LOUVAIN DRUG RESEARCH INSTITUTE

Membrane interactions of lipopeptides and saponins, two groups of natural amphiphiles

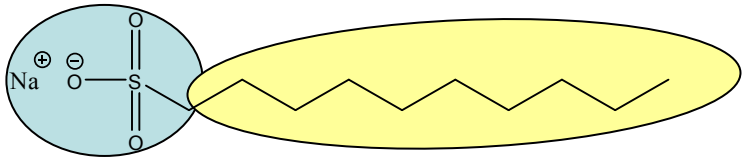
J. Lorent
(public defence)

Main supervisor : M-P. Mingeot-Leclercq (FACM)
Co-supervisor : J. Quetin-Leclercq (GNOS)

Introduction : physico-chemical properties of amphiphiles

Polar part (hydrophilic)

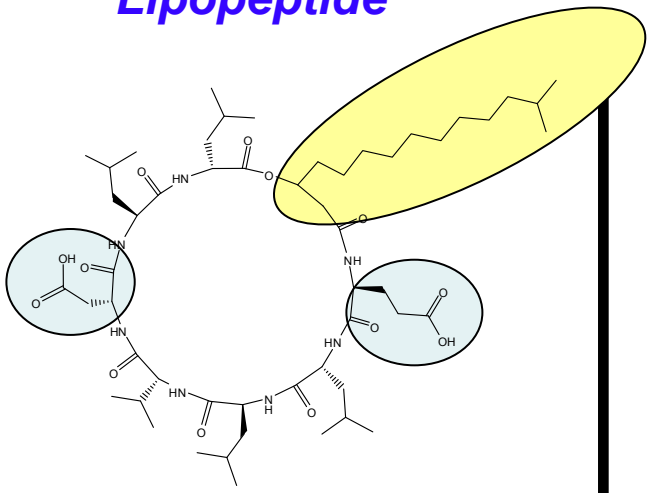
Apolar part (hydrophobic)



Sodium dodecylsulfate (detergent)

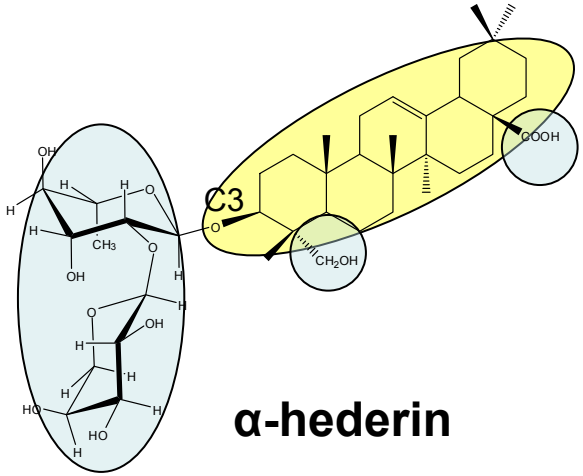
Introduction : selected amphiphiles of natural origin

Lipopeptide



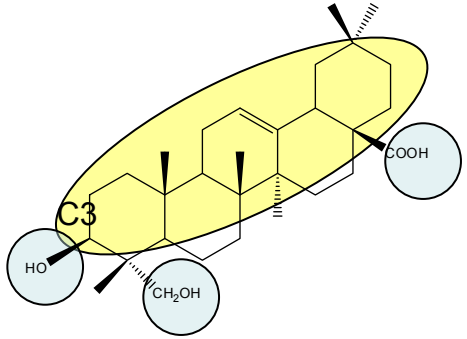
surfactin

Saponin

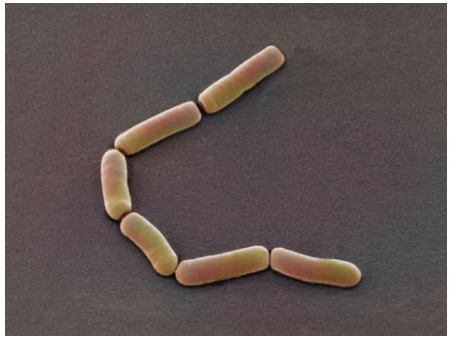


α-hederin

Triterpenic acid



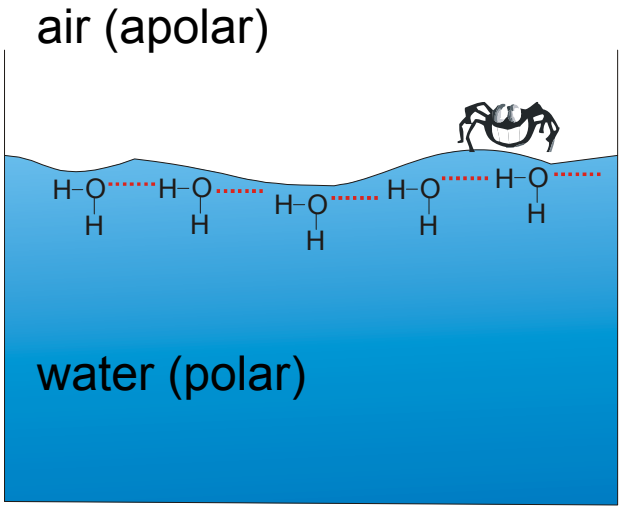
hederagenin



Isolated from *Bacillus subtilis*

Isolated from *Hedera helix* L.

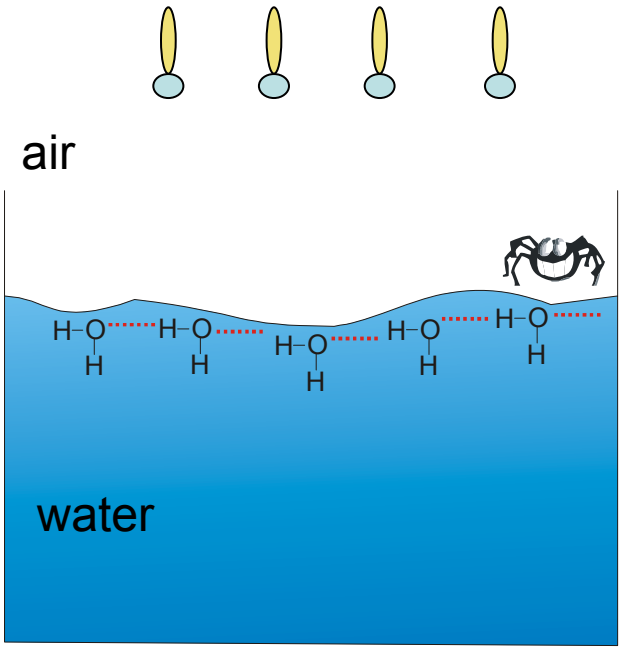
Introduction : amphiphiles (effect on interfaces)



Interfacial /
Surface tension or energy

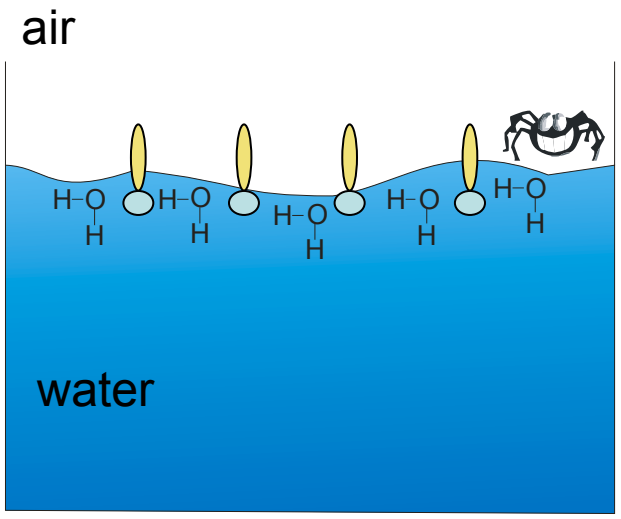
$\gamma = (F / l)$ or (E / A)

Introduction : amphiphiles (effect on interfaces)



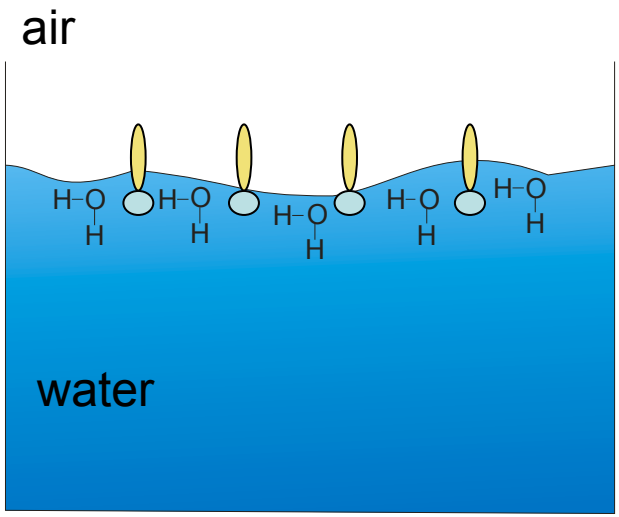
Interfacial /
surface tension
$\gamma = (F / l)$ or (E / A)

Introduction : amphiphiles (effect on interfaces)



Interfacial /
surface tension
γ = (F / l) or (E / A)

Introduction : amphiphiles (effect on interfaces)



Interfacial energy

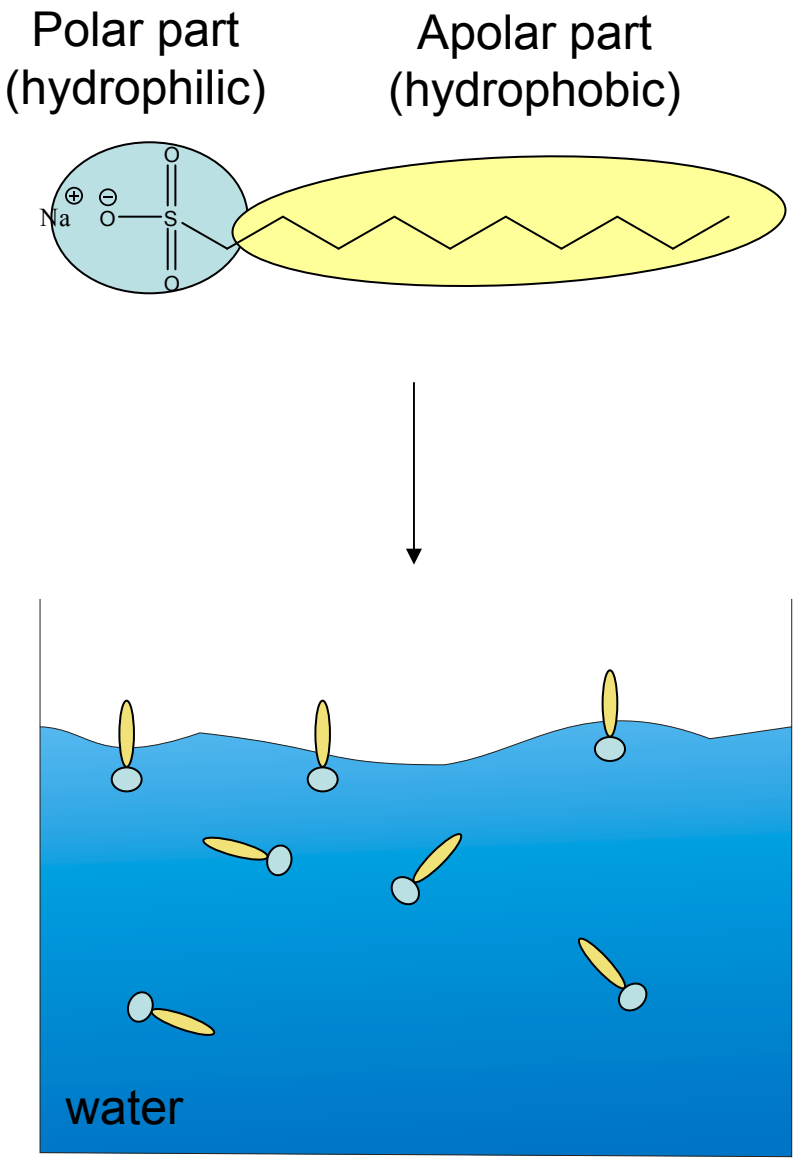
Emulsions / suspensions

• liquid / air (foam)

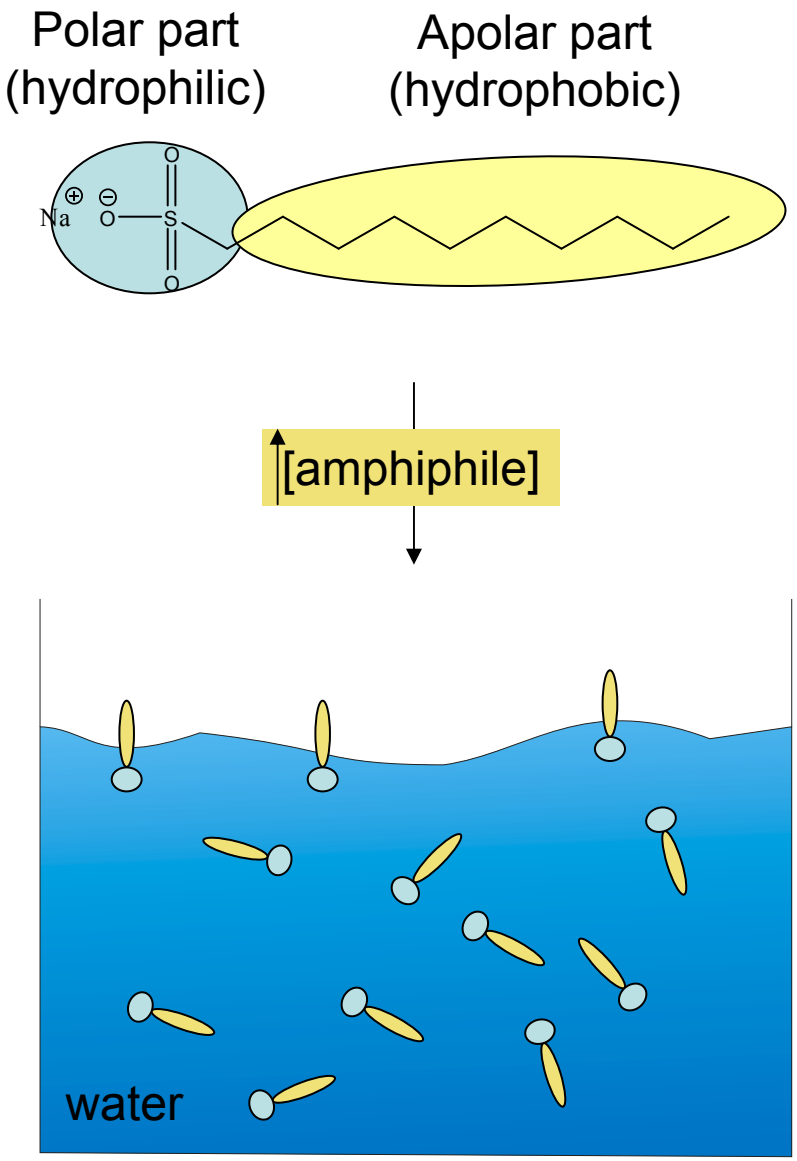
• liquid (apolar) / liquid (polar)

creams

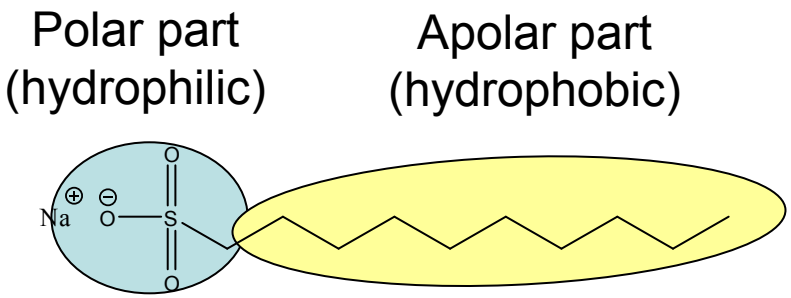
Introduction : amphiphiles (self-aggregation)



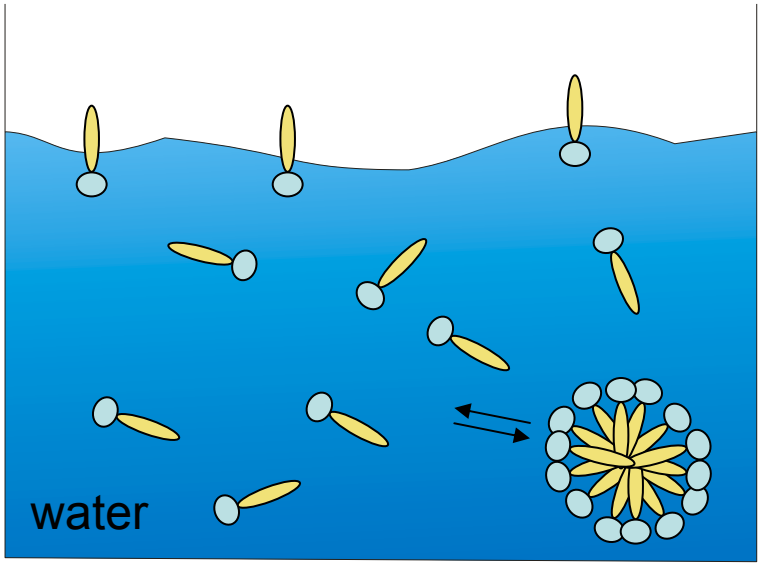
Introduction : amphiphiles (self-aggregation)



Introduction : amphiphiles (effect on interfaces)



[amphiphile] > Critical micellar concentration (CMC)

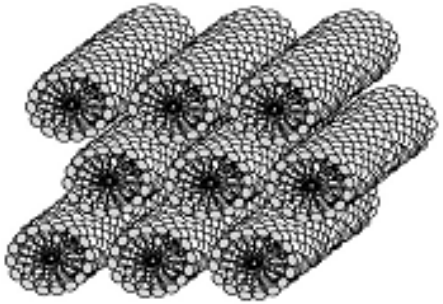


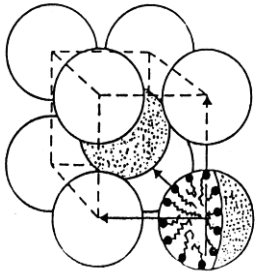
Micelles or other aggregates

Introduction : amphiphiles (self-aggregation, intrinsic curvature and polymorphism)

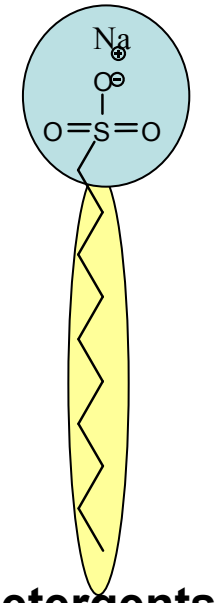
Intrinsic molecular curvature

$\varsigma > 0$

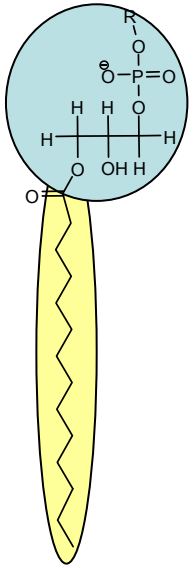




Cone shape **Normal hexagonal H_I** **Micelles** **Cubic (C_I)**

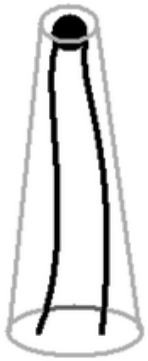


Detergents

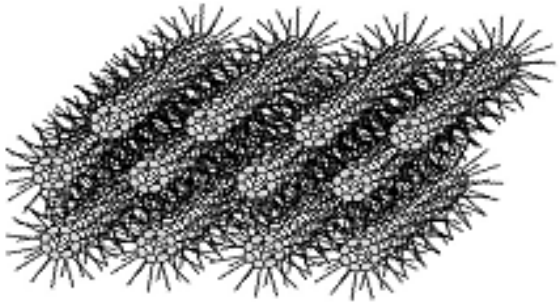


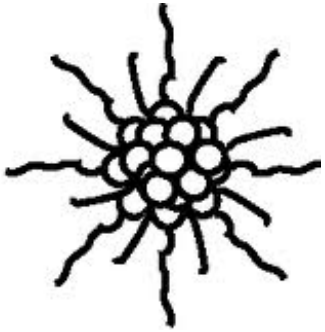
Lysophospholipids

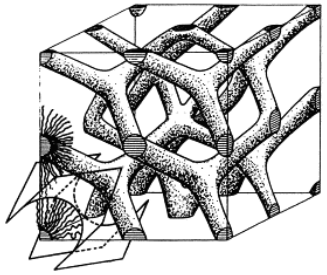
Introduction : amphiphiles (self-aggregation, intrinsic curvature and polymorphism)



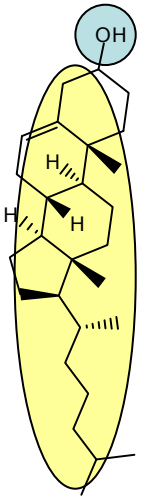
$\varsigma < 0$





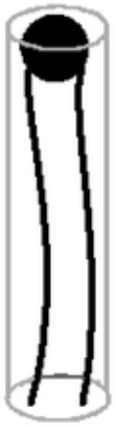


Inverted cone shape | **Inverse hexagonal H_{II}** | **Inverted micelles** | **Inverted cubic (C_{II})**



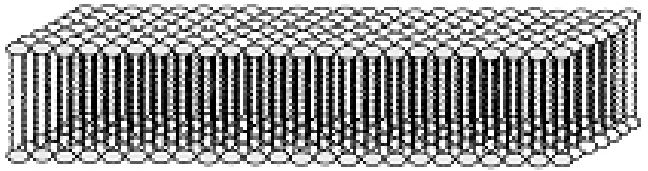
Cholesterol

Introduction : amphiphiles (self-aggregation, intrinsic curvature and polymorphism)

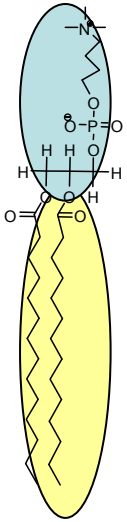


Cylinder shape

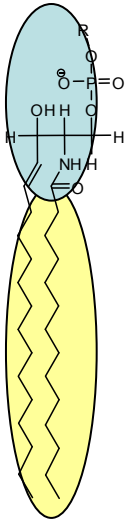
$\varsigma = 0$



Lamellar L_α, L_β, L_o (*bilayers or lipid membranes*)

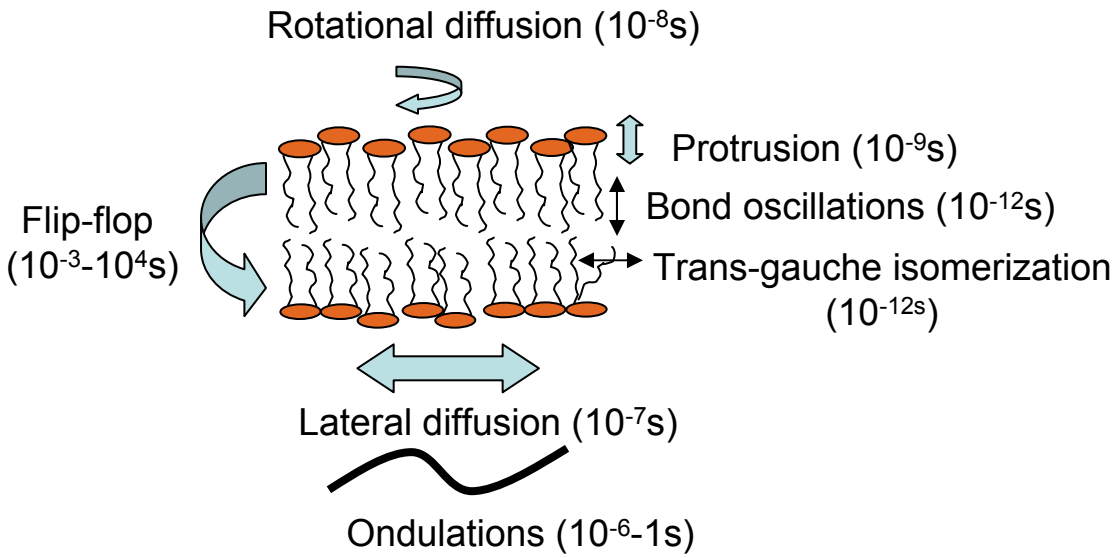


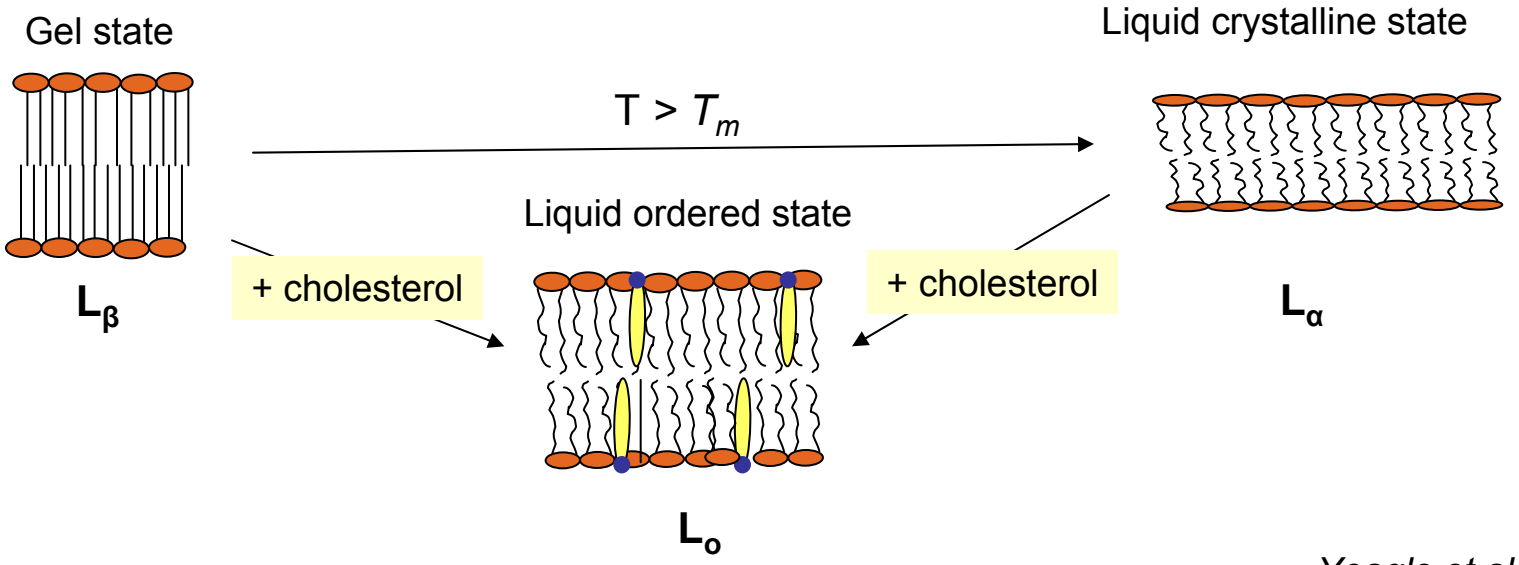
Phosphatidylcholines



Sphingomyelins

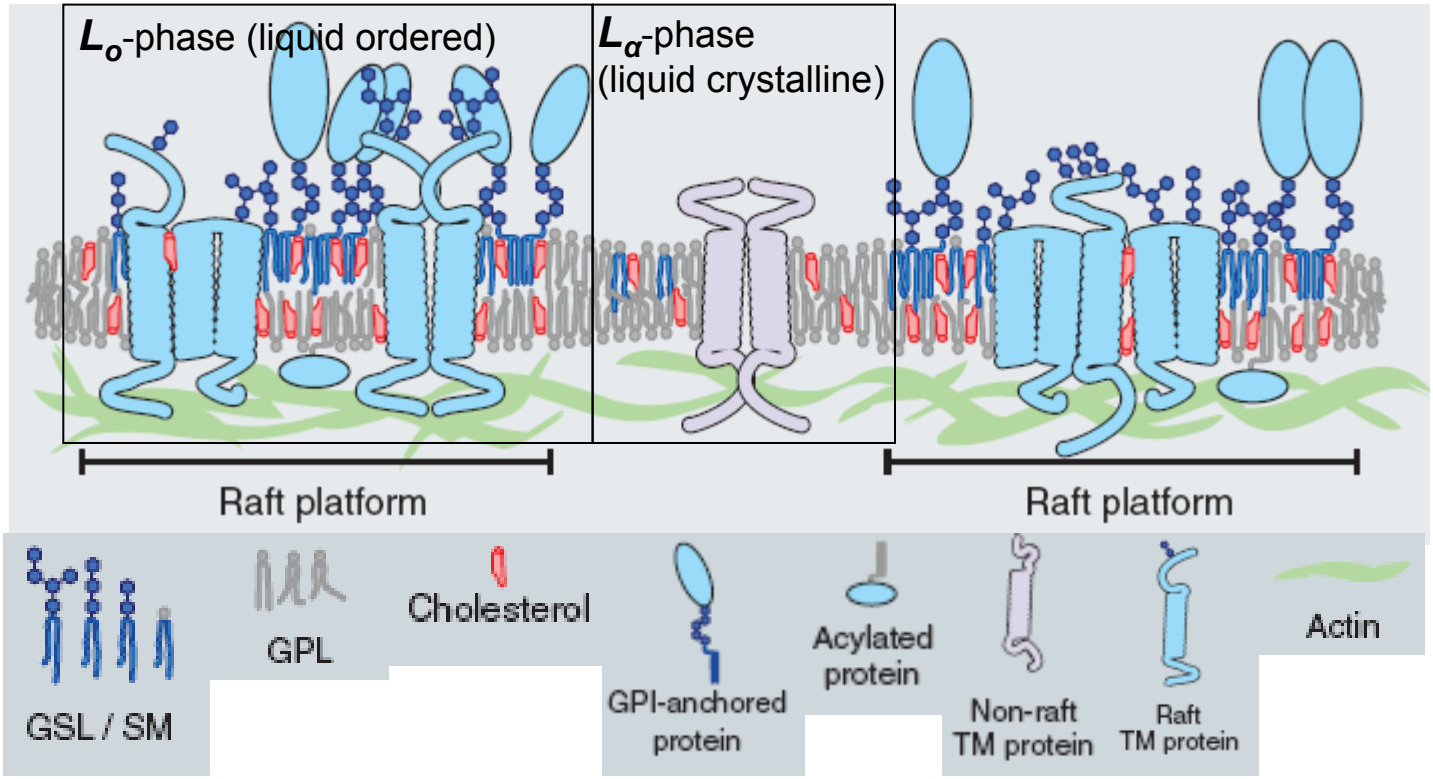
Introduction : bilayer dynamics and cholesterol effects





Yeagle et al. (2005)

Introduction : the biological membrane (lateral heterogeneity and lipid rafts)

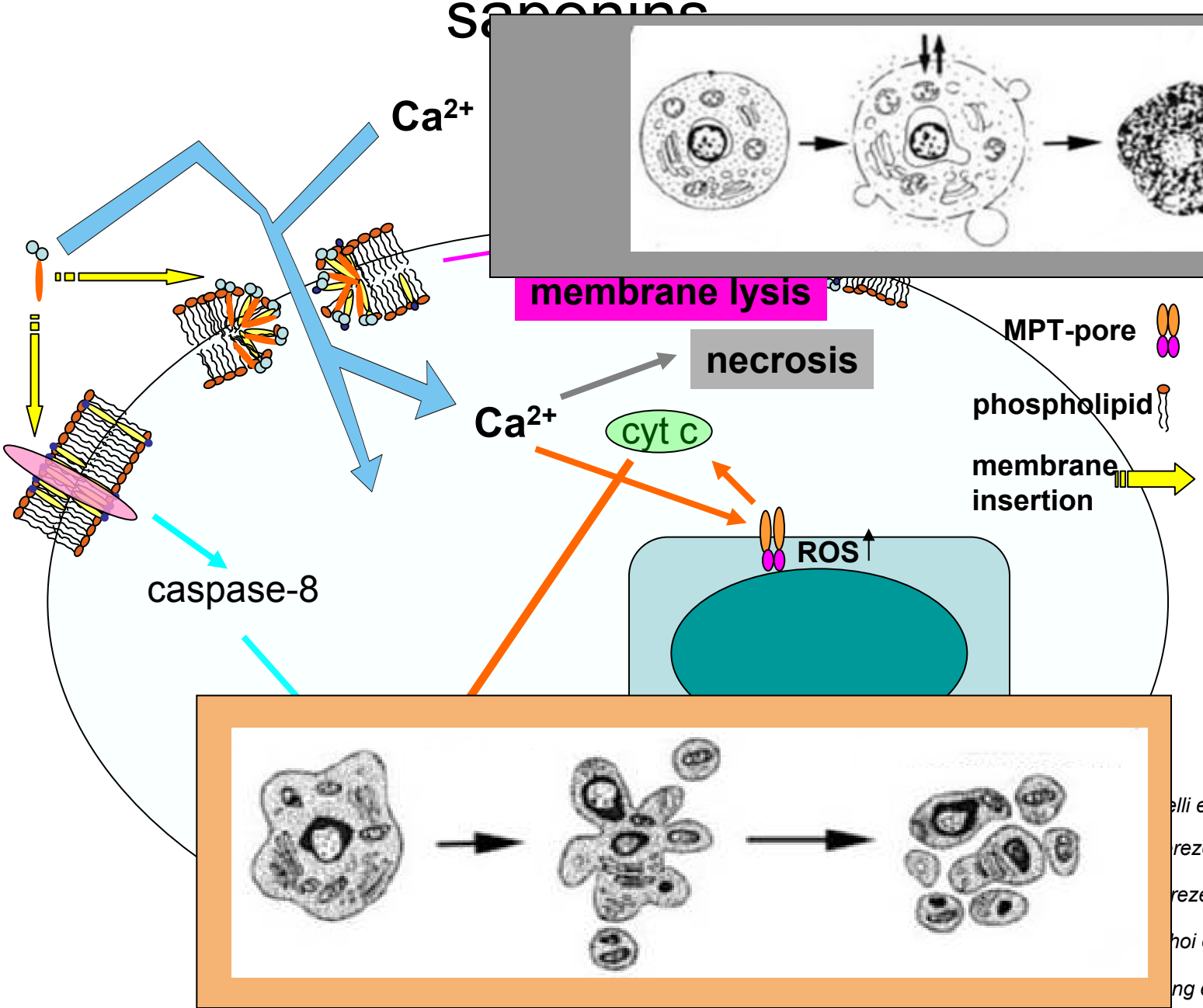


Rafts (L_o, liquid ordered phase)

- enriched in **cholesterol**, **glyco-sphingolipids**, **saturated phospholipids**
- Variable size (20-200nm)
- **functional platforms**: receptor **Fas**, **DISC** assembly (**death receptor**)

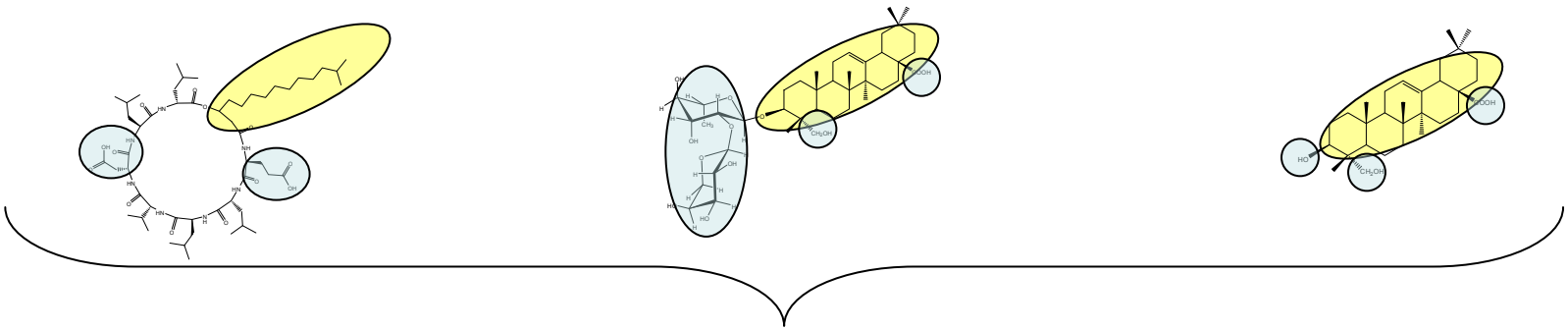
Lingwood (2011)

Introduction : different cell deaths induced by sa



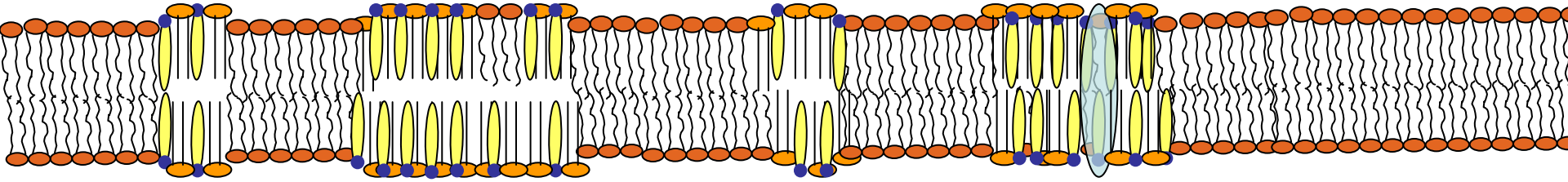
elli et al., 2008
rezesky, 1995
rezesky, 1995
hoi et al., 2008
ng et al., 2010

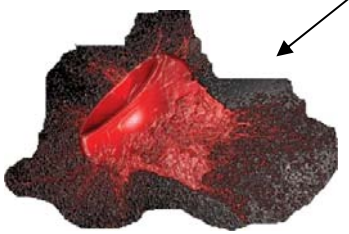
Aim of the study

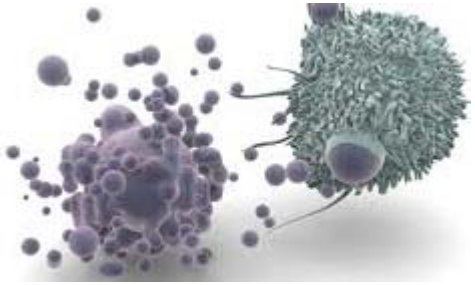


?

Raft located death receptor







Materials and methods : Membrane models

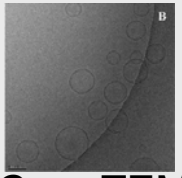
- LUV (large unilamellar vesicles)/ MLV (multilamellar vesicles)
  - Size = 100 nm-1 µm
  - Easy determination of lipid concentration
    ➔ **Quantification** of effects and amphiphilie/lipid ratio

- GUV (giant unilamellar vesicles)
  - Size ~ 10-50 µm
  - Difficult determination of lipid concentration
    ➔ Easy **observation** of effects

- SPB (supported planar bilayers)
  - Difficult determination of lipid concentration
    ➔ **Nanoscopic** effects

- Cancer cells (monocytes from leukemia, U937)
  - **Cytotoxicity**, determination of cell death

LUV/MLV

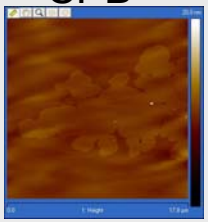


Cryo-TEM

GUV

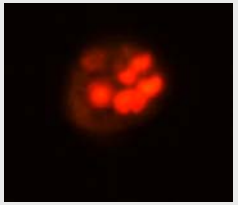
Light microscopy

SPB



AFM

Cells

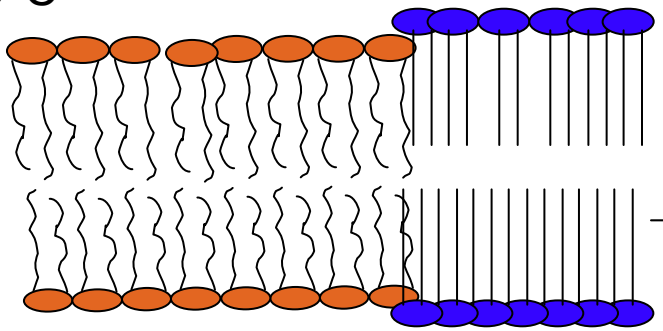


Materials and methods: phase separation models

DOPC : DPPC (1:1)

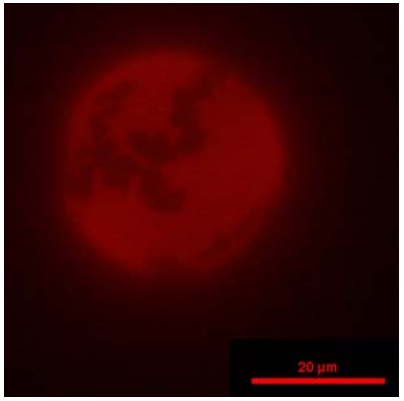
T_m= -3°C

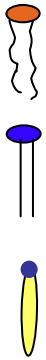
T_m= 42°C



L_α (liquid crystalline) / L_β (gel state)

TR-DPPE (L_α) labeled GUVs





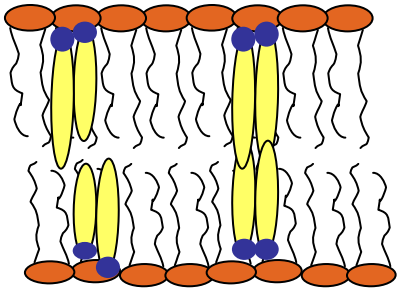
DOPC,DMPC

DPPC

Chol

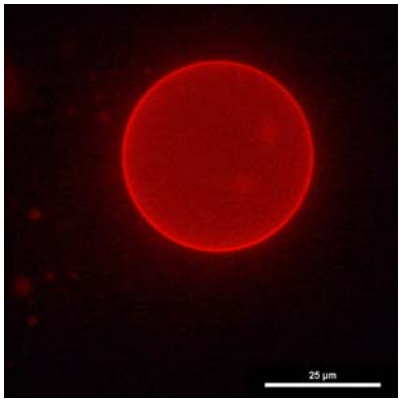
DMPC:Chol (3:1)

T_m=24°C

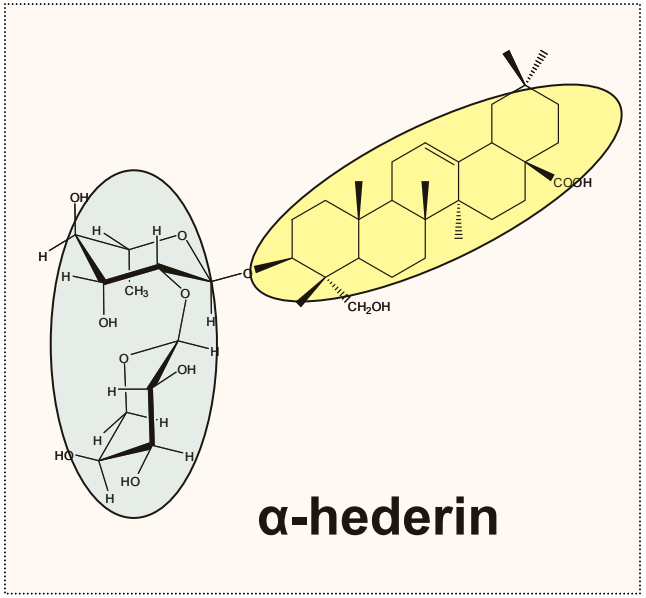


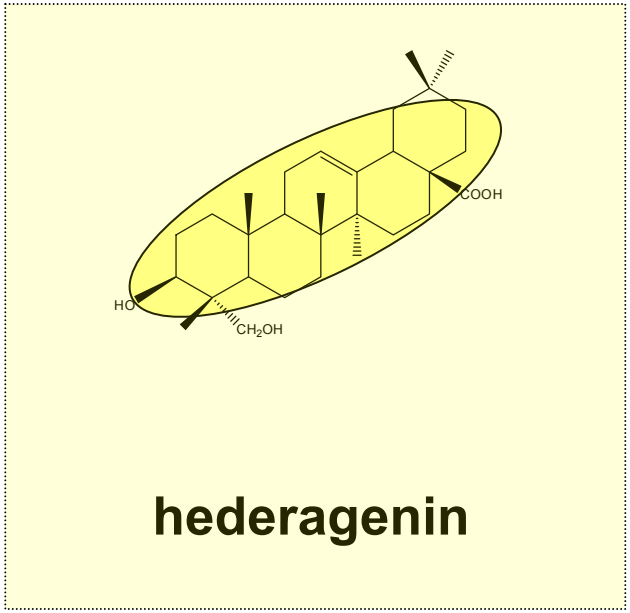
L_o L_α L_o

liquid ordered / liquid crystalline



Interaction of α−hederin and hederagenin with lipids and effects on membrane models





Journal of Biological Chemistry 2013, 288, pp. 14000-14017

Induction of Highly Curved Structures in Relation to Membrane Permeabilization and Budding by the Triterpenoid Saponins, α- and δ-hederin

Joseph Lorent[1,2], Cécile S. Le Duff[3], Joelle Quetin-Leclercq[2] and Marie-Paule Mingeot-Leclercq[1]*

[1]Université catholique de Louvain, Louvain Drug Research Institute, Cellular and Molecular Pharmacology, Avenue E. Mounier 73, UCL B1.73.05, B-1200 Bruxelles, Belgium.

[2]Université catholique de Louvain, Louvain Drug Research Institute, Pharmacognosy, Avenue E. Mounier 73, UCL B1.72.03, B-1200 Bruxelles, Belgium.

[3]Université catholique de Louvain, - Institute of Condensed Matter and Nanosciences, Molecules, Solids and Reactivity, Place Louis Pasteur 1, UCL L4.01.04, B-1348 Louvain-la-Neuve, Belgium.

Langmuir, submitted

Phase separation and permeabilization induced by the saponin α-hederin and its aglycone hederagenin in a raft mimicking bilayer

Joseph Lorent[1,4], Laurence Lins[2], Domenech Òscar[3], Joelle Quetin-Leclercq[4], Robert Brasseur[2] and Marie-Paule Mingeot-Leclercq[1]

[1]Université catholique de Louvain, Louvain Drug Research Institute, Cellular and Molecular Pharmacology, UCL B1.73.05, Avenue E. Mounier 73, B-1200 Brussels, Belgium.

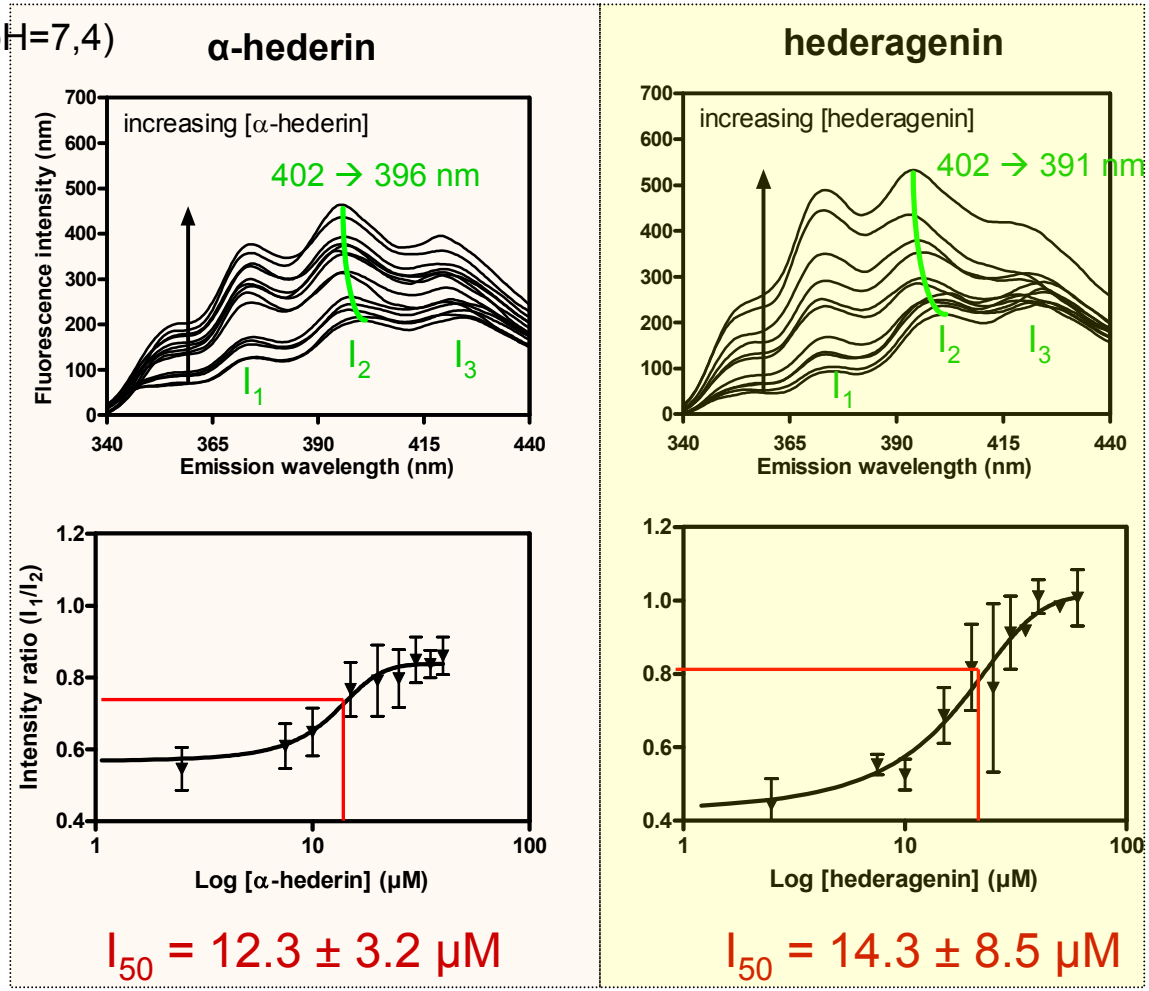
[2]ULg, Centre de Biophysique Moléculaire Numérique, Agro-BioTech Gembloux, Passage des Déportés, 2, B- 5030 Gembloux, Belgium.

[3]University of Barcelona, Departament de Fisicoquímica, Facultat de Farmàcia, UB and Institut de Nanociència i Nanotecnologia IN, 08028 Barcelona, Spain.

[4]Université catholique de Louvain, Louvain Drug Research Institute, Pharmacognosy, B1.72.03, Avenue E. Mounier 72, B-1200 Brussels, Belgium.

Interaction with dehydroergosterol (DHE) in aqueous solution : DHE spectroscopy

TRIS.HCl (10 mM, pH=7,4)

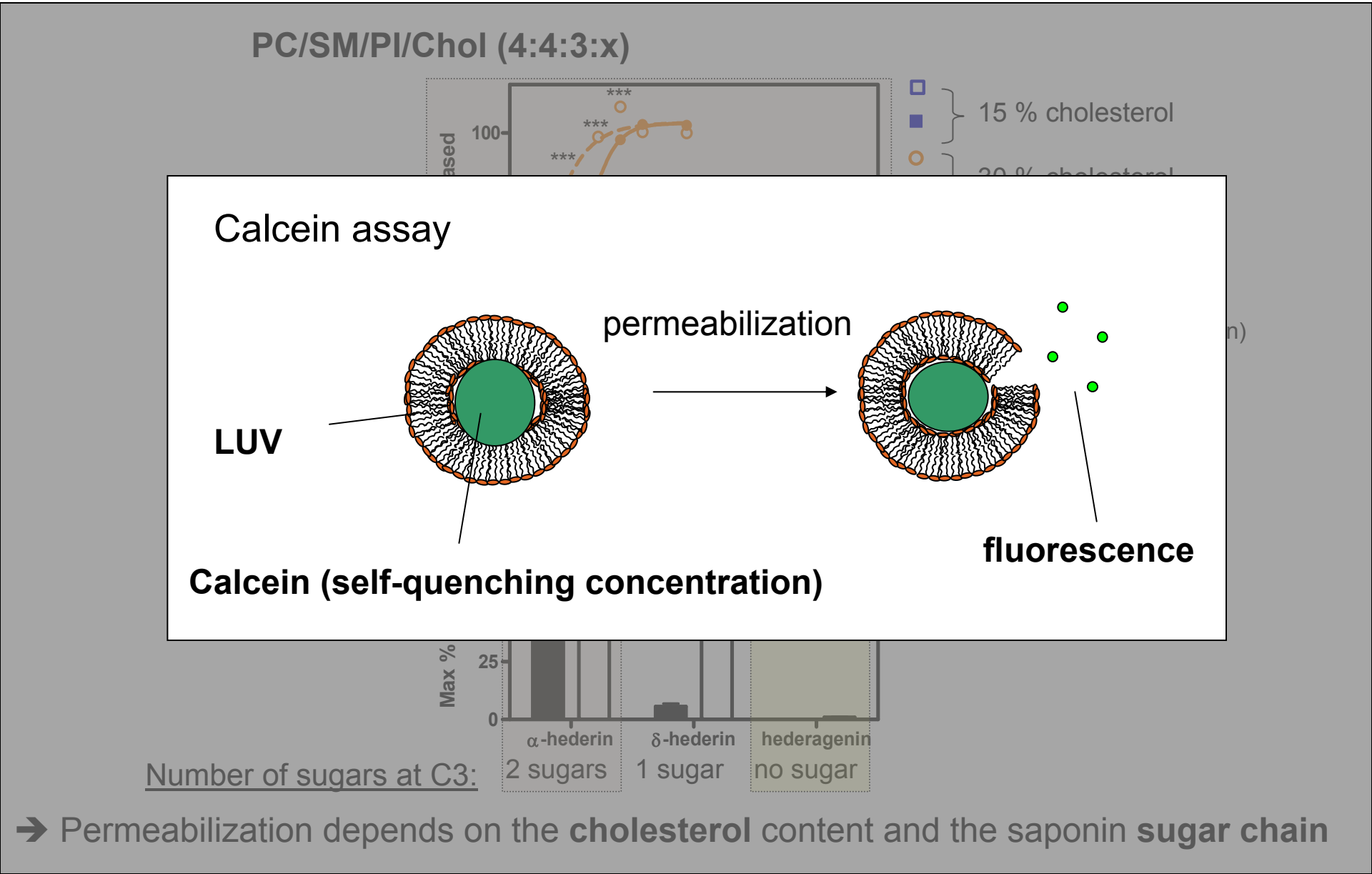


I_1 = monomeric peak (=monomeric DHE)
I_2,I_3 = structured emission (=crystalline DHE)

➔Both molecules bind to DHE forming some type of mixed aggregates (micelles)

➔ I_{50} (inflexion point) of α-hederin corresponds to its CMC (13 μM)

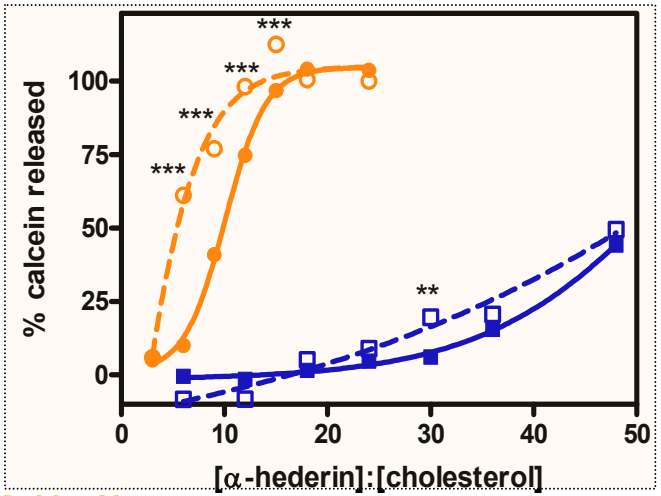
Permeabilization of LUV : cholesterol dependence



➔ Permeabilization depends on the **cholesterol** content and the saponin **sugar chain**

Permeabilization of LUV : cholesterol dependence

PC/SM/PI/Chol (4:4:3:x)



15 % cholesterol

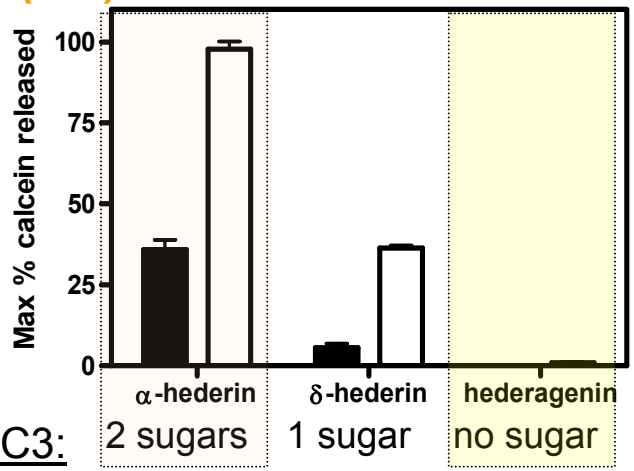
30 % cholesterol

18h

30min

p<0.05, ** p<0.01, *** p<0.001
two-way ANOVA (18h vs 30min)

DMPC/Chol (3:1)



[compound] / [lipid] ratio

4

12

Number of sugars at C3:

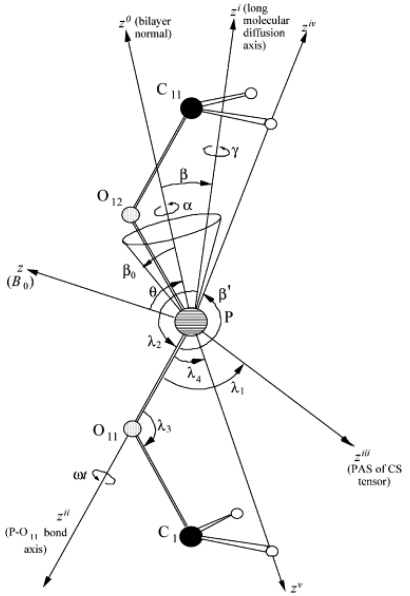
➔ Permeabilization depends on the **cholesterol** content and the saponin **sugar chain**

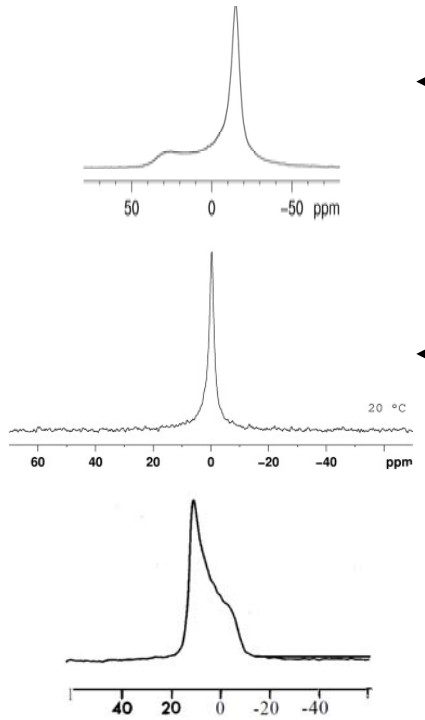
Reorganization of MLV : cholesterol dependence

DMPC/Chol (3:1)

^{31}P-NMR

Pic plus large ➔ vesicule plus grand

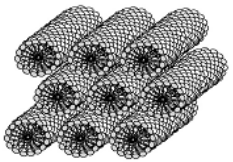




MLV (low curvature)

micelles

(high curvature)



C_1 = high

C_2 = 0

Chemical shift tensor of the phosphore atom

Malcolm et al. 2003

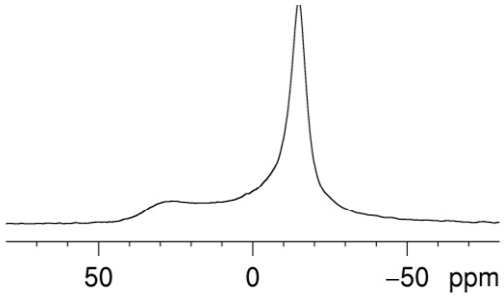
➔ [illegible]

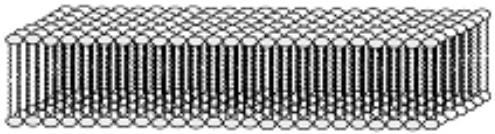
➔ Increase of isotropic motion and appearance of hexagonal pattern with α-hederin

Reorganization of MLV : cholesterol dependence

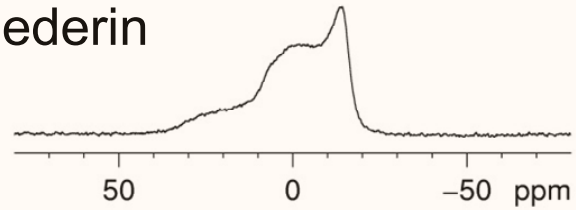
DMPC/Chol (3:1)

Control



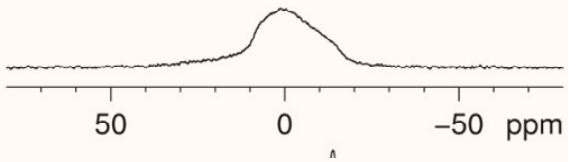


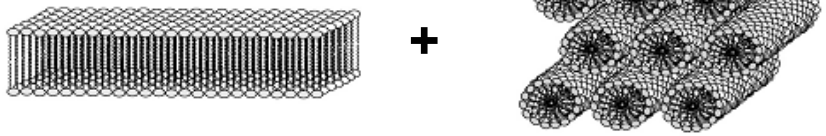
10% α-hederin

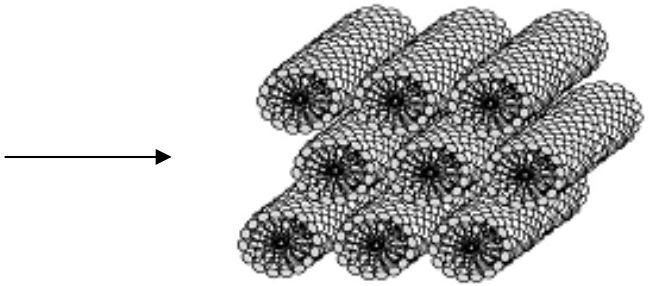


E

20% α-hederin





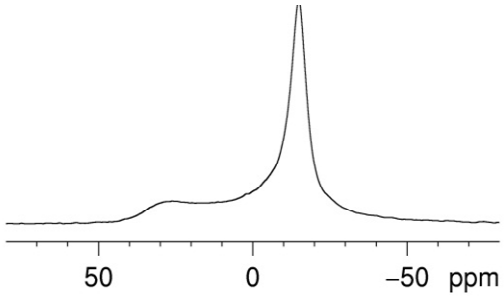


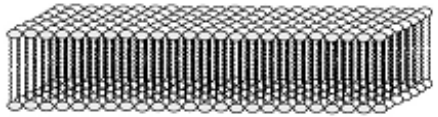
➔ Disruption of bilayer structure depends on **sugar chain** and **cholesterol** (not shown)
➔ Increase of isotropic motion and appearance of hexagonal pattern with α-hederin

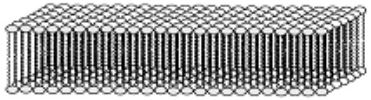
Reorganization of MLV : cholesterol dependence

DMPC/Chol (3:1)

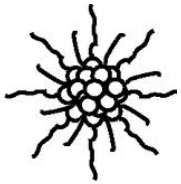
Control



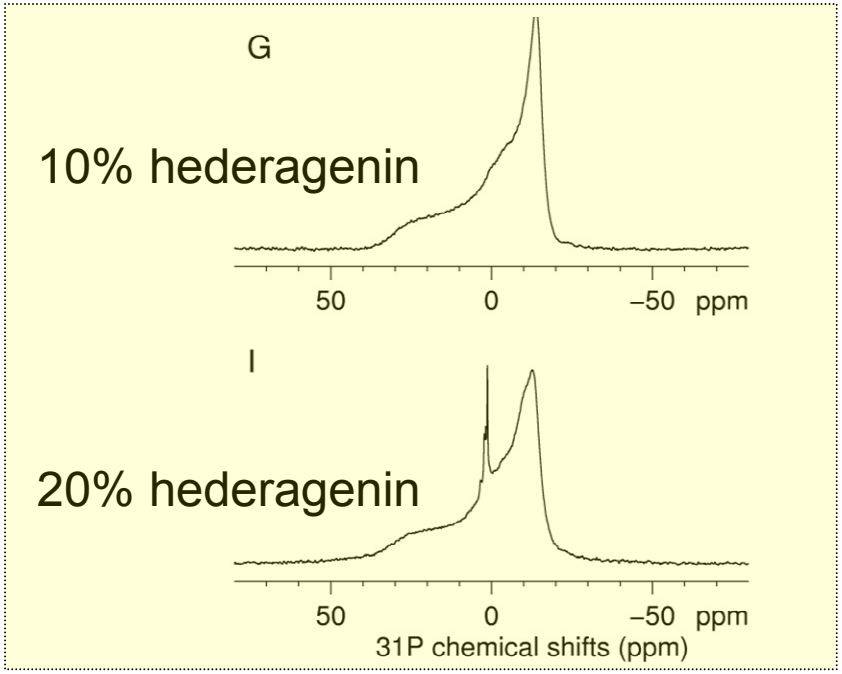




>



< 100 nm



➔ Disruption of bilayer structure depends on **sugar chain** and **cholesterol** (not shown)
➔ Increase of isotropic motion and appearance of hexagonal pattern with α-hederin

Sterol/phospholipid phase separation in MLV

DMPC/Chol (3:1)

α-hederin | hederagenin

[compound]/[lipids]

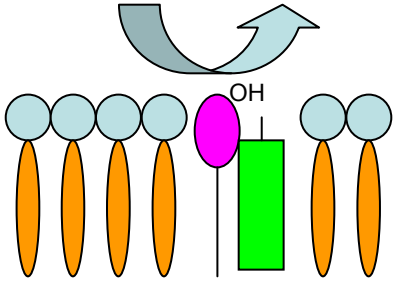
Control
0.25
0.6
0.8
1.2

*p < 0.05, one-way ANOVA (vs. Control)

Förster energy resonance transfert (FRET)

High energy transfert

High FRET ratio

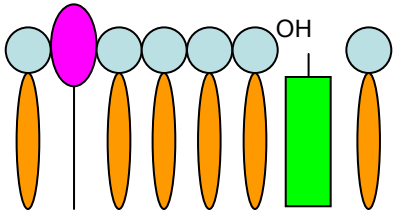


DPH-PC

DHE

Low energy transfert

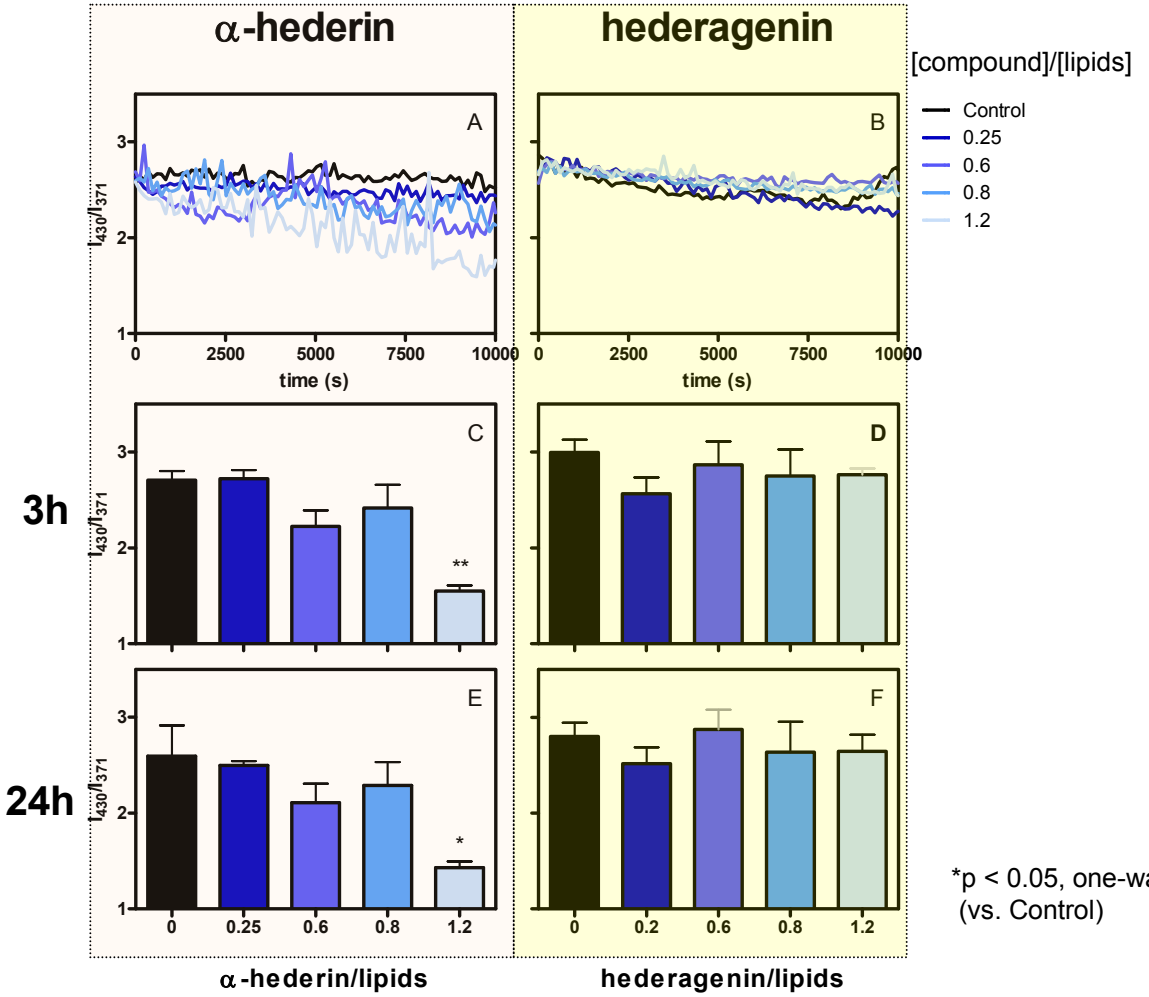
Low FRET ratio



➔ Decrea… compound/lipid ratios

Sterol/phospholipid phase separation in MLV

DMPC/Chol (3:1)



*p < 0.05, one-way ANOVA (vs. Control)

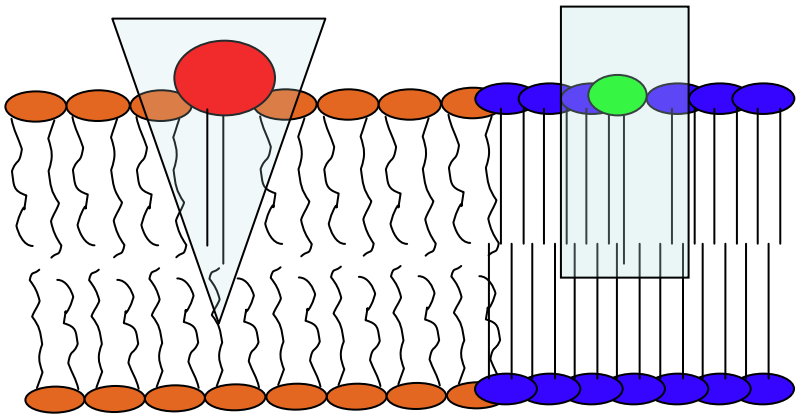
➔ Decrease of FRET efficiency is significant only with α-hederin at high compound/lipid ratios

Effects on GUV

DMPC/Chol (3:1)

Fluorescence microscopy

TR-DPPE NBD-DPPE

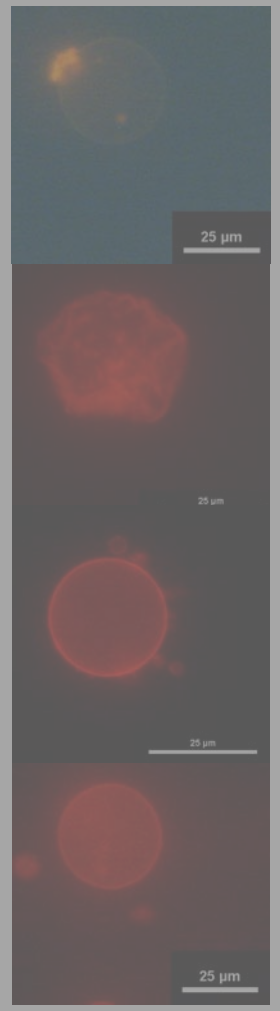


phase separation

wrinkled

budding

spherical

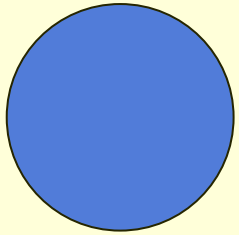
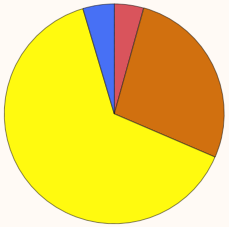
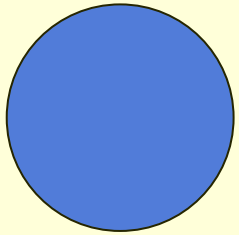
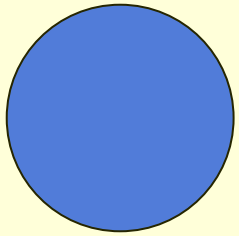


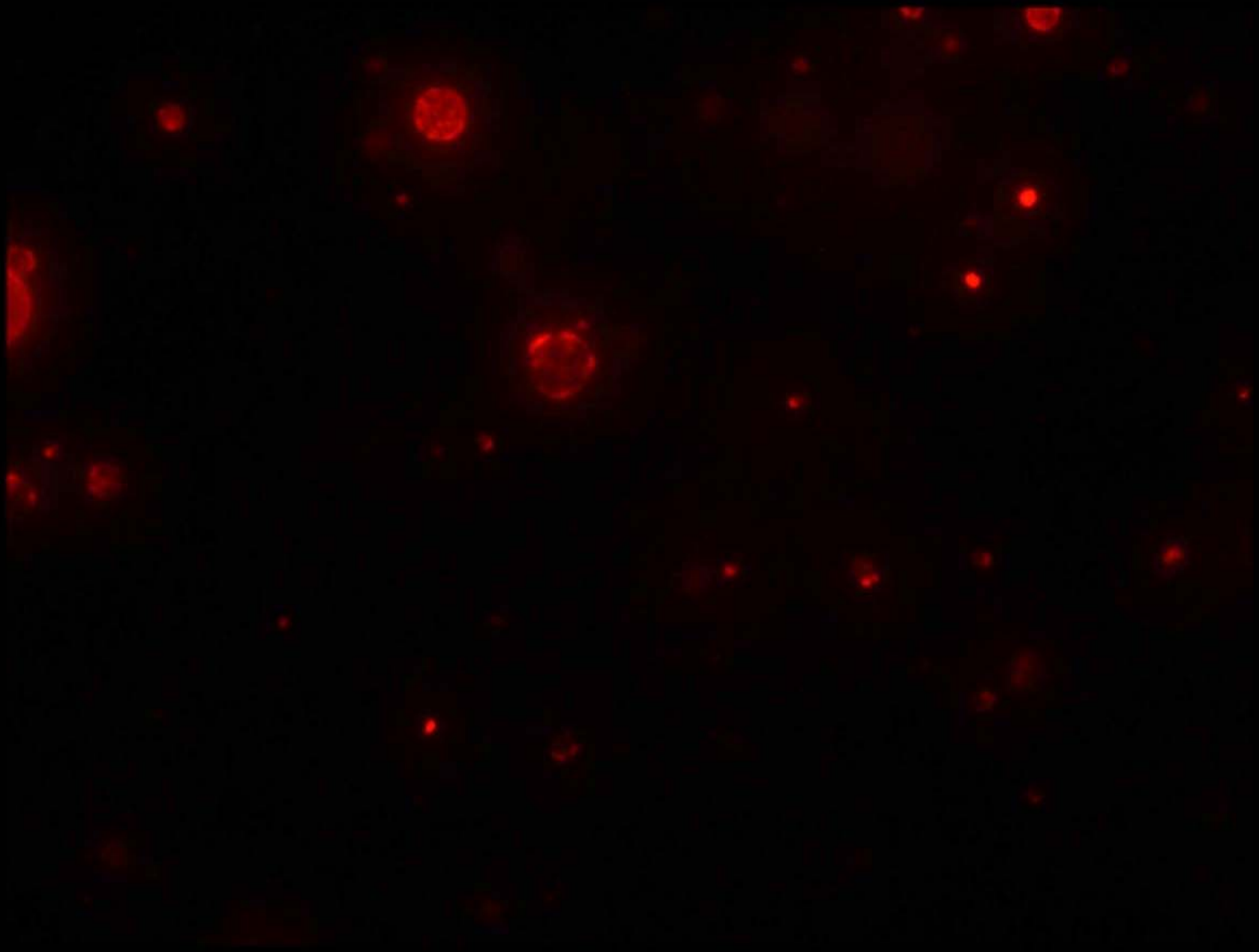
Effects on GUV

DMPC/Chol (3:1)

10 µM

< CMC
(α-hederin)

Incubation time	α-hederin	hederagenin
1h		
2h		
48h	Complete transformation of GUV	



Phase separation in GUV

Confocal microscopy

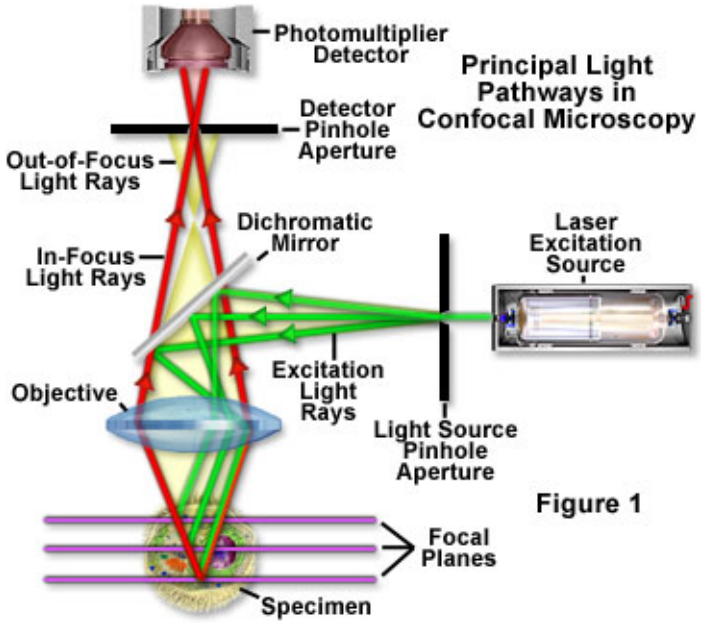
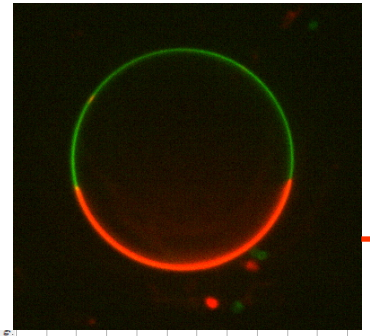


Figure 1

GUV (DOPC:DPPC:Chol)

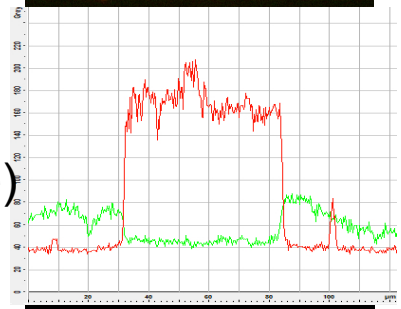
Confocal slide



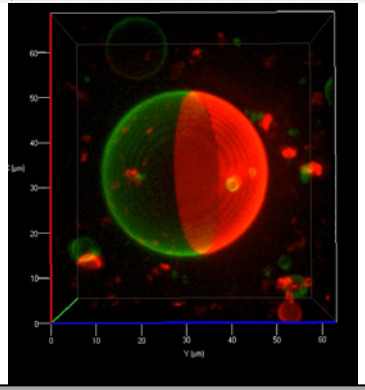
NBD-DPPE (L_o)

TR-DPPE (L_α)

Profile (cirumference)

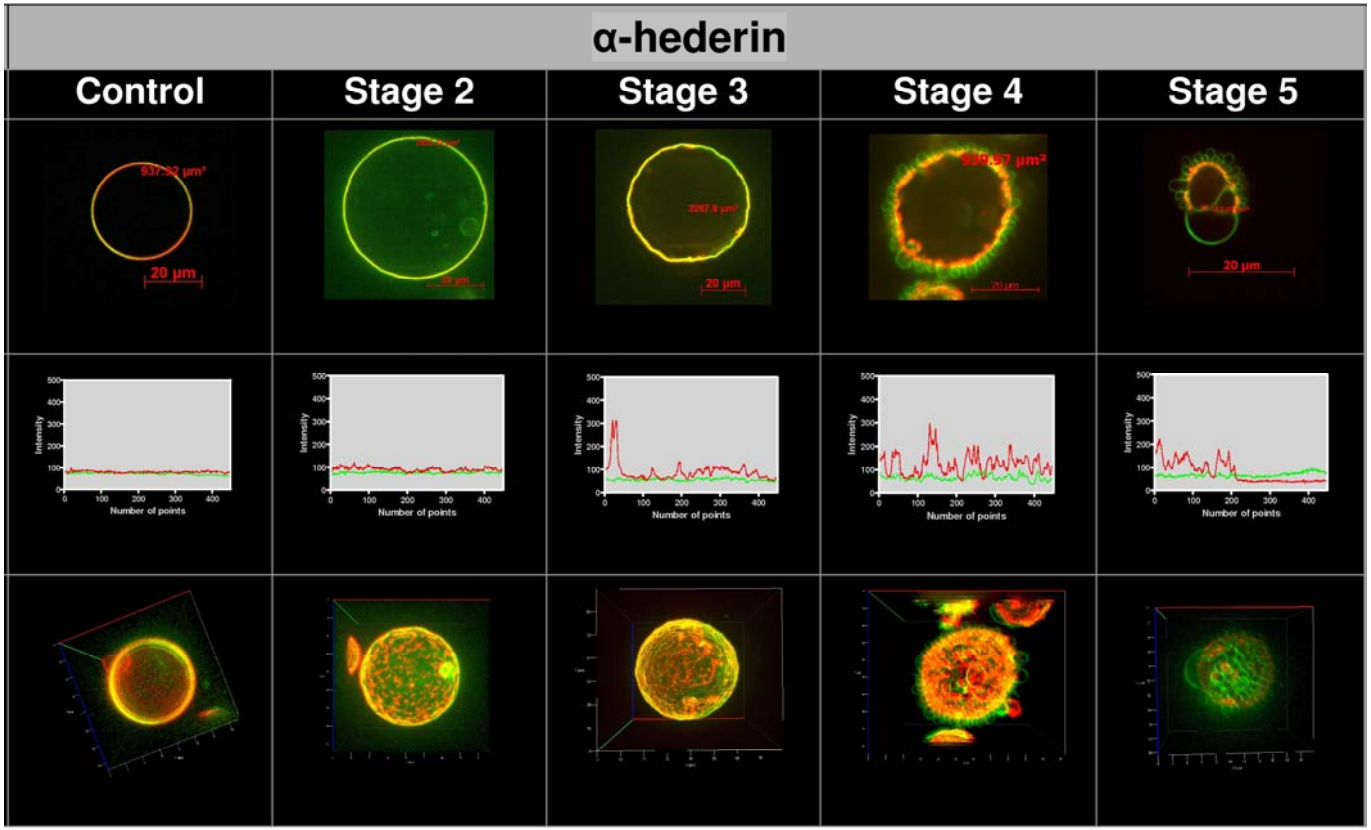


3D reconstruction (all slides)



Phase separation in GUV

α-hederin (10 µM)



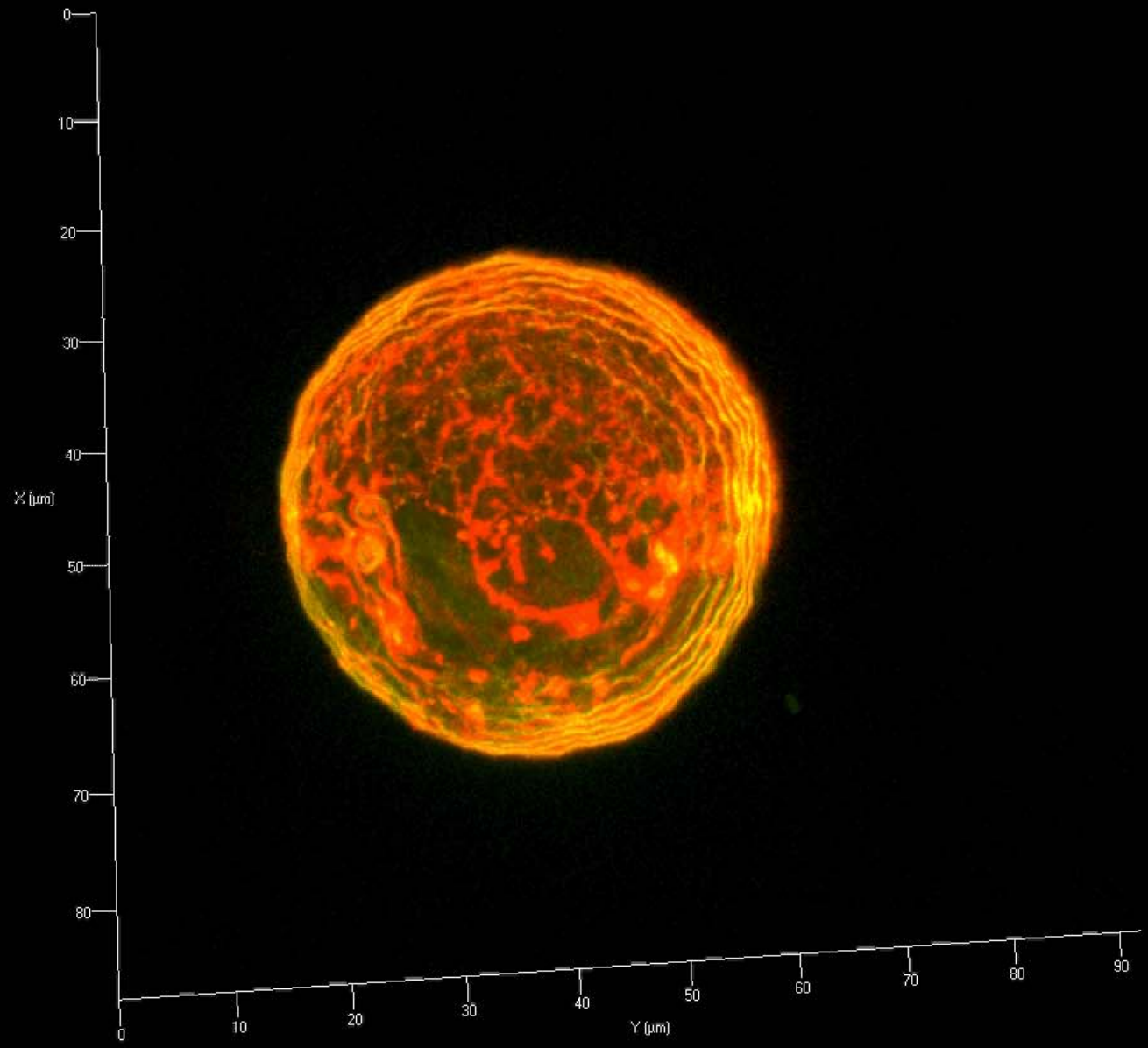
Confocal (1 slide)

Confocal (profile, circumference)

Confocal (3D)

➔ Accumulation of mainly TR-DPPE into worm-like domains
➔ At later stages : separation of TR-DPPE and NBD-DPPE

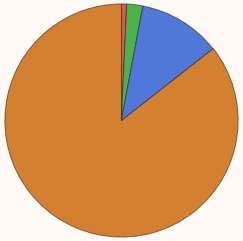
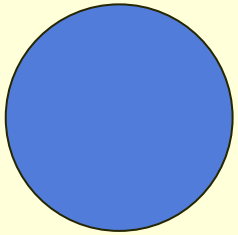
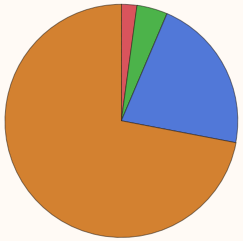
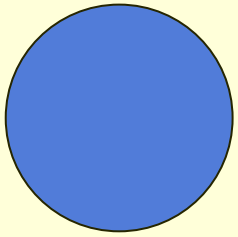
NBD-DPPE (green)
TR-DPPE (red)

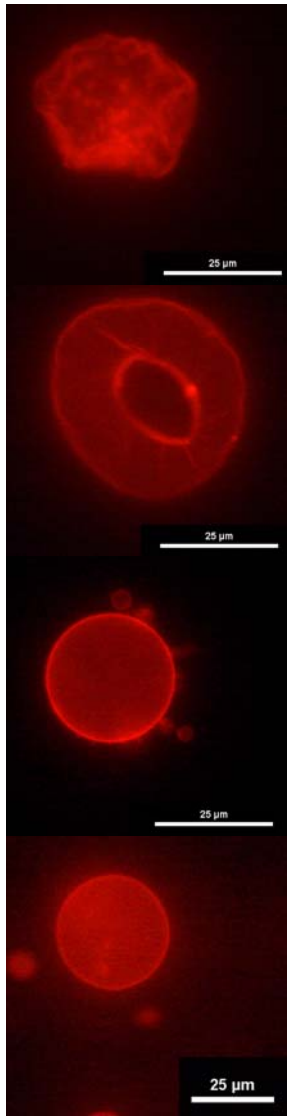

0
10
20
30
40
50
60
70
80
X (µm)
0
10
20
30
40
50
60
70
80
90
Y (µm)

Effects on GUV

40 µM

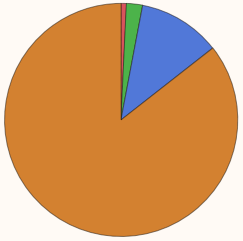
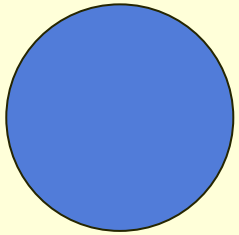
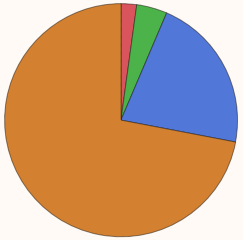
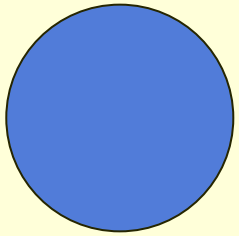
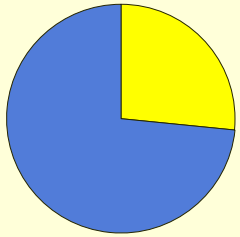
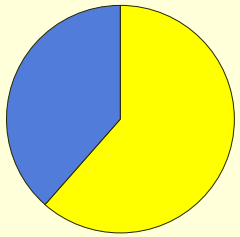
> CMC
(α-hederin)

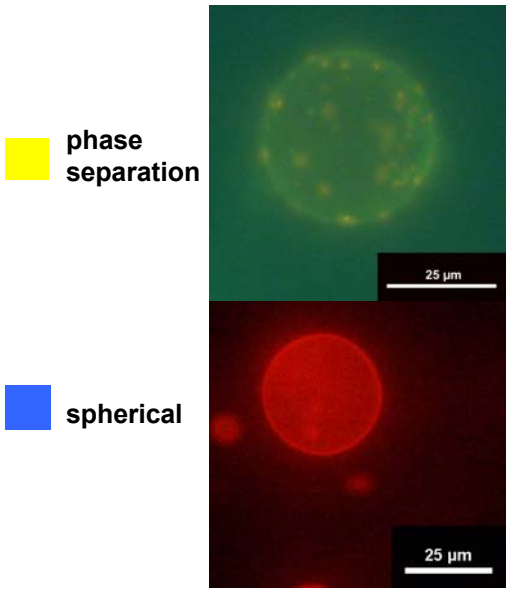
Incubation time	α-hederin	hederagenin
30 min		
1h		



Effects on GUV

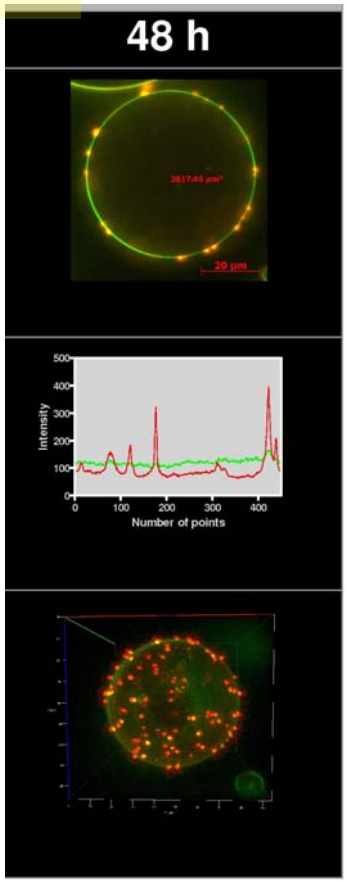
40 µM

Incubation time	α-hederin	hederagenin
30 min		
1h		
4h	N.D.	
48h	N.D.	



Phase separation in GUV

hederagenin (40 µM)



Confocal (1 slide)

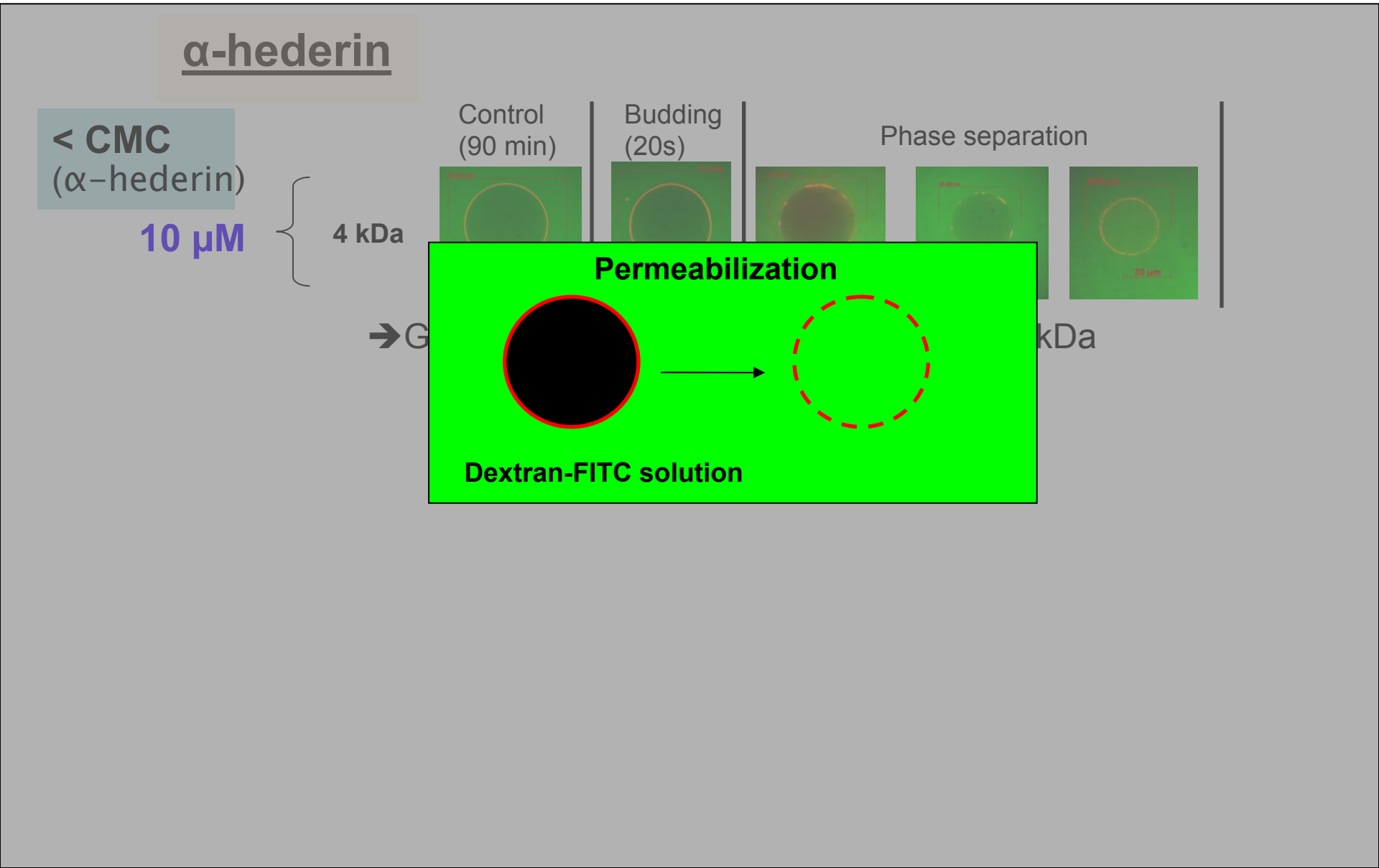
Confocal (profile, circumference)

Confocal (3D)

NBD-DPPE (green)
TR-DPPE (red)

➔ Formation of small circular domains with hederagenin

Permeation of GUVs to FITC-dextran



Permeation of GUVs to FITC-dextran

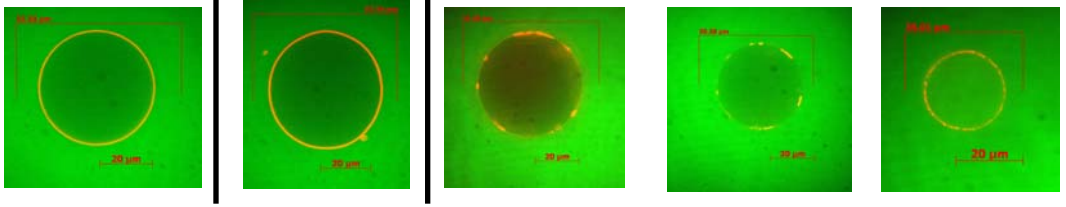
α-hederin

< CMC
(α-hederin)

10 μM

4 kDa

Control (90 min) | Budding (20s) | Phase separation (30, 60, 90 min)



➔**Gradual** permeation to FITC-dextran of 4 kDa

Permeation of GUVs to FITC-dextran

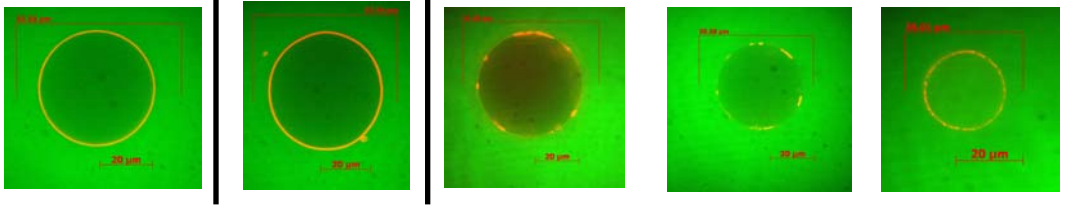
α-hederin

< CMC
(α-hederin)

10 μM

4 kDa

Control (90 min) | Budding (20s) | Phase separation



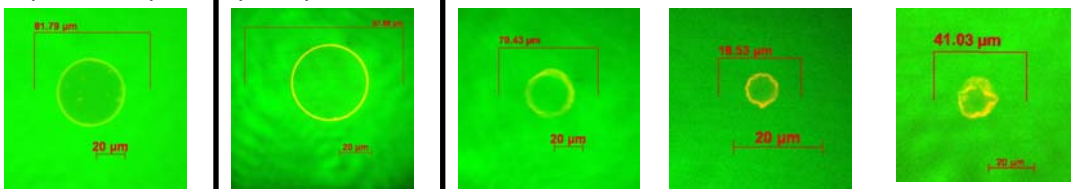
➔Gradual permeation to FITC-dextran of 4 kDa

> CMC
(α-hederin)

40 μM

4 kDa

Control (90 min) | No wrinkling (20s) | Increasing wrinkling



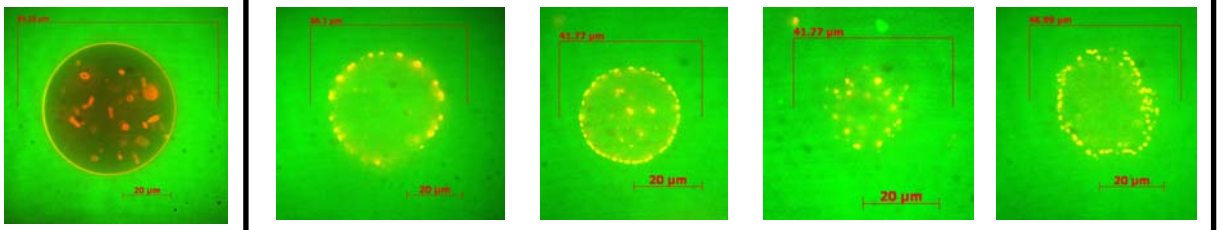
➔**Immediate** permeation to FITC-dextran of 4 kDa

Permeation of GUVs to FITC-dextran

hederagenin

40 µM — **4 kDa**

Control (48h) | Phase separation (48h)



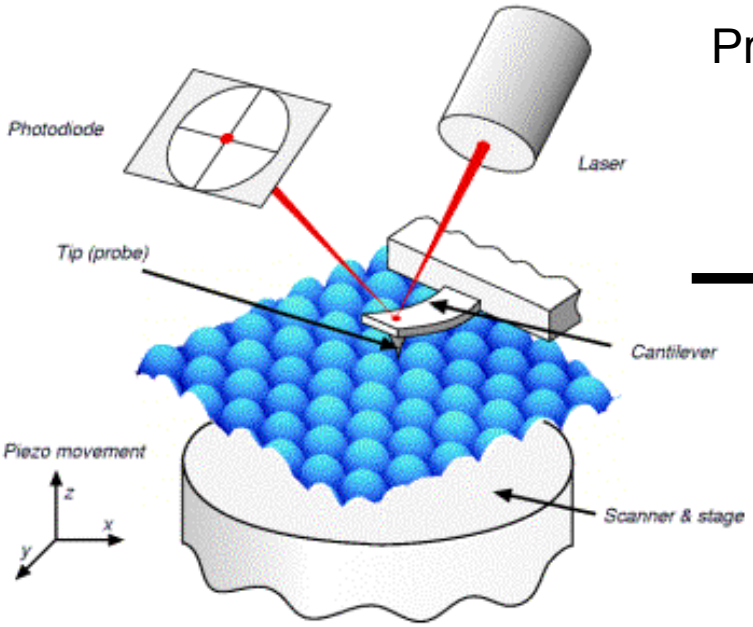
➔Permeation to FITC-dextran of 4 kDa after 48h

Nanoscopic effects of α-hederin on supported planar bilayer

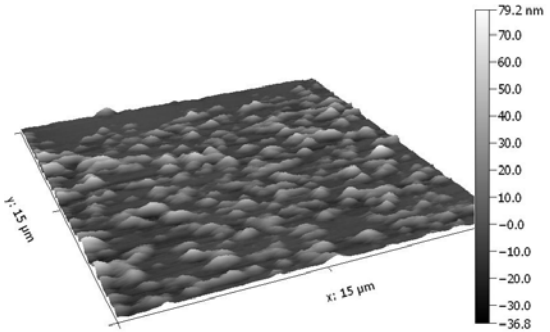
40 µM

DMPC/C

Atomic force microcopy



Profile (supported planar bilayer)



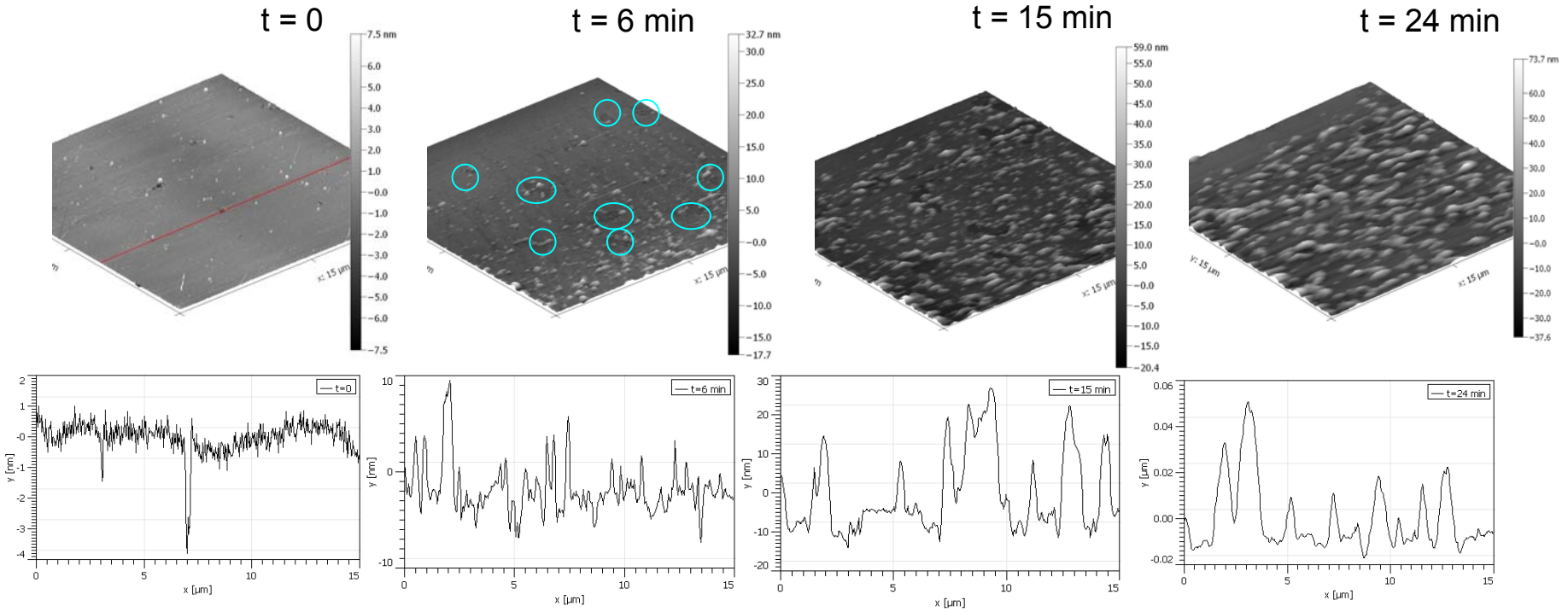
24 min

- ➔ Accumulation of membrane material into worm-like structures (new mesophase)
- ➔ Formation of holes with increasing size upon time

Nanoscopic effects of α-hederin on supported planar bilayer

40 µM **α-hederin**

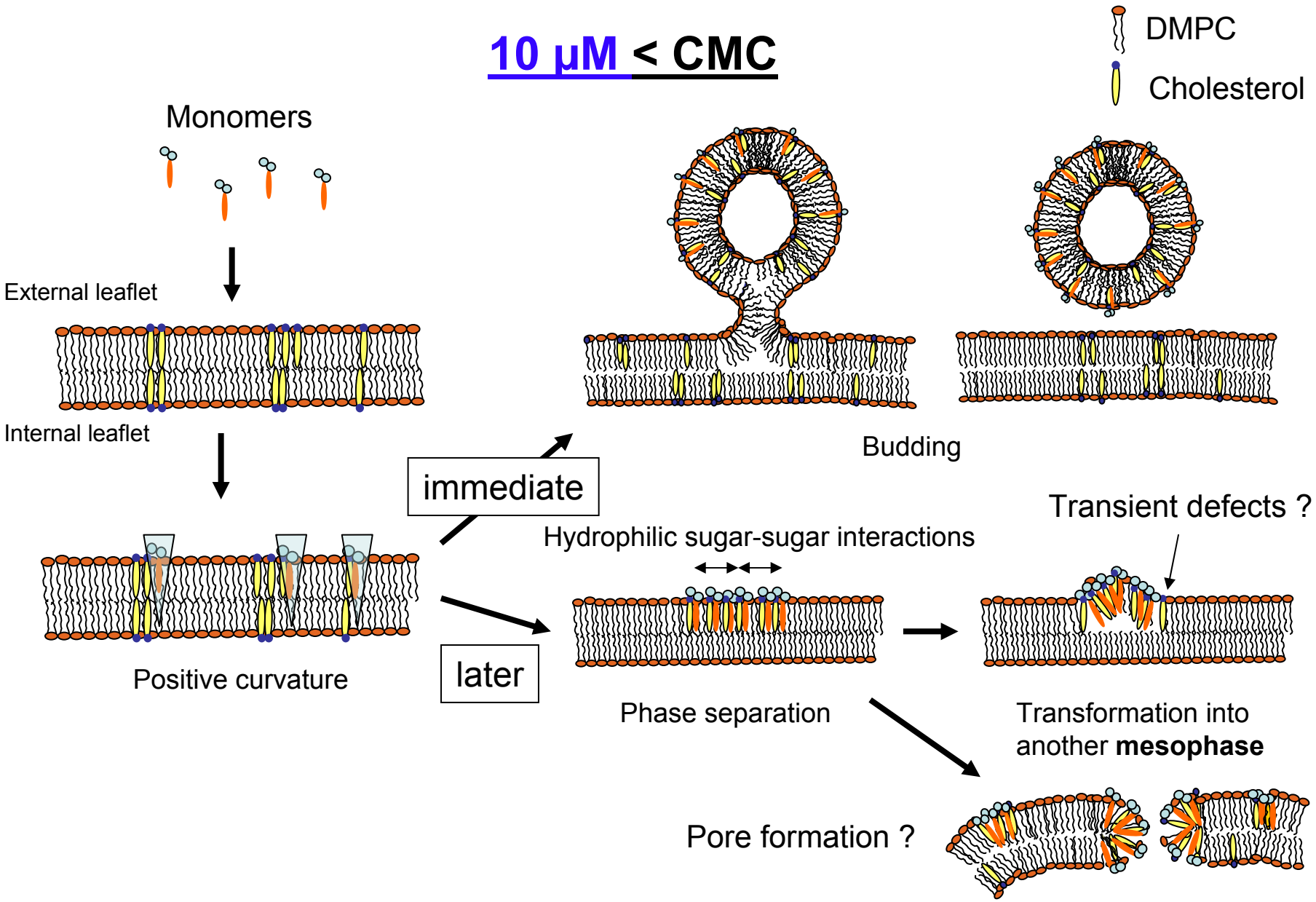
DMPC/Chol (3:1)



➔ Accumulation of membrane material into worm-like structures (new mesophase)

➔ Formation of holes with increasing size upon time

Conclusions : Membrane interactions of α-hederin



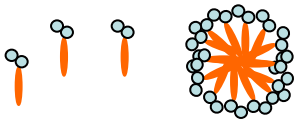
Conclusions: Membrane interactions of α-hederin

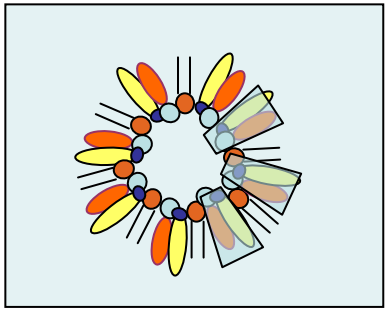
40 µM > CMC

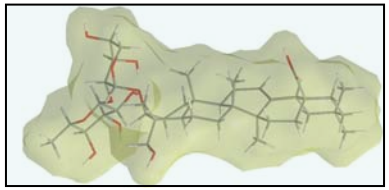
DMPC

Cholesterol

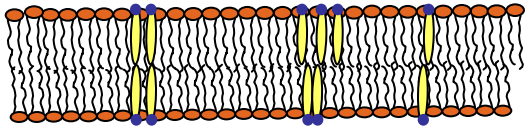
Aggregates and monomers



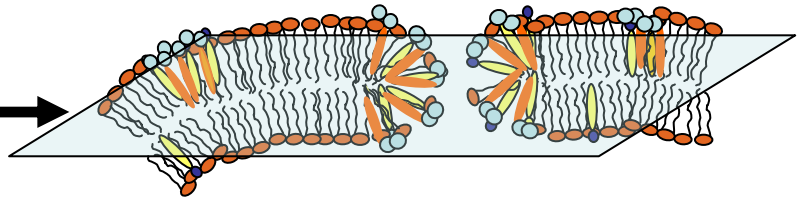


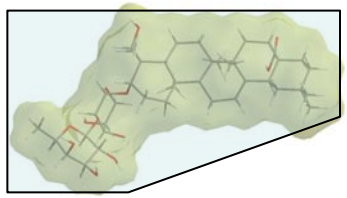


External leaflet



Internal leaflet





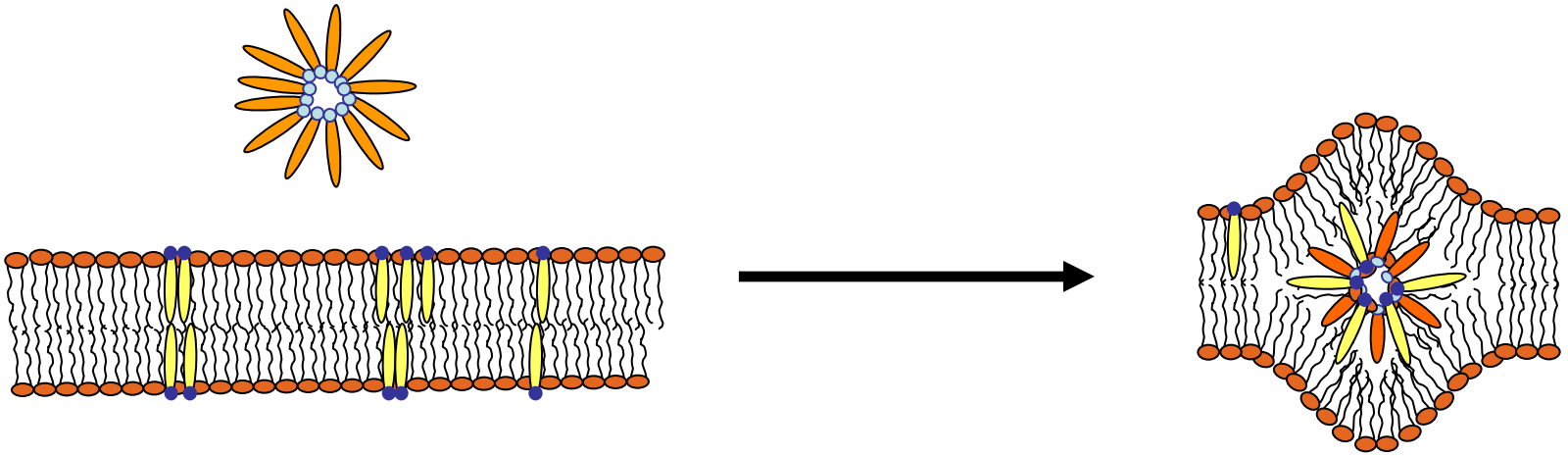
toroidal macroscopic pore

➔immediate permeabilization without transformation into new mesophase (at least at short incubation periods)

Conclusions : Membrane interactions of hederagenin

40 µM > CMC ??

Inverse aggregates ?



➔ accumulation into round spots
➔ transformation into inverse structures ?
➔ transient defects ➔ permeabilization ?

Investigations on cells

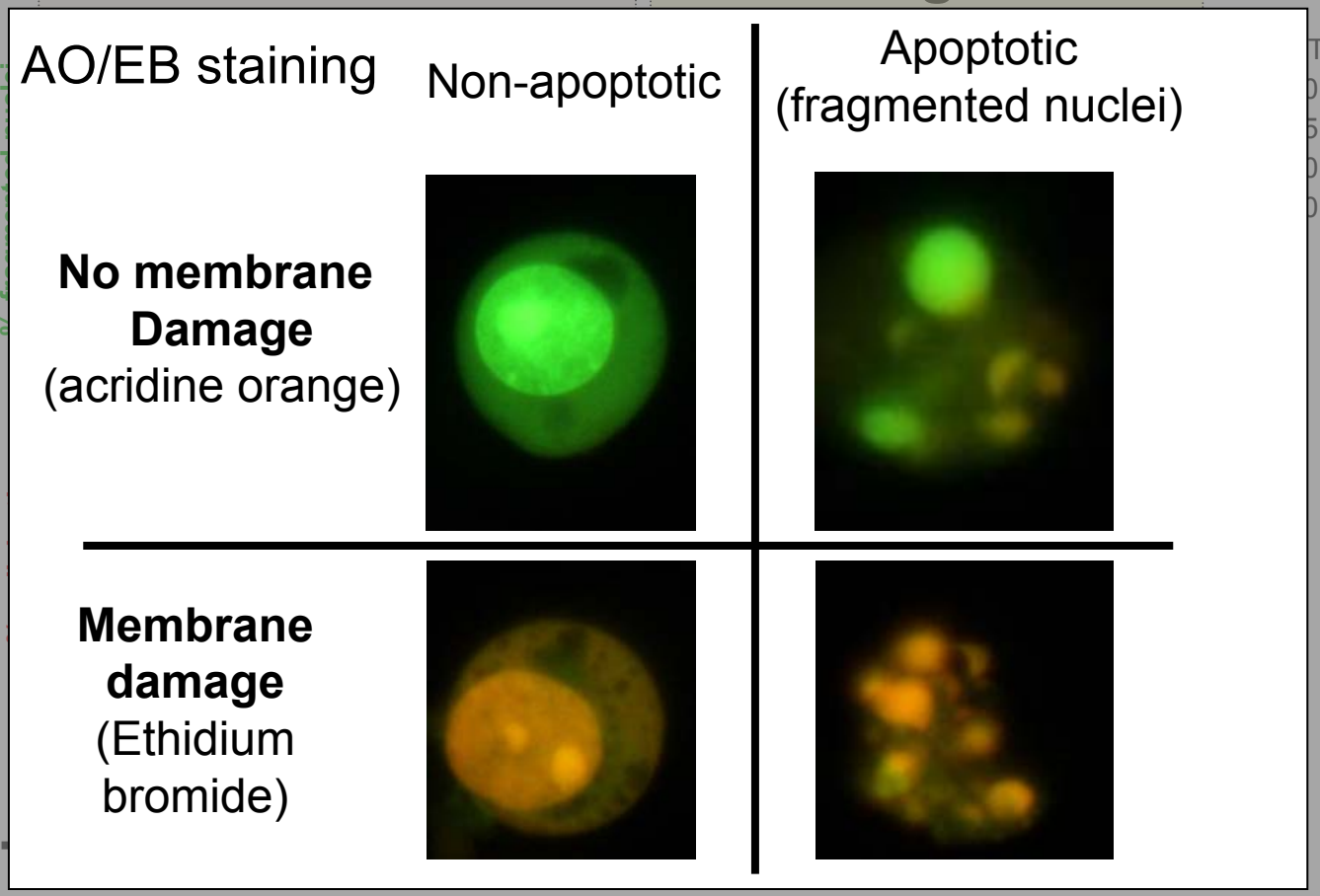
α-hederin and hederagenin induce apoptosis and non-apoptotic cell death in U937 and THP-1 cells in a cholesterol-dependent manner

Joseph Lorent[1,2], Joelle Quetin-Leclercq[2] and Marie-Paule Mingeot-Leclercq[1]*

[1]Université catholique de Louvain, Louvain Drug Research Institute, Cellular and Molecular Pharmacology, UCL B1.73.05, avenue E. Mounier 73, B-1200 Bruxelles, Belgium.
[2]Université catholique de Louvain, Louvain Drug Research Institute, Pharmacognosy, UCL B1.72.03, avenue E. Mounier 73, B-1200 Bruxelles, Belgium.

Cell death : Acridine orange/Ethidium bromide in U937 cells

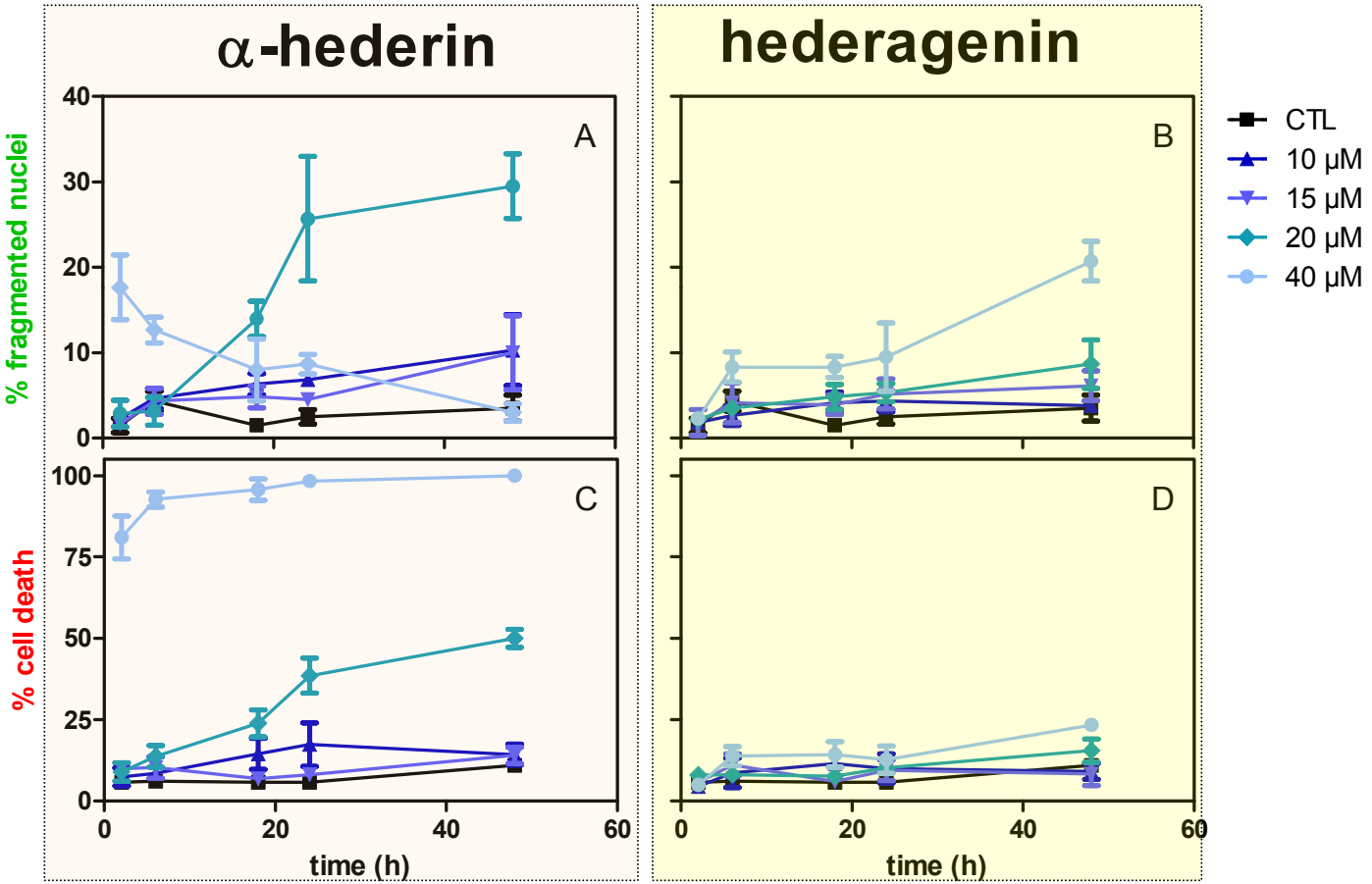
α-hederin | hederagenin



➔ Non-apoptotic cell death (very important at high α-hederin concentrations)

+ **Q-VD-O-Ph** (general caspase inhibitor) ➔ inhibition of apoptosis for both compounds
➔ no inhibition of non-apoptotic cell death

Cell death : Acridine orange/Ethidium bromide in U937 cells



➔ Apoptosis induction = faster with α-hederin than hederagenin

➔ Non-apoptotic cell death (very important at high α-hederin concentrations)

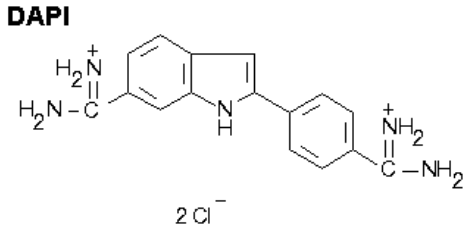
+ **Q-VD-O-Ph** (general caspase inhibitor) ➔ inhibition of apoptosis for both compounds
➔ no inhibition of non-apoptotic cell death

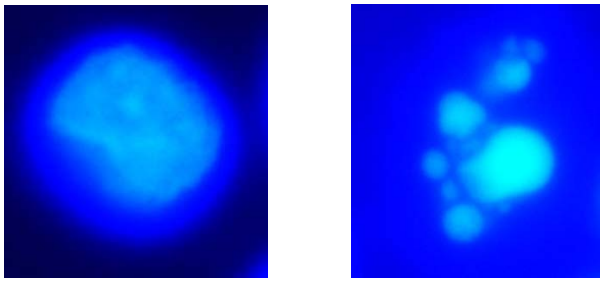
Cholesterol dependence of total cell death / apoptosis in U937

α-hederin

DAPI (apoptosis assay)

Cell nuclei (fluorescence microscopy)





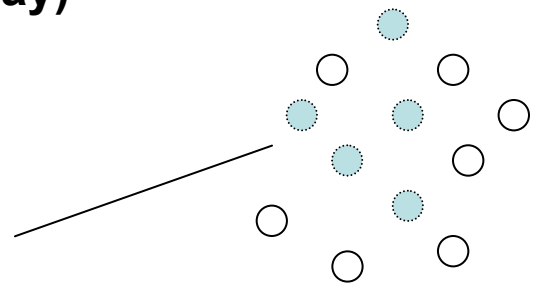
Fluorescent DNA intercalator

normal

apoptotic

Trypan blue staining (cell death assay)

➔ accumulates only in death cells



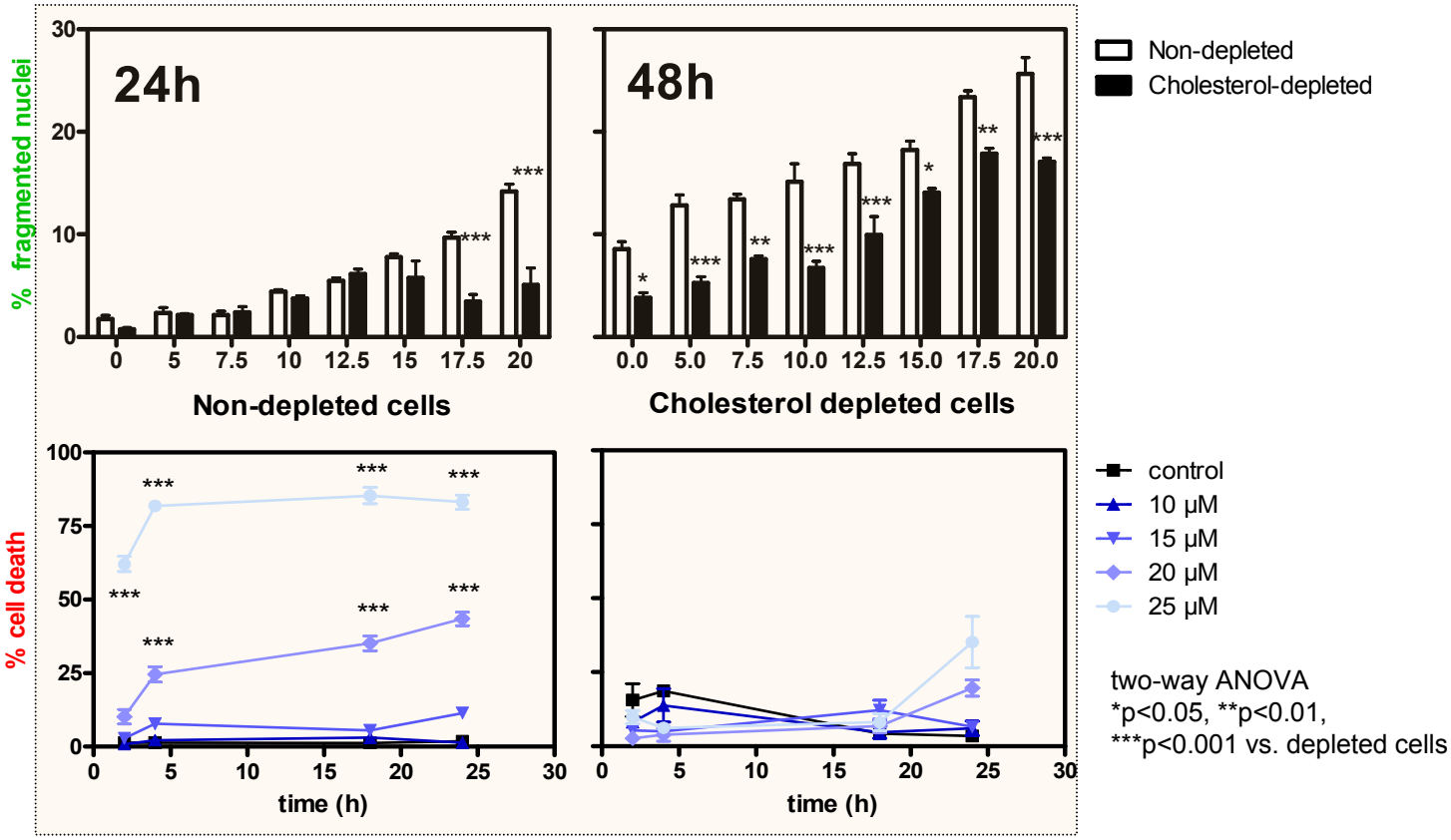
death cell

Microscope

death and apoptosis in depleted cells

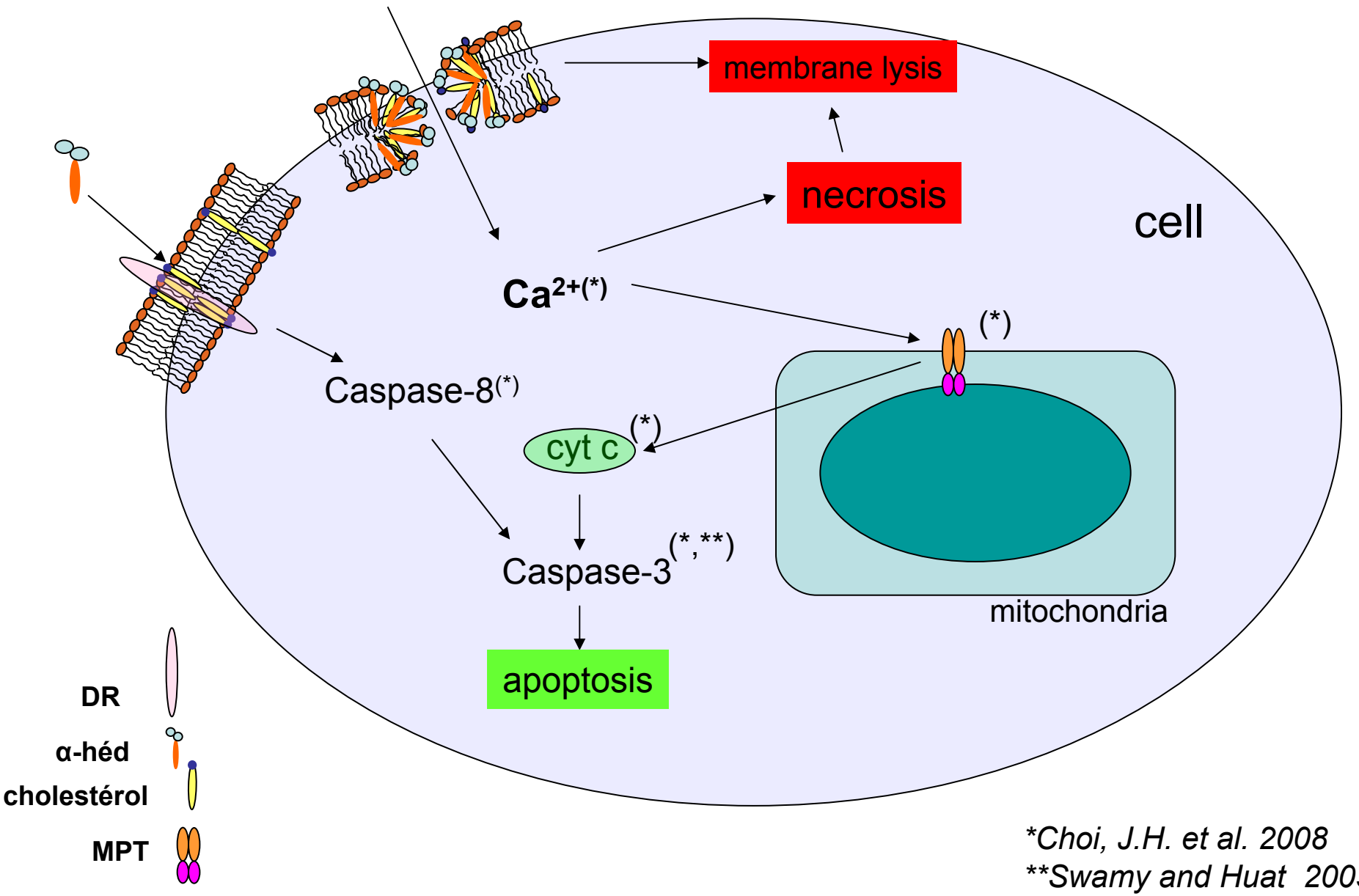
Cholesterol dependence of total cell death / apoptosis in U937

α-hederin



➔ Cholesterol dependence of cell death : decrease of α-hederin induced total cell death and apoptosis in depleted cells

Conclusions : Potential interactions



*Choi, J.H. et al. 2008

**Swamy and Huat 2003

BBA, Biomembranes, 2013, 1828, pp. 801-815

Effect of surfactin on membrane models displaying lipid phase separation

Magali Deleu[1°*], Joseph Lorent[2°], Laurence Lins[3], Robert Brasseur[3], Nathalie Braun[4], Karim El Kirat[4], Tommy Nylander[5], Yves F. Dufrêne[4] and Marie- Paule Mingeot-Leclercq[2]

[1]Université de Liège Gembloux Agro-Bio Tech, Unité de Chimie Biologique Industrielle, Passage des Déportés, 2, B-5030 Gembloux, Belgium

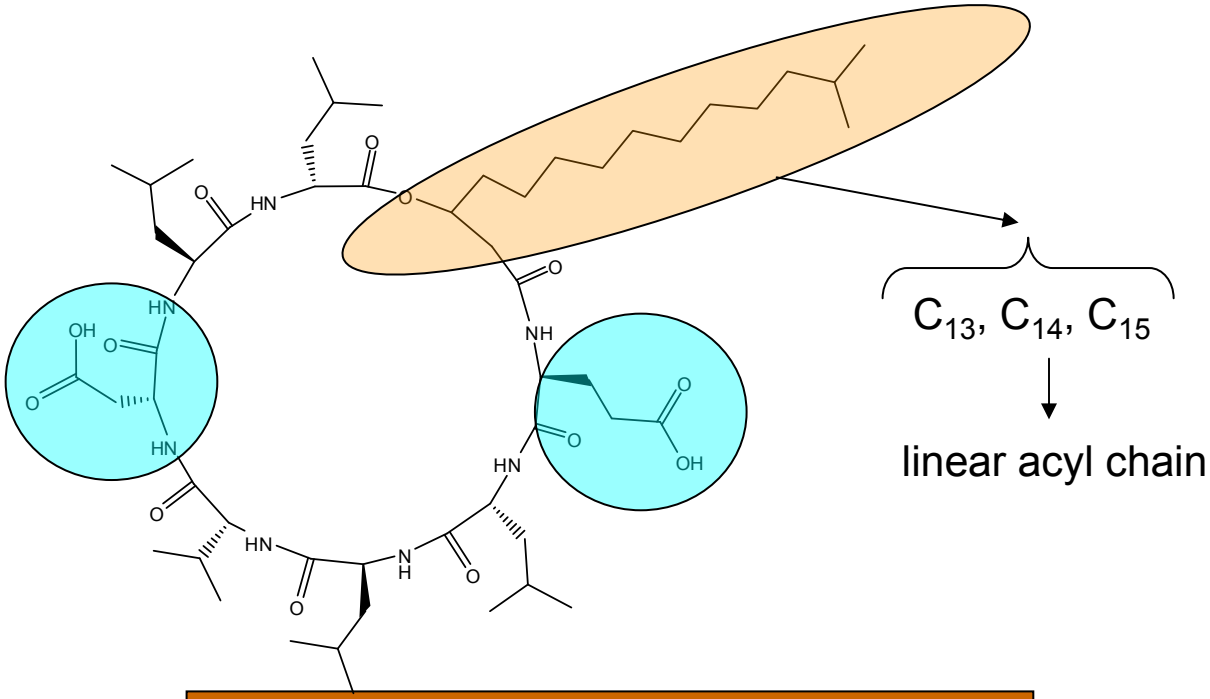
[2]Université catholique de Louvain, Louvain Drug Research Institute, Cellular and Molecular Pharmacology, Avenue E. Mounier 73, B1.73.05, B-1200, Brussels, Belgium

[3]Université de Liège Gembloux Agro-Bio Tech, Centre de Biophysique Moléculaire Numérique, Passage des Déportés, 2, B-5030 Gembloux, Belgium

[4]Université catholique de Louvain, Institute of Condensed Matter and Nanosciences, Bio and Soft Matter, Croix du Sud 1, L7.04.01, B-1348 Louvain-la-Neuve, Belgium

[5]Lund University, Center for Chemistry and Chemical Engineering, Physical Chemistry, 1S-221 00 Lund, Sweden
°Equal First author

Molecular structure, CMC and activity of surfactin



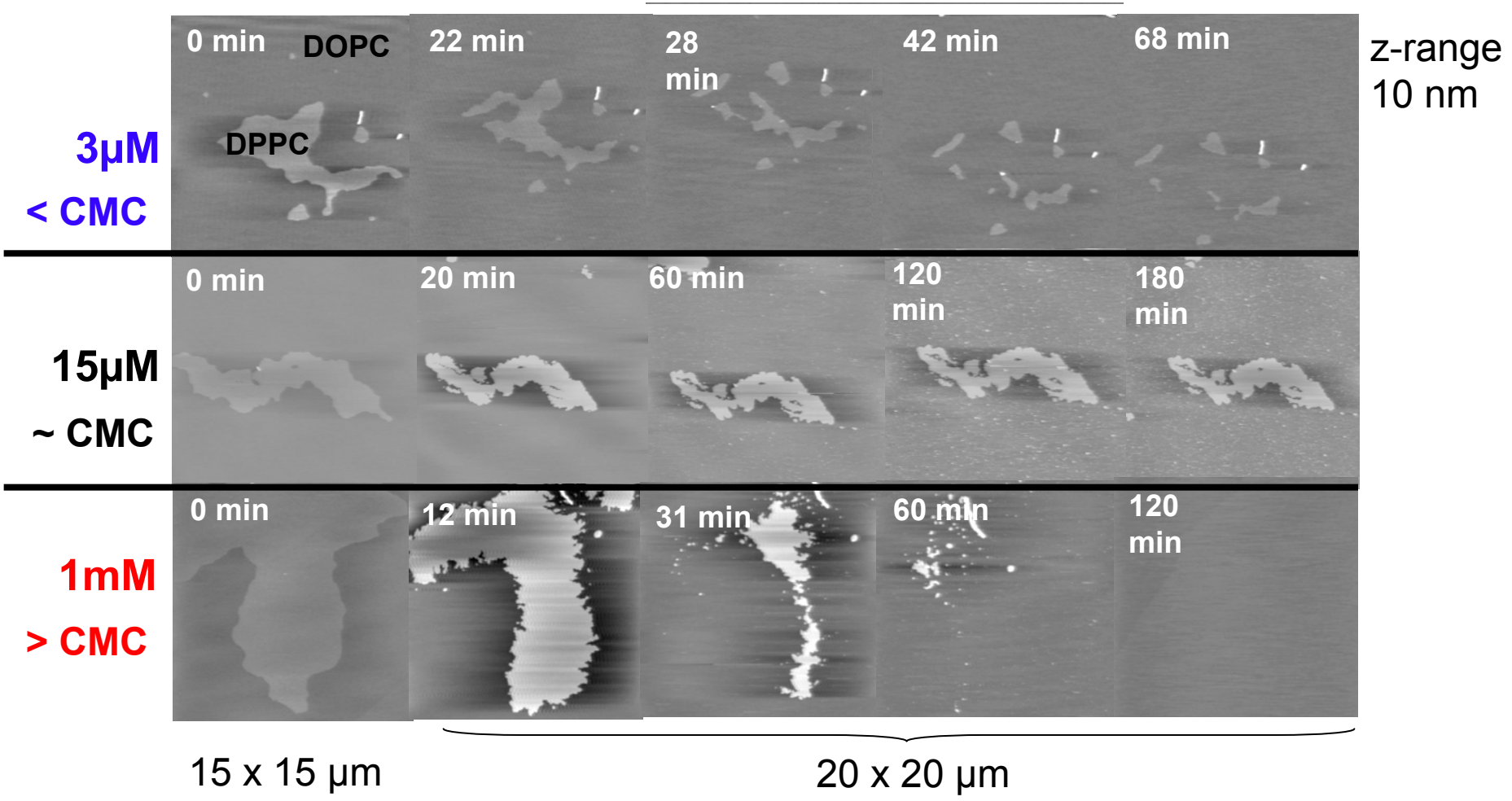
➔ CMC = **12.85 ± 0.05 µM** (pH = 8.5)

(determined by isothermal titration calorimetry)

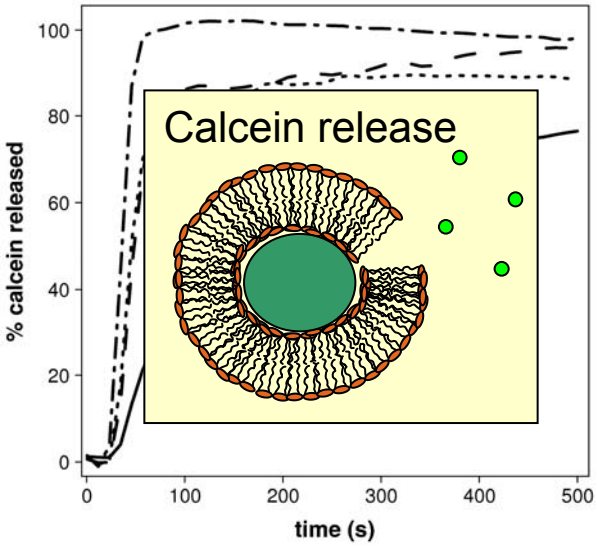
➔ Tested on **DOPC/DPPC** (1:1) bilayer at pH =8,5

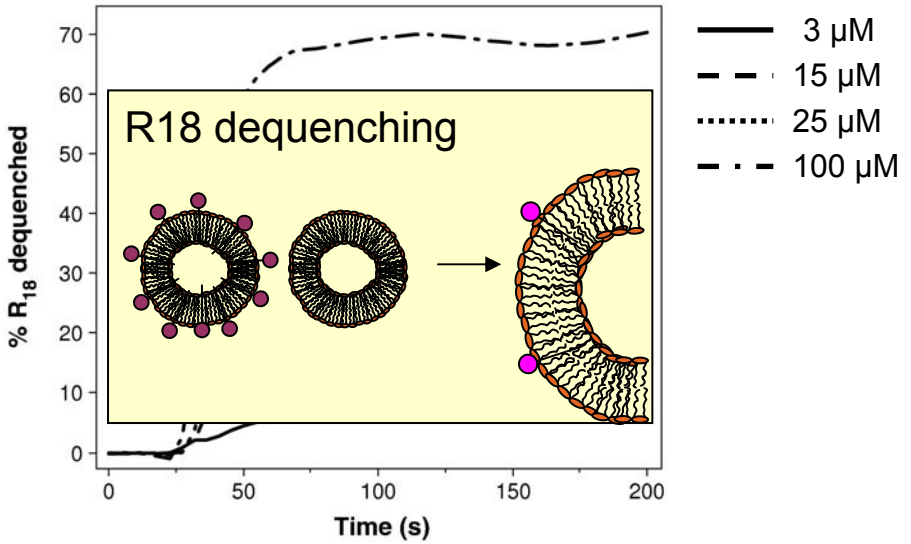
Integrity of SPB : AFM

Supported planar bilayer : **DOPC/DPPC (1:1) (25°C)**



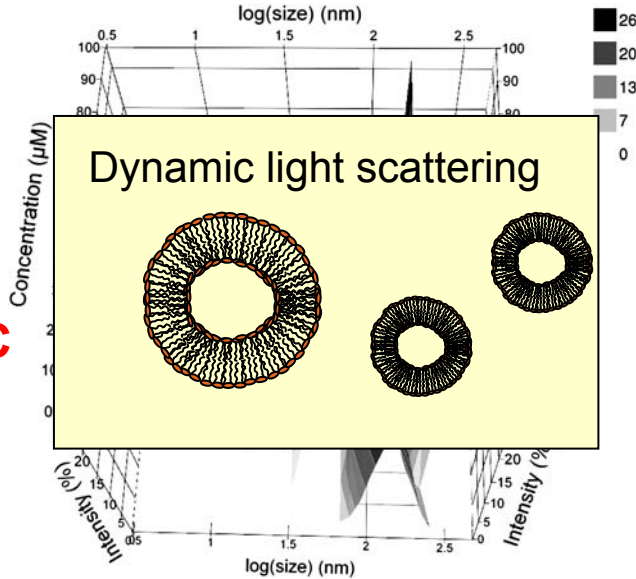
Permeabilization and remodelization of LUV: calcein release, R_{18} dequenching and dynamic light scattering (DLS)



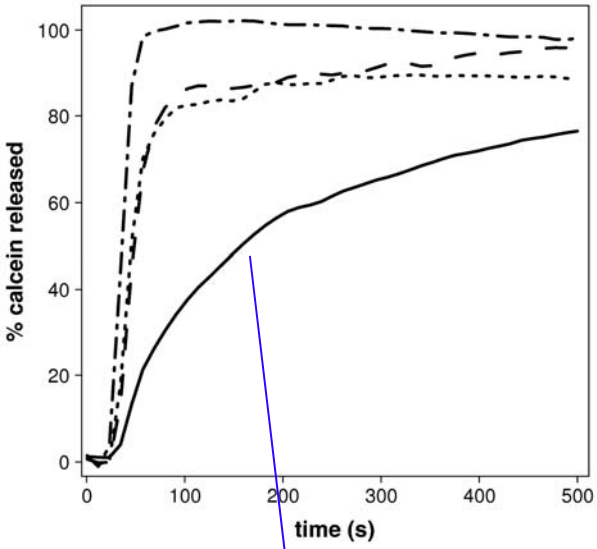


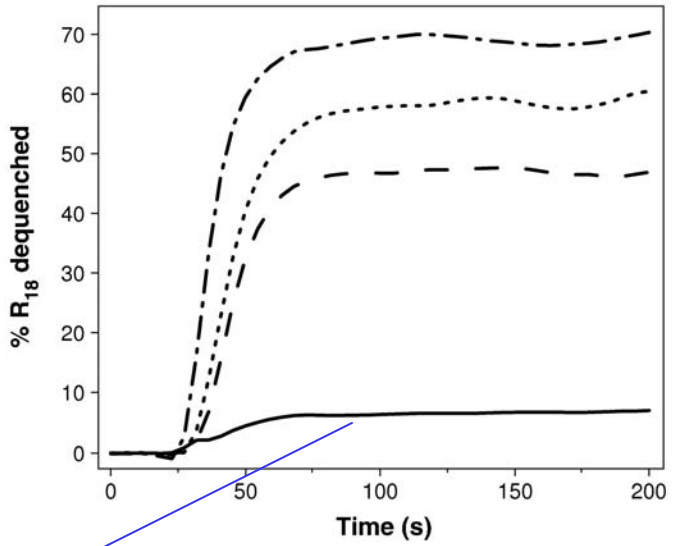
$$\%calcein_{released} = y^{1}_{\max}(1-\exp^{-k_1 t})$$

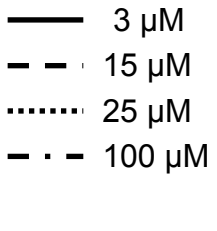
CMC



Permeabilization and remodelization of LUV: calcein release, R_{18} dequenching and dynamic light scattering (DLS)

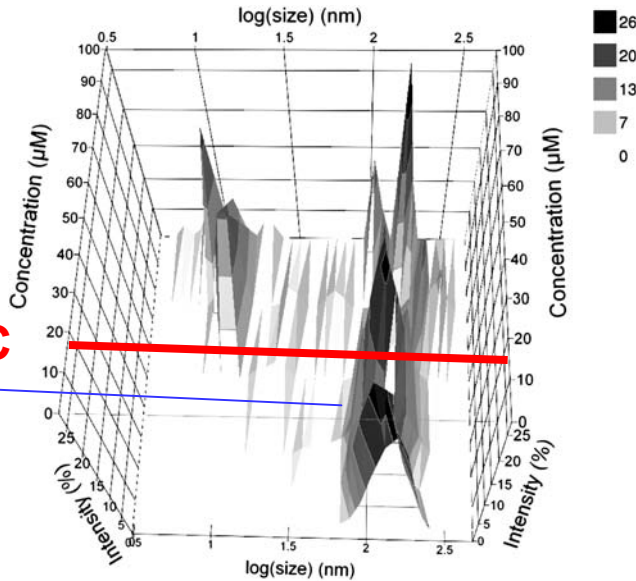




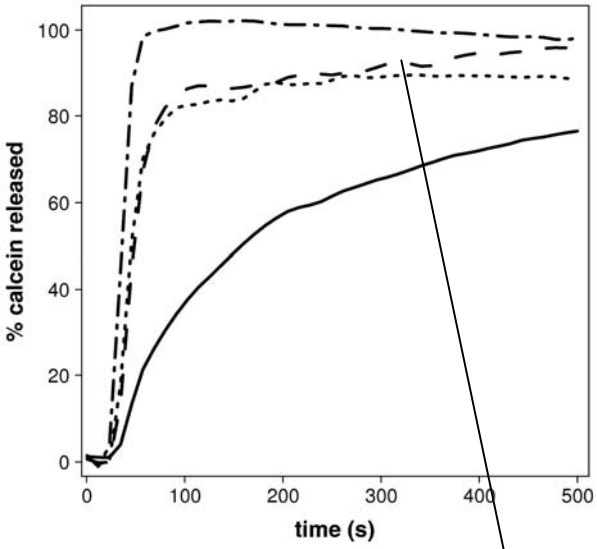


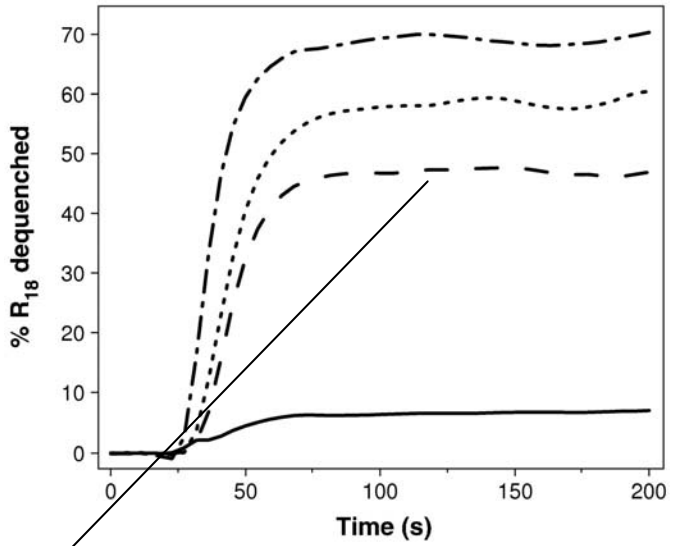
< CMC

$$\%calcein_{released} = y^{1}_{\max}(1-\exp^{-k_1 t})$$



Permeabilization and remodelization of LUV: calcein release, R_{18} dequenching and dynamic light scattering (DLS)

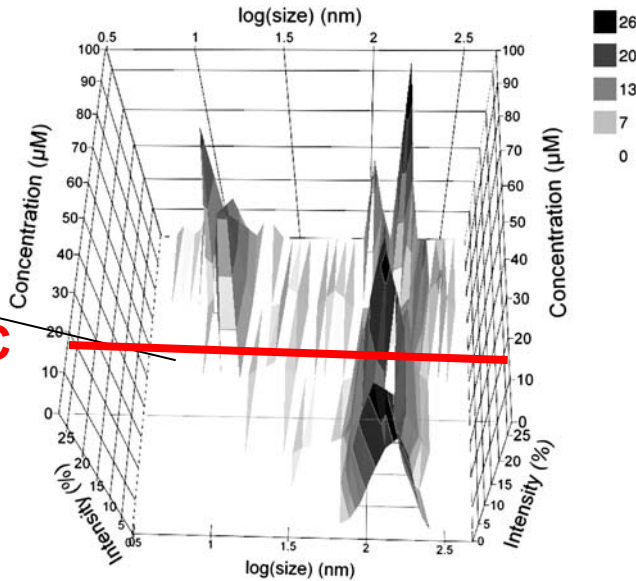




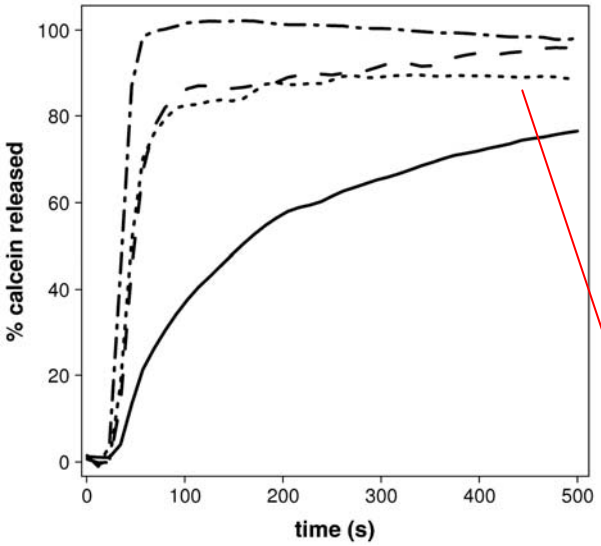
3 μM
15 μM
25 μM
100 μM

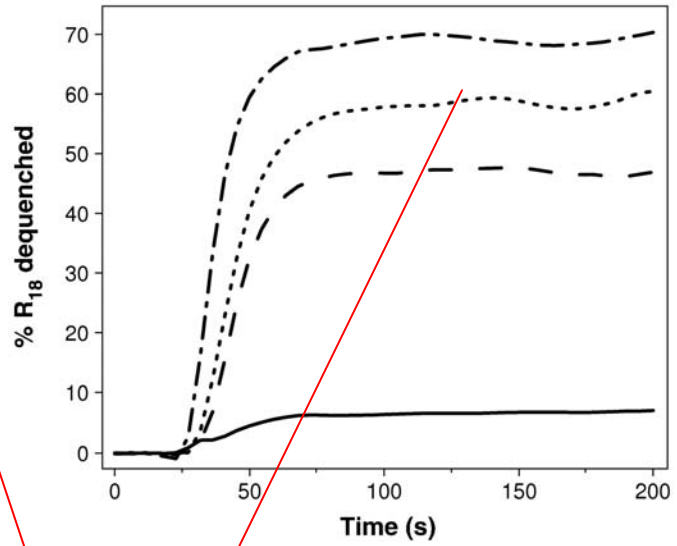
~ CMC

$$\%calcein_{released} = y^1_{\max}(1-\exp^{-k_1 t}) + y^2_{\max}(1-\exp^{-k_2 t})$$



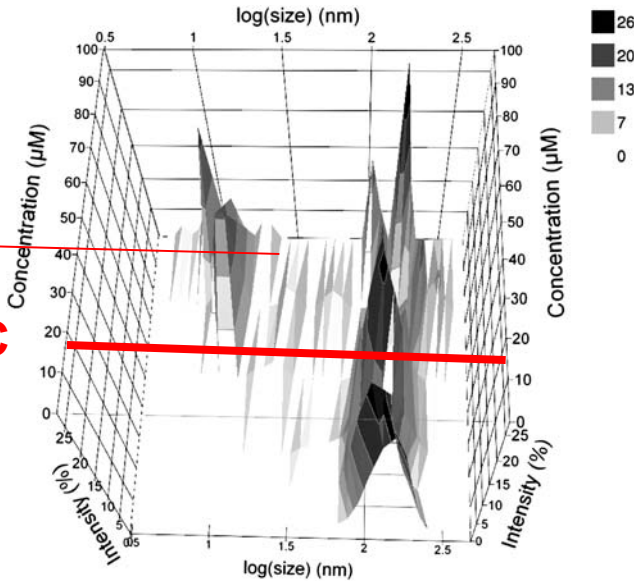
Permeabilization and remodelization of LUV: calcein release, R_{18} dequenching and dynamic light scattering (DLS)





3 μM
15 μM
25 μM
100 μM

> CMC

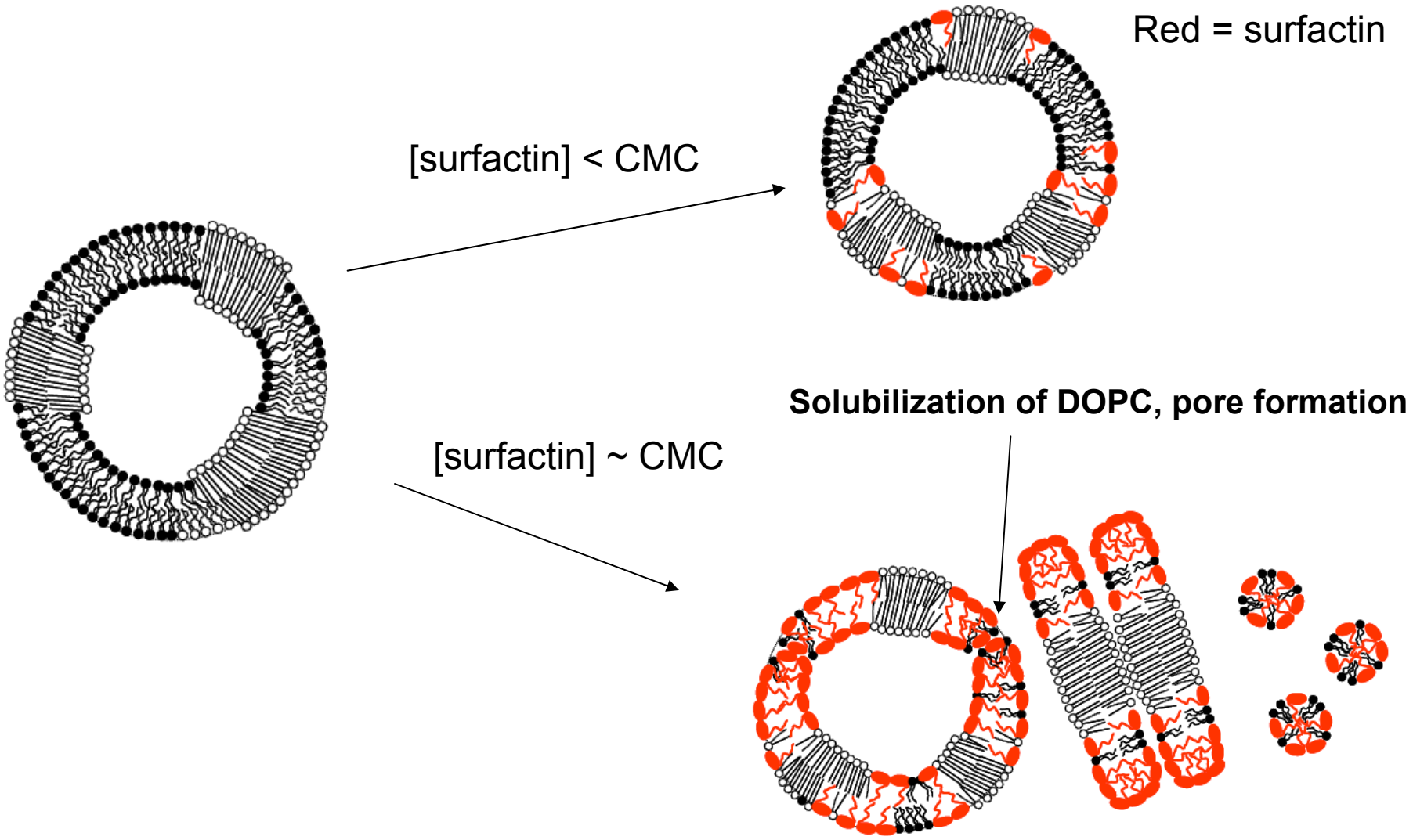


$$\%calcein_{released} = y^{1}_{\max}(1-\exp^{-k_1 t}) + y^{2}_{\max}(1-\exp^{-k_2 t})$$

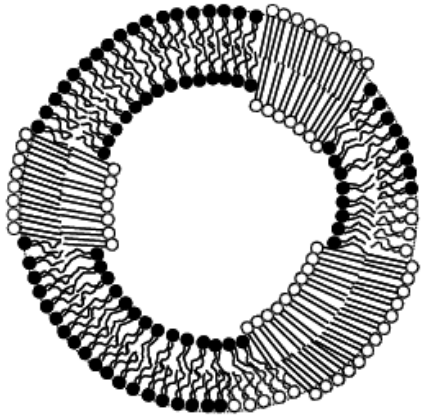
CMC

Two release mechanisms

Conclusions : Surfactin / membrane interaction

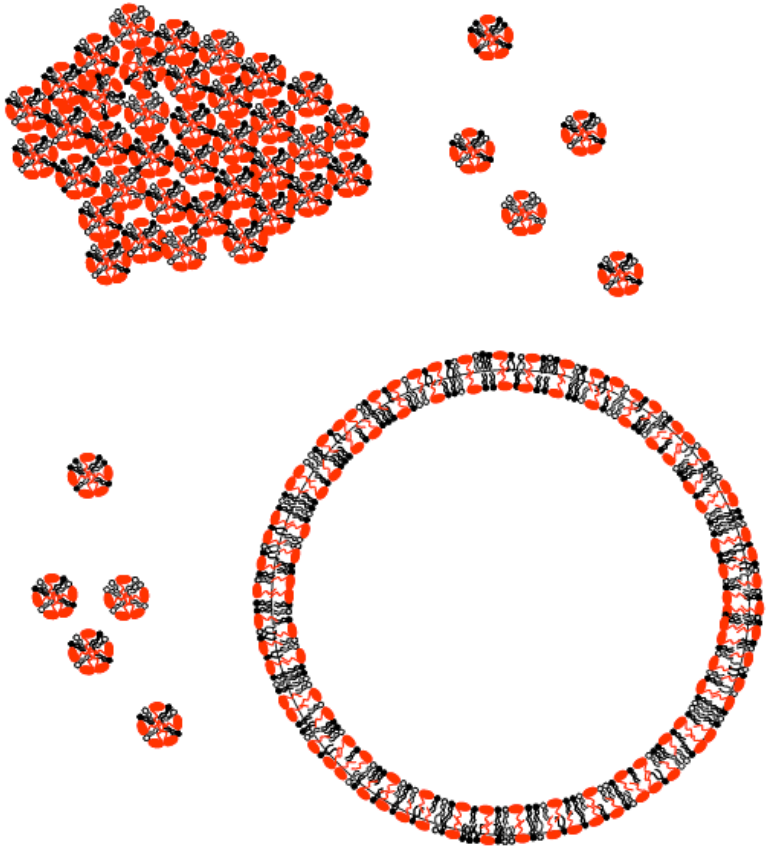


Conclusions : Surfactin / membrane interaction

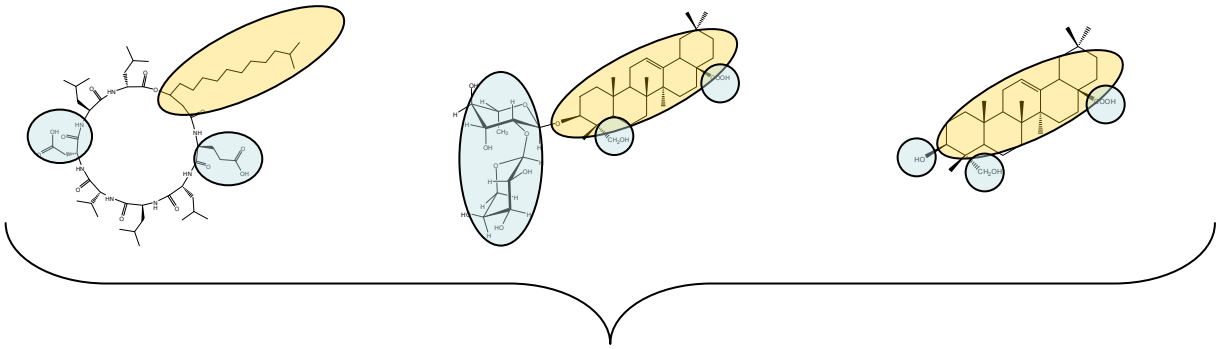


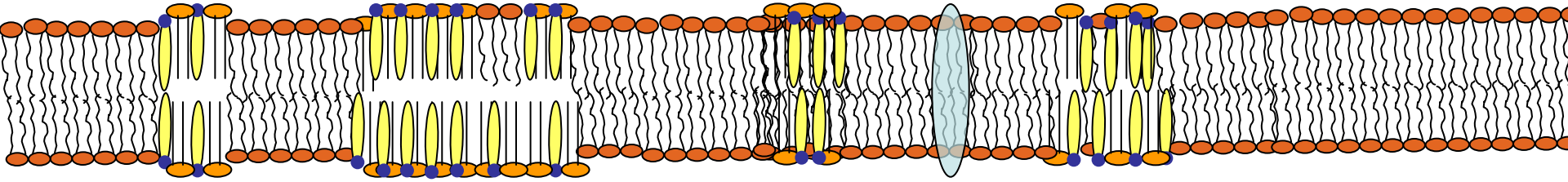
[surfactin] >> CMC

Transformation into micelles, Fusion ?

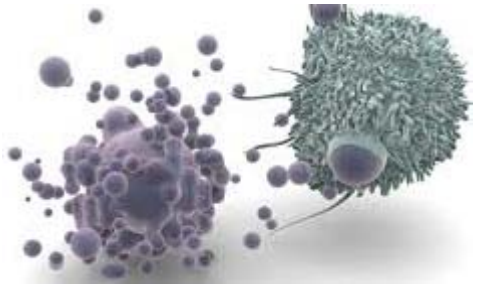


General conclusions



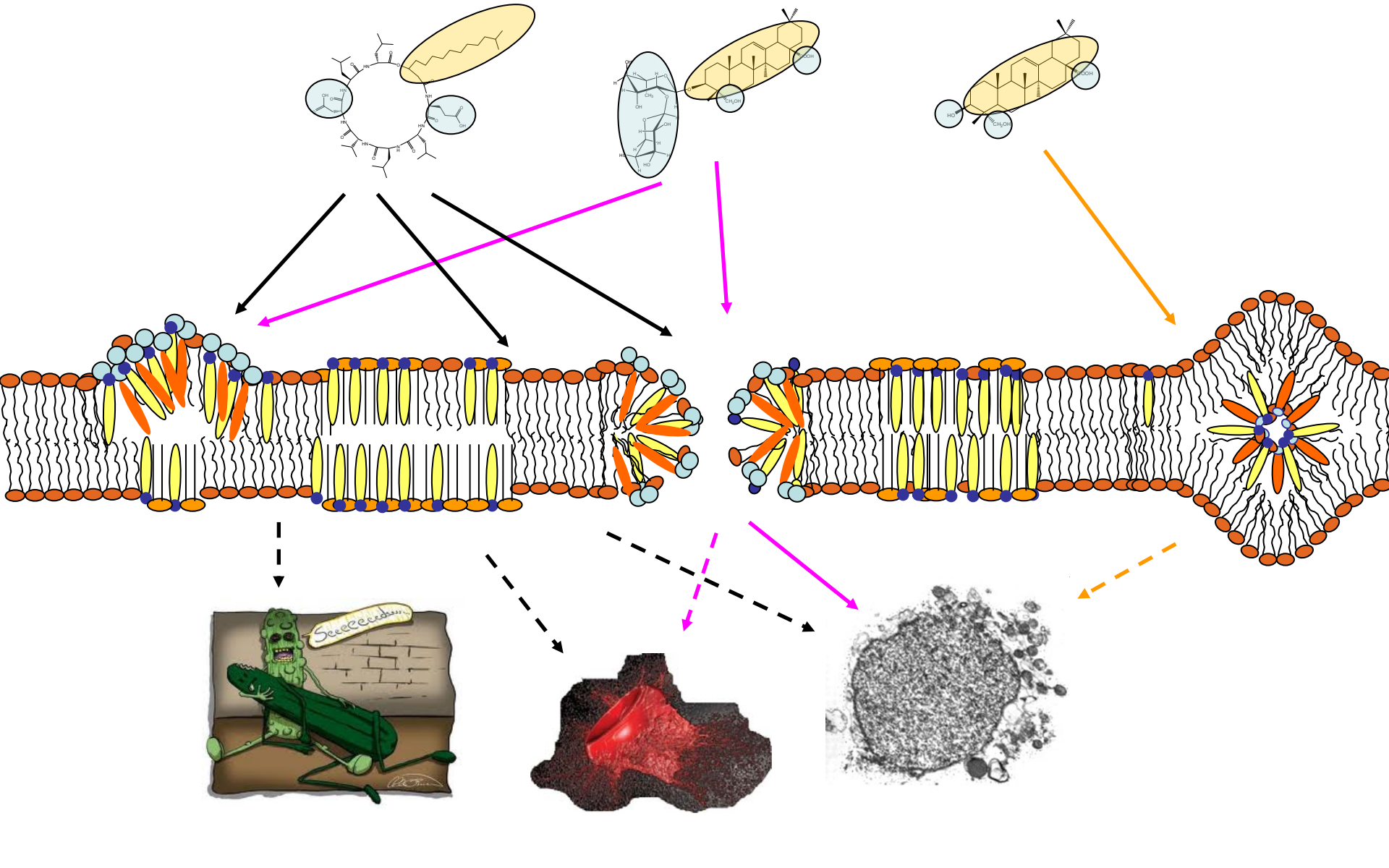


Membrane raft receptors (Fas,.) ?



Other receptor dependent activities

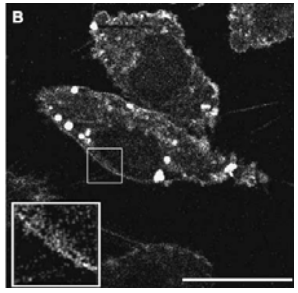
General conclusions

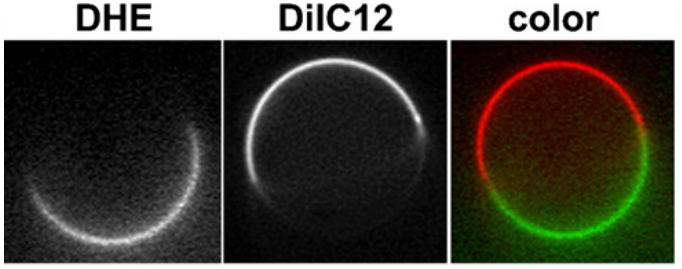


Perspectives

Short term-perspectives

Biphoton microscopy of DHE

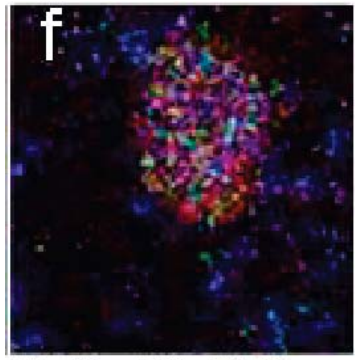




GUV : DOPC:DPPC:DHE (1:1:1)

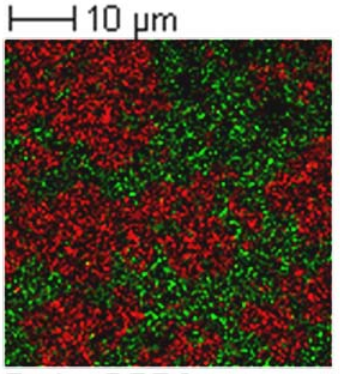
Cell staining *(McIntosh 2008, Garvik 2009)*

Tof-SIMS



R=Chol G=PLP
B=Hcol-A1

Cells
(*Mazzucchelli 2008*)

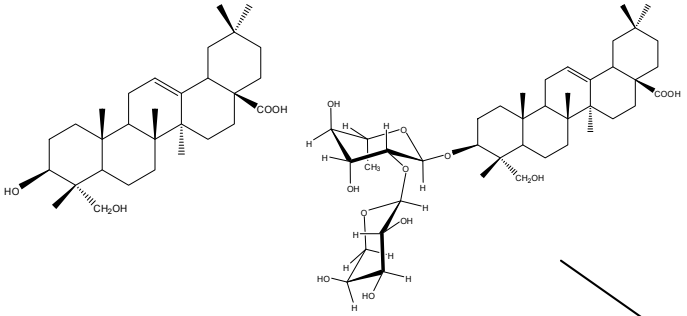


Red = DPPC-
Green = DOPC-

SPB (*Lorent*)

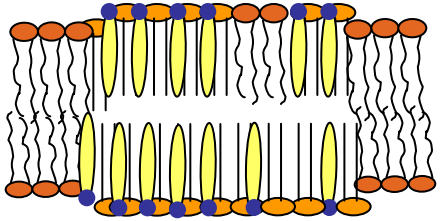
Perspectives

Long term-perspectives



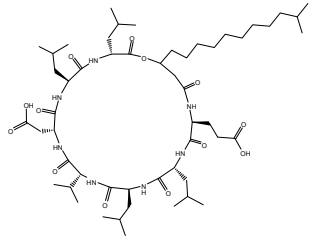
Hemisynthesis

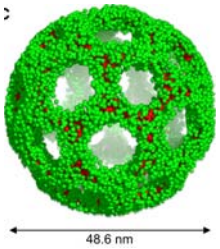
(QSAR)



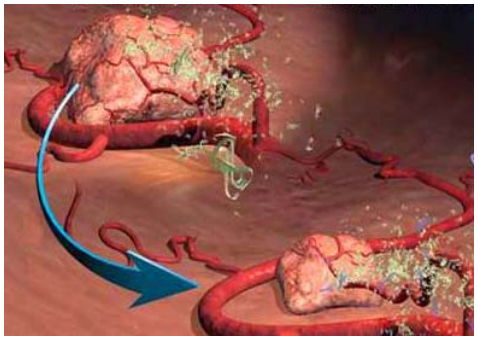
↓ Hemolysis

↑ Activity on cancer cells





New nanoparticles



New anticancer drugs ?