

Neutron reflectometry for studying interactions between bioactive molecules and biomimetic membranes

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FNRS Senior Research Associate

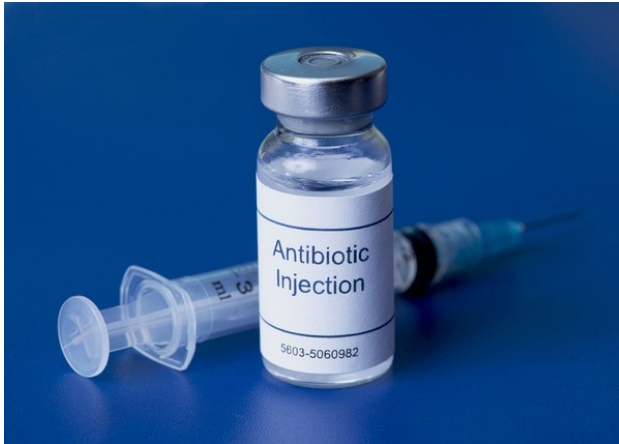
Laboratory of Molecular Biophysics at Interfaces

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<http://www.gembloux.ulg.ac.be/biophysique-moleculaire-aux-interfaces/>

Continuous emergence of
microbial resistance

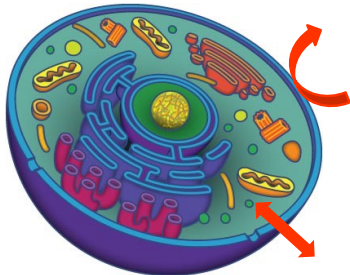
⇒ Need of new active drugs



Harmful effects of
pesticides on human health
and environment

⇒ Need of biopesticides
or other alternatives

⇒ Decipher the molecular mechanism of bioactive
molecules is a *sine qua non* condition to optimize their use
and to design optimal compounds

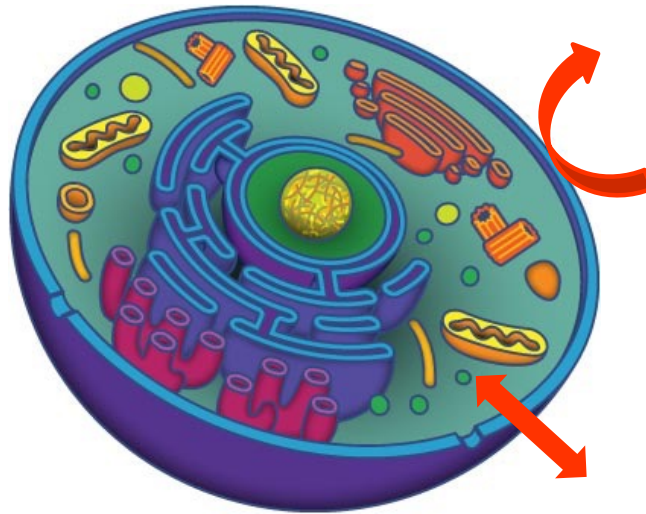


Perception by the cells is a critical step
Plasma membrane plays a **central role** in
signaling processes

Laboratory of Molecular Biophysics at Interfaces

Main research topics

- ✓ Study of the structure-function relationships of biomolecules
- ✓ Understanding of the mechanisms involved in biological phenomena and occurring at the level of a biological membrane



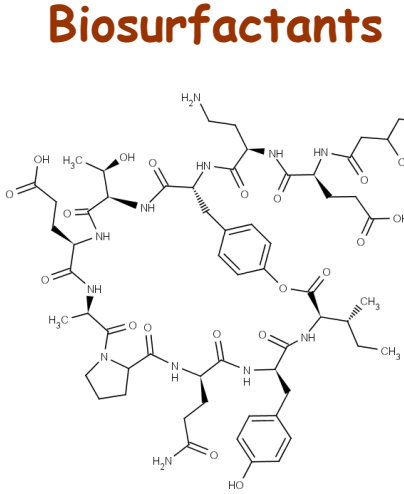
Perception of structurally-different biomolecules by the lipid fraction of plasma membrane
Complementary experimental and *in silico* biophysical techniques

Human health (Antimicrobial properties)

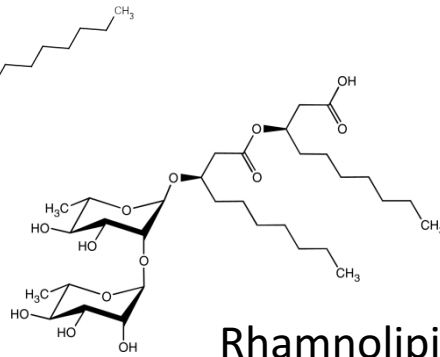
Plant protection (Eliciting properties)

Different biomolecules

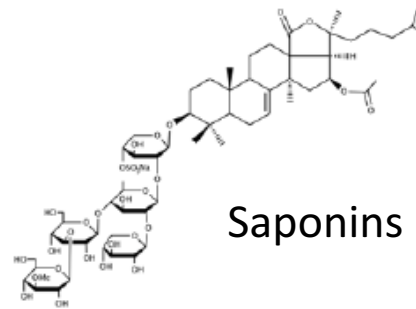
Biosurfactants



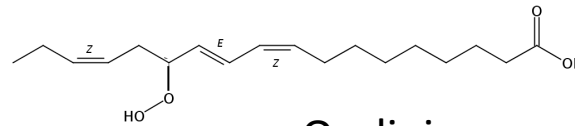
Lipopeptides



Rhamnolipids

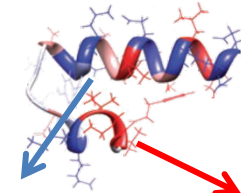


Saponins



Oxylipin

Peptides



Domain 1

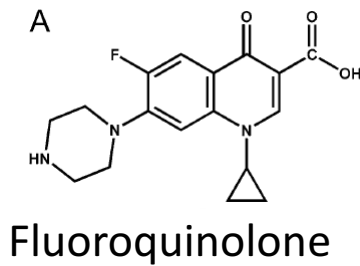
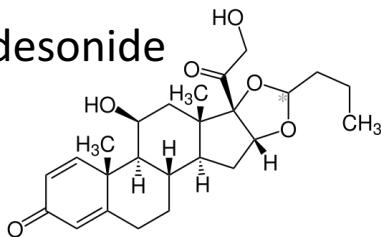
Domain 2



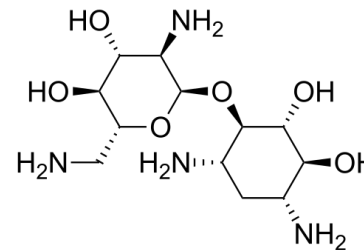
Remorin C-terminal peptide

Drugs

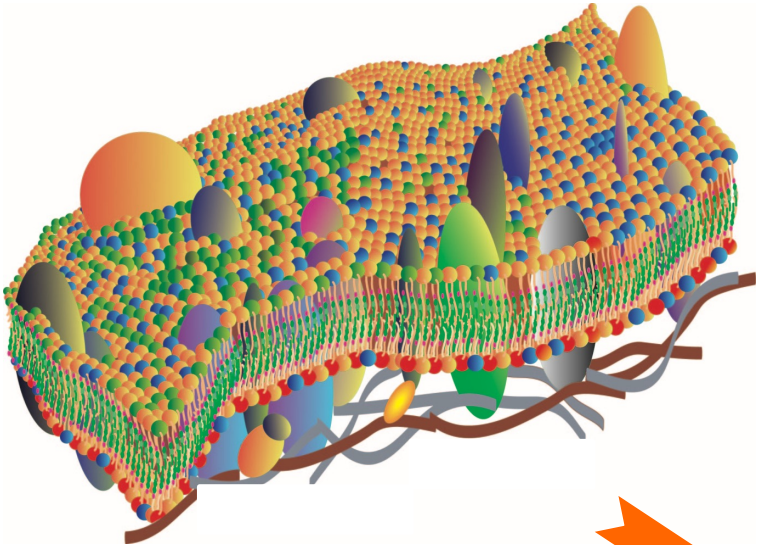
Budesonide



Neamine



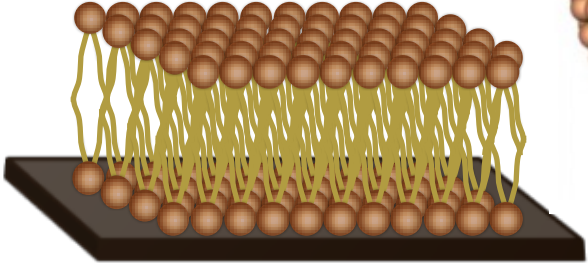
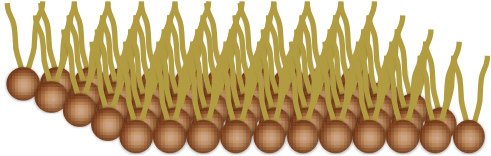
Different membrane models



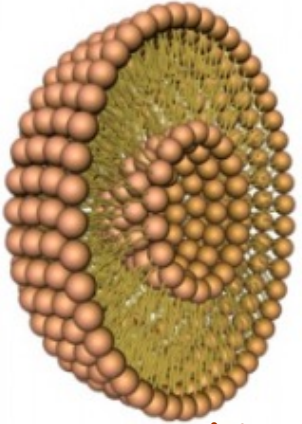
Complex dynamic entity



Monolayers



Bilayers

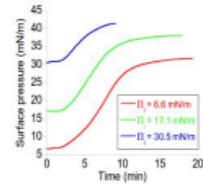


Liposomes

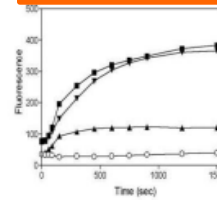
Complementary of biophysical techniques

Global

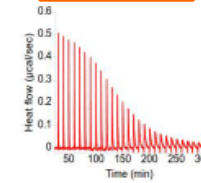
Langmuir trough



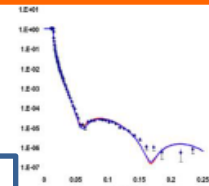
Fluorescence



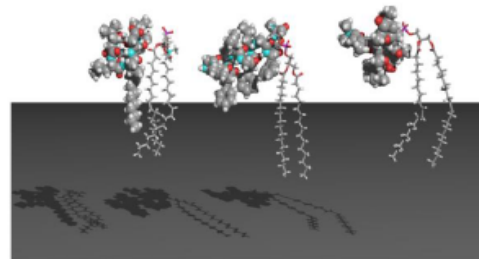
ITC



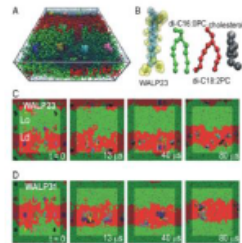
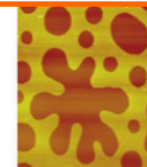
Neutron reflectivity



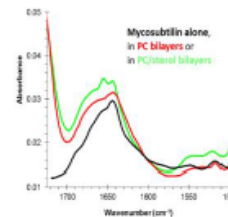
Molecular-specific



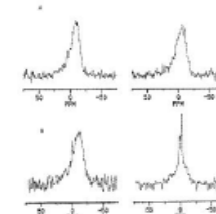
AFM



Molecular modelling

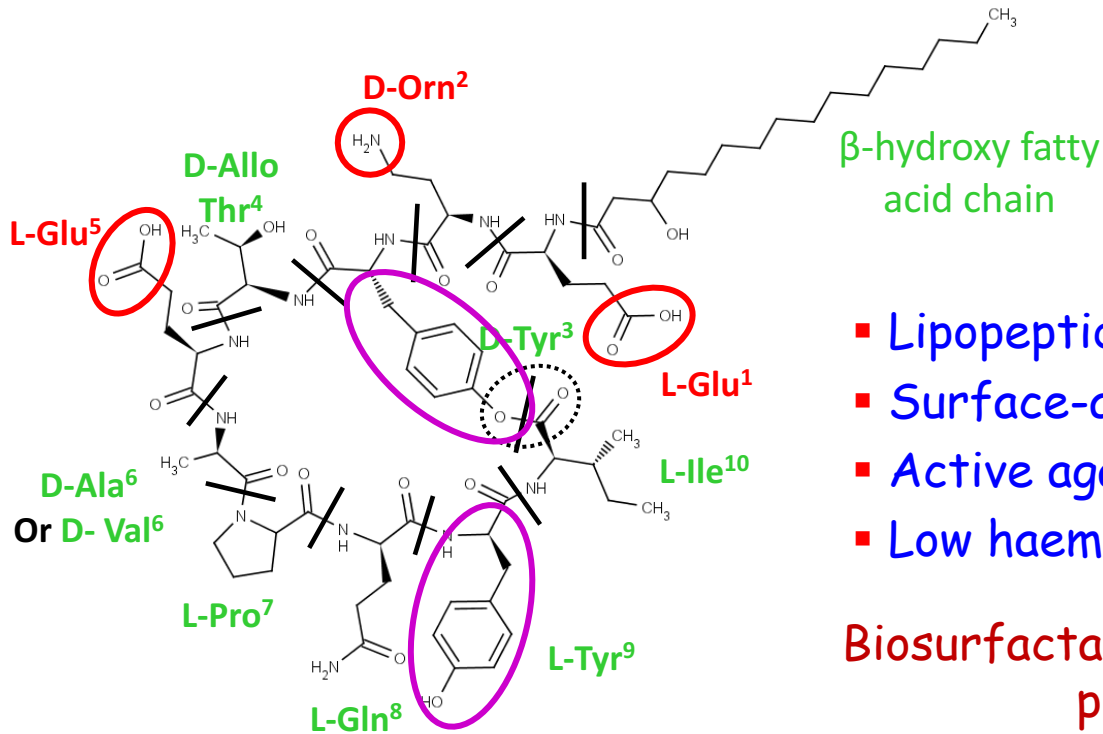


IR spectroscopy



NMR

Interaction between the antifungal lipopeptide fengycin and lipid membranes



- Lipopeptide excreted by *B. subtilis*
- Surface-active properties (CMC~ 5 μ M)
- Active against fungi
- Low haemolytic activity

Biosurfactant with high potential for plant protection

MW : ~1460 daltons

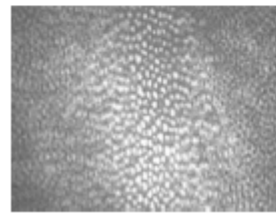
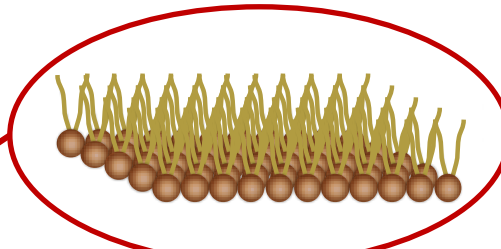
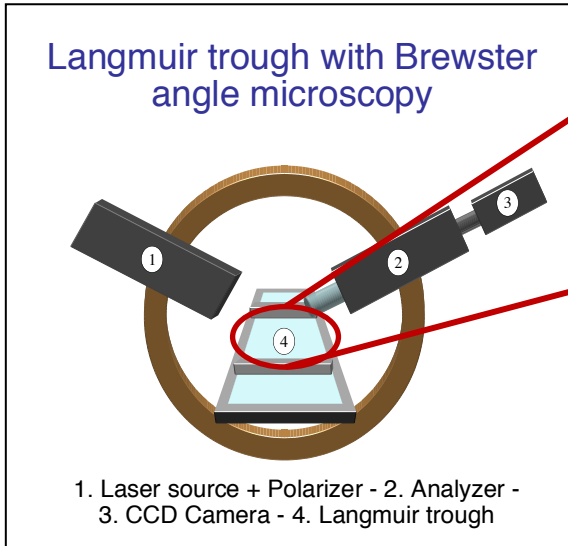
Host cell membrane interaction
Molecular details not known



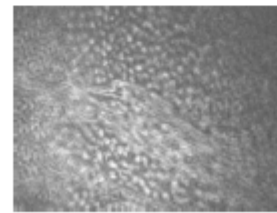
Vanittanakom et al., 1986, *J. Antibiot.*, 39(7), 888–901 - Nishikiori et al., *J. Antibiot.*, 39, 755–761 - Deleu et al., 1999, *Colloids Surfaces A*, 152, 3-10

Two main questions

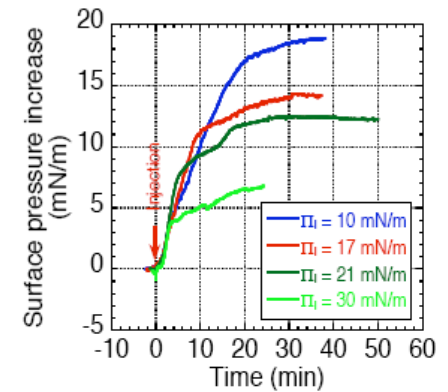
1. What is the effect of fengycin on biological membranes (lipid lateral organization, depth of insertion, solubilization) ?
2. What is the importance of the fengycin conformation in this interaction ?



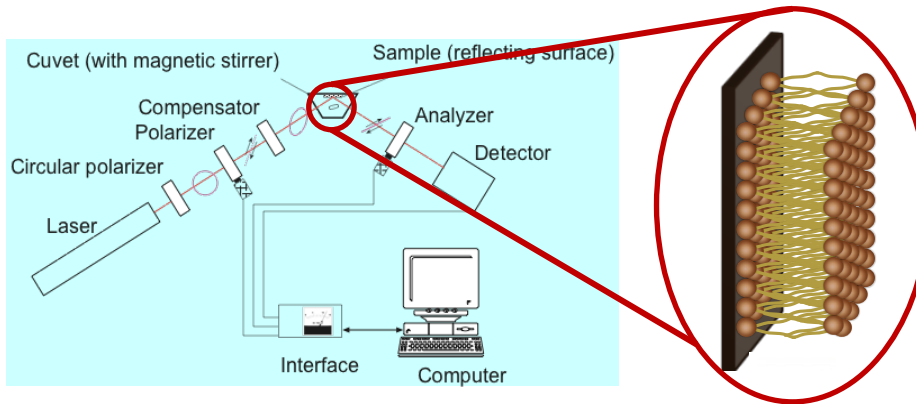
0 min



5 min

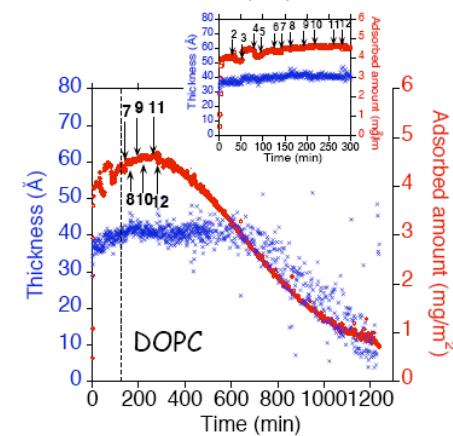
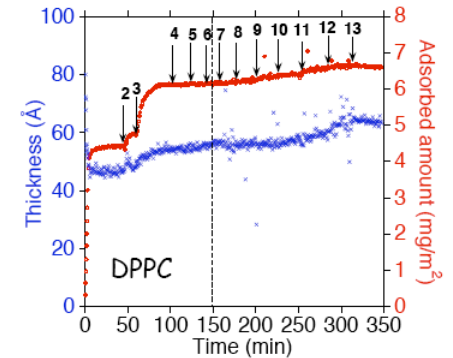


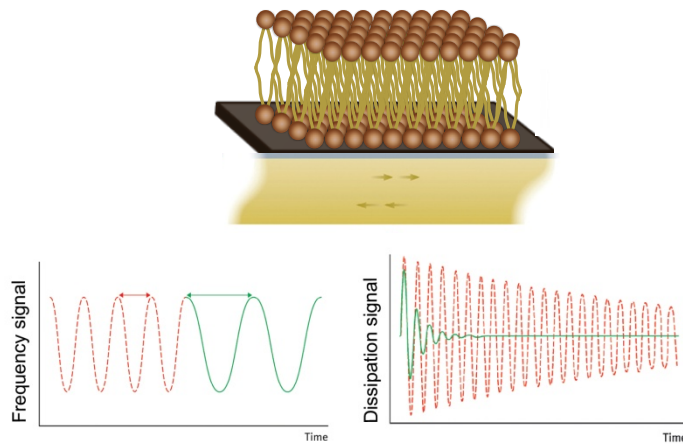
Fengycin inserts into lipid monolayer and modifies its lateral organization



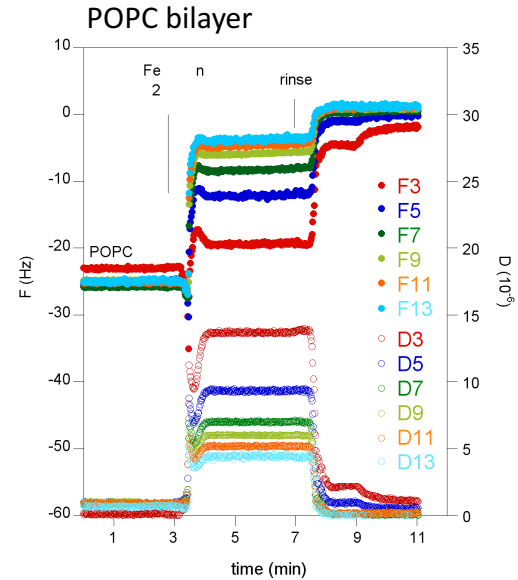
Ellipsometry gives “optical” thickness and “dry” mass versus time

Fengycin adsorbs into lipid bilayers and differently affects their integrity according to their physical state

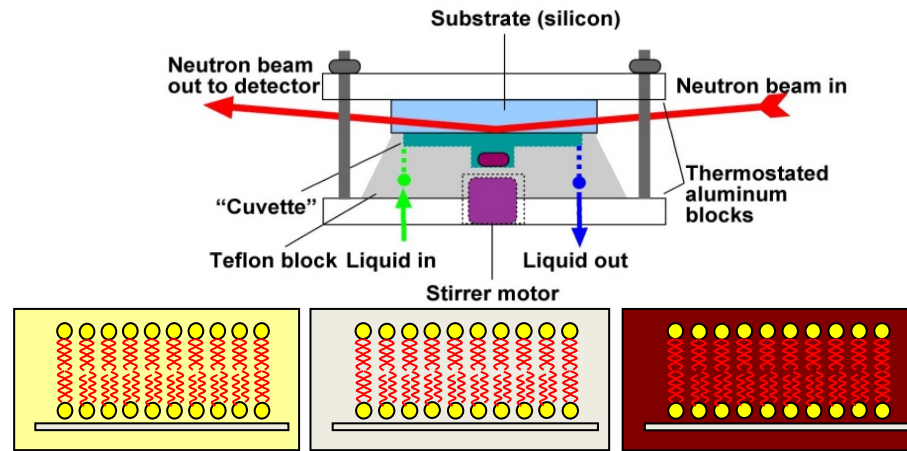




QCM-D gives “wet” mass with coupled water and dissipation measures the viscoelastic properties of the film



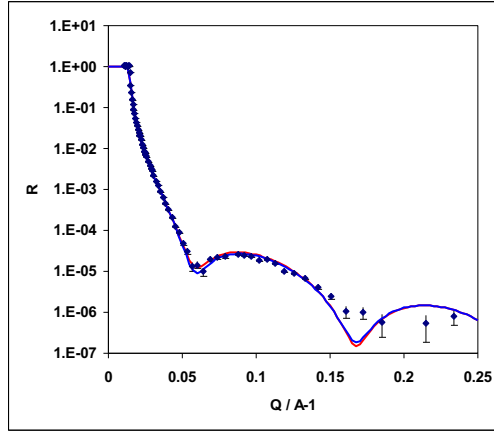
Binding of fengycin to a fluid lipid bilayer causes its fluidification leading to its destabilization



Neutron Reflectometry gives density profile of the interfacial layer

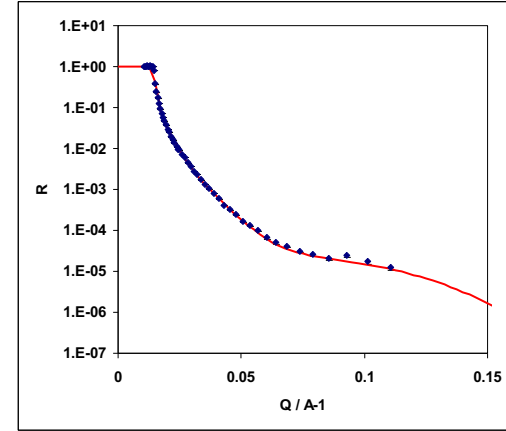
Selective deuteration + contrast matching gives composition perpendicular to the interface

Neutron reflectivity profiles for d-P-h-O-PC bilayers from vesicle dispersion in D₂O buffer (Tris 10mM, NaCl 150 mM, pH 7.4).



Effect of 20 μ M fengycin on d-P-h-O-PC bilayer in D₂O

← After 3h
After 10h →



Calculated properties of pure d-P-h-O-PC bilayer

Layer	d (Å)	R (Å)	SLD (10 ⁻⁶ Å ⁻²)	Solvent %
Head group	8	4	0.93	25
Tail	27	10	3.01	20

d = thickness, R = Roughness, SLD = Scattering length density

Bilayer coverage decreases from 80% to 65% after 3h and to 25% after 10h when 20 μ M fengycin are added

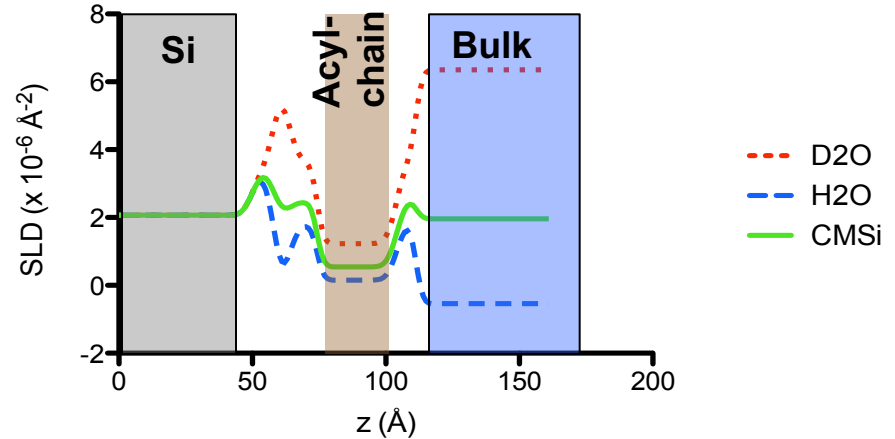
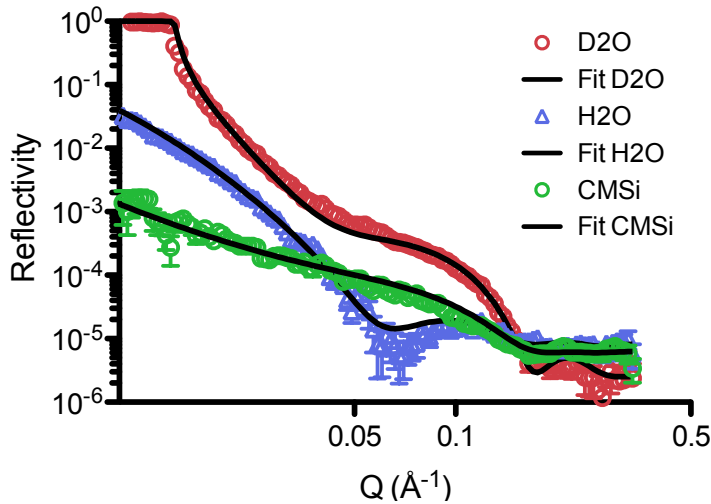
Calculated properties of d-P-h-O-PC bilayer after 3 hours and 10 hours of fengycin adsorption

	Layer	d (Å)	R (Å)	SLD (10 ⁻⁶ Å ⁻²)	Solvent %
3 h	Head group	8	4	0.93	40
	Tail	27	15	2.81	35
10 h	Head group	8	4 inner 6 outer	0.93	80
	Tail	27	20	3.01	75

A low fengycin concentration is able to destabilize a fluid phospholipid bilayer with a very slow kinetics

Local concentration threshold

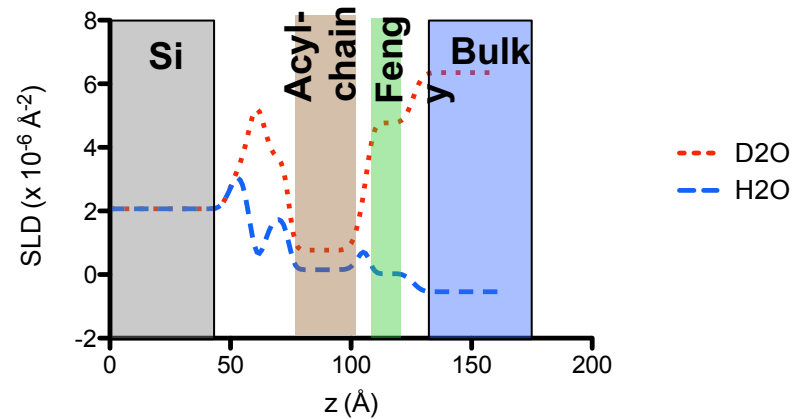
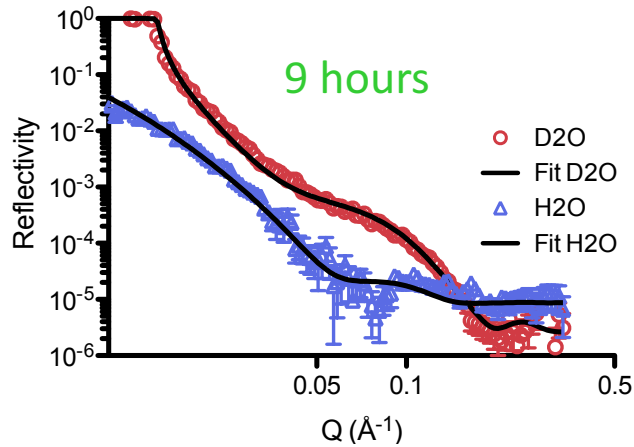
h-POPC/*h*-POPS bilayer



	Sub- strate	Water	Head group 1	Acyl chain	Head group 2	Bulk solution
Thick- ness, Å		5	11	30	7	
SLD $\times 10^{-6} \text{Å}^{-2}$		bulk	2.65	0.28	2.80	
Solvent vol. %		100	27	16	27	
Rough- ness		3	2	3	2	

Total thickness = 48 Å
 Total surface coverage = 84%
 PS mainly located in the outer leaflet

h-POPC/*h*-POPS bilayer + addition of Fengycin (20 μM)

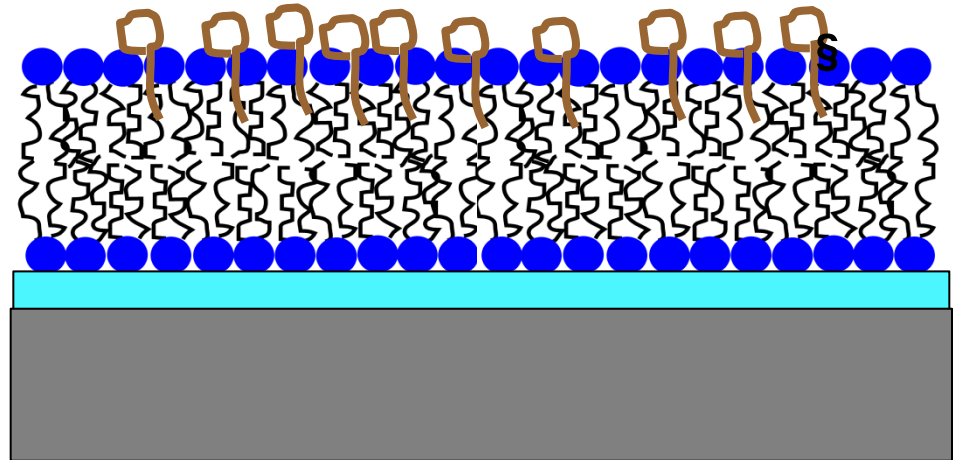


	Substrate	Water	Head group 1	Acyl chain	Head group 2	Fengycin	Bulk solution
Thickness, \AA		5	11	30	3	19	
SLD $\times 10^{-6} \text{ \AA}^{-2}$		bulk	2.65	0.22	2.00	1.17 H_2O 1.63 D_2O	
Solvent vol. %		100	27	9	27	67	
Roughness		3	2	3	2	3	

- Additional sublayer of fengycin
- Fatty chain of fengycin is inserted into the Pho core of the bilayer

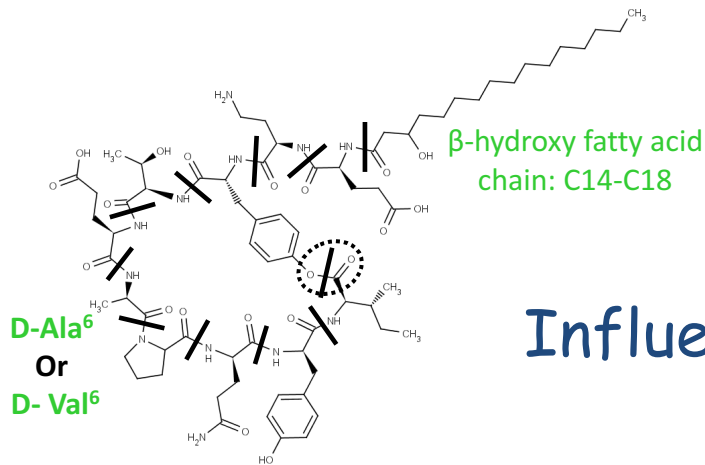
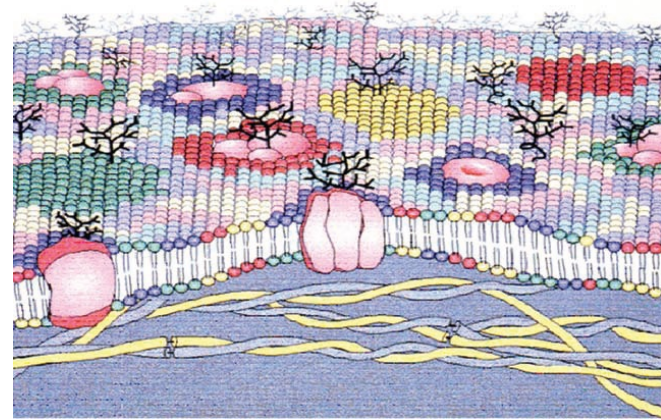
Organization of Fengycin within PC/PS bilayer

- ✓ Penetration into the acyl chain region of bilayer
- ✓ Due to electrostatic interactions: Rather dense layer in outer head group region.
- ✓ Inner head group region not affected



Further questions

Effects of membrane organization
(nanodomains)
of specific lipids (ergosterol)



Influence of fengycin homologs and variants

Conclusion

NR = method of choice for a detailed picture of a biomimetic system in the plane perpendicular to the interface

- ⇒ Information about the **depth of insertion** (not available with other techniques)
- ⇒ Information about **kinetics of the destabilization**

Acknowledgements

Monolayers studies

Dr. Nail Nasir (Gx-ABT, LBMI)
Prof. Laurence Lins (Gx-ABT, LBMI)

QCM-D studies

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NR studies

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Dr. R. Campbell (ISIS-Didcot)
Dr. M. Hellsing (Uppsala University)

Ellipsometry studies

Prof. M. Cardenas (Denmark University)



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