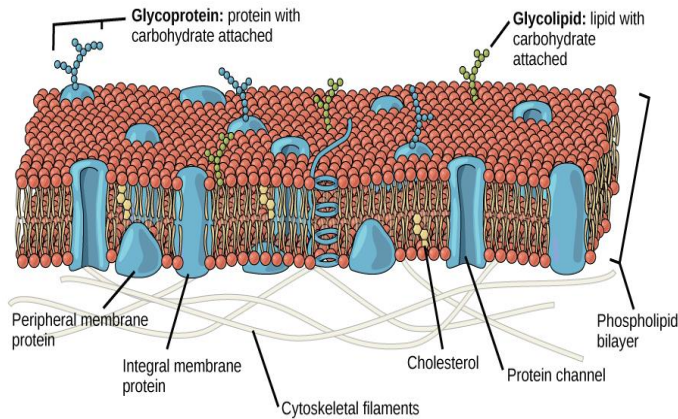


Model Biological Membrane Platform: Current State and Progress

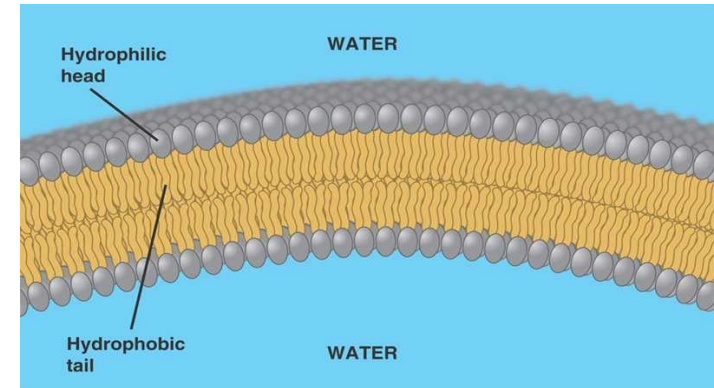
Irena Kiesel

Metting Sacley
28th-29th May 2015

- Deuterated Lipids Extraction (Robin Delhom)
- Polymer Tethered Membranes (Irena Kiesel)
 - Idea
 - Test with commercially available lipids
 - Current state with natural lipids
- Liquid/Liquid interface (Ernesto Scoppola)



Real system: complex

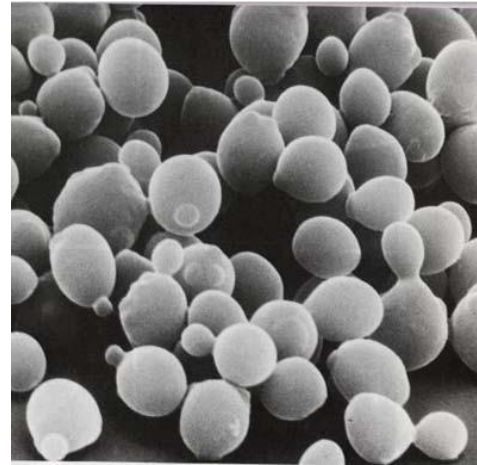


Model system: Mimic real system with controlled conditions

- Investigate transport processes through membranes (drugs, proteins, diseases)
 - Natural, deuterated lipids
 - Tethered membranes for neutron reflectometry
- Create a recipe for reproducible and stable model membranes

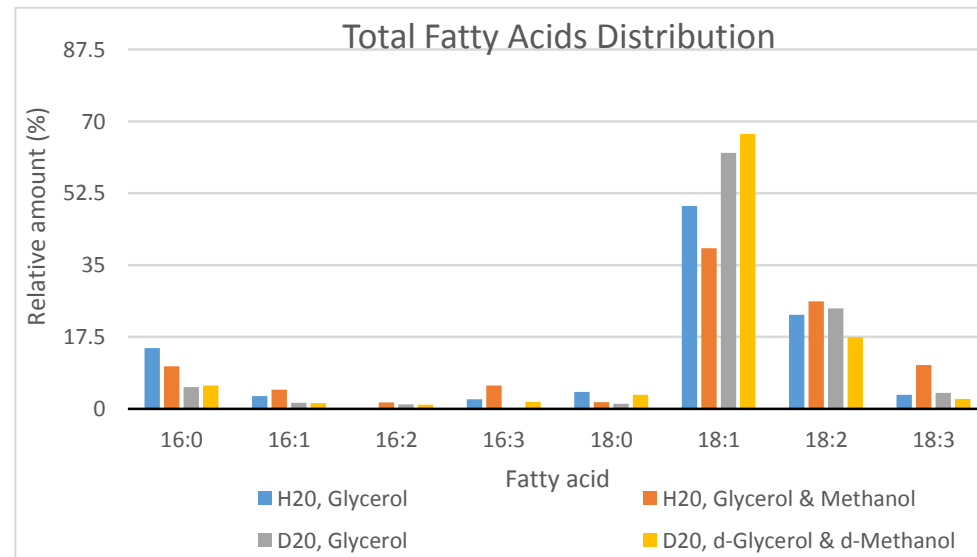
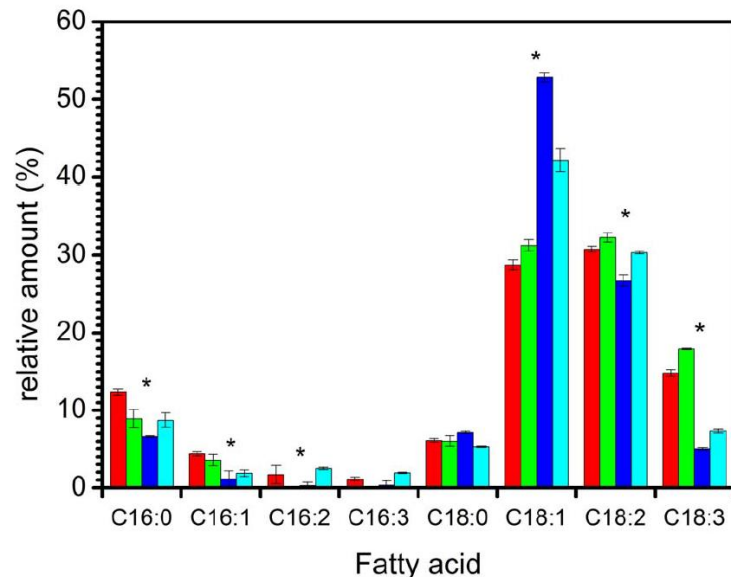
Pichia pastoris yeast

Composition
h-lipids = d-lipids ?!



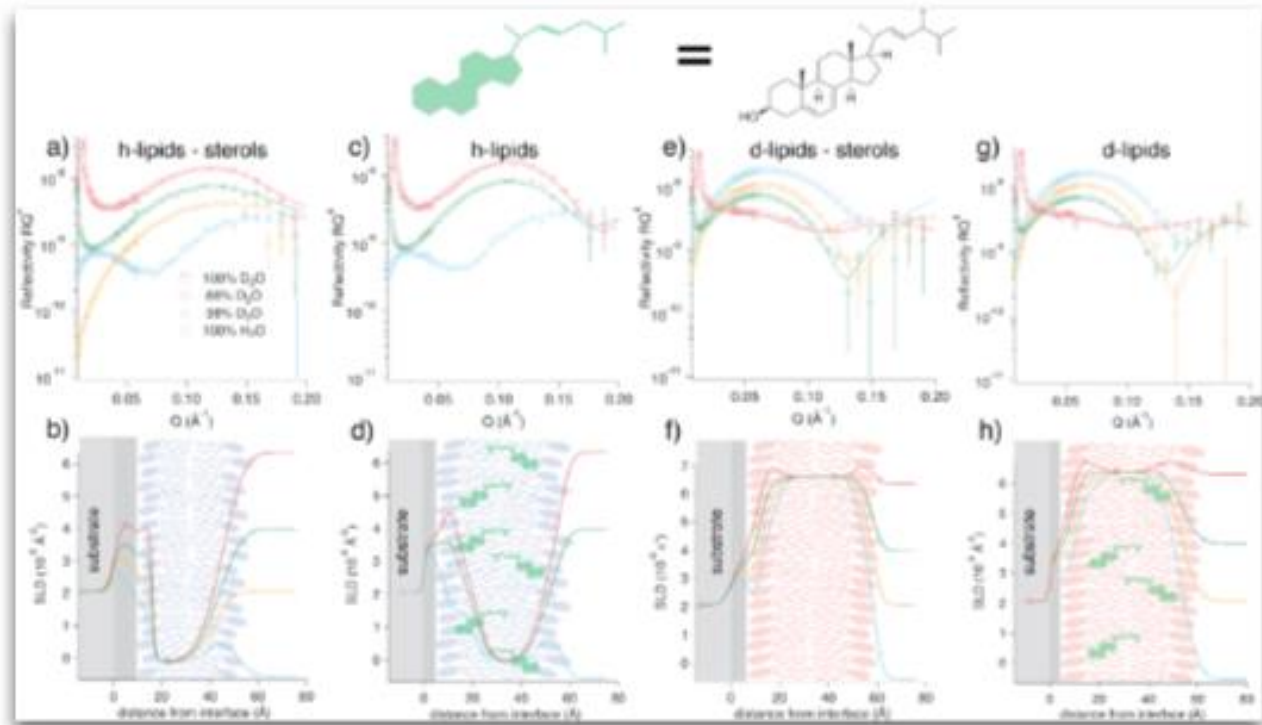
Robin Delhom

Δ Growth temperature Δ Carbon source



DEUTERATED NATURAL LIPID EXTRACTION - STRUCTURAL CHARACTERISATION AND INTERACTION WITH AN ANTIBIOTIC (SINE2020)

PhD A. de Ghellinck, R. Delhom

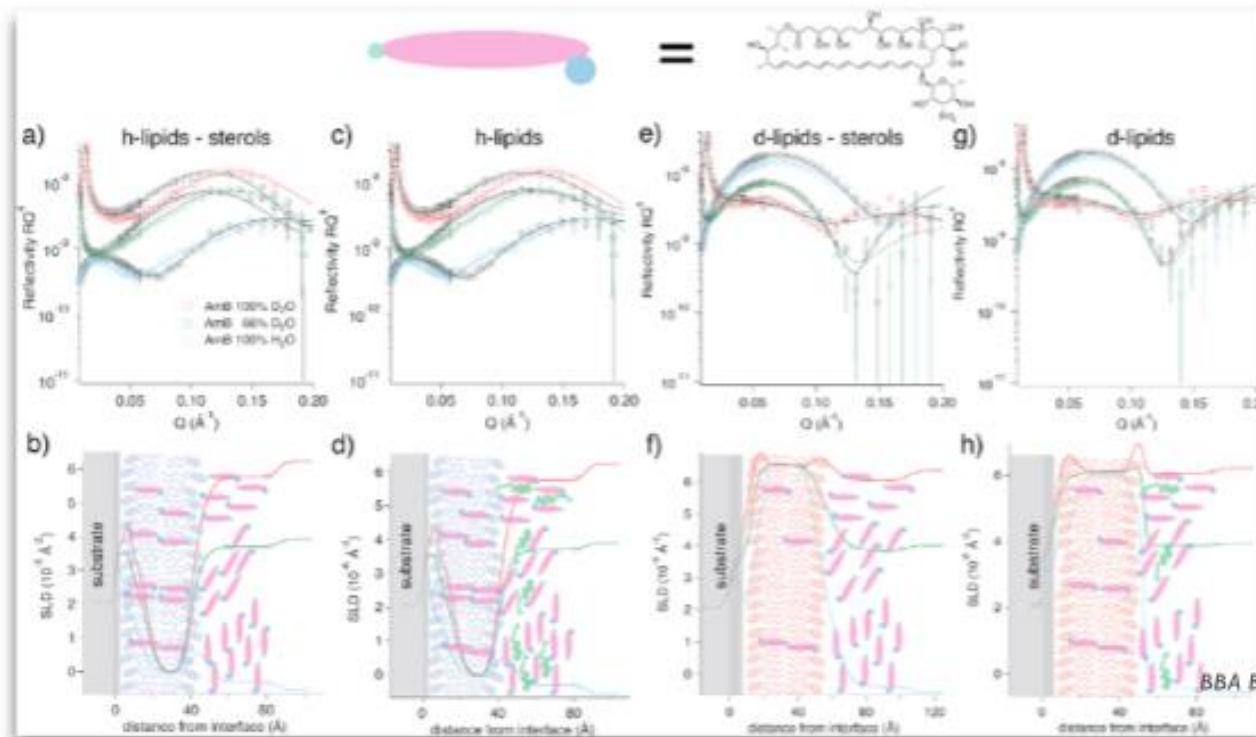


PlosONE 2014

BBA Biomembranes under revision

DEUTERATED NATURAL LIPID EXTRACTION - STRUCTURAL CHARACTERISATION AND INTERACTION WITH AN ANTIBIOTIC (SINE2020)

PhD A. de Ghellinck, R. Delhom



PlosONE 2014

BBA Biomembranes under revision

Characterization by Neutron Diffraction



Q-range $0.02 - 2.5 \text{ \AA}^{-1}$
Times ~min



research papers

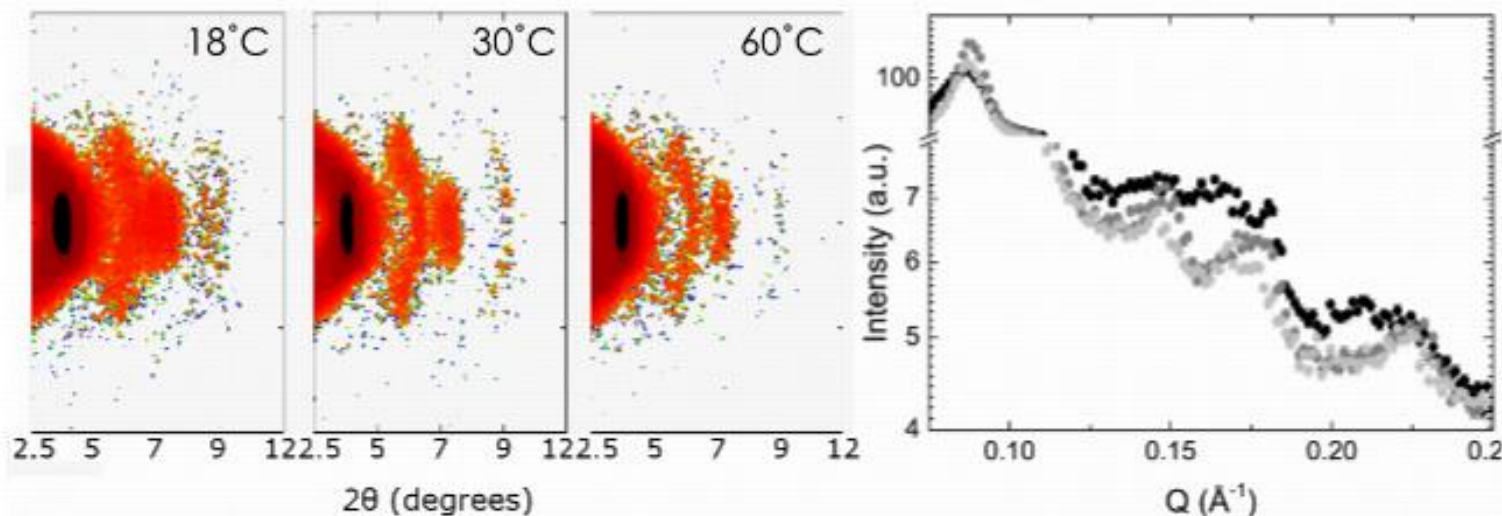
Acta Crystallographica Section D
Biological
Crystallography
ISSN 1399-0047

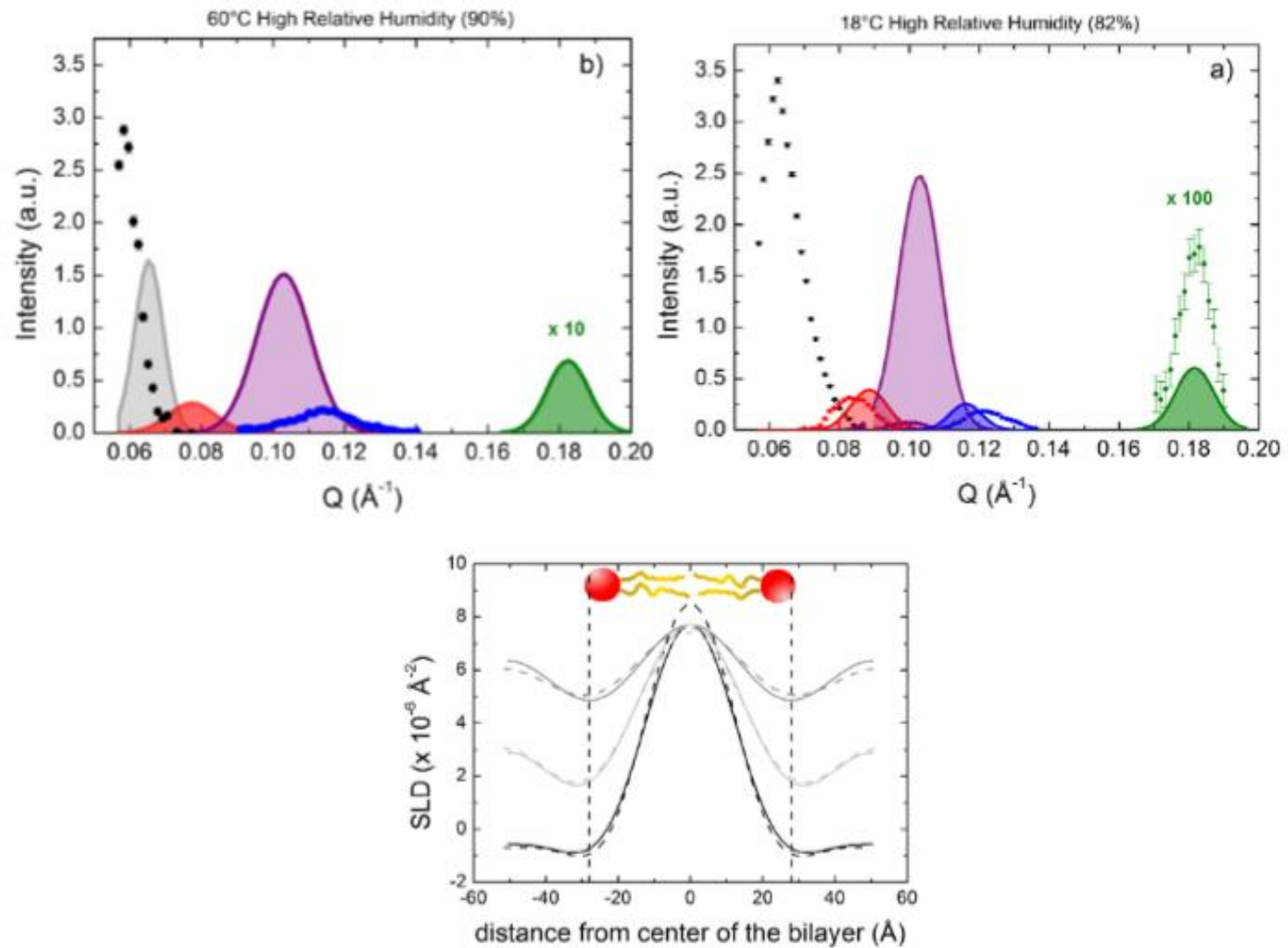
Multi-lamellar organization of fully deuterated lipid extracts of yeast membranes

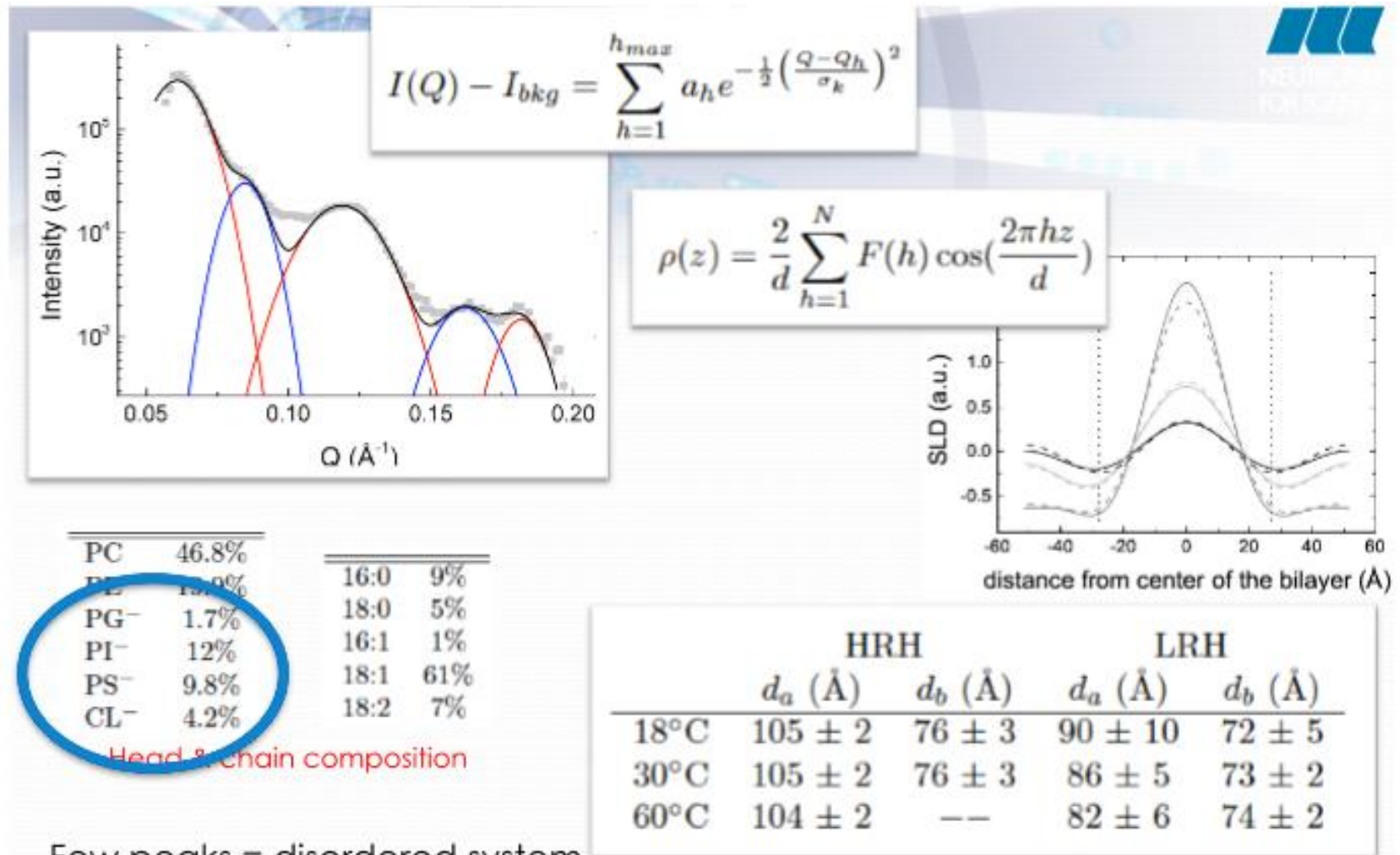
Yuri Gerelli,^{a*} Alexis de
Ghellinck,^{a,b} Juliette Joubert,^c
Valérie Laux,^a Michael
Haertlein^a and Giovanna
Fragneto^{a*}

Neutron scattering studies on mimetic biomembranes are currently limited by the low availability of deuterated unsaturated lipid species. In the present work, results from the first neutron diffraction experiments on fully deuterated lipid extracts from the yeast *Pichia pastoris* are presented. The structural features of these fully deuterated lipid stacks are compared with those of their hydrogenous analogues

Received 29 April 2014
Accepted 18 October 2014



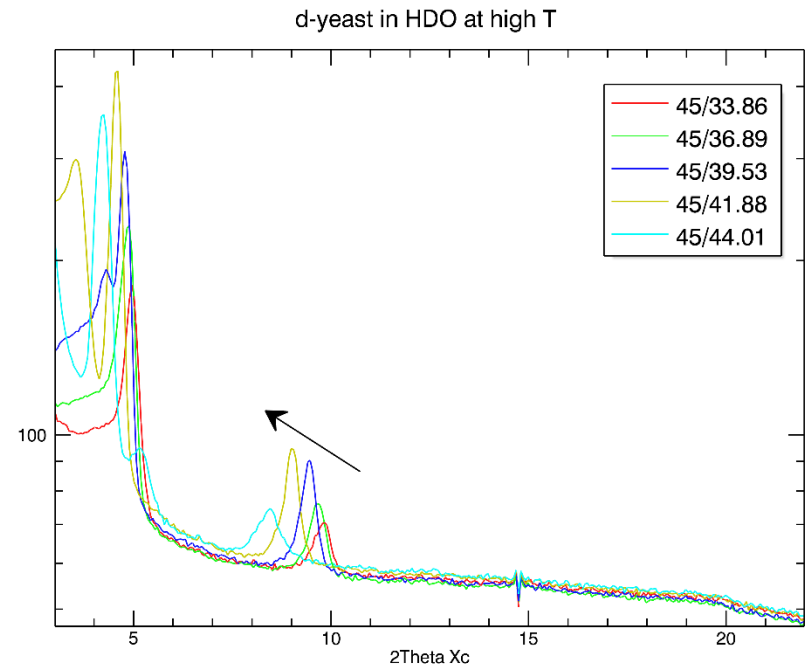
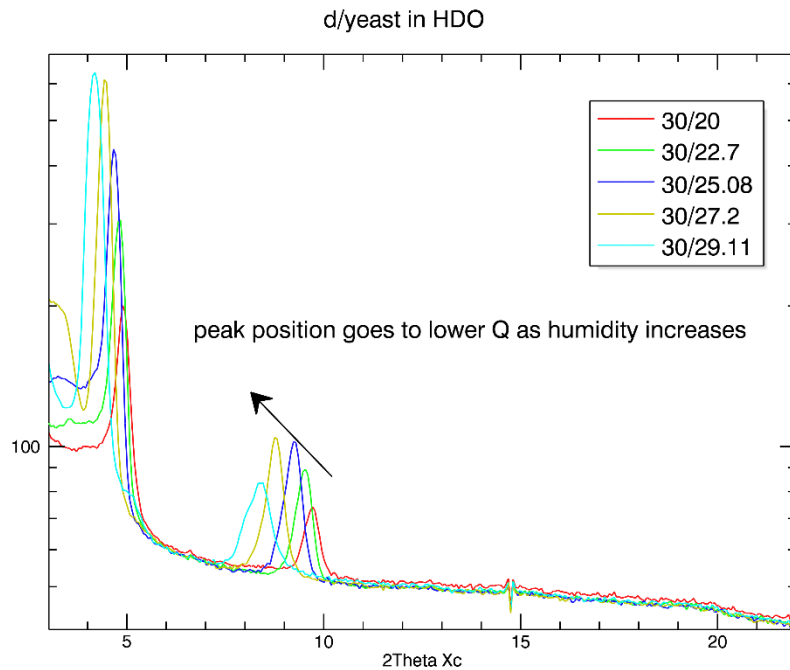




Few peaks = disordered system

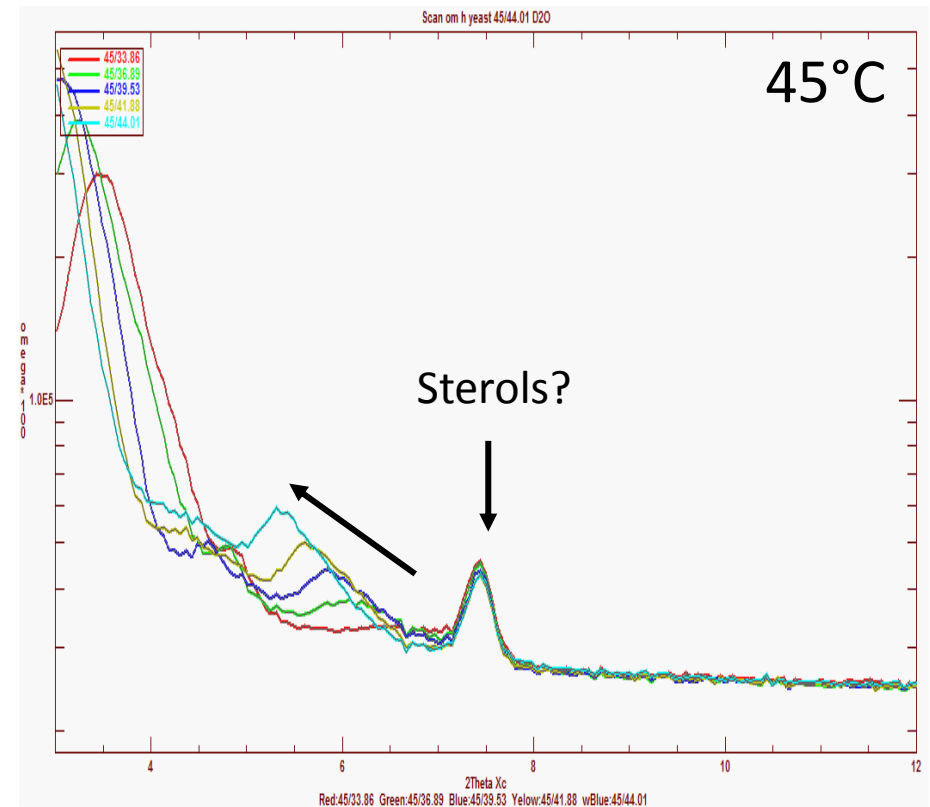
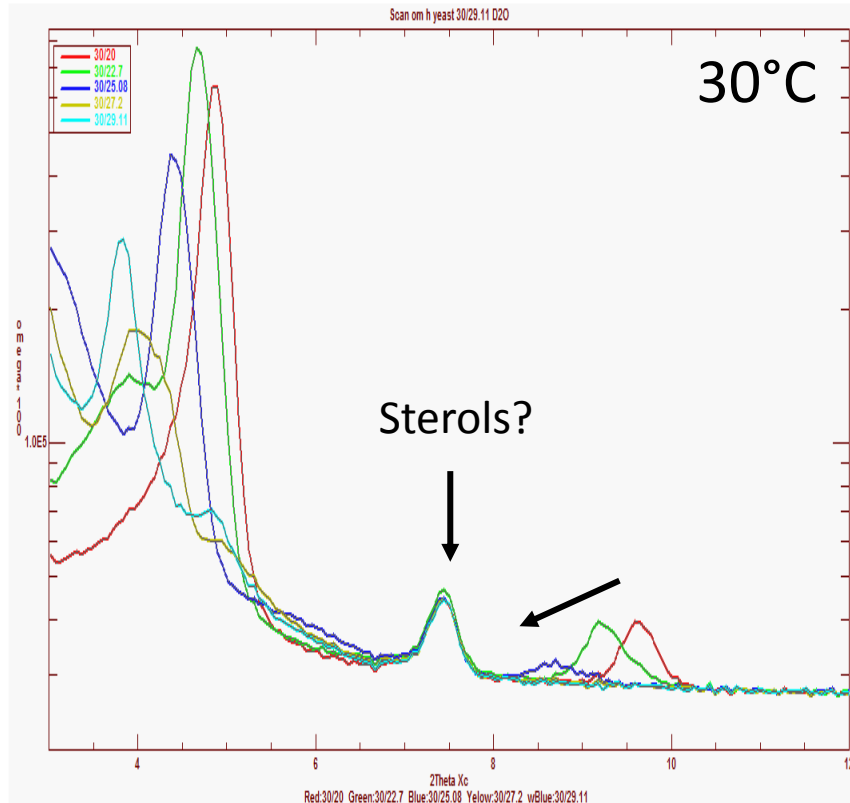
Two d-spacings due to inhomogeneity of sample

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



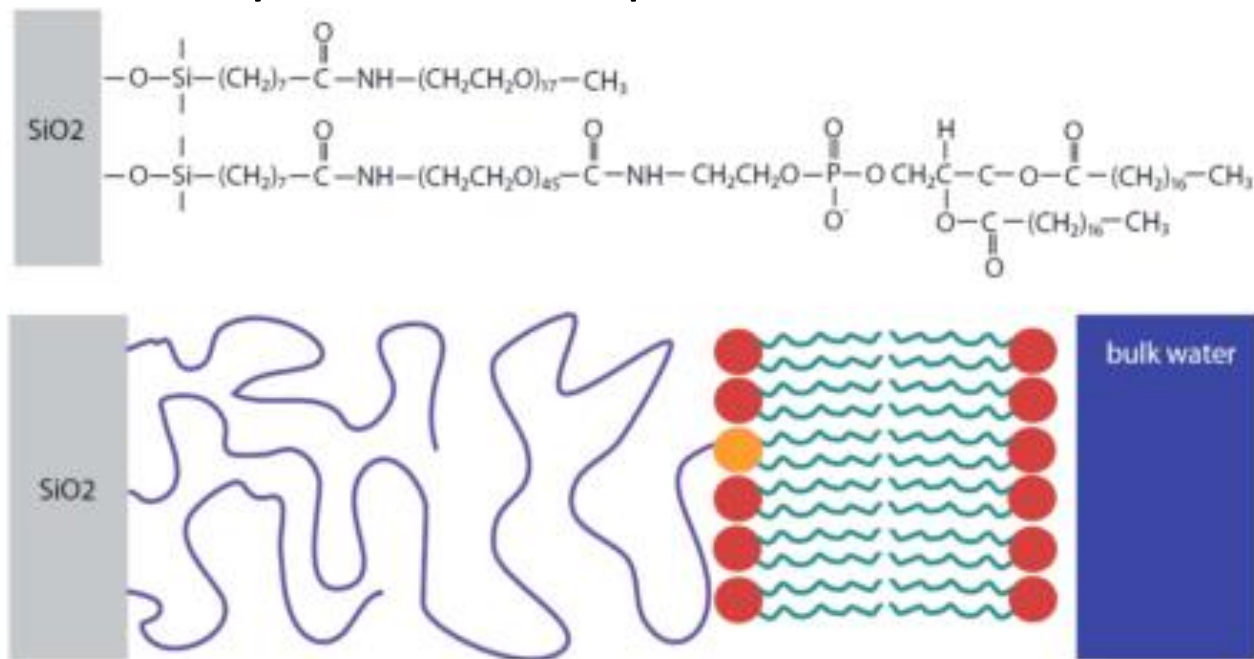
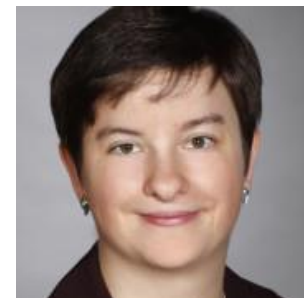
Spincoated multilayer of deuterated yeast lipids extracts on silicon

- Humidity chamber
- Different temperatures (30°C and 45°C)
- Grating incident diffraction:
 - peak → Order of the lipid-stacks, distance in between



Multilayer hYeast at 30°C and 45°C and different relative humidities
Differences between hYeast and dYeast
Analysis in progress

Collaboration with AG Nickel from Munich:
Polymertethered spincoated membranes



Hertich et al.,
Langmuir 2014, 30, 9442-9444

Deuterated and natural lipids extracted from yeast (de Ghellnick, current project PhD Robin Delhom)

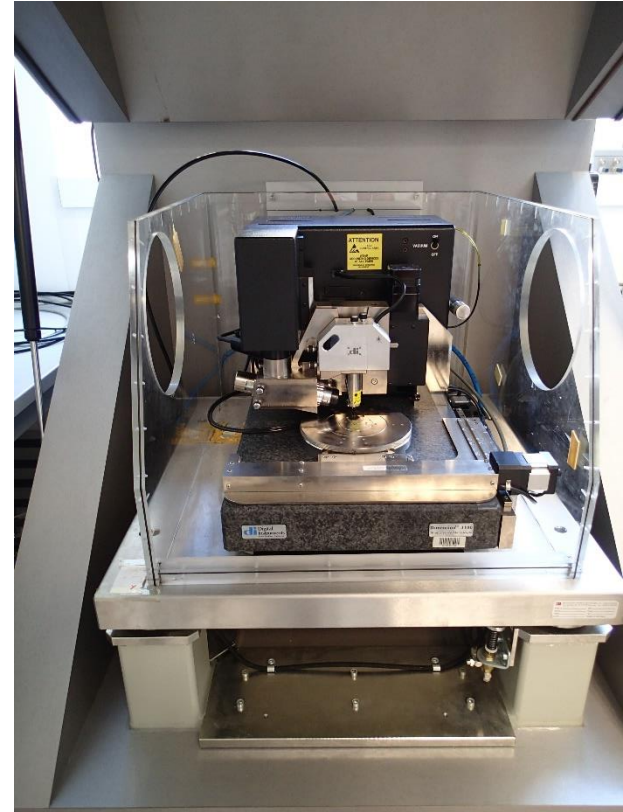
Advantage:

- Fast easy sample preparation with spin-coating
- Reusable substrates



Fluorescence Microscopy

+ Ellipsometer

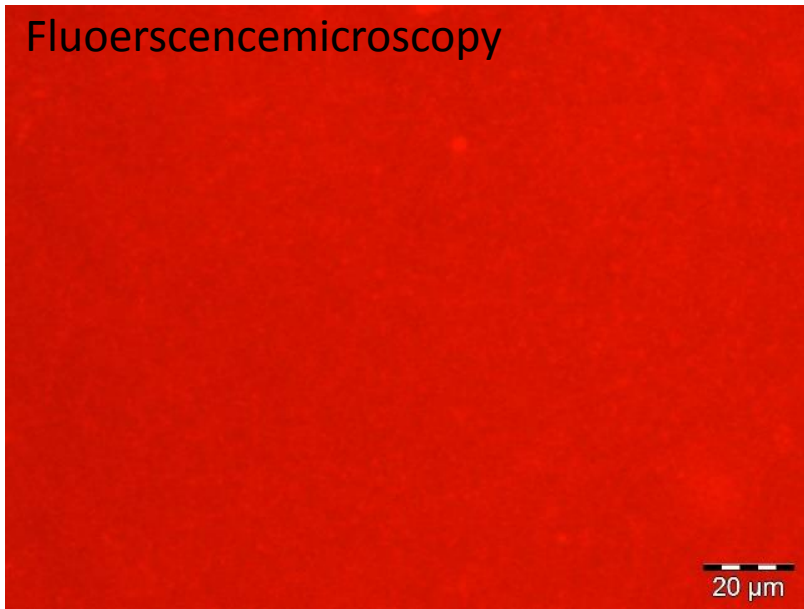


AFM (Veeco)

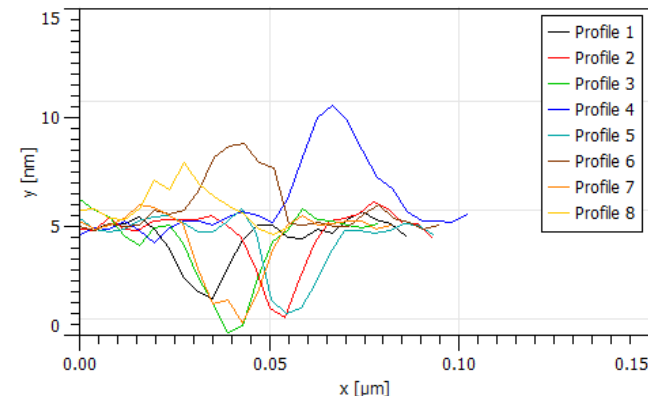
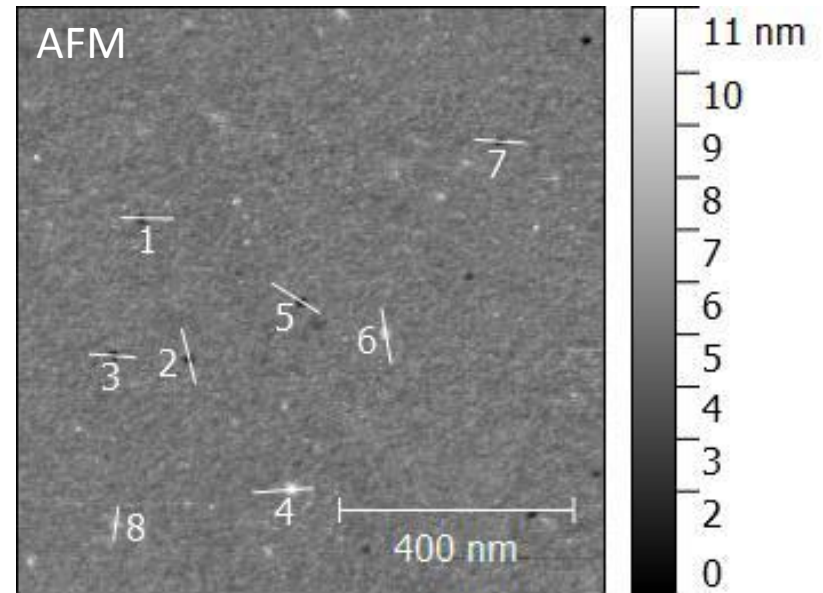
- Ordered a liquid head for
measurements in water

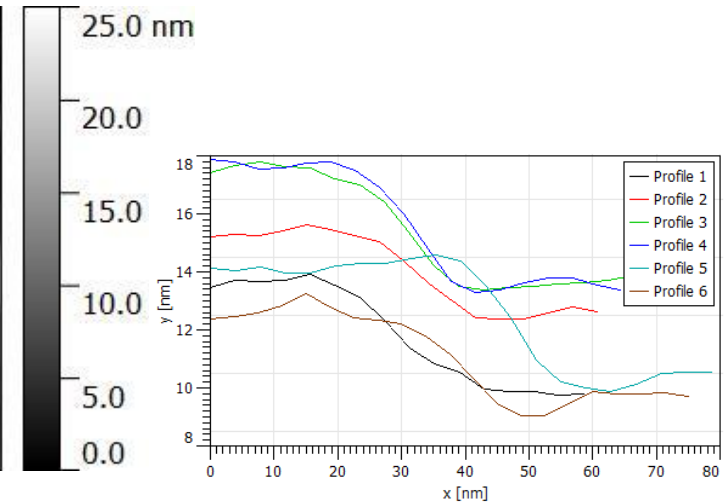
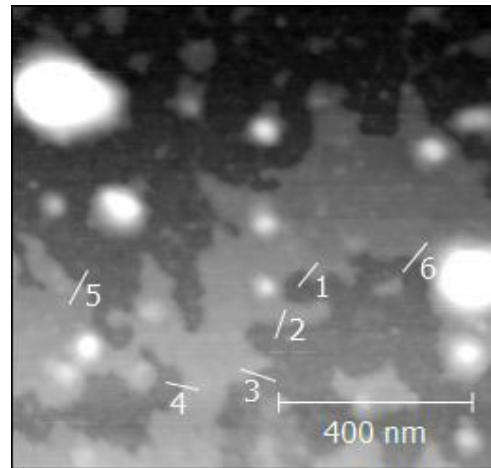
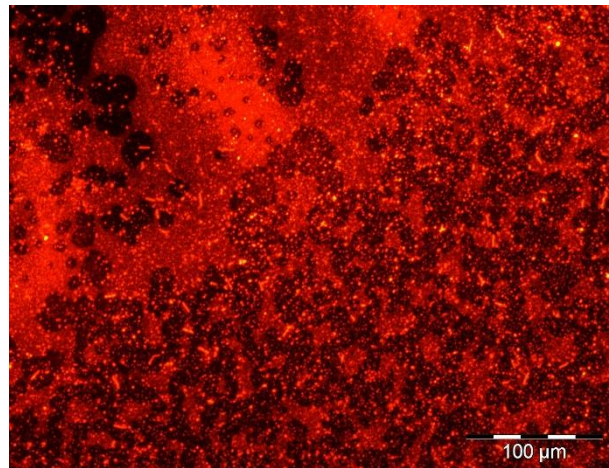
- First substrates successfully prepared
- Test with synthesized lipids (SOPC on PEG/Silicon)
 - Different concentrations

Fluorescence microscopy

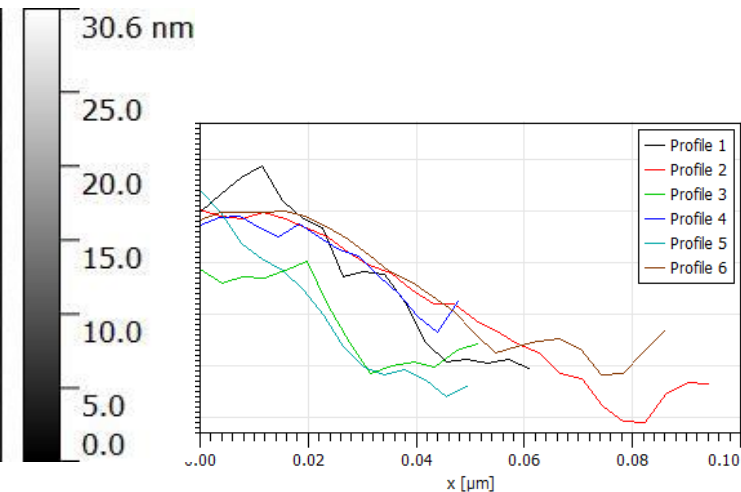
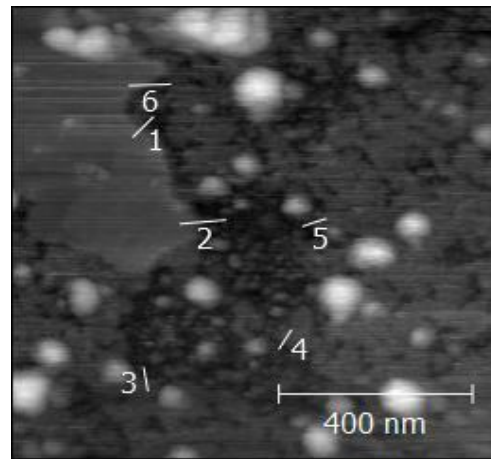
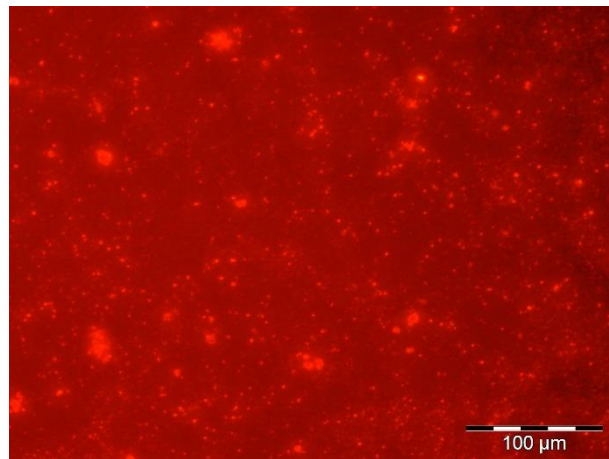


2 mg/mL SOPC/Isopropanol
measurements in water





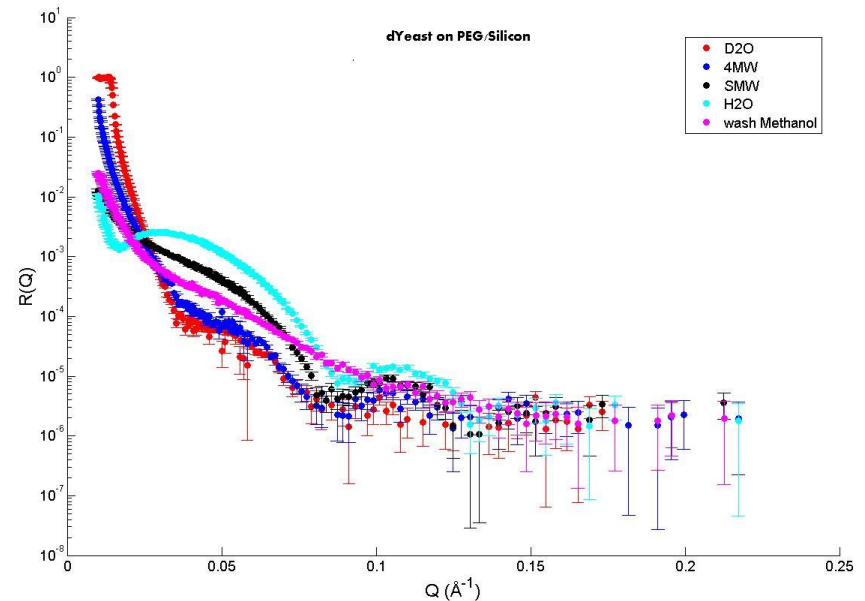
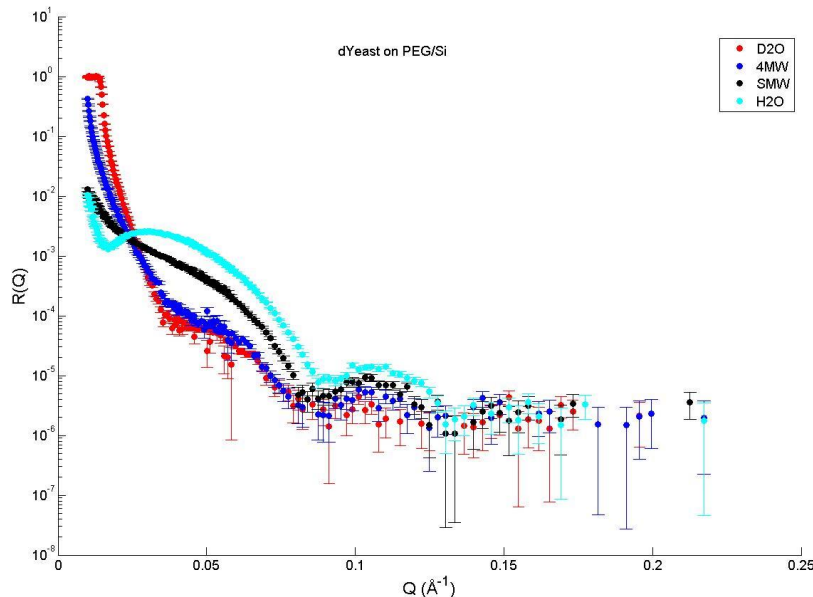
Air



Water

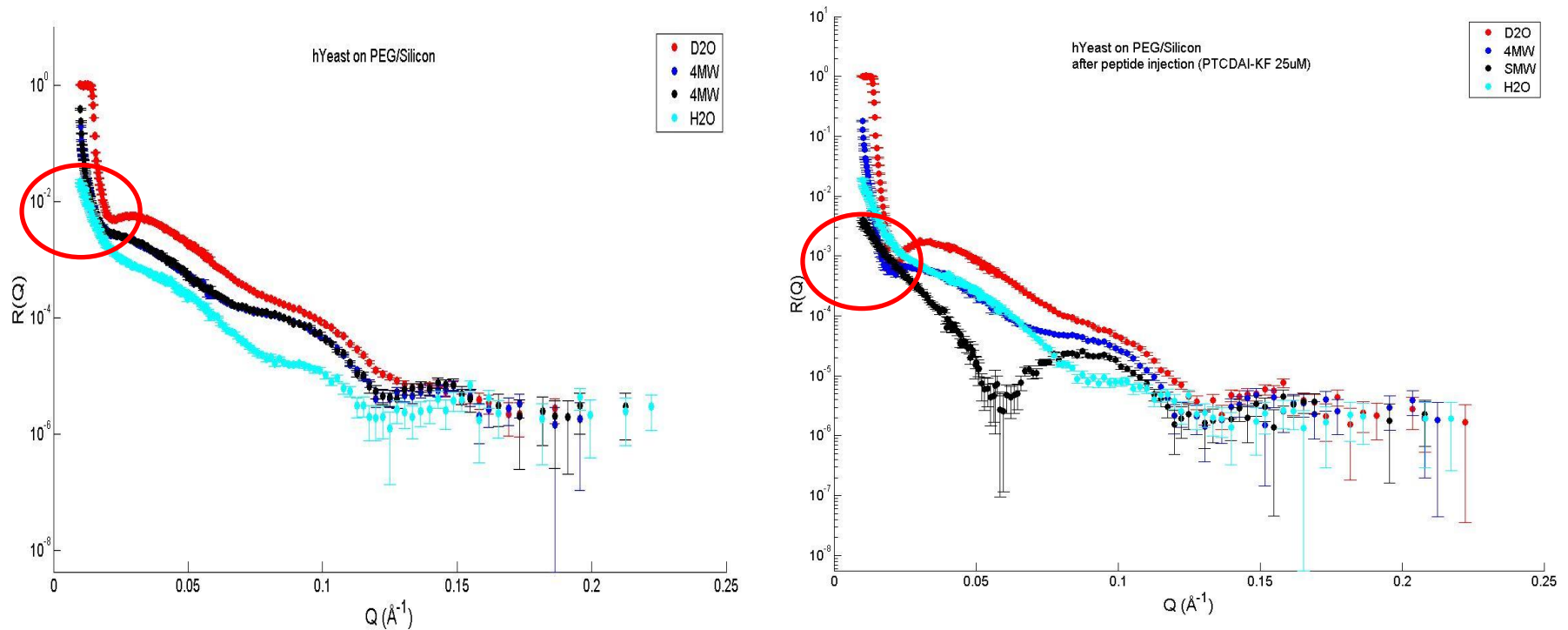
1.5 mg/mL hYeast/Methanol+Chloroform → Solubility

- Natural Yeast lipids, deuterated, spincoated on PEG/Silicon



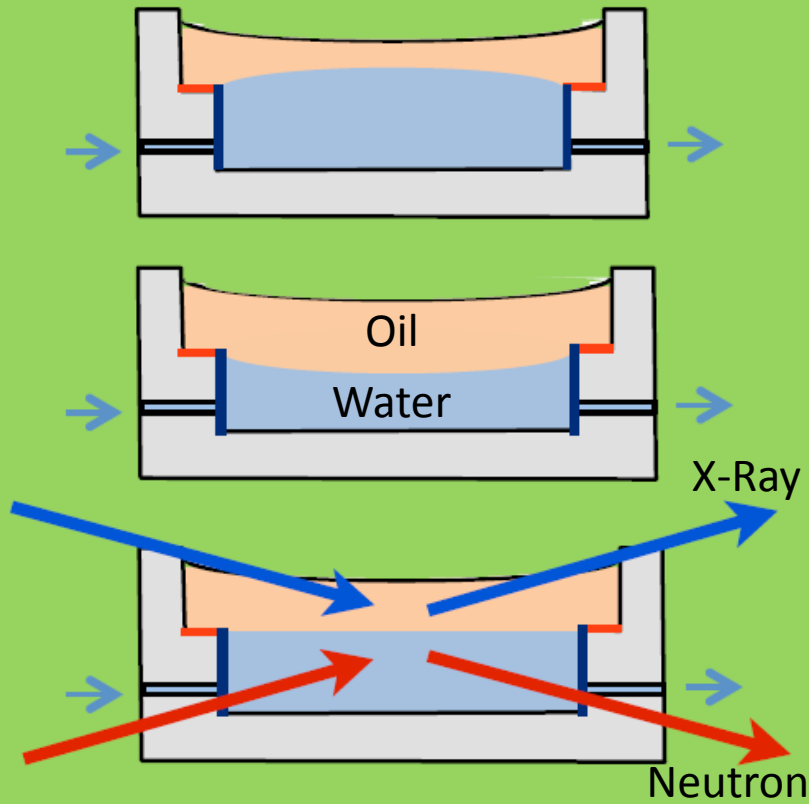
- Nice features in the reflectivities for different contrasts
- Features fits to the size of the PEG Layer
- Proof, that we can wash away the lipids with methanol
- Analysis is work in progress...

- Test measurement with neutron reflectometry at Figaro

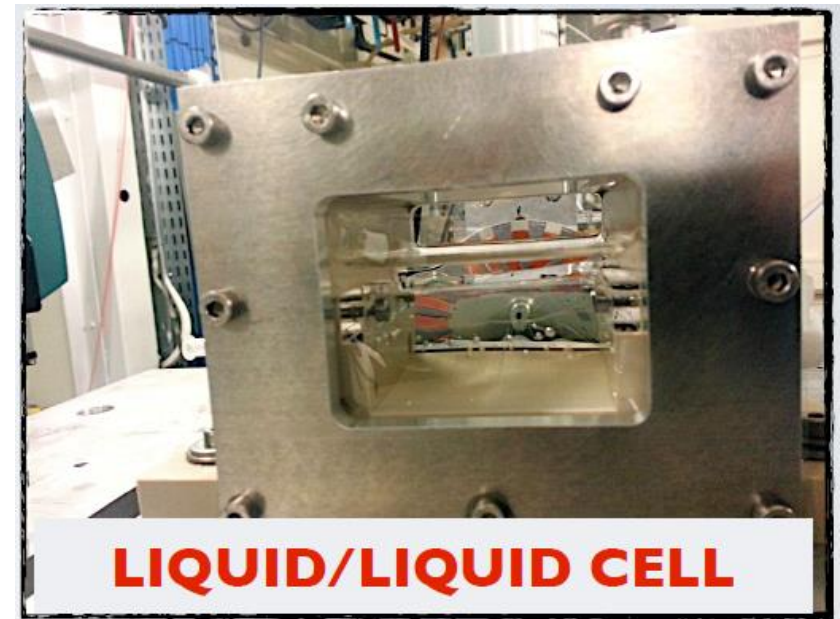


- Same substrate, but with hYeast
- First test with a peptide from cooperation partner (Jean-Philippe Michel, Paris)
- Significant change in the reflectivity curve, especially for D2O

- Lipid extraction from yeast: Find parameters for similar composition
- First successful substrate preparations
- Find optimal solvent and spincoating parameters for the natural lipids
- Precharacterization with (Liquid)AFM and Fluorescence-Microscopy
- Experiment in June at DELTA (x-ray reflectivity)
- Experiment in July at FIGARO (neutron reflectivity)

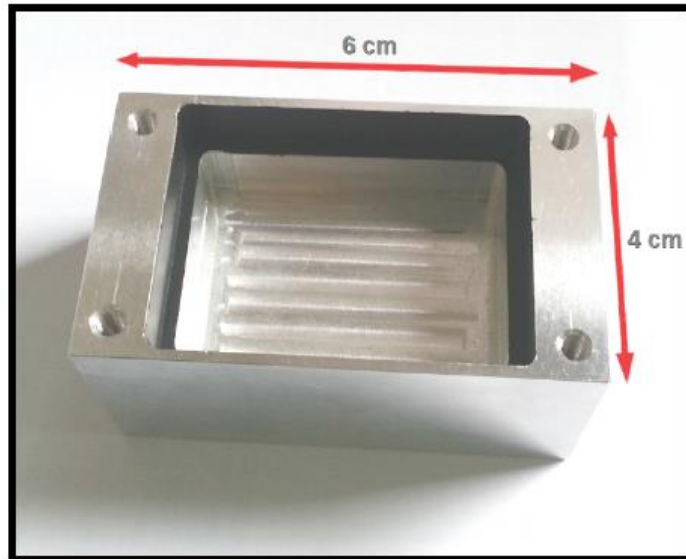
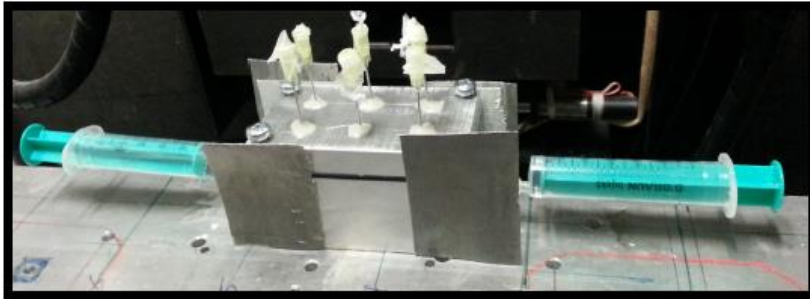
Flat Interface and controlled sizes

Ernesto Scoppola

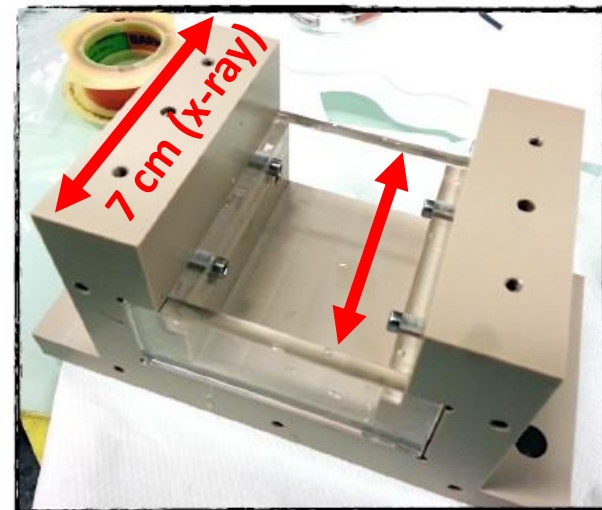


- Low Curvature with controlled sizes
- Lower Attenuation

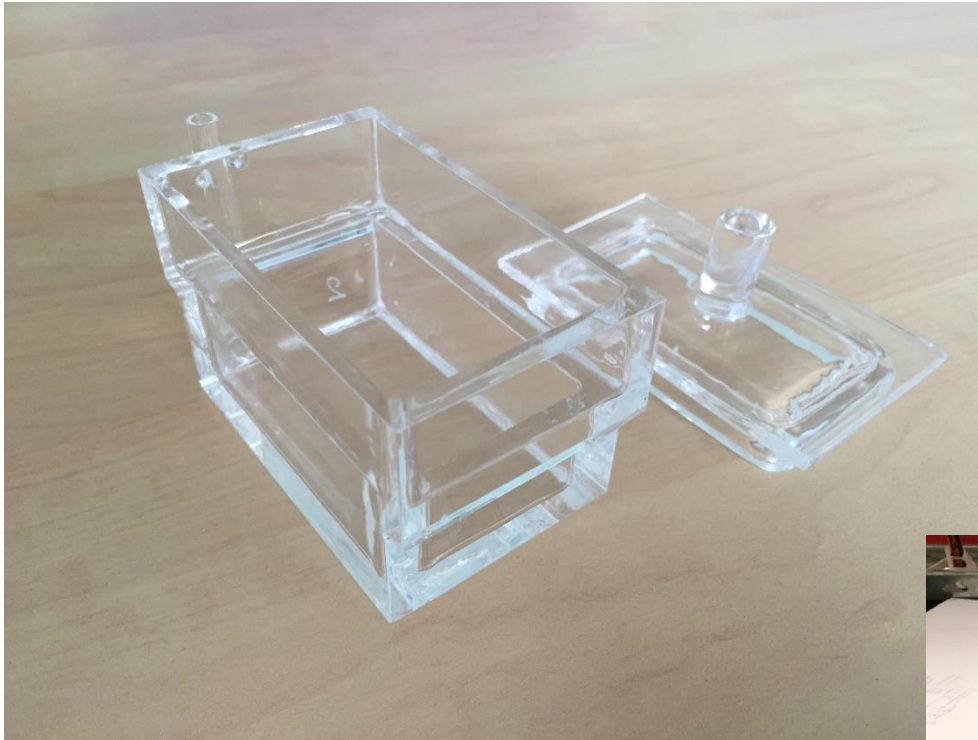
Prototype Cell



Peek/Quartz Cell



5 cm /
3.5 cm
(neutron)



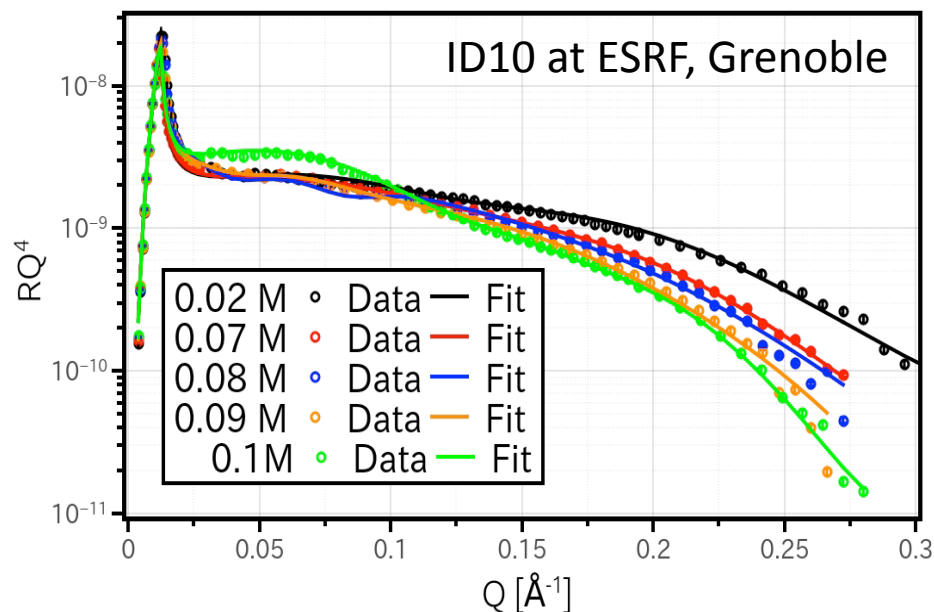
Current cells

- Quartz (One piece)
- Different sizes

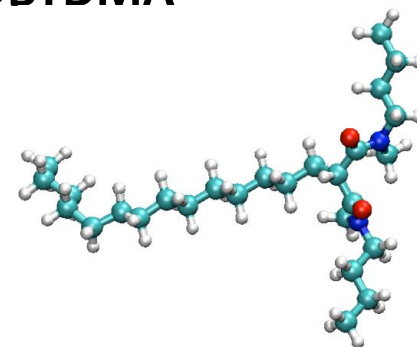
Combination of two methods:

- Neutrons: Nuclei composition
(Extractant)
- X-Ray: Electron Density **(Ions)**





- Water with **Lithium Nitrate (2 M)** and **Neodymium Nitrate (0.25 M)**
- Dodecane
- Extractant: **DMBTDMA**



Increasing extractant concentration

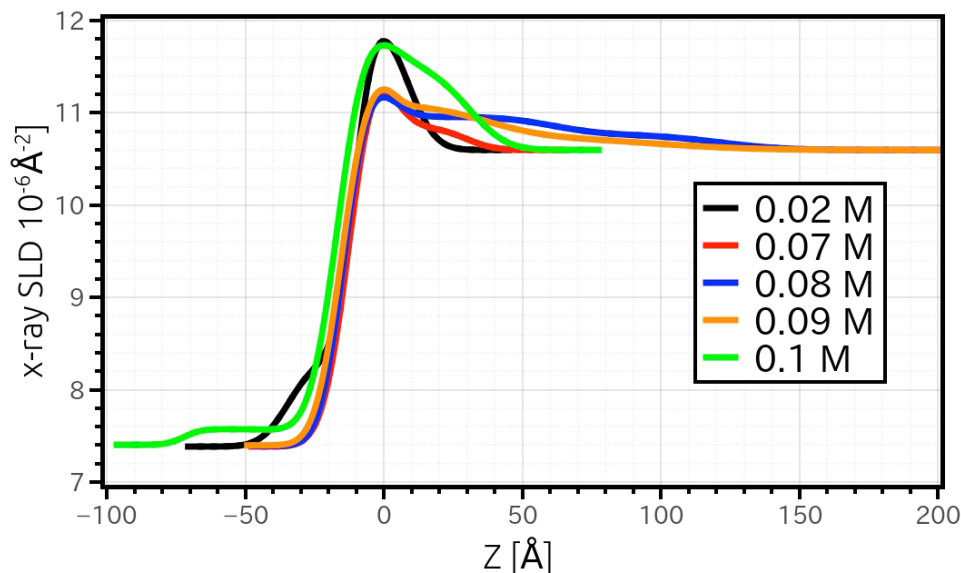
→ Change in slope

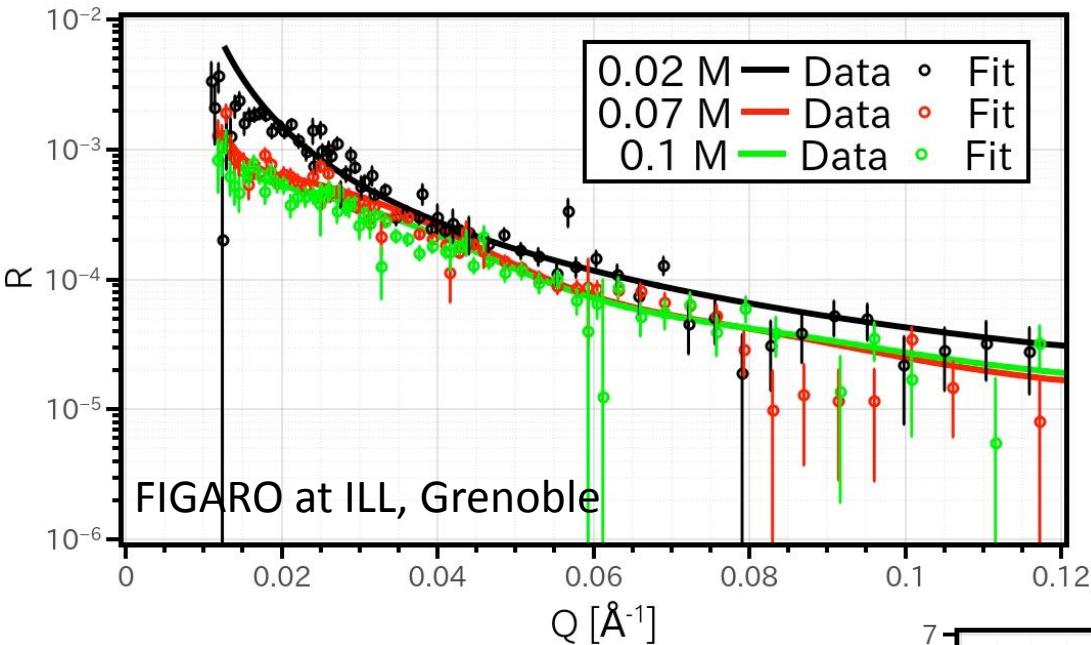
At the CAC

→ higher intensity in the low Q region

Higher SLD at the interface

→ adsorption of ions





Lower SLD at the interface
→ Adsorption of Diamide.

