ILL works on model membranes

towards natural systems

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1st JRA Meeting
"Adanced Neutron Tools for Soft and Biomaterials"

7th December 2012
Garching - D

Outline

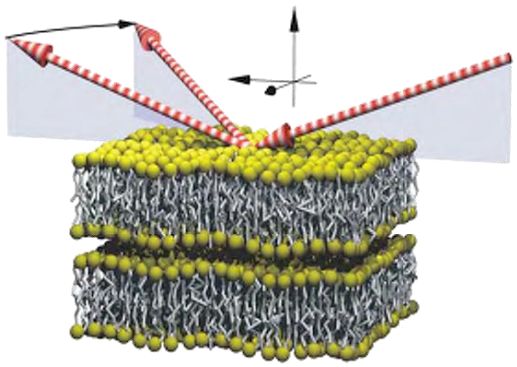
- Introduction
- Model systems
  - Bilayer compositional asymmetry
    - Lipid Flip-Flop
    - Cholesterol distribution
- Fully deuterated natural lipid membranes
  - Multilamellar organization by diffraction
  - Bilayer structure by NR
- Future work

Introduction

- Aim of the work
  - Moving from simple systems to complex or natural ones
- Tests required
  - different lipid mixtures
  - different deposition techniques
    - *Langmuir Blodgett-Schaefer, vesicles spreading, solvent exchange*
  - polymeric and lipid cushions

Neutron Reflectometry

- Information derived :



Salditt et al. Langmuir 19, 2003, 7703

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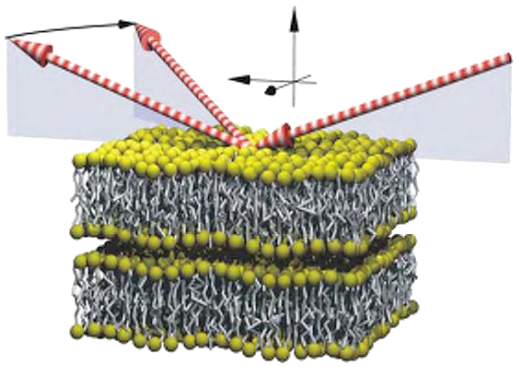
  - **Profile** of the **structure** along the normal of the bilayer



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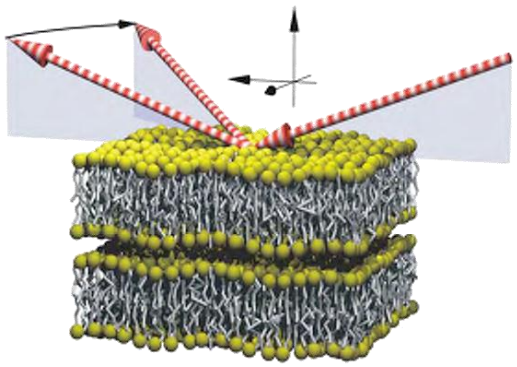

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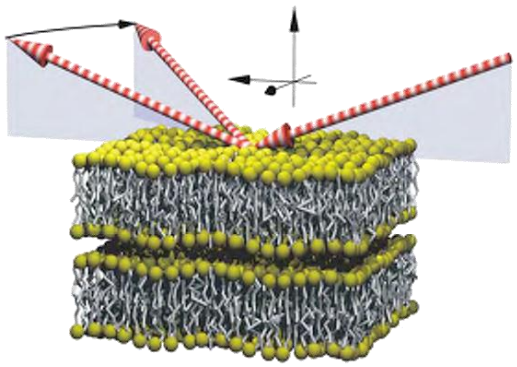
  - Different **composition** of the **leaflets**
    - migration of material, flip-flop
    - inclusion of peptides, drugs etc...



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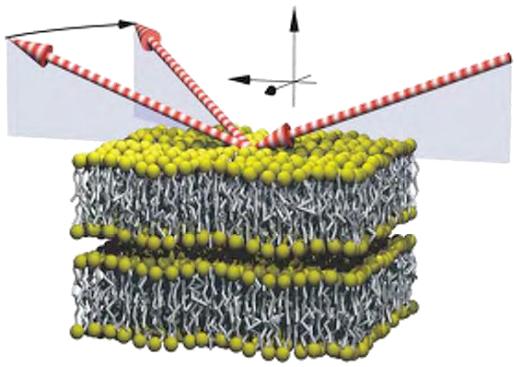
  - **Profile** of the **structure** along the normal of the bilayer
  - **Water penetration**
  - Different **composition** of the **leaflets**
    - migration of material, flip-flop
    - inclusion of peptides, drugs etc...
  - **Modifications** iduced by interactions
    - Holes, channels, pores ...



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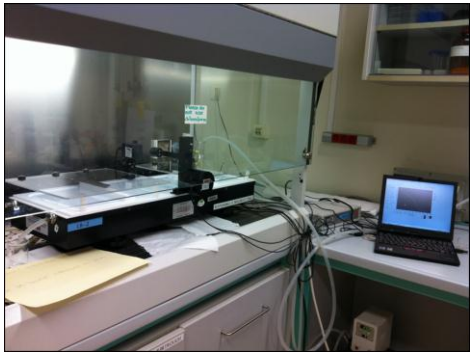
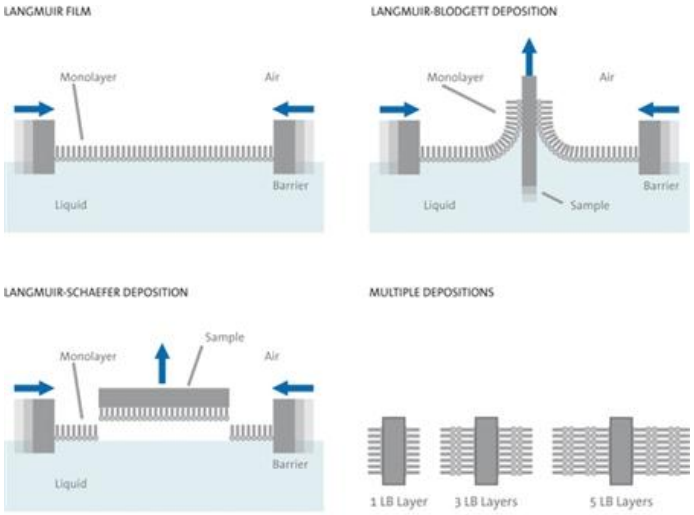
Lipid Flip-Flop

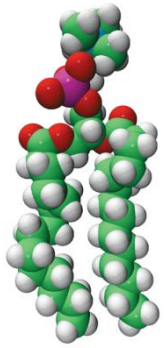
Langmuir Blodgett - Langmuir Schaefer techniques



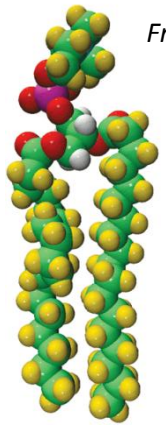
Partnership for soft condensed matter, ILL, Grenoble



From NIMA



14:0PC DMPC



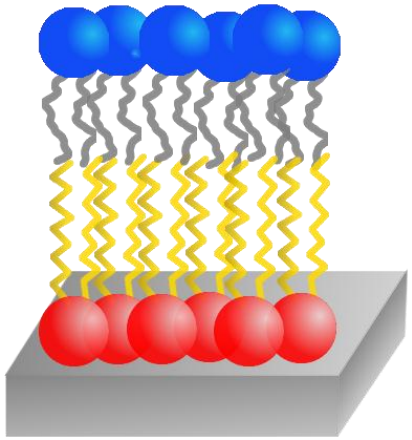
18:0PC DSPC-d83

From Avanti Polar Lipids

Starting depositions 14°C 40 mN/m lateral pressure

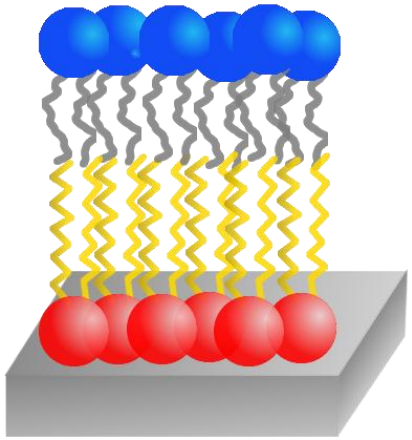
- **Symmetric**
  - 1:1 DSPC:DMPC in both leaflet
- **Asymmetric**
  - DSPC by LB (inner)
  - DMPC by LS (outer)

Measurements



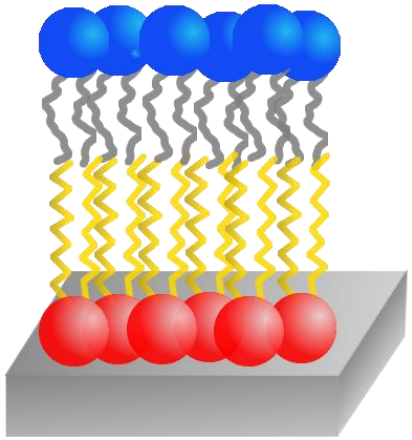
Measurements

- Laminar flow cells



Measurements

- Laminar flow cells
- Mono-crystalline Silicon as solid support



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- Laminar flow cells
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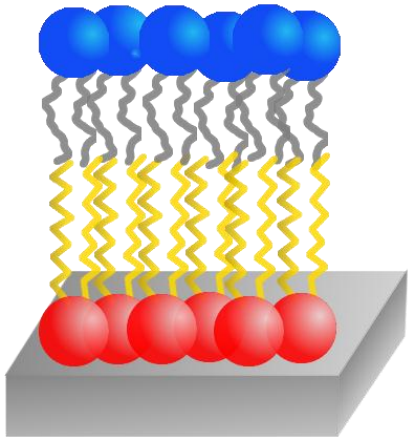
  - D2O, H2O and 34:66 HDO



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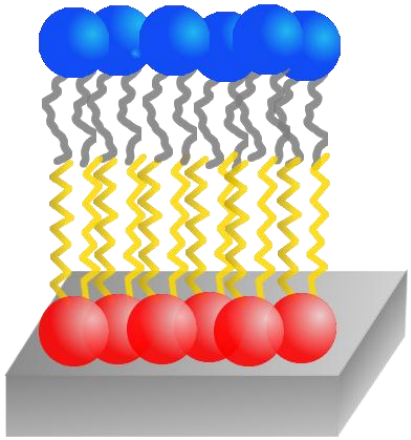
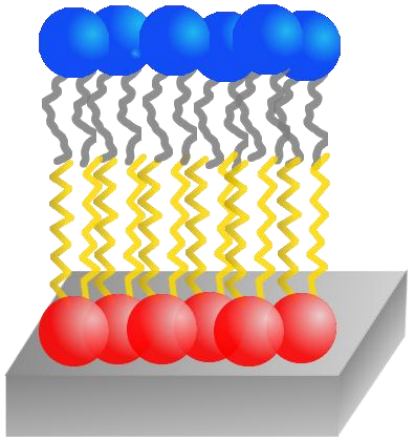


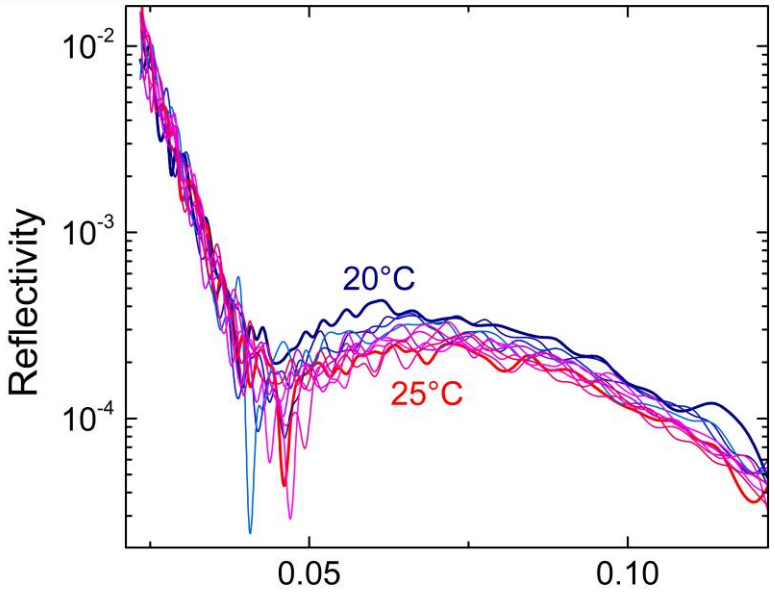
Measurements

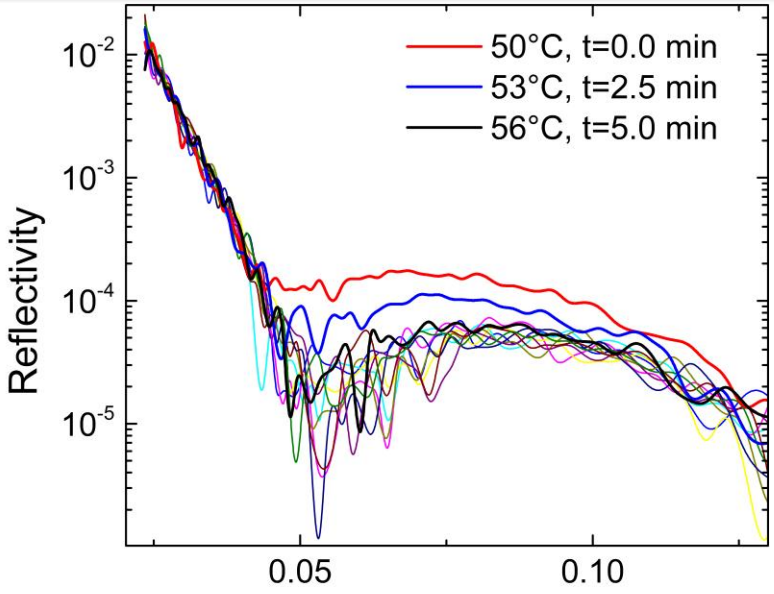
- Laminar flow cells
- Mono-crystalline Silicon as solid support
- 3 different solvents for contrast variation
  - D2O, H2O and 34:66 HDO
- 2 reflectometers
  - D17 and FIGARO @ILL
- Thermal cycle 14°C→60°C→14°C
  - T_m DSPC-d13 52.5°C
  - T_m DSPC-d83 50.5°C
  - T_m DMPC 23.5°C

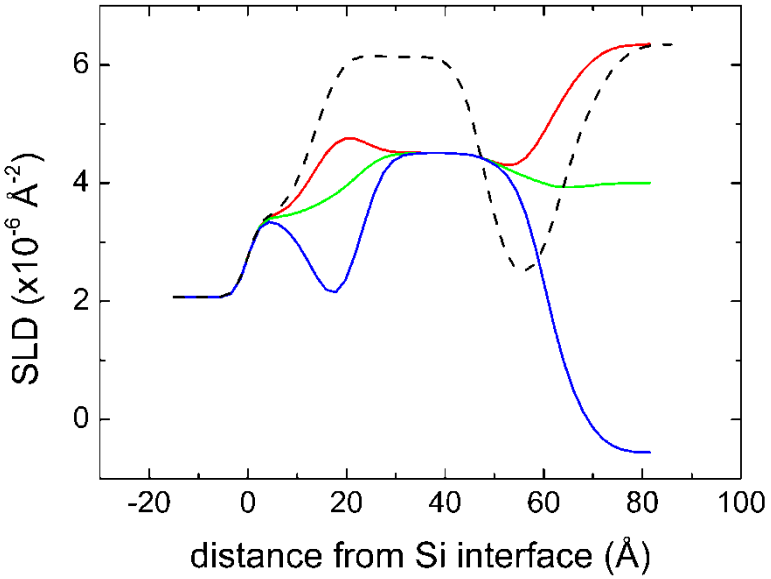


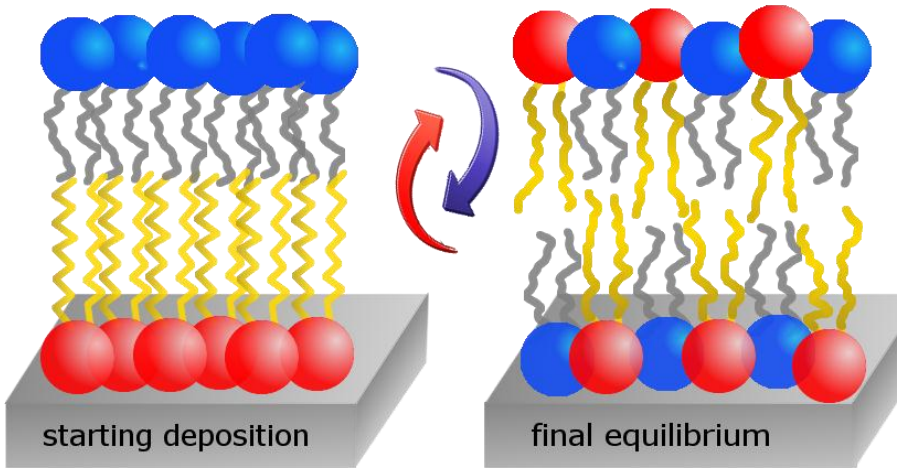
Results

Y. Gerelli et al. – *Langmuir*, **2012**









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)

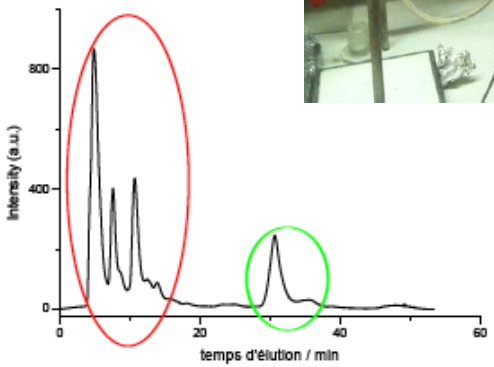
Lipids extracted with Folch method

Phospholipids separated by 2D TLC

Lipids separation (HPLC):

Apolar lipids (chloroform-acetic acid)

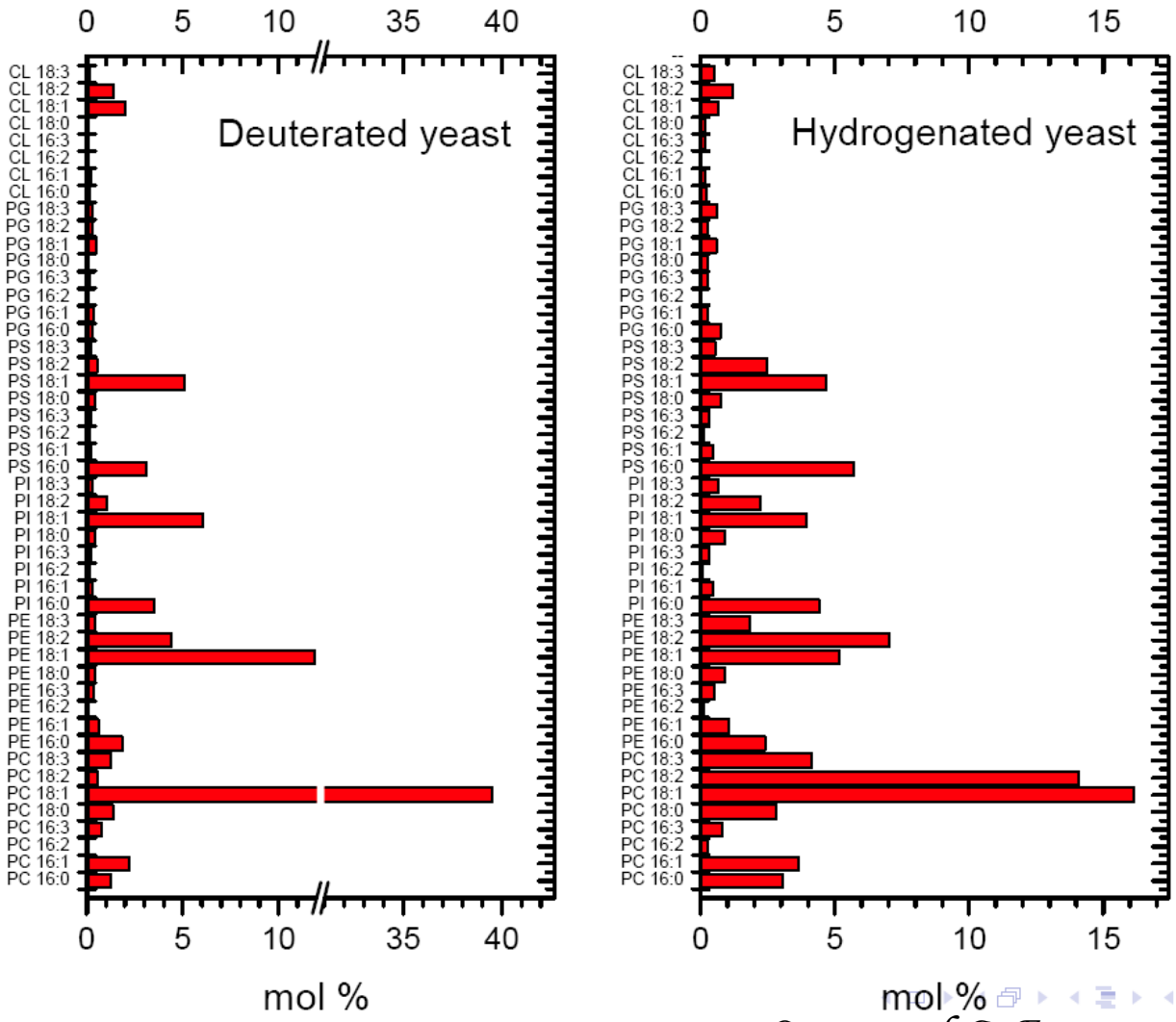
Polar lipids (methanol)



Separation of sterols from apolar

Courtesy of G. Fragneto and A. de Ghellinck

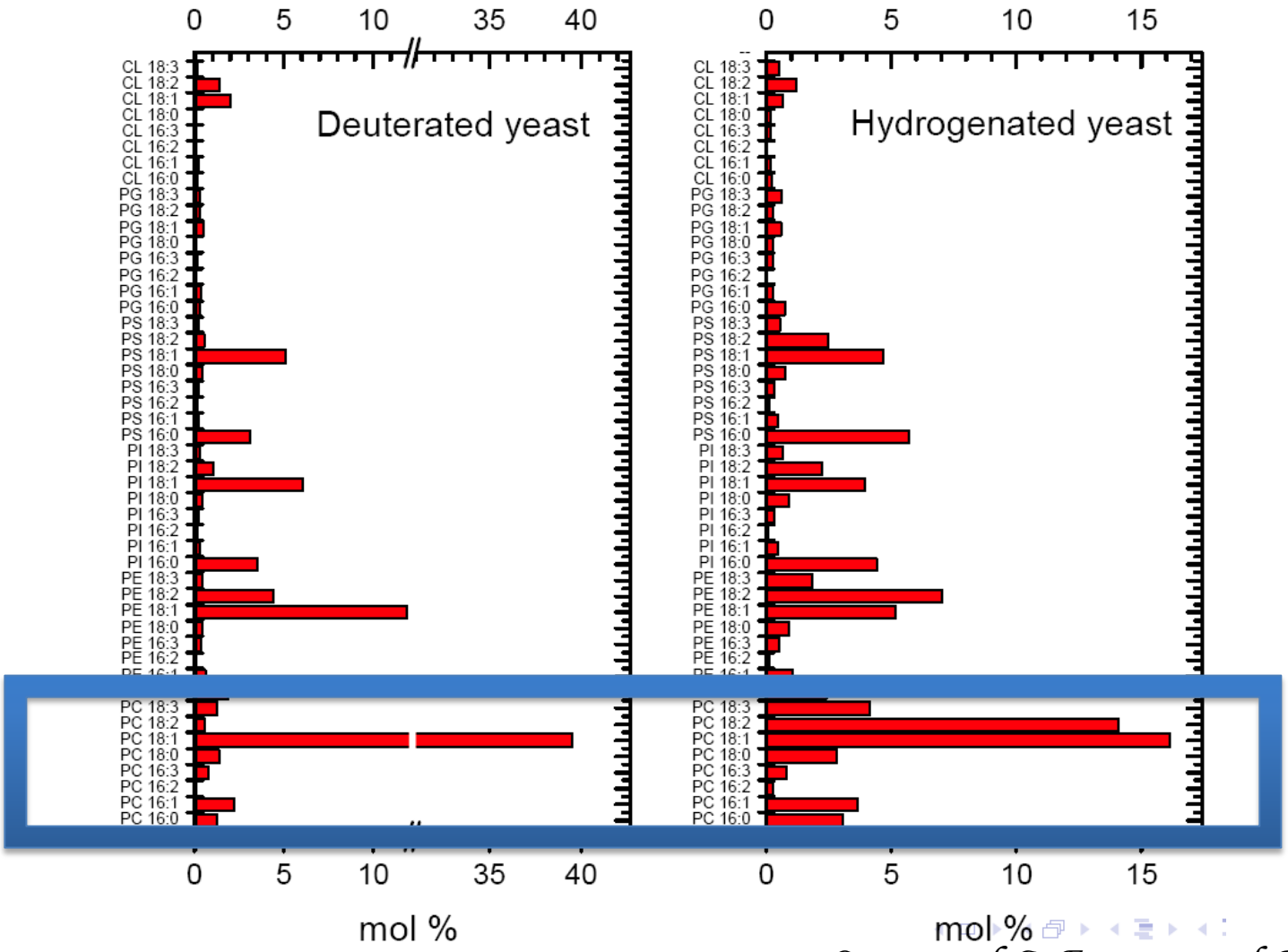
Lipid composition



PC

Courtesy of G. Fragneto and A. de Ghellinck

Lipid composition

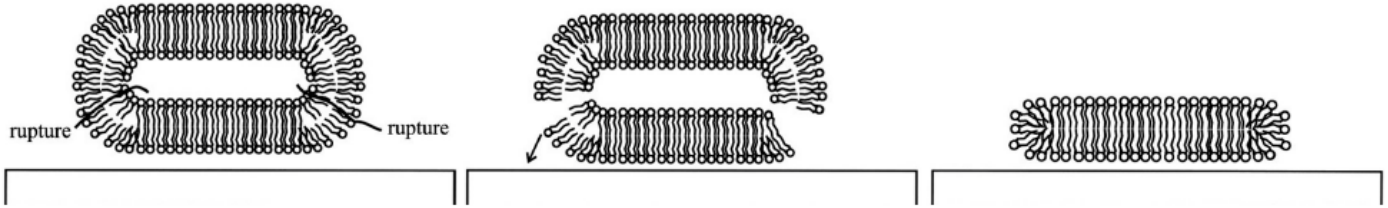


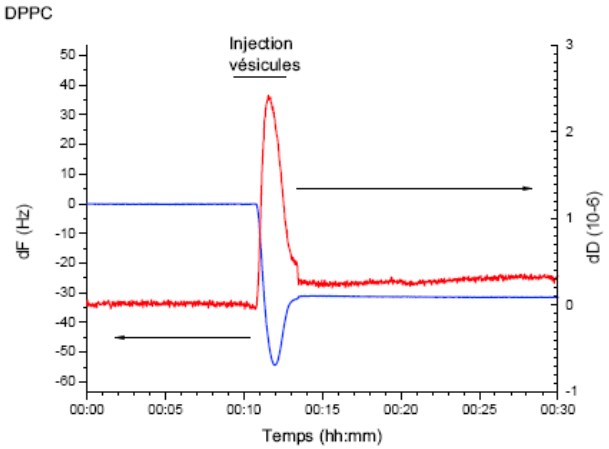
PC

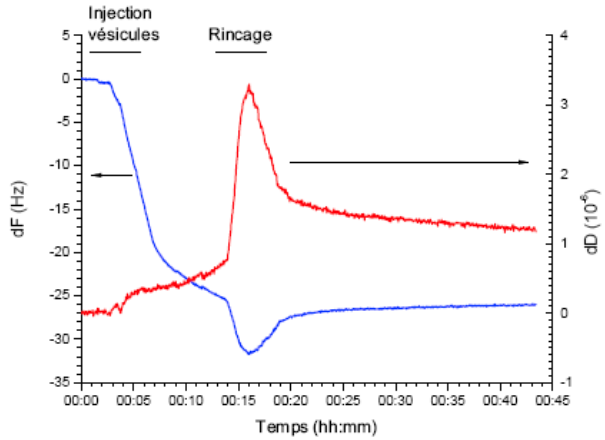
Courtesy of G. Fragneto and A. de Ghellinck

Deposition Method

Unilamellar vesicles prepared by sonication forms spontaneously a supported planar bilayer

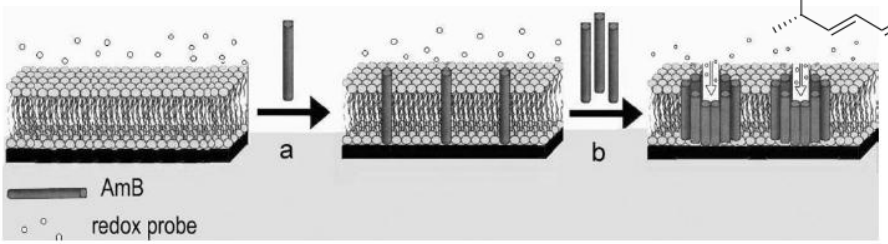


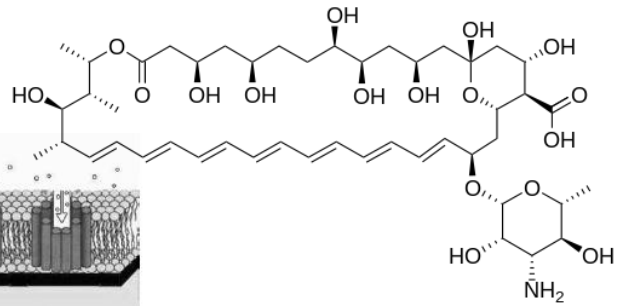


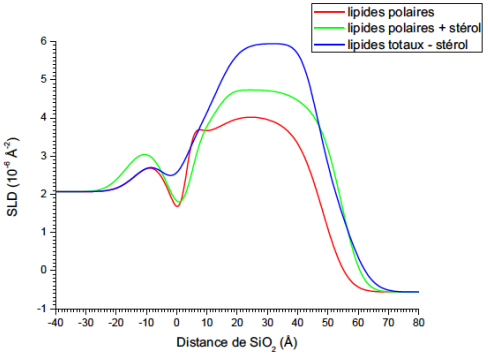


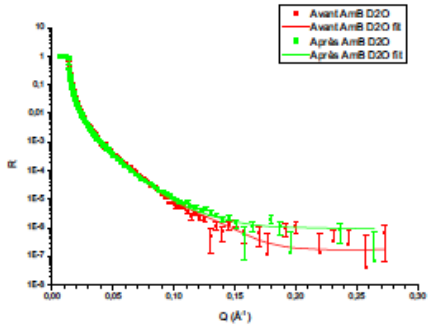
Courtesy of A. de Ghellinck

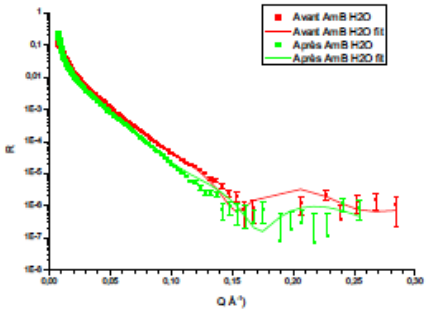
Interaction with amphotericin

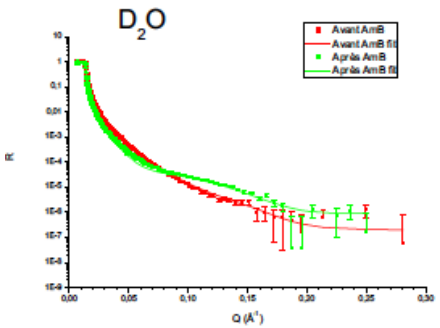


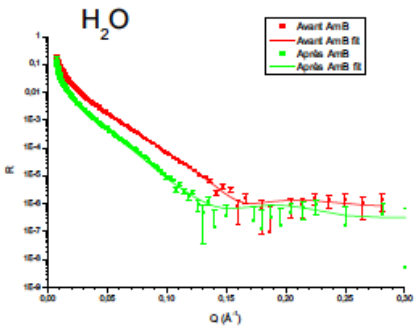


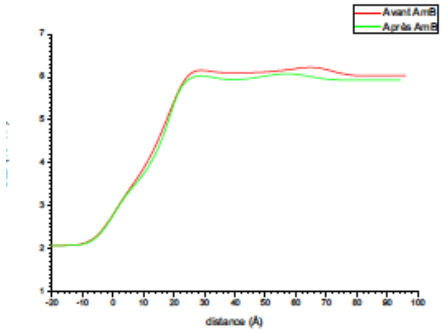


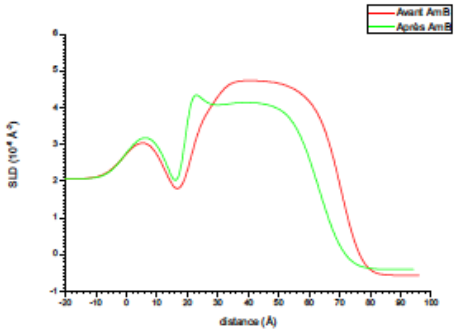


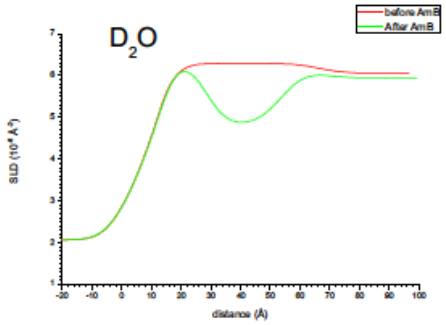


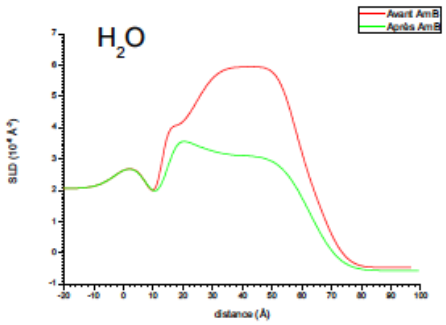




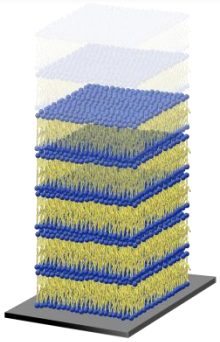


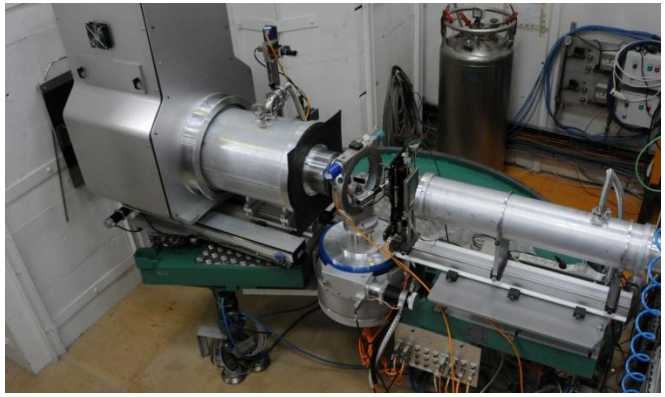


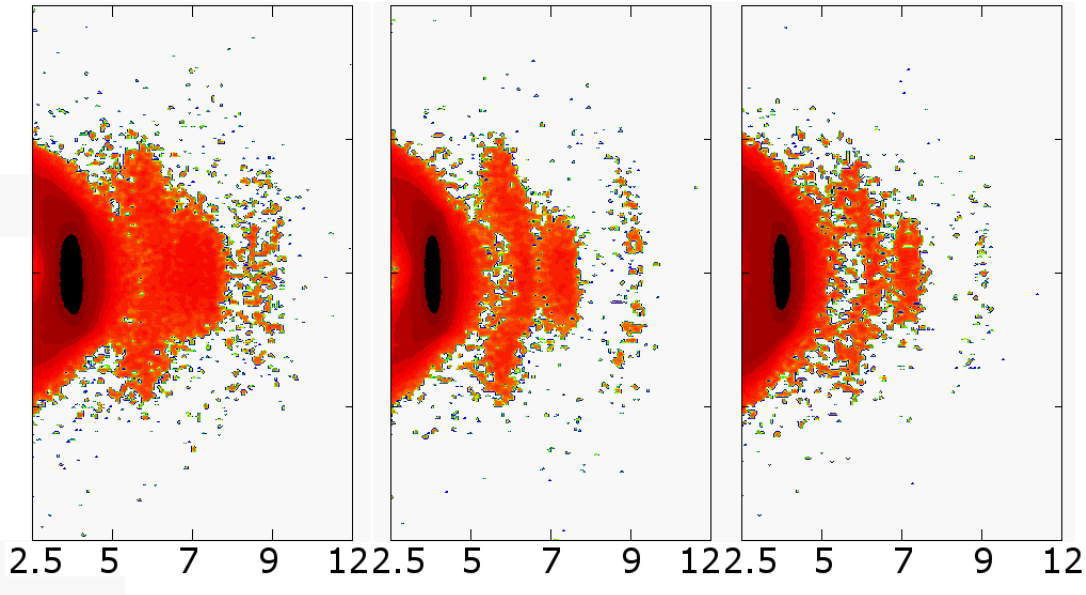


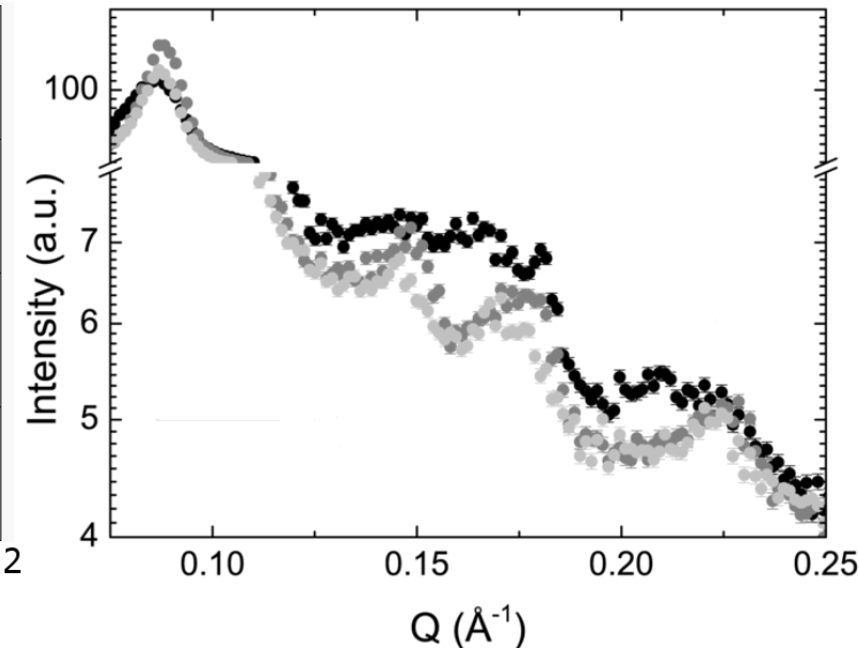


Neutron diffraction on *P. Pastoris*









Courtesy of G. Fragneto

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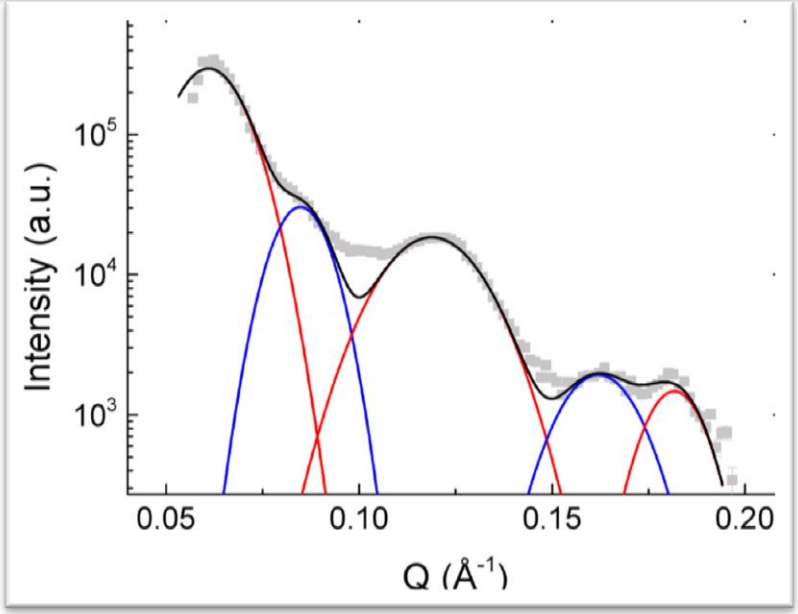
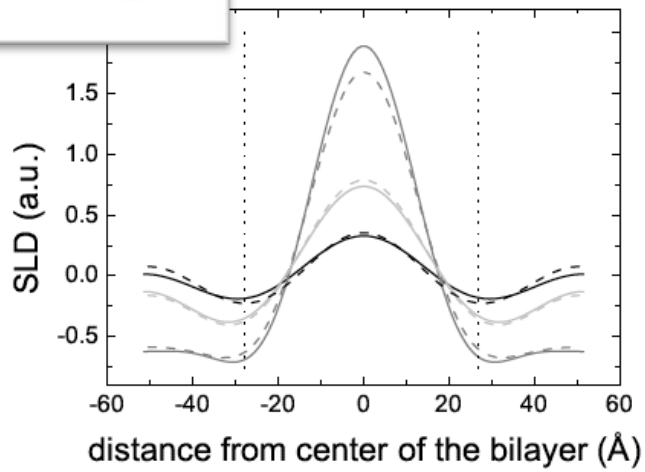


$$\rho(z) = \frac{2}{d}\sum_{h=1}^{N} F(h)\cos\left(\frac{2\pi h z}{d}\right)$$



PC	46.8%
PE	19.9%
PG$^-$	1.7%
PI$^-$	12%
PS$^-$	9.8%
CL$^-$	4.2%

16:0	9%
18:0	5%
16:1	1%
18:1	61%
18:2	7%

	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

Courtesy of G. Fragneto

Future work

- Better understanding of flip-flop processes in charged bilayers, in presence of third agents (cholesterol and proteins) and in more complex systems
- Development of defects-free deposition methods (solvent exchange)
- Comparison between different types of cushions, from polymer to cross-linked molecules, highly compatible with many lipid species

Acknowledgements

Main contributors

Alexis De Ghellinck, Giovanna Fragneto

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Thank for your attention !!!