

ILL works towards biological relevant membranes

from synthetic to natural systems

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NMI3-II/FP7 Satellite Meeting

“Advanced Neutron Tools for Soft and Biomaterials”

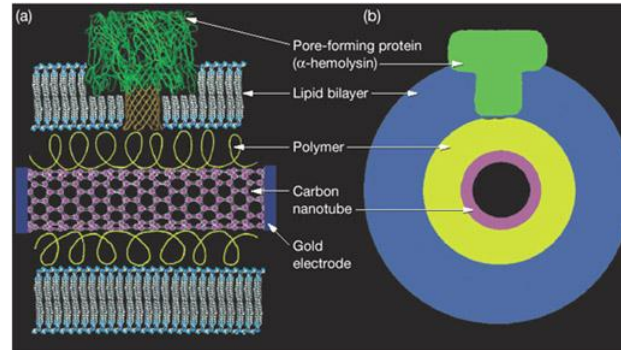
21st June 2013 Berlin
and 28th November Munich

Outline

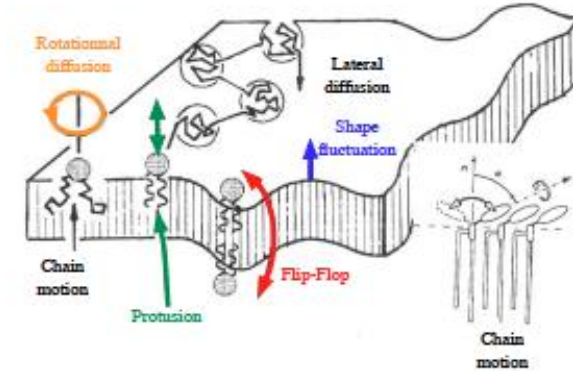
- Introduction
- ✓ First goal : platform for lipid extraction
 - ✓ Optimization of the growth process
 - ✓ Separation of polar and apolar components
 - ✓ Characterization by neutron diffraction and reflectometry of the bilayer structure
- Second goal: biological relevant membranes
 - ✓ Sample Environment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

Introduction

<https://www.llnl.gov/>

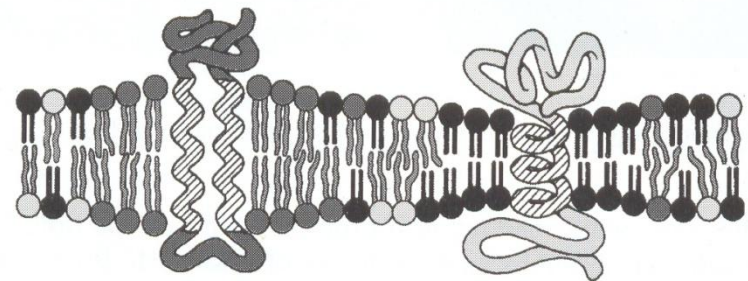
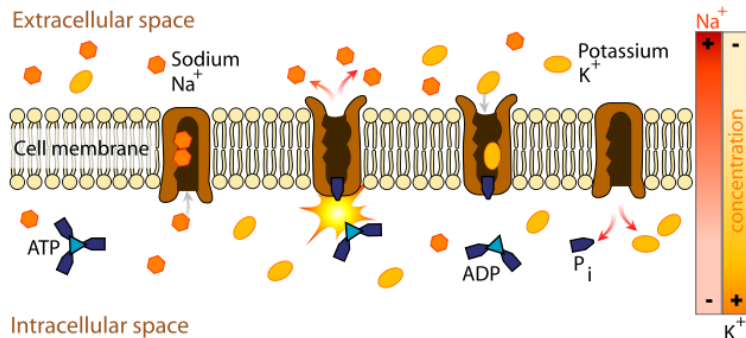


Sackmann HBP (1995)



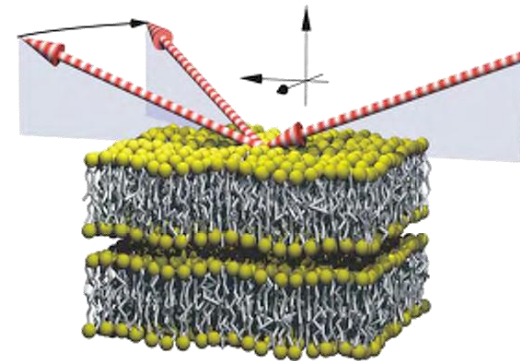
"Understanding membrane structure and how this relates to the biological function of membrane components currently represents one of the grand challenges in structural biology research. **By knowing the impact of structure on function we can hope to manipulate structure to our advantage...**"

Caffrey & Wang 1995



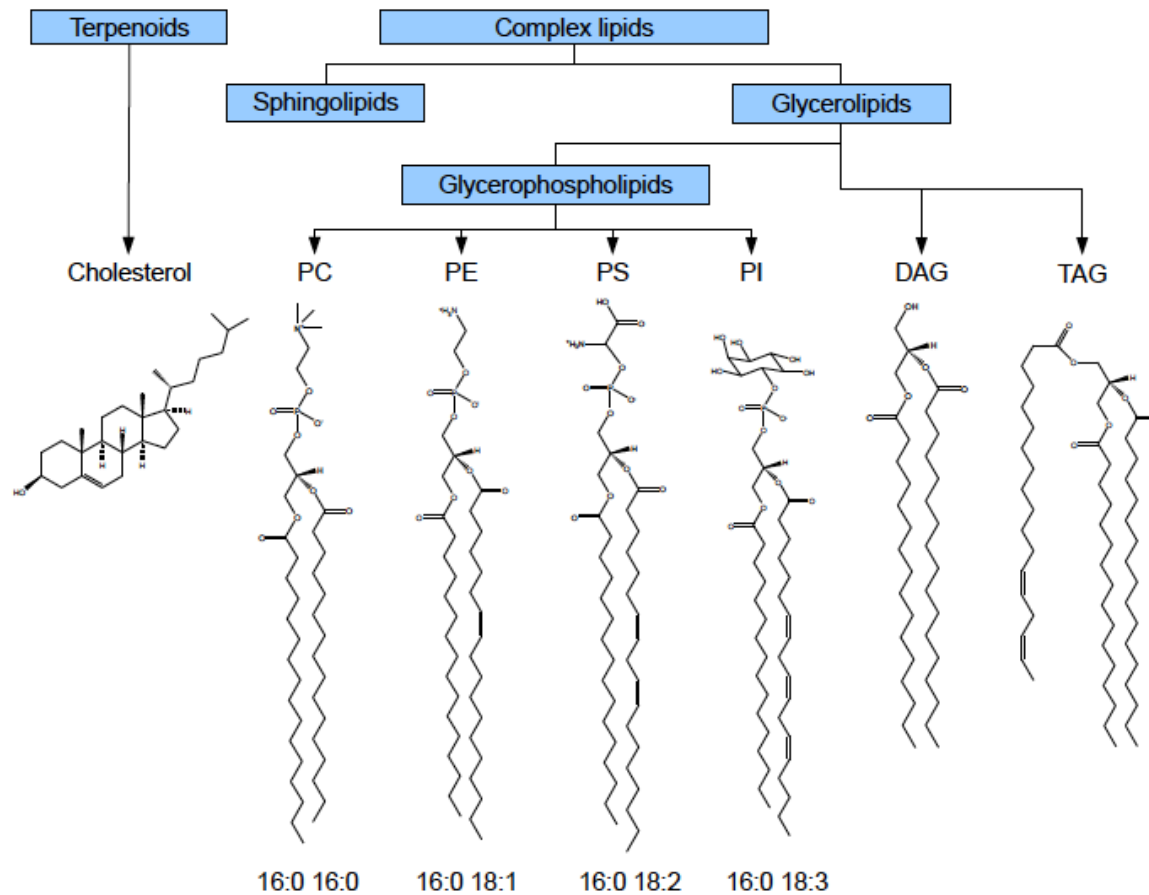
Neutron Reflectometry

- 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** induced 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

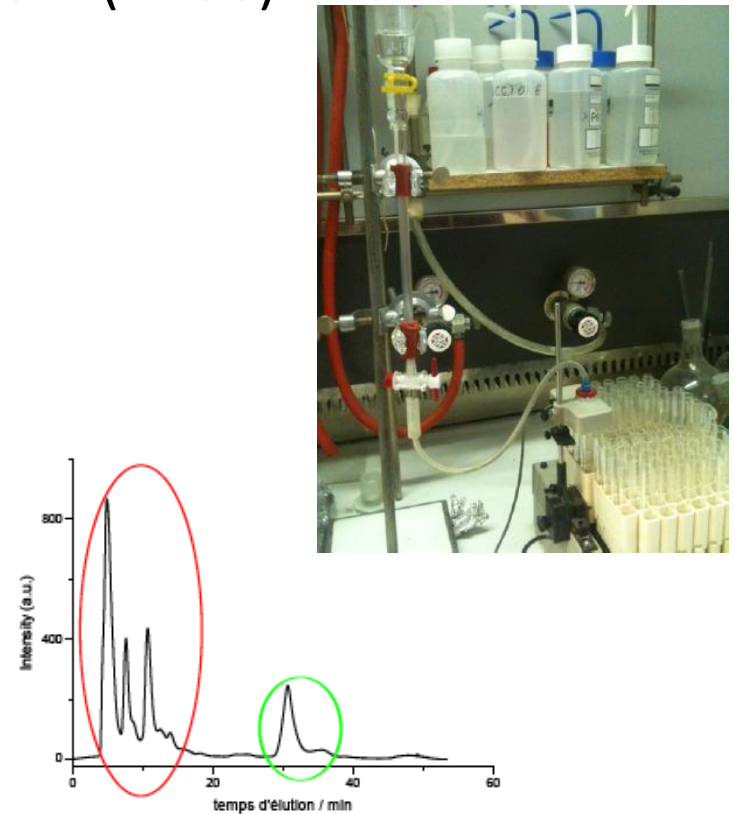
Phospholipids separated by 2D TLC

Lipids separation (HPLC):

a-polar lipids (chloroform-acetic acid)

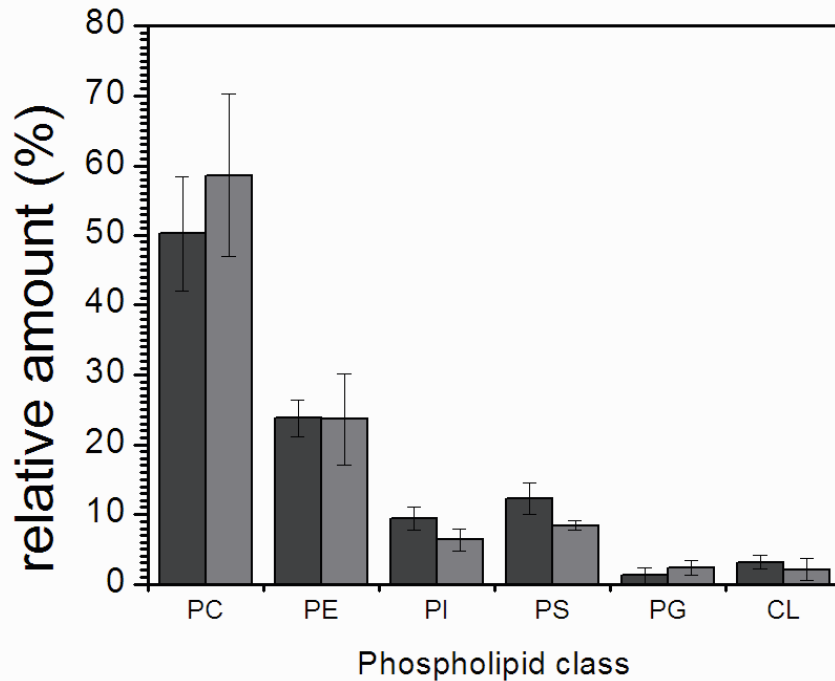
Polar lipids (methanol)

Separation of sterols from a-polar

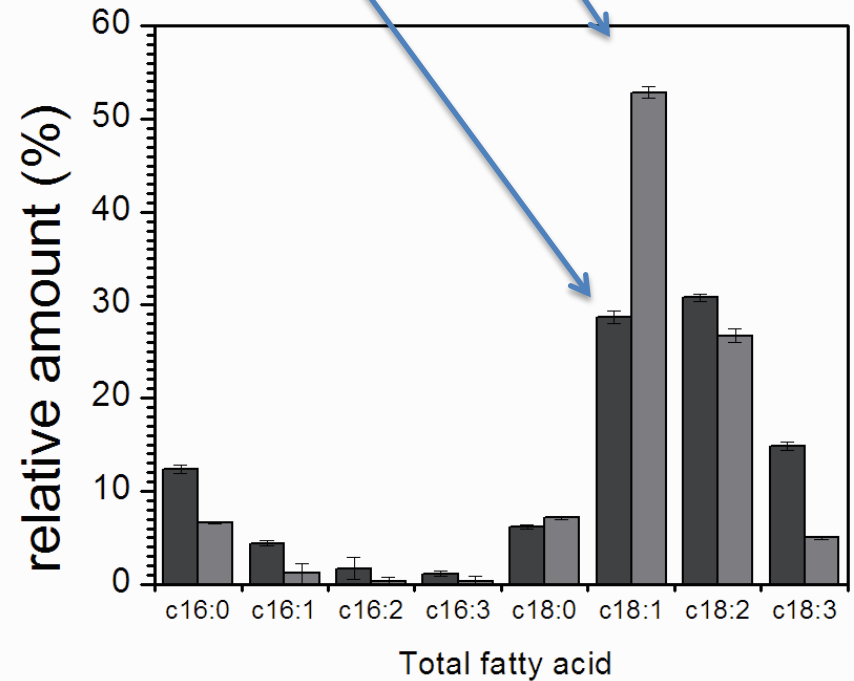


Courtesy of G. Fragneto and A. de Ghellinck

Analysis of lipid composition in H and D extracts



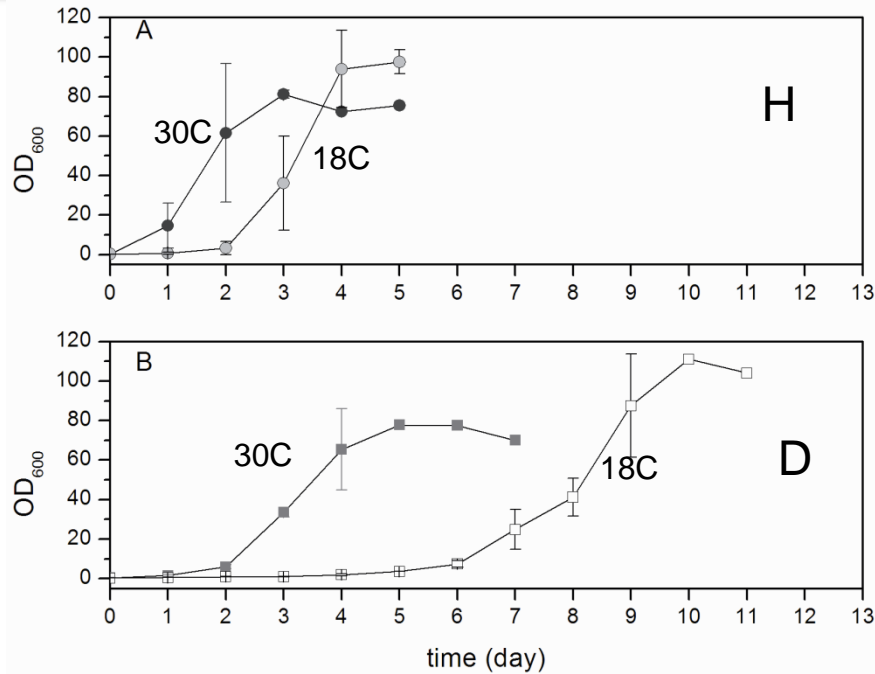
Similar head-group distribution



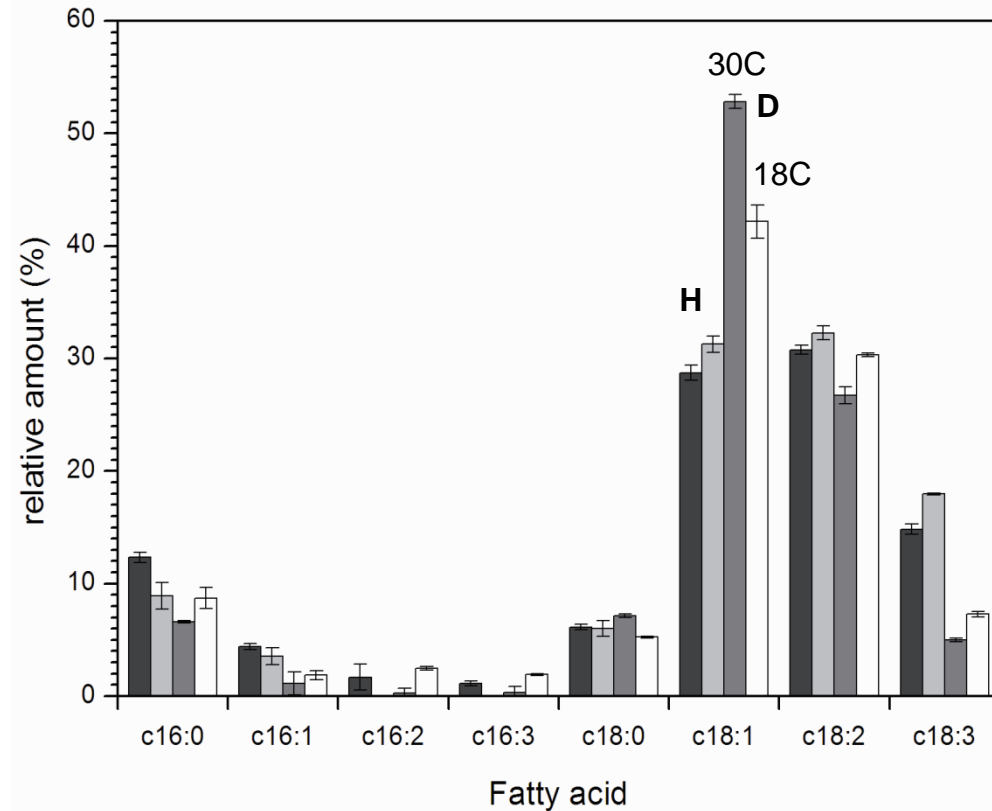
Lower degree of unsaturation for d-lipids

A. de Ghellinck et al., in preparation

Growth method – Temperature effect

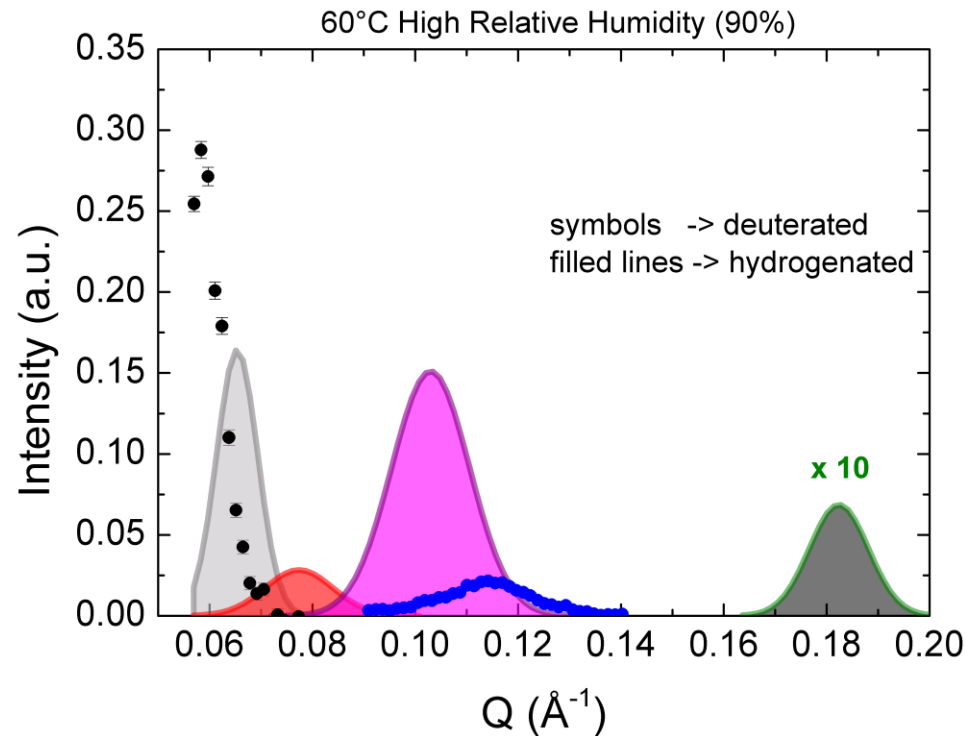


Growth of yeast at different temperature can be used to modulate chain composition



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Neutron diffraction on *P. Pastoris*



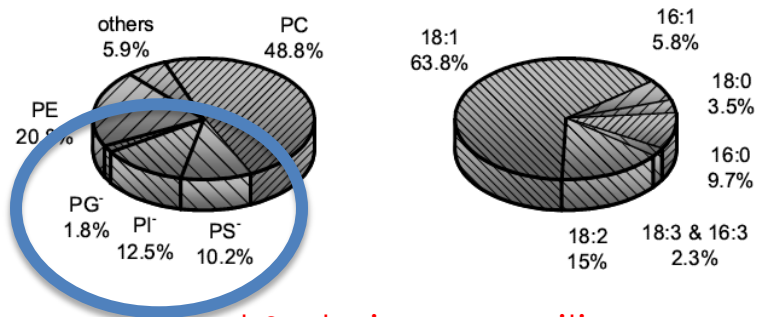
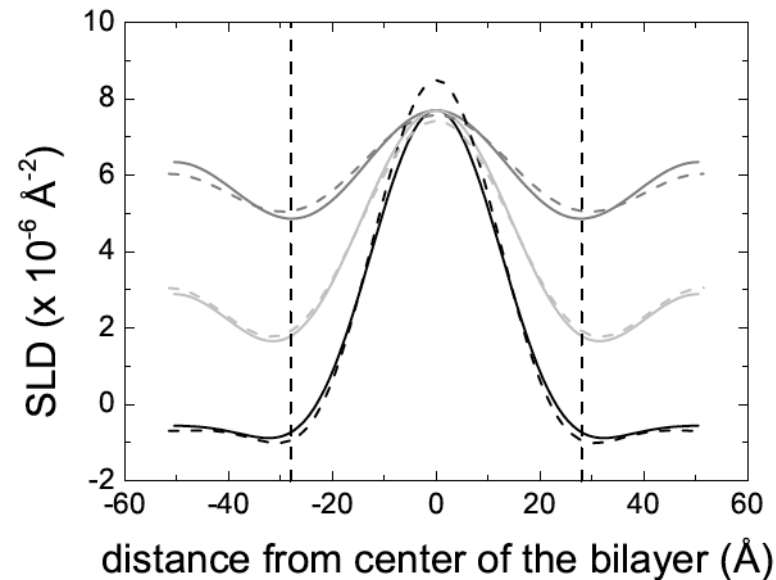
Some compositional differences lead to complete different diffraction patterns

Pre-characterization of deuterated extracts is essential

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Neutron diffraction on *P. Pastoris*

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



	HRH		LRH	
	d_a (\AA)	d_b (\AA)	d_a (\AA)	d_b (\AA)
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

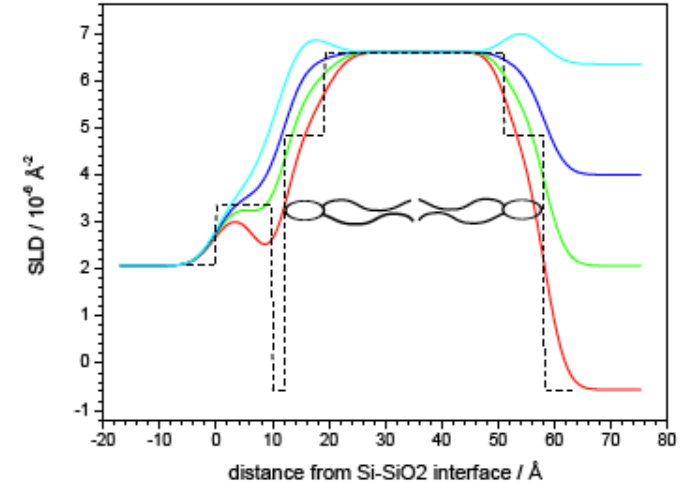
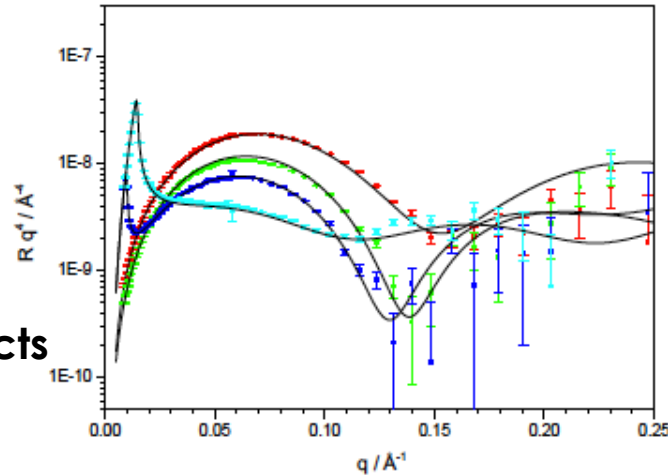
Few peaks = disordered system

Two d-spacings due to heterogeneity of sample

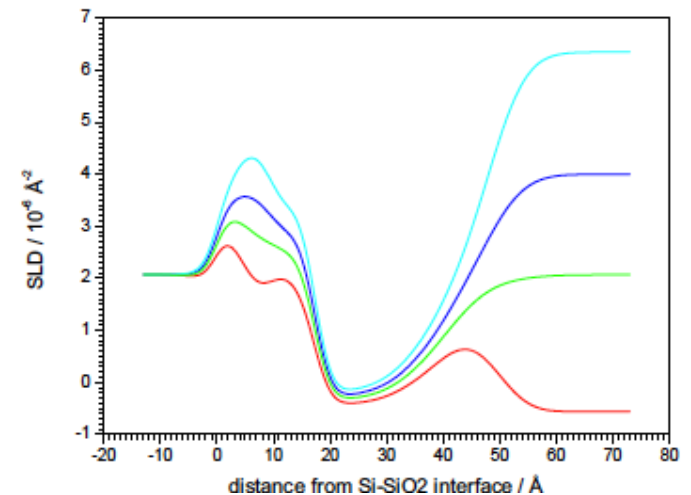
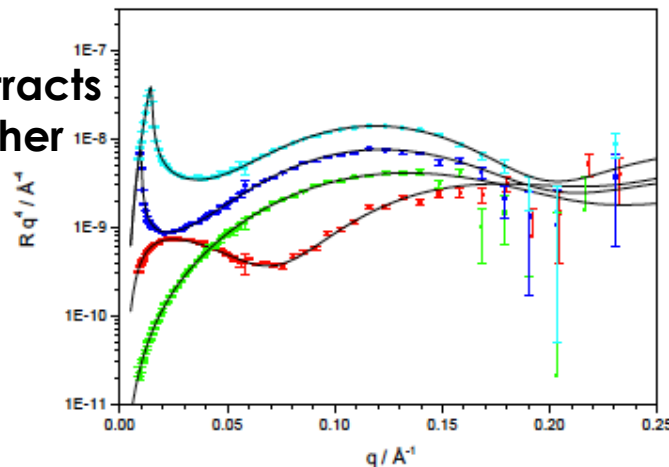
Large d-spacing due to large presence of negatively charged lipids

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

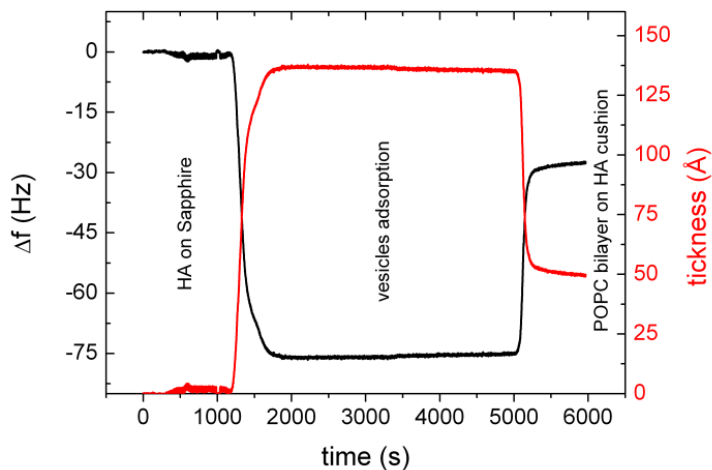
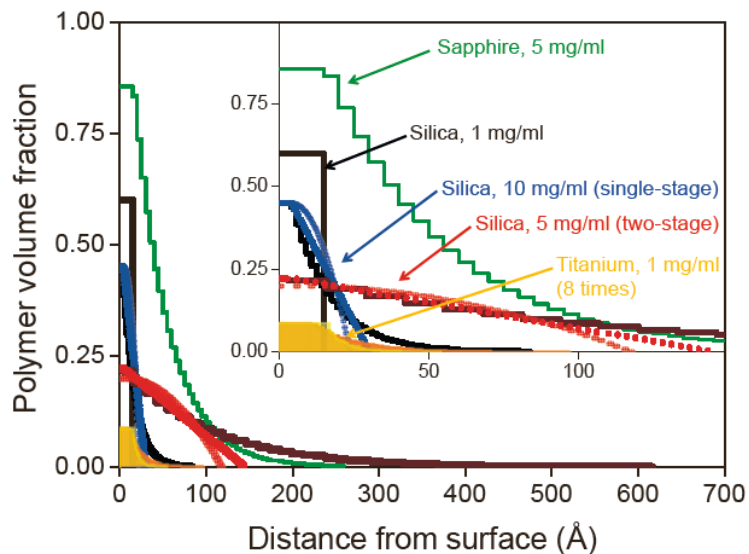
- AmB forms a layer on the top of the bilayer that is not removed by rinsing
- AmB inserts into the bilayer in presence of sterols

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

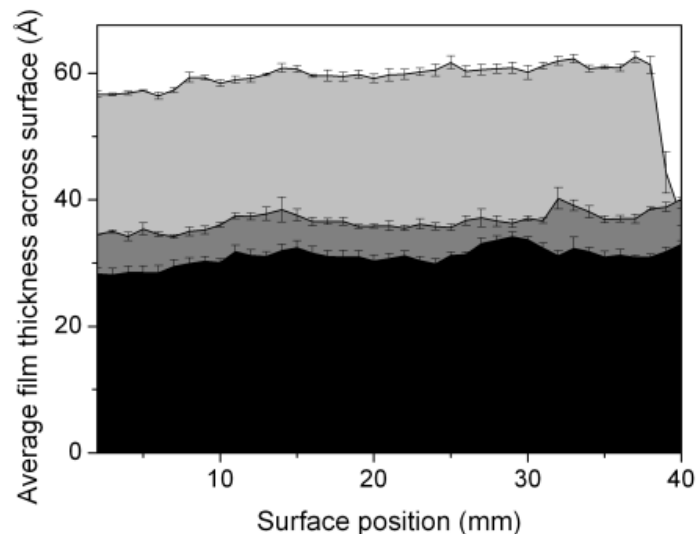
Profiles from NR data



QCM-D :

Frequency shift proportional
adsorbed mass

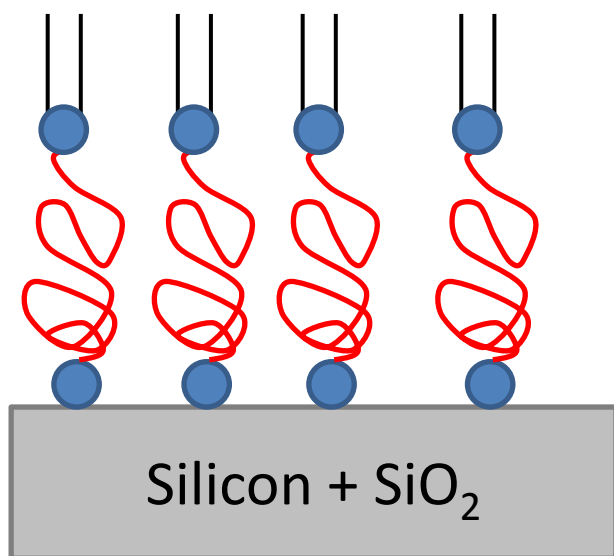
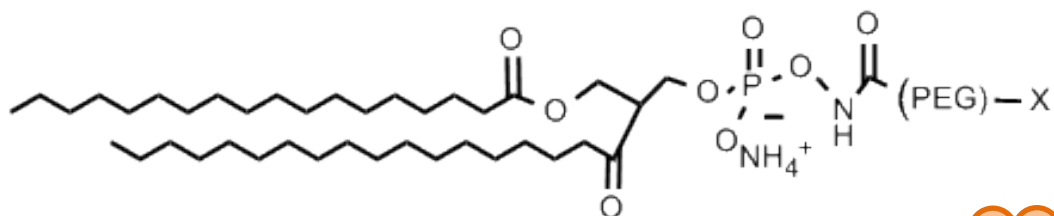
Ellipsometry data



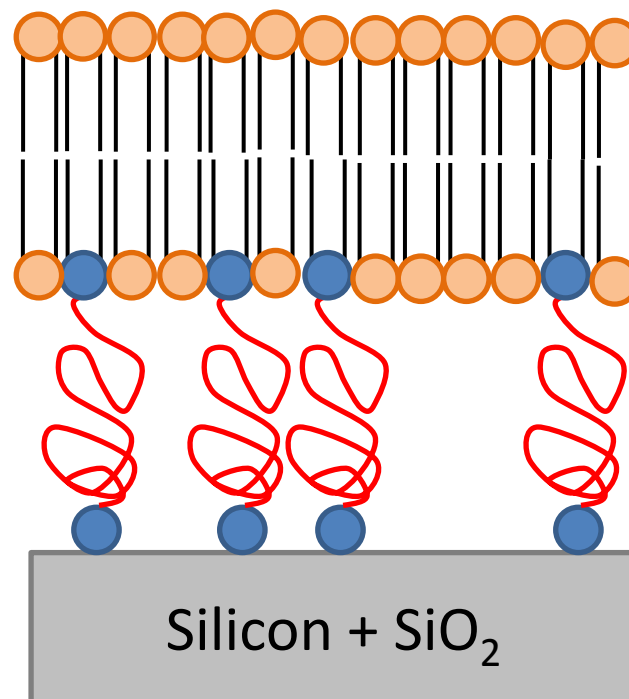
Thickness increase during grafting of HA and Langmuir-Blodgett deposition of DSPC. The black area is the silane linker, followed by a thin layer of grafted HA (dark grey) and the light grey represents the lipid monolayer.

I. Berts PhD thesis

Silane-PEG-DLPE spacer

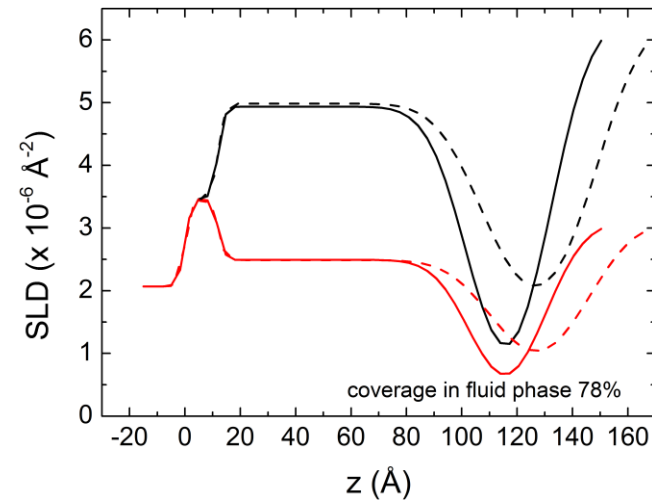
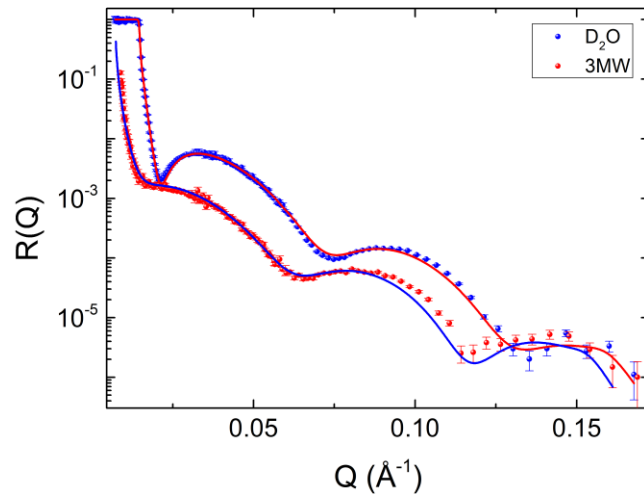


Surface polymer MAXIMUM density
is limited by PEG hindrance



Bilayer obtained by solvent exchange
or LB-LS depositions

Reflectivity from DPPC bilayers on PEG cushions: LB-LS and vesicle fusion

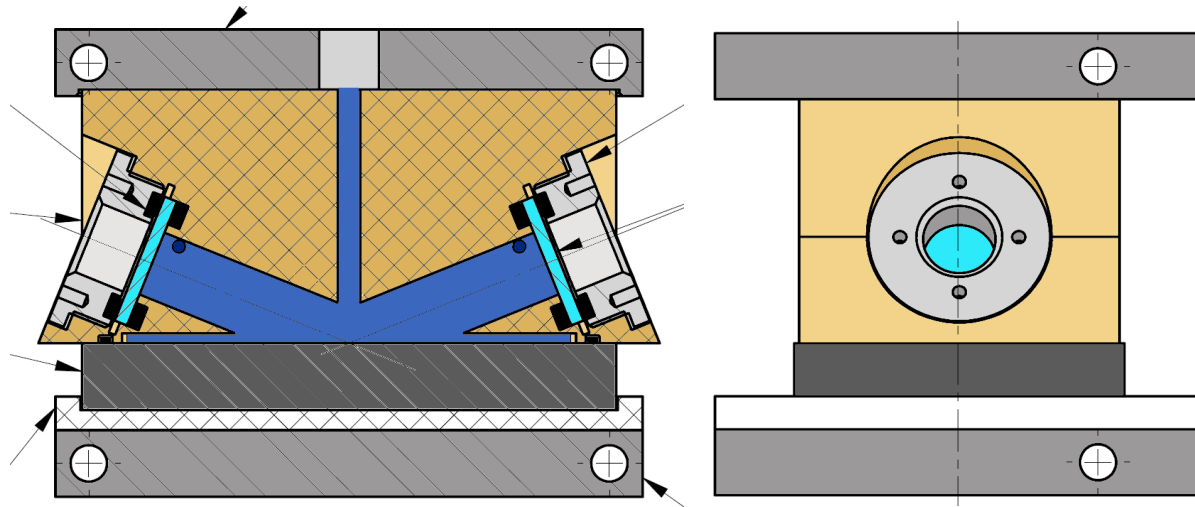


Low coverage (78%) and rough layer

Experiment in progress at HZB on shorter PEG (2000 vs. 5000)
and higher concentration

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*

Thanks to Simon Baudoin (ILL-SANE) for technical drawings

Acknowledgements

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