ILL works towards biological relevant membranes

from synthetic to natural systems

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"Advanced Neutron Tools for Soft and Biomaterials"

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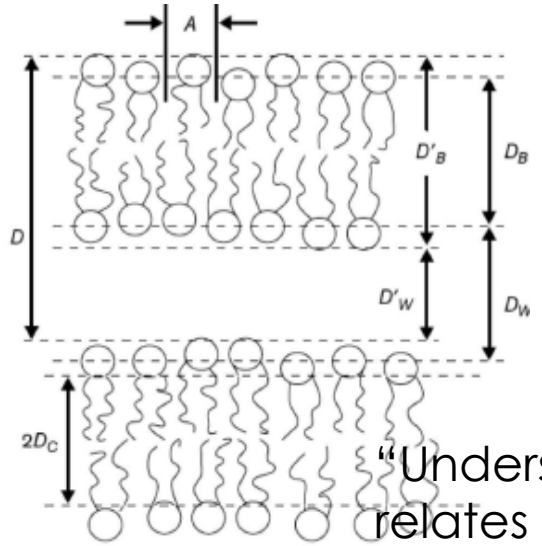
Outline

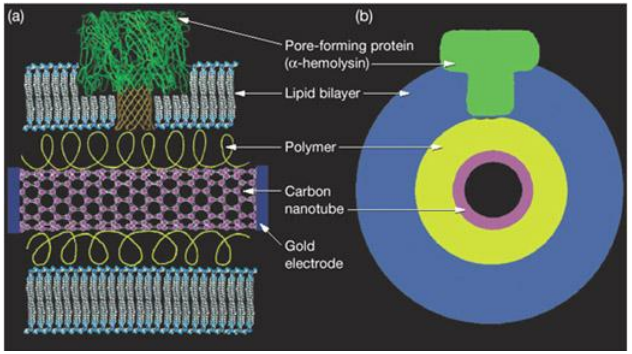
- Introduction
- ✓ First goal : platform for lipid extraction
  - ✓ Optimization of the growth process
  - ✓ Separation of polar and apolar components
  - ✓ Characterization by neutron diffraction and reflectometry of the bilayer structure
- ➢ Second goal: biological relevant membranes
  - ✓ Sample Environment for pre-characterization
  - ➢ New sample preparation protocol

Introduction

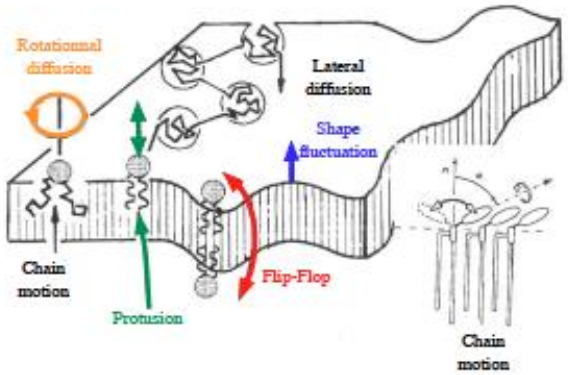


Nagle BBA (2000)

https://www.llnl.gov/

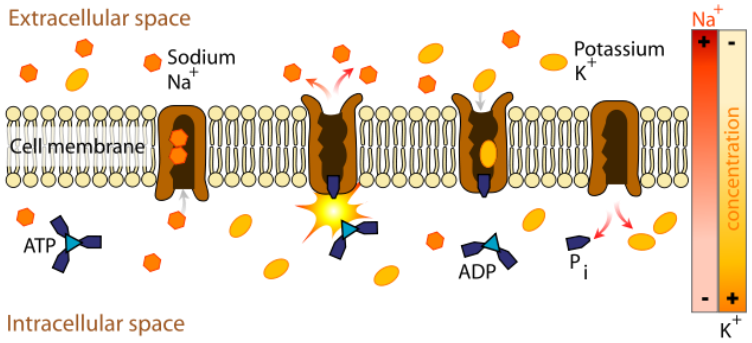


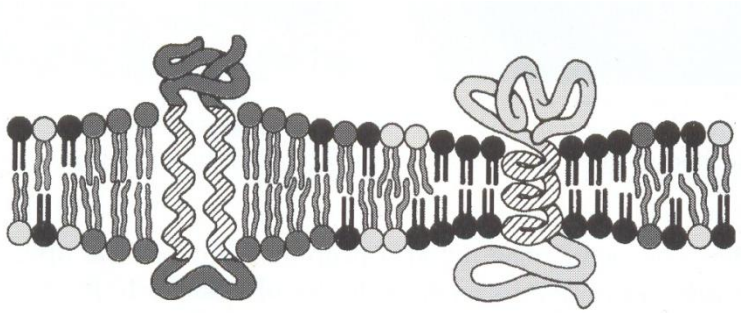
Sackmann HBP (1995)



"Understanding membrane structure and how this relates to the biological function of membrane components currently represents one of the grand challenges in structural biology research. **By knowing the impact of structure on function we can hope to manipulate structure to our advantage**…"

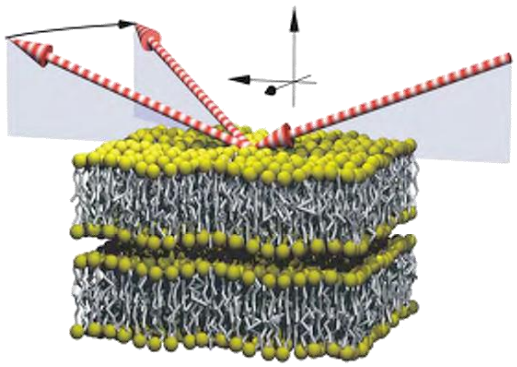
Caffrey & Wang 1995





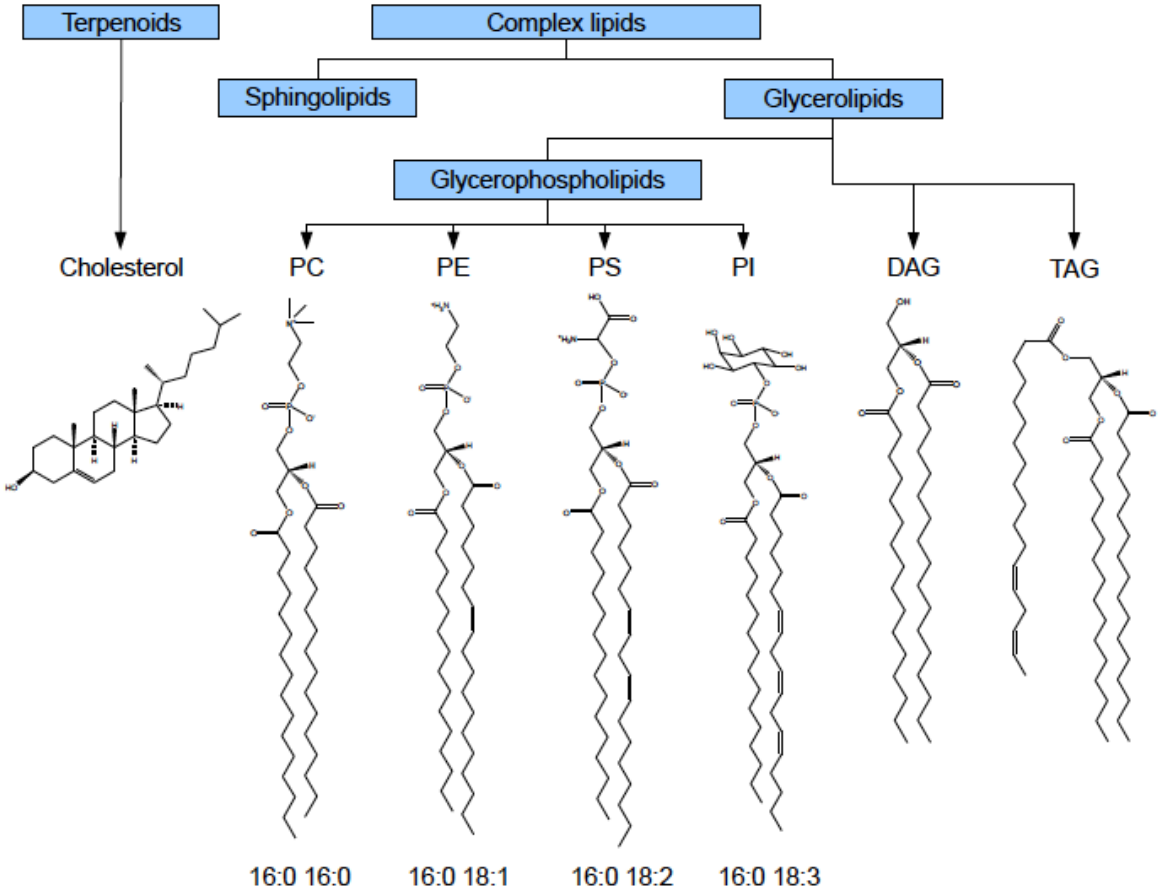
Neutron Reflectometry

- Information derived :
  - **Profile** of the **structure** along the normal of the bilayer
  - **Water penetration**
  - Different **composition** of the **leaflets**
    - migration of material, flip-flop
    - inclusion of peptides, drugs etc...
  - **Modifications** iduced by interactions
    - Holes, channels, pores ...



Salditt et al. Langmuir 19, 2003, 7703

Natural Membranes



Courtesy of G. Fragneto and A. de Ghellinck

Natural Membranes: *Pichia Pastoris*

A. de Ghellinck ILL – M. Sferrazza ULB – H. Wacklin ESS – V. Laux Dlab – J. Johuet CEA – G. Fragneto, ILL

Yeast cells grown in a deuterated medium (D-lab)

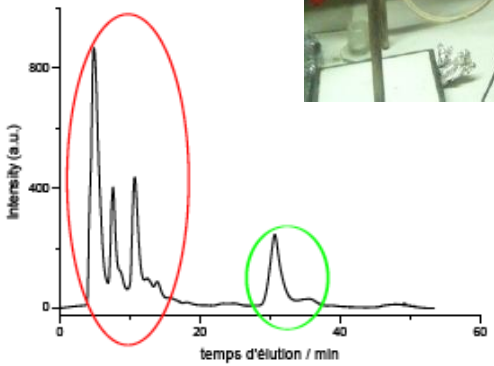
Lipids extracted with Folch method

Phospholipids separated by 2D TLC

Lipids separation (HPLC):

a-polar lipids (chloroform-acetic acid)

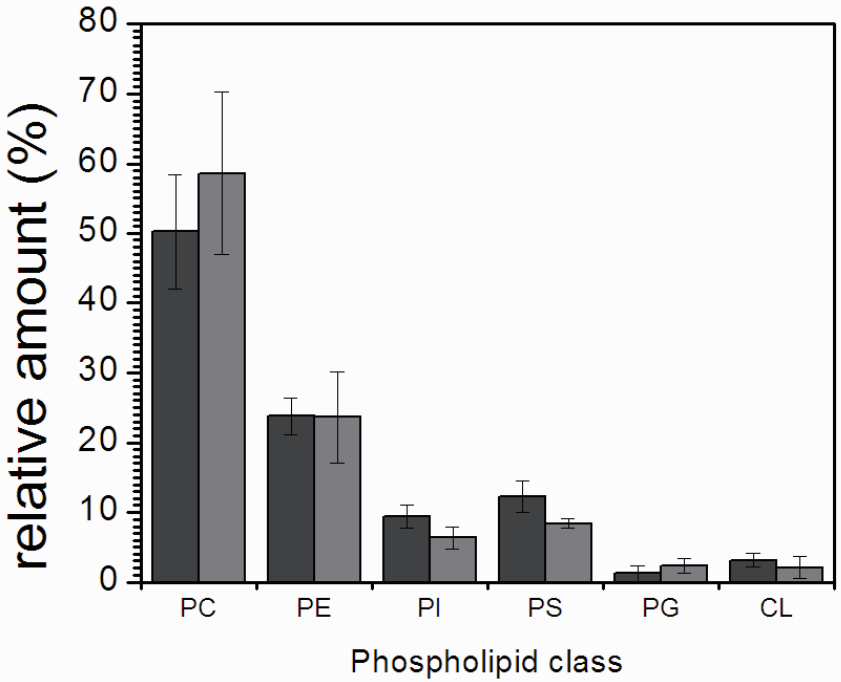
Polar lipids (methanol)

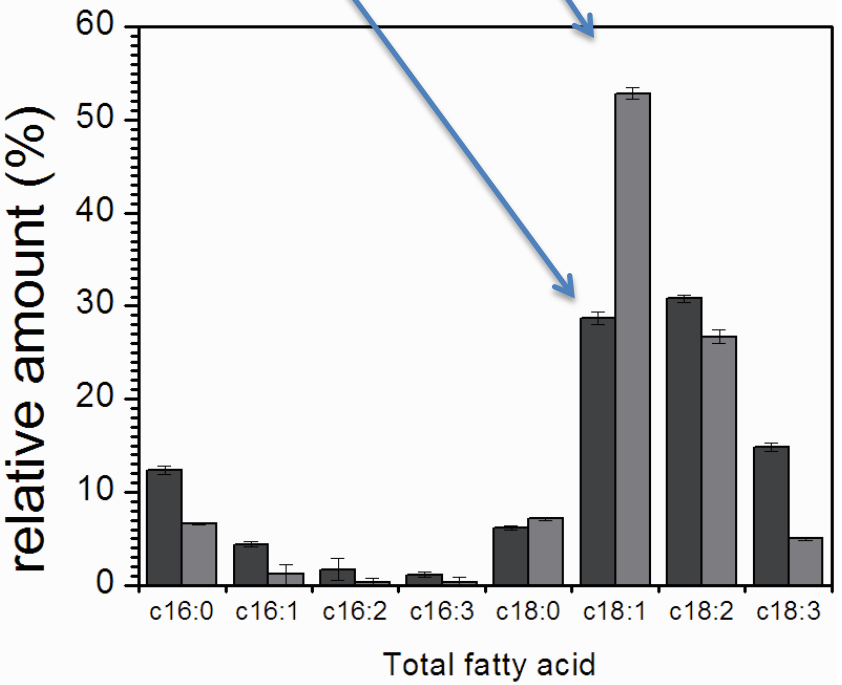


Separation of sterols from a-polar

Courtesy of G. Fragneto and A. de Ghellinck

Analysis of lipid composition in H and D extracts





Similar head-group distribution

Lower degree of unsaturation for d-lipids

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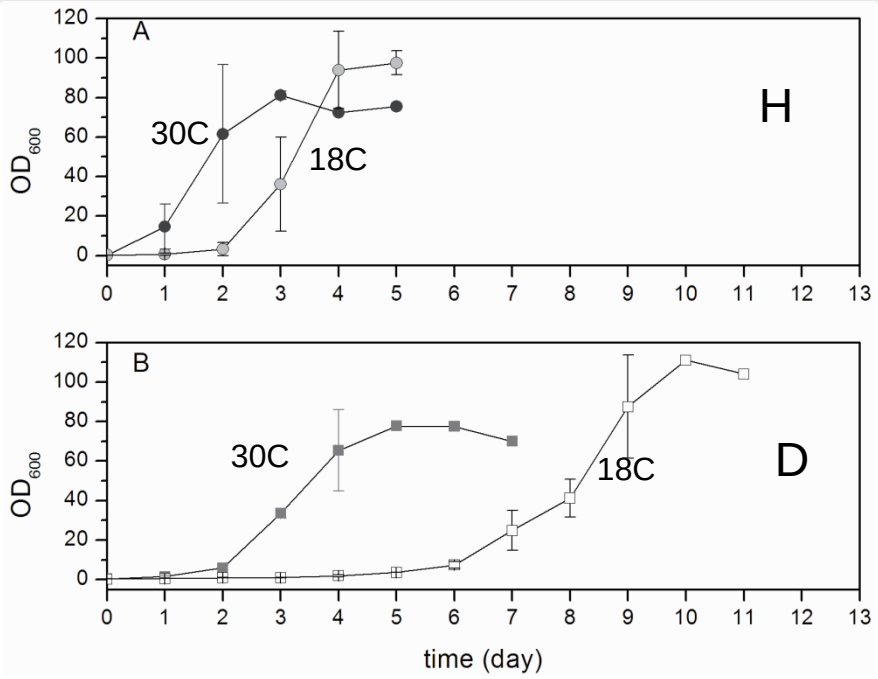
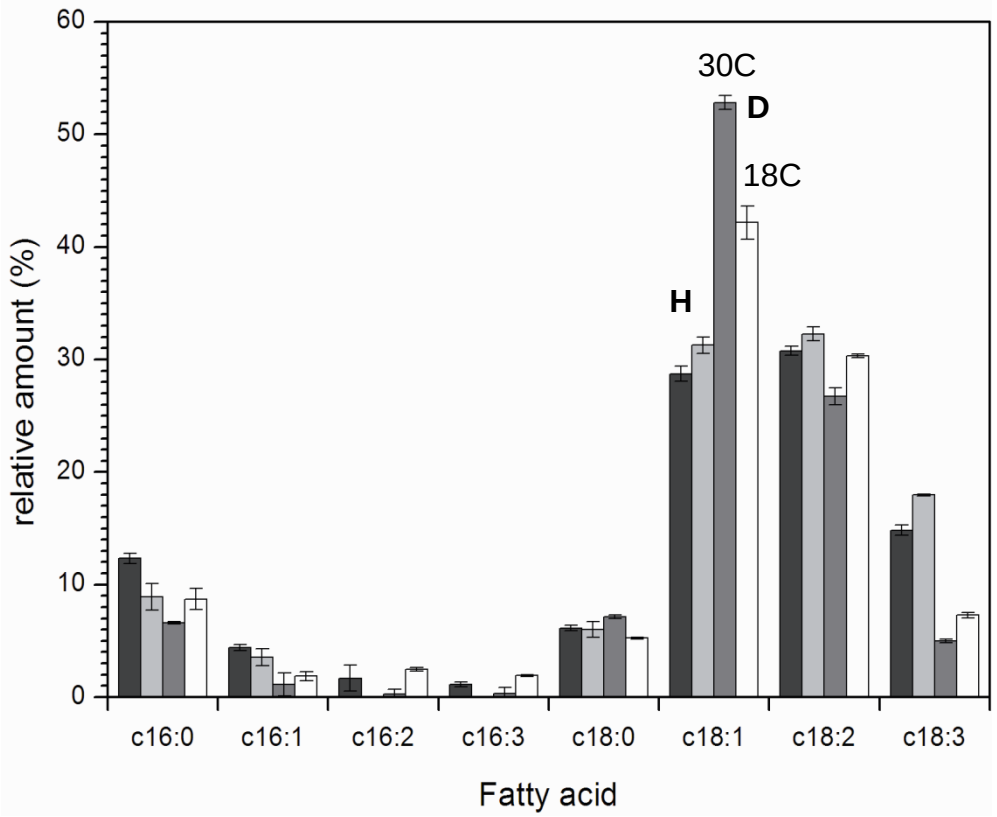
Growth method – Temperature effect



Growth of yeast at different temperature can be used to modulate chain composition



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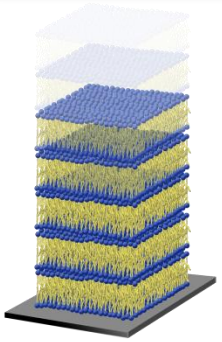
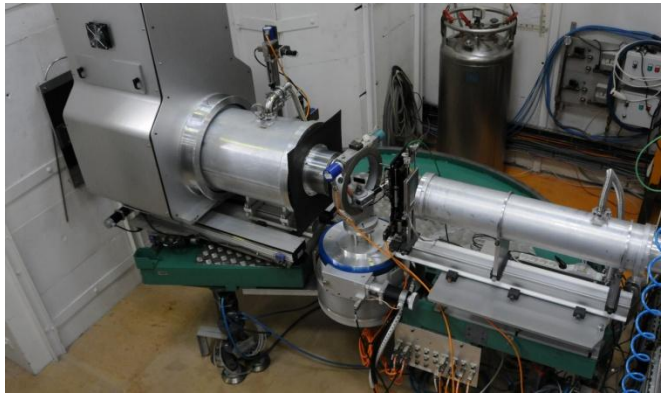
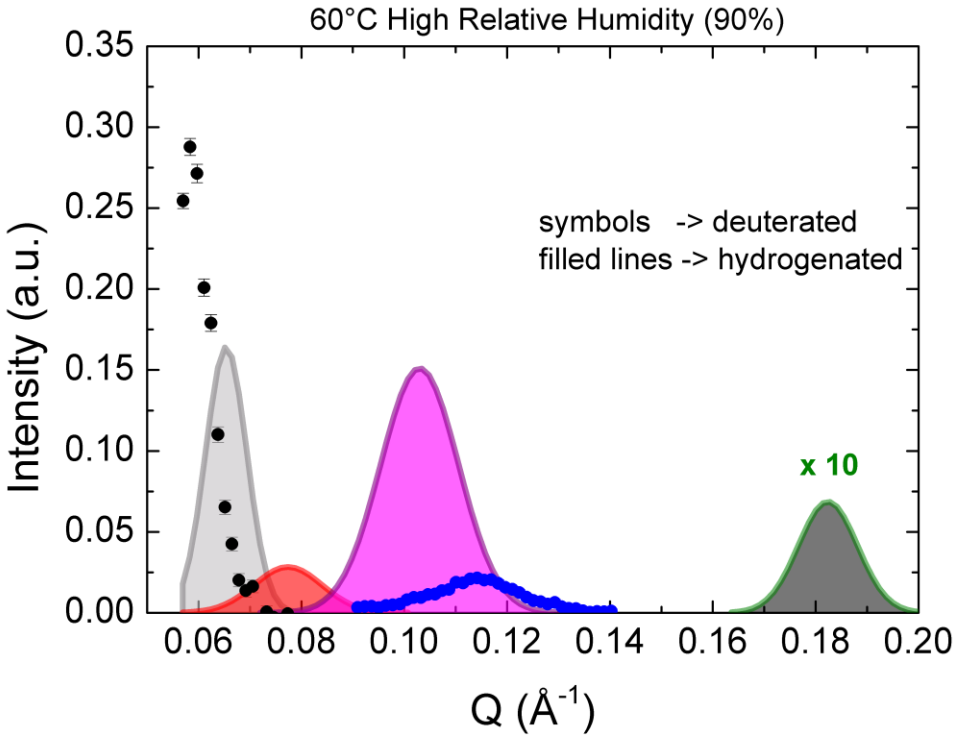
Neutron diffraction on *P. Pastoris*







Some compositional differences lead to complete different diffraction patterns

Pre-characterization of deuterated extracts is essential

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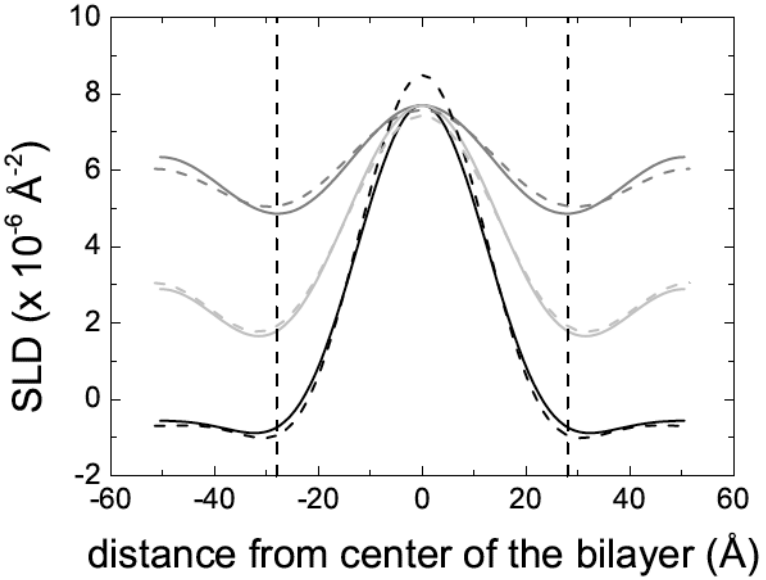
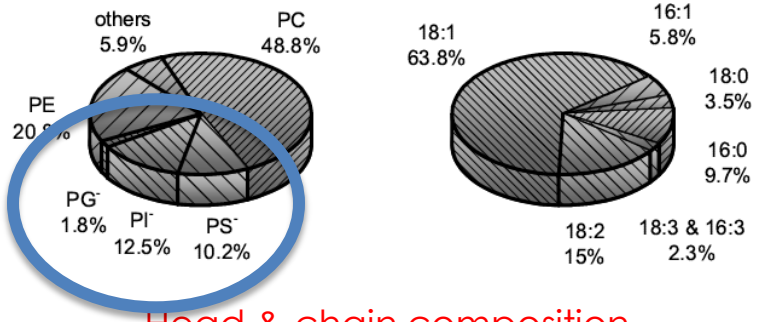
Neutron diffraction on *P. Pastoris*

$$\rho(z) = \frac{2}{d}\sum_{h=1}^{N} F(h)\cos\left(\frac{2\pi h z}{d}\right)$$





Head & chain composition

	HRH		LRH	
	d_a (Å)	d_b (Å)	d_a (Å)	d_b (Å)
18°C	105 ± 2	76 ± 3	90 ± 10	72 ± 5
30°C	105 ± 2	76 ± 3	86 ± 5	73 ± 2
60°C	104 ± 2	−−	82 ± 6	74 ± 2

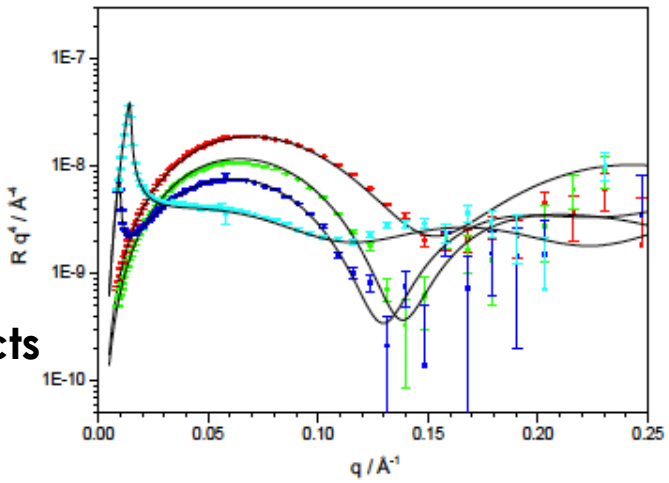
Few peaks = disordered system

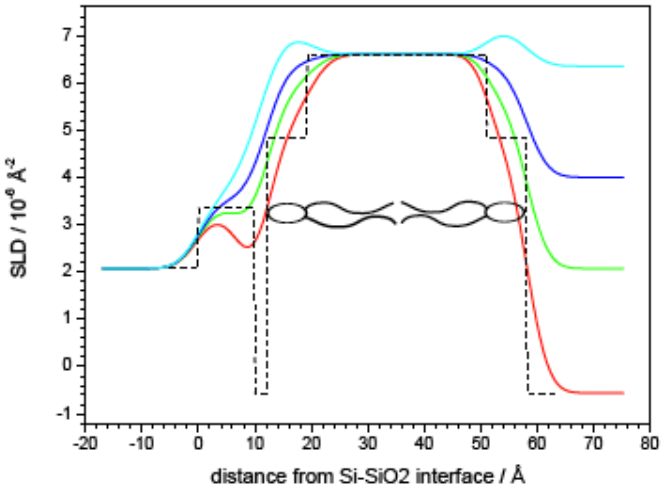
Two d-spacings due to heterogeneity of sample

Large d-spacing due to large presence of negatively charged lipids

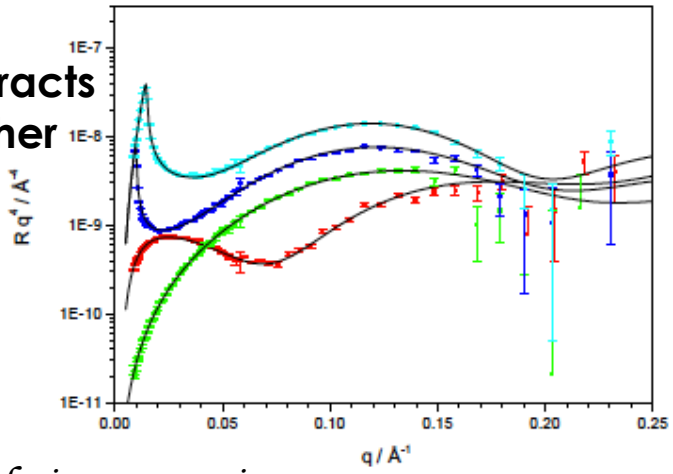
Neutron Reflectometry of *P. Pastoris*

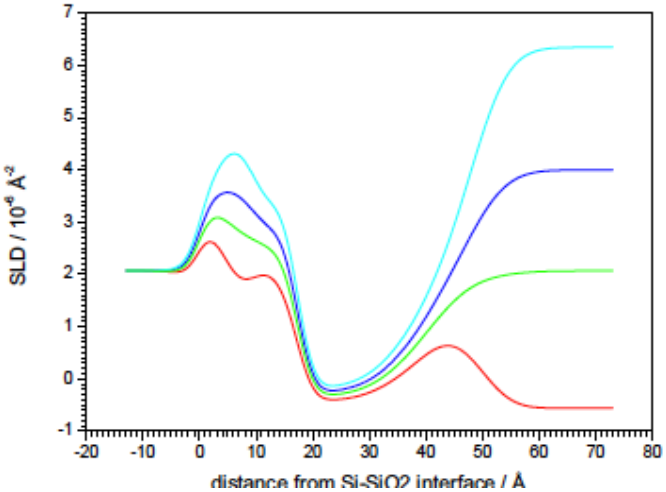
Deuterated extracts
Similar to DOPC





Hydrogenous extracts
Thinner and rougher





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Interaction with Amphotericin B

FIRST USE OF THE NATURAL EXTRACTS

- AmB forms a layer on the top of the bilayer that is not removed by rinsing

- AmB inserts into the bilayer in presence of sterols

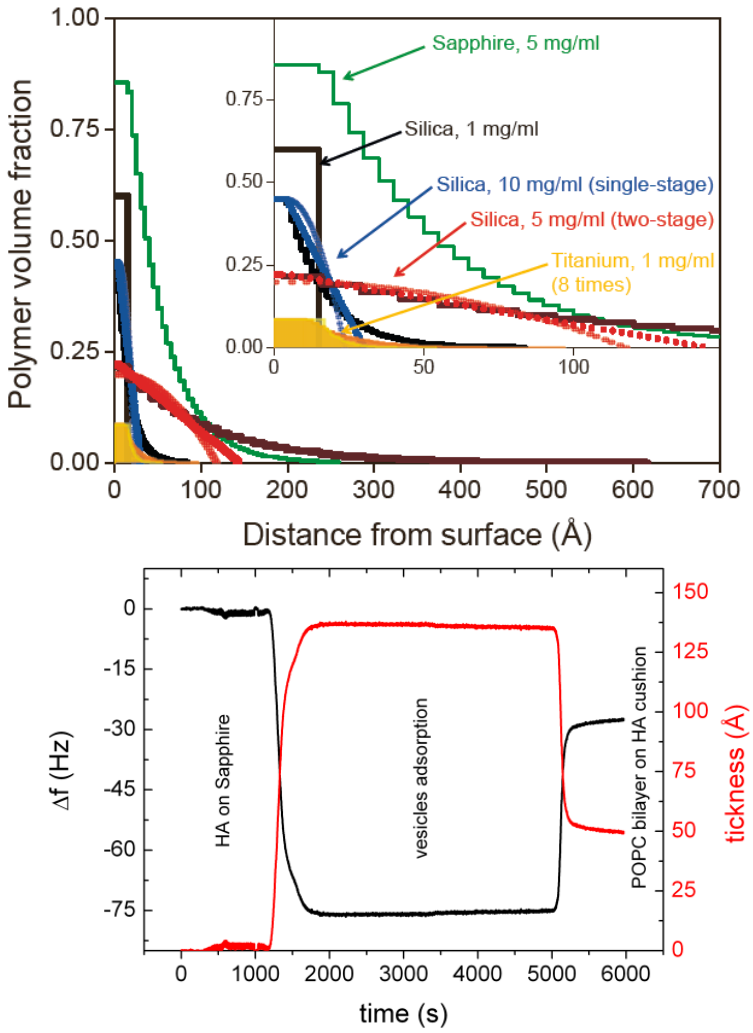
Development of a better cushion

- Limitations :
  - almost no complementary techniques to check to goodness of the cushion
  - Tests only during beamtime

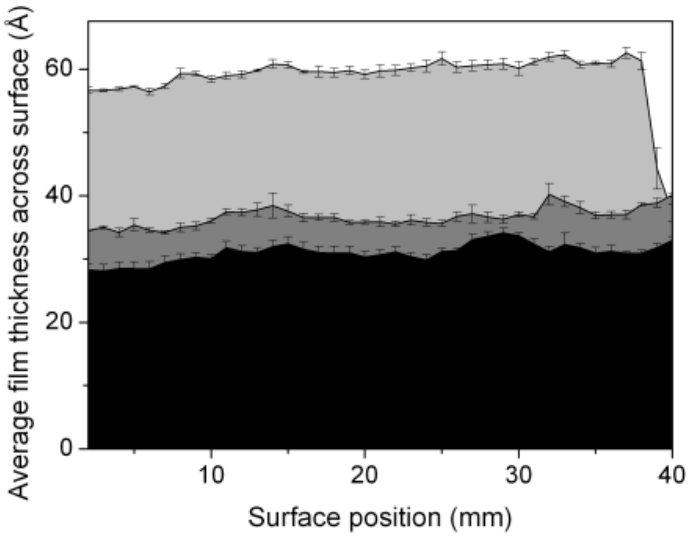
- System tested and to be tested next week
  - **Hyaluronic acid** physically AND chemically adsorbed
  - **Chitosan** cushion
  - **PEG-LIPID derivatives**

Polymeric cushions

Profiles from NR data



Ellipsometry data

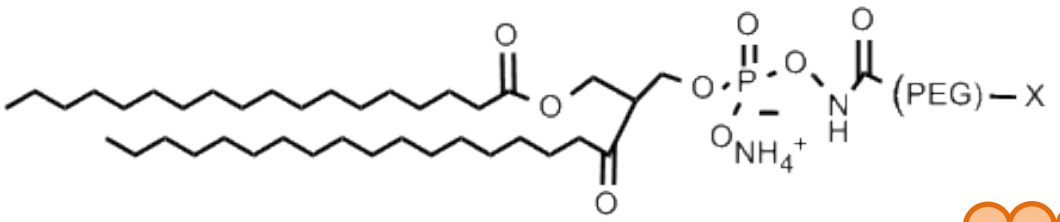


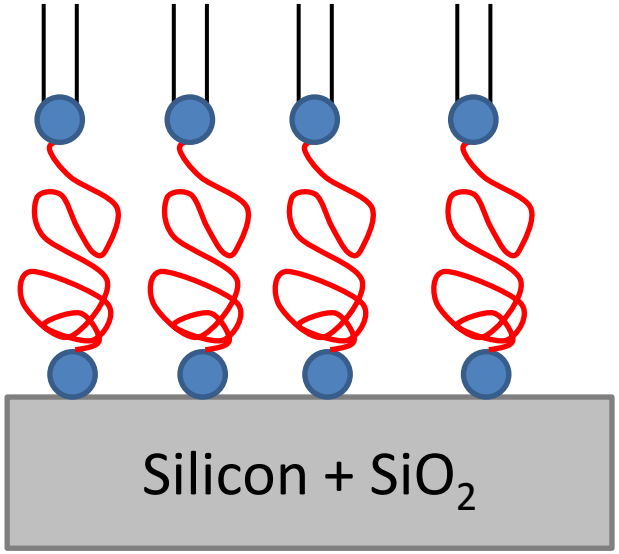
Thickness increase *during grafting of HA and Langmuir-Blodgett deposition of DSPC. The black area is the silane linker, followed by a thin layer of grafted HA (dark grey) and the light grey represents the lipid monolayer.*

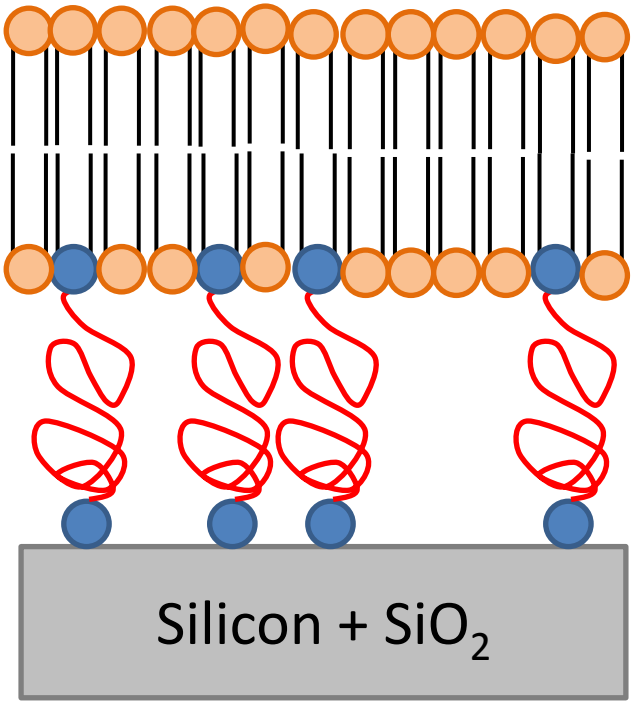
QCM-D :
Frequency shift proportional adsorbed mass

I. Berts PhD thesis

Silane-PEG-DLPE spacer





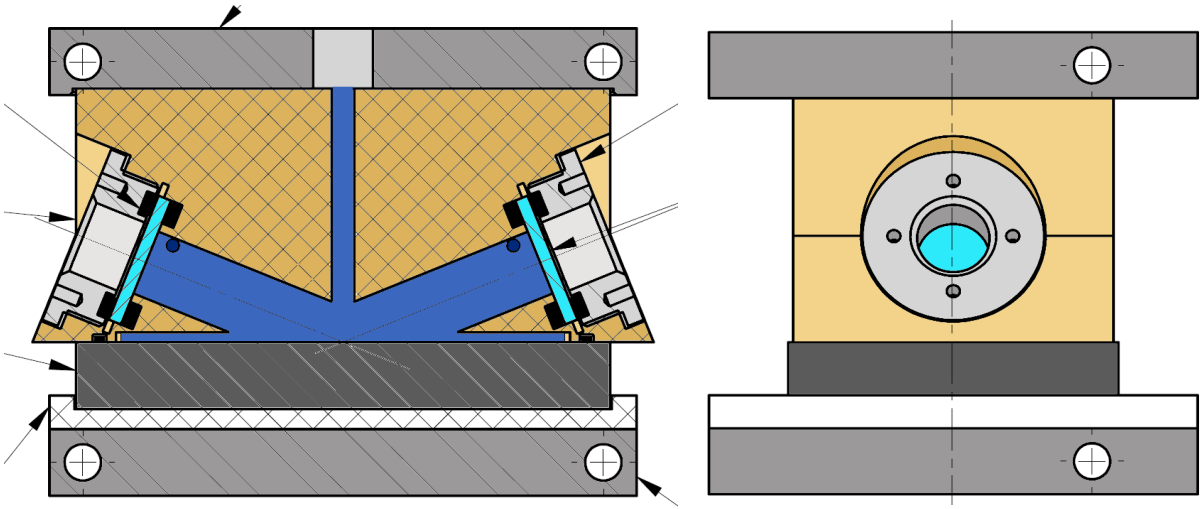


Surface polymer MAXIMUM density is limited by PEG hindrance

Bilayer obtained by solvent exchange or LB-LS depositions

Combined Reflectometry-Ellipsometry measurements

- The cell fits into normal Langmuir troughs
- Samples can be deposited from solution directly inside the cell
- Exactly the same sample can be measured by reflection of neutrons and of visible light



✘ *Almost impossible to control the temperature*

Acknowledgements

Main contributors

Alexis De Ghellinck, Giovanna Fragneto

Simon Baudoin for the technical drawing of the Ell.-Refl. Flow cell

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Thank for your attention !!!