 10  
mM, gPLRP2 = 3,3 mg/L)

	MGDG/DGDG/SQDG/PG		
	T <sub>0</sub>	T <sub>5min</sub>	
FFA	-	54,3 ± 2,4	↗
MGDG	65,4 ± 1,0	10,6 ± 0,1	↘ -84%
MGMG	-	31,3 ± 2,2	↗
DGDG	34,6 ± 1,0	3,7 ± 0,1	↘ -90%
DGMG	<i>nq</i>	<i>nq</i>	
SQDG	24,7 ± 0,4	2,3 ± 0,3	↘ -91%



In the presence of biliary salts:  
Galactolipase activity  
Phospholipase activity

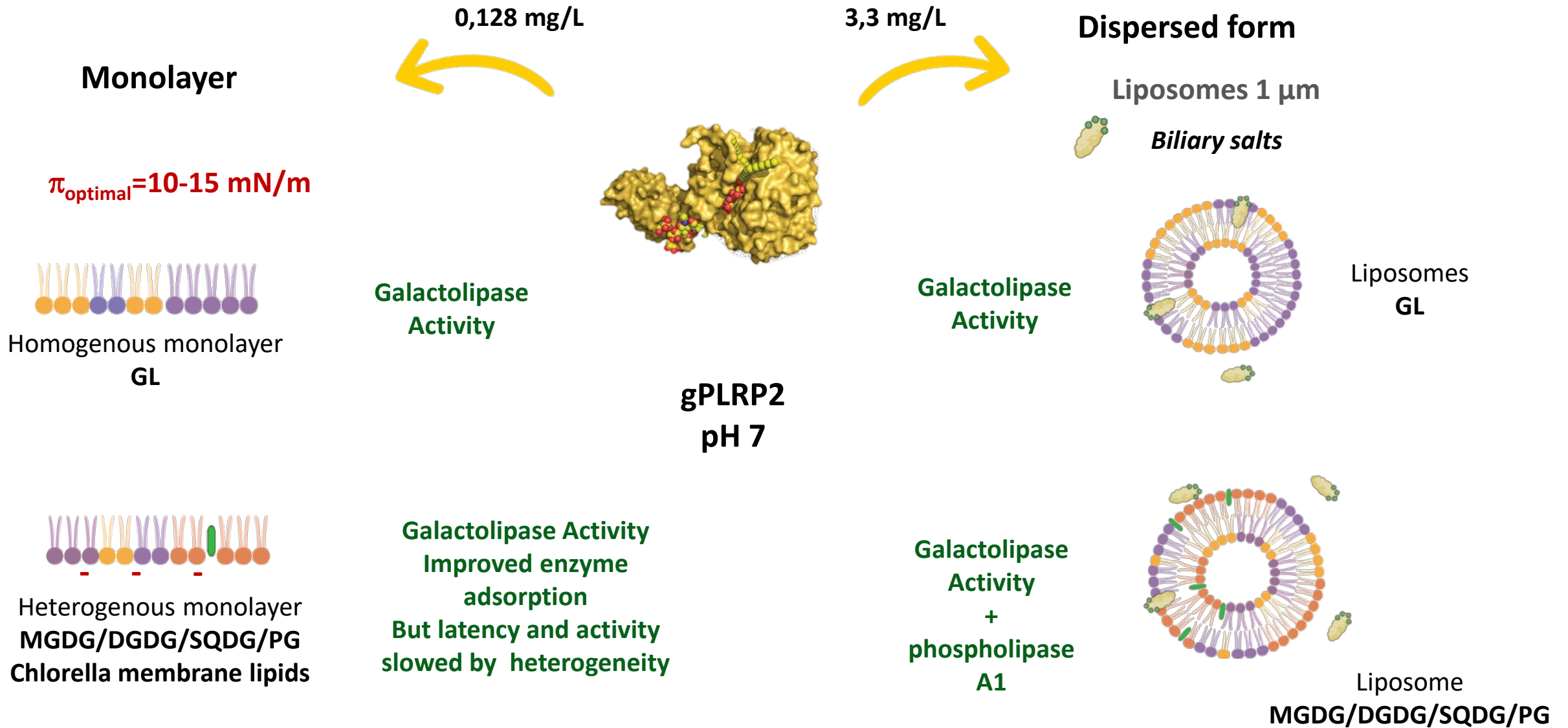
→ Generation of new surfactant molecules

Ab: Pancreatic lipase related protein 2 (PLRP2), BSSL : bile salt stimulated lipase, DGDG: digalactosyldiacylglycerol, DGMG: digalactosylmonoacylglycerol, FFA: free fatty acids, MGDG: monogalactosyldiacylglycerol, MGMG: monogalactosylmonoacylglycerol, SQDG: sulfoquinovosyldiacylglycerol, SQMG: sulfoquinovosylmonoacylglycerol, PG: Phosphatidylglycerol

(Kergomard et al, 2022  
PHD unsubmitted work)



### 3) Digestion of microalgae galactolipids



## Conclusions

- ✓ Microalgae galactolipids can be an interesting complementary source of omega 3 PUFA possibly esterified under the galactolipids form

Microalgae sources	Terrestrial sources (green leafy vegetables)
VLC omega 3 PUFA	Omega 3 PUFA precursors only
Higher PL/GL ratios in photoautotrophy, adjustable with environmental conditions	Ratio PL/GL =45:55 in leaves and 30:70 in chloroplasts
~10-15 % (DM) of lipids of which 70-80 % can be GL	2-30 g/kg (DM) MGDG-DGDG
No arable land use / Concentration/ Stabilization energy consuming	Arable land use

- ✓ These galactolipids have specific interfacial behaviour compared to glycerolPL
- ✓ Can be easily digested by close analogue of human PLRP2
- ✓ Lots of work remained to be done to understand assembly behaviour in the digestive tract

Work to stabilize biomass and maintain molecular form by Maeva Subileau



Preclinical trial on GL terrestrial sources by C. Vors

Thanks to all contributors !!



Jeanne Kergomard  
Gilles Paboef  
Véronique Vié

Nathalie Barouh  
Bruno Baréa  
Amy Joy Carpentier  
Pierre Villeneuve

Frédéric Carrière

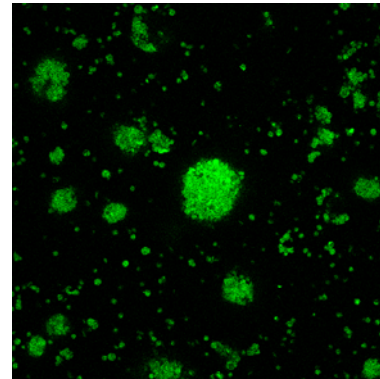
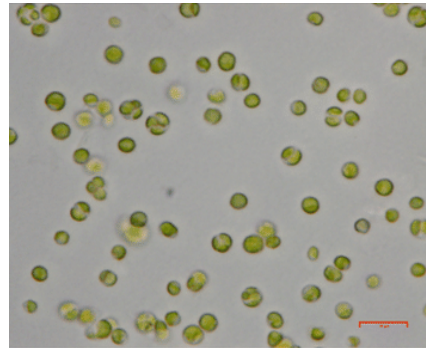
Claire Vigor  
Thierry Durand

Pierre-Emmanuel Millet  
Luca Costa

Maeva Subileau  
Juliette Wind  
Claire Bourlieu-Lacanal

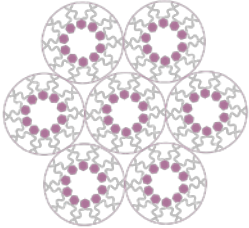
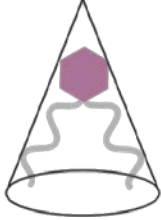
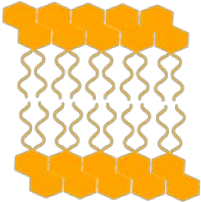
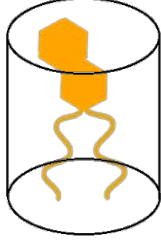
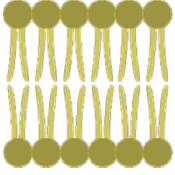
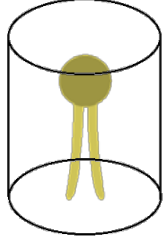
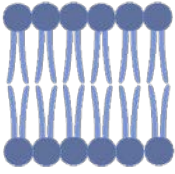
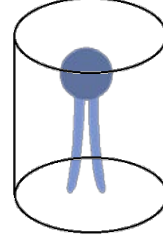


# Thank you for your attention



EXTRA-SLIDES

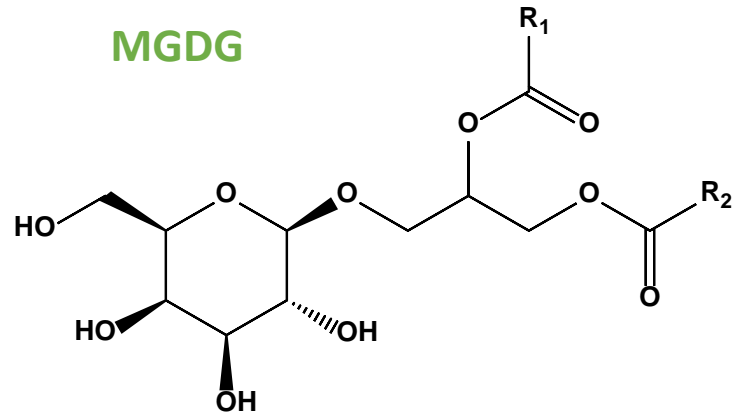
Lipid	Phase	Molecular arrangement	Molecular arrangement
-------	-------	-----------------------	-----------------------

<b>MGDG</b>	<b>Hexagonal inverse (HII)</b>		 <b>Conic</b>
<b>DGDG</b>	<b>Lamellar (L<math>\alpha</math>)</b>		 <b>Cylindrical</b>
<b>SQDG</b>	<b>Lamellar (L<math>\alpha</math>)</b>		 <b>Cylindrical</b>
<b>PG</b>	<b>Lamellar (L<math>\alpha</math>)</b>		 <b>Cylindrical</b>

*Formes moléculaires et phases polymorphiques du quartet lipidique des thylakoïdes*

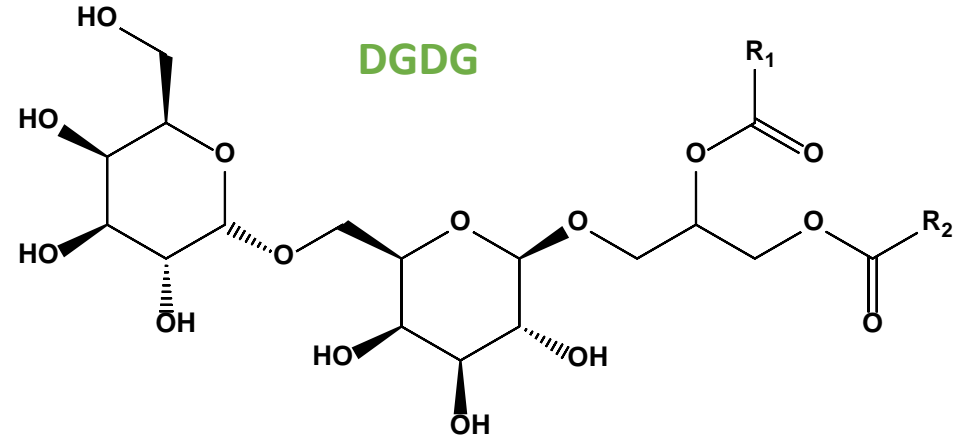


MGDG



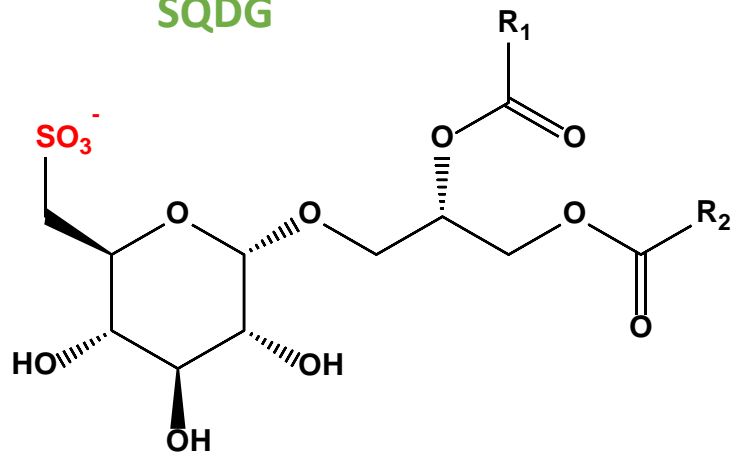
Monogalactosyldiacylglycerol

DGDG



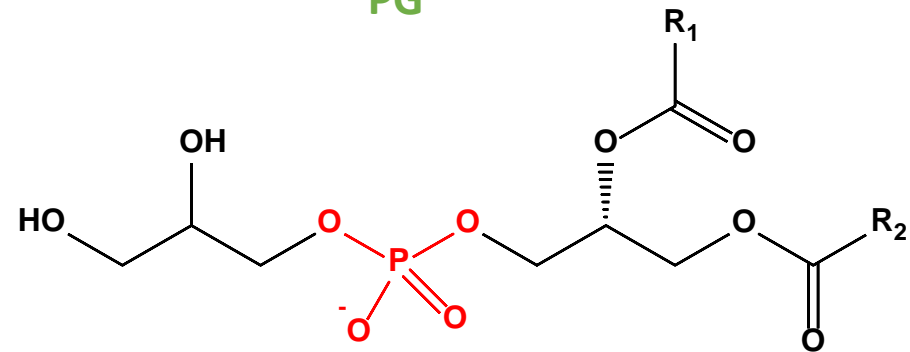
Digalactosyldiacylglycerol

SQDG



Sulfoquinovosyldiacylglycerol

PG



Phosphatidylglycerol