NMI3-II WP20 - Task 1
Model Membranes Platform

Giovanna Fragneto

15th May 2014 NMI3-II JRA meeting Grenoble

Up-date on staff resources

- A post-doc has been hired at ISIS in 2013
- Yuri Gerelli (post-doc at ILL) - end of NMI3-II contract Dec 2013 (model membrane systems)
- Alexis de Ghellinck - PhD obtained Dec 2013 (lipid extraction - not funded by NMI3-II but contributed to project)
- PhD studentship awarded by ILL co-financed by ESS to continue work on lipid extraction towards deuterated lipid platform setting up. Start Oct 2014. ILL part financed by NMI3-II.

Results: Lipid extraction paper out last month

OPEN ACCESS Freely available online

PLOS | ONE

Production and Analysis of Perdeuterated Lipids from *Pichia pastoris* Cells

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Abstract

Probing molecules using perdeuteration (i.e deuteration in which all hydrogen atoms are replaced by deuterium) is extremely useful in a wide range of biophysical techniques. In the case of lipids, the synthesis of the biologically relevant unsaturated perdeuterated lipids is challenging and not usually pursued. In this work, perdeuterated phospholipids and sterols from the yeast *Pichia pastoris* grown in deuterated medium are extracted and analyzed as derivatives by gas chromatography and mass spectrometry respectively. When yeast cells are grown in a deuterated environment, the phospholipid homeostasis is maintained but the fatty acid unsaturation level is modified while the ergosterol synthesis is not affected by the deuterated culture medium. Our results confirm that the production of well defined natural unsaturated perdeuterated lipids is possible and gives also new insights about the process of desaturase enzymes.

Citation: de Ghellinck A, Schaller H, Laux V, Haertlein M, Sferrazza M, et al. (2014) Production and Analysis of Perdeuterated Lipids from *Pichia pastoris* Cells. PLoS ONE 9(4): e92999. doi:10.1371/journal.pone.0092999

- 1110 views in 3 weeks….

Results: Characterisation of multilayers from deuterated lipids (submitted)

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

A neutron diffraction study

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Received: date / Accepted: date

Abstract. The last few decades have seen the development of a strong interest in the structural characterization of model lipid membranes typically composed by a limited variety of synthetic lipids. Membrane properties, like rigidity, permeability, phase diagram, are nowadays intensively studied in many scientific fields. Nevertheless, it is known that synthetic model systems do not reproduce all the structural features of a natural lipid membrane as those are often induced by the synergistic action of several and different constituents of the bilayer. Among the techniques used for bilayer structural characterization an important role is played by neutron scattering. The use of deuterated natural lipid molecules is expected to contribute greatly to the next years advancements in structural studies with neutron scattering techniques. In the present work the first neutron diffraction experiment on fully deuterated lipid extracts from *Pichia pastoris* yeast is presented and the results are compared to those obtained from their hydrogenated analogous. The features of the membrane stacks as the number of *d*-spacings and the derived inter-bilayer distances confirm that large differences exist between model and real systems.

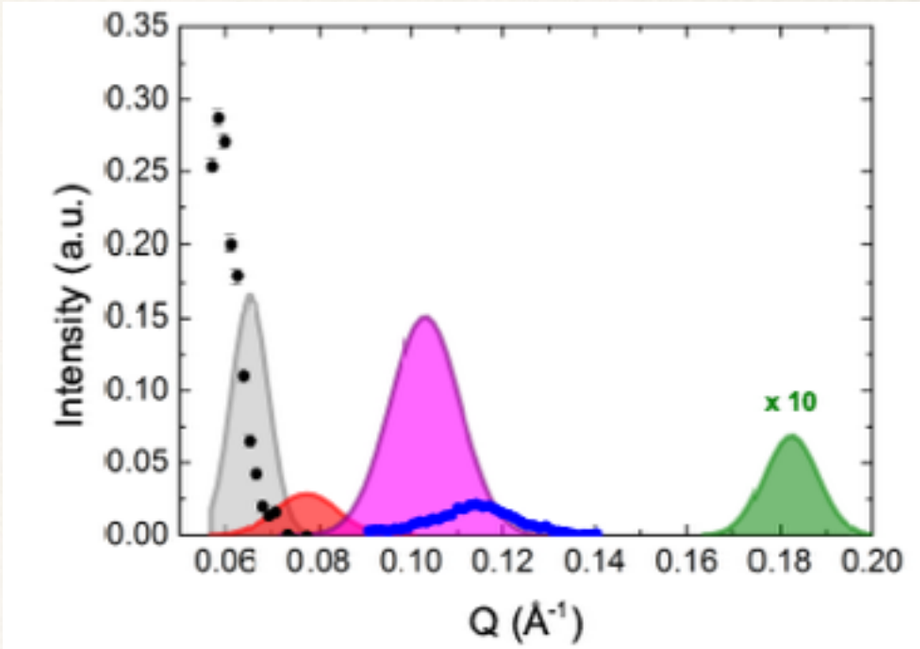


Fig. 4. Comparison of high relative humidity diffraction data collected at 60°C for deuterated (full symbols) and hydrogenated (shaded area) lipid extracts.

Results: Interaction of the natural lipids with an antibiotic (in preparation)

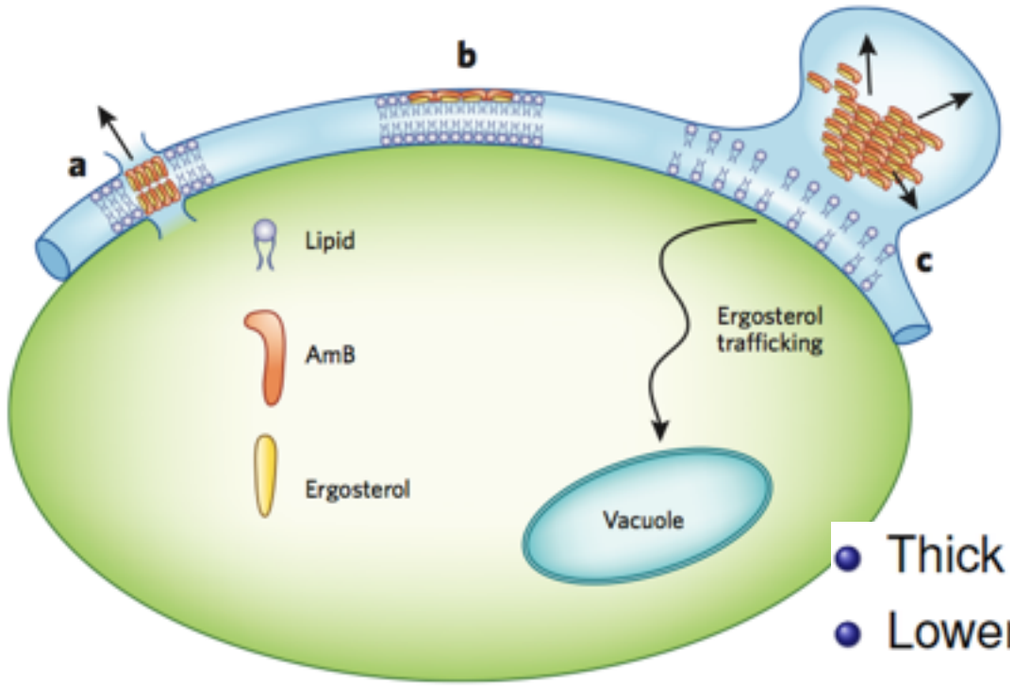
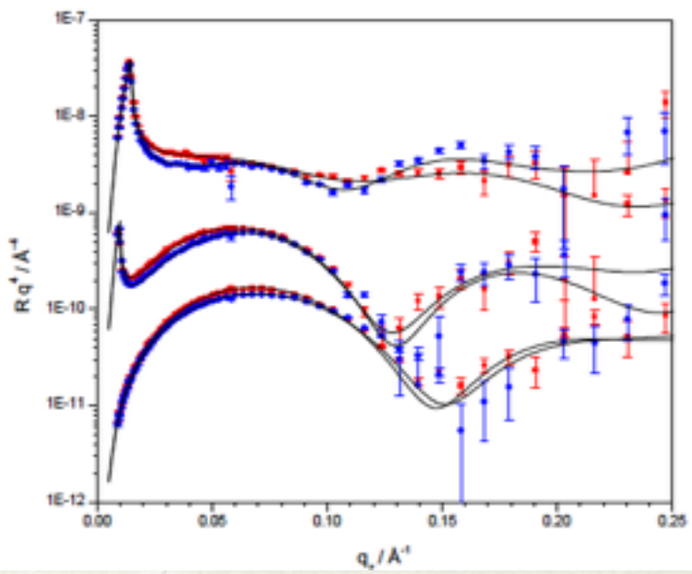
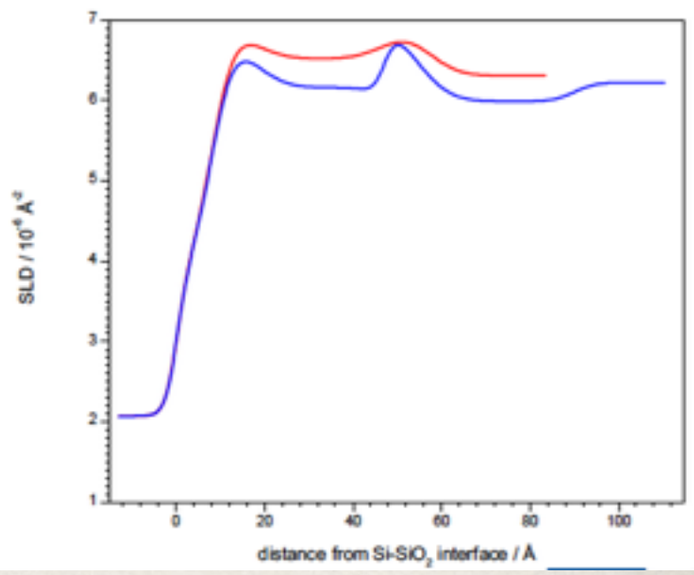


Figure 1 | The antifungal mode of action of AmB. (**a**,**b**) Various models have be
interaction of AmB with the fungal plasma membrane component ergosterol. E
formation (**a**) and surface adsorption (**b**) result in membrane damage. Recentl
extramembranous sterol sponge model—was described by Anderson *et al.*[2]. (**c**
membrane surface and physically extracts ergosterol, which leads to depletion
constituent and eventually membrane disruption. This process may cause addi
membrane protein dysfunction and/or vacuole fragmentation because of chan

- Neutron reflectivity measurements on hydrogenated and deuterated extracts with and without sterols

- Thick and diluted layer on the top of the lipid bilayer
- Lower SLD of the tail region





- Based on these results and in collaboration with ESS, ISIS and other research centres, European grant applications have been made earlier this year or are in progress

Model membranes: study of lipid exchange (out end 2013)

Langmuir

additional featu

Article

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Lipid Exchange and Flip-Flop in Solid Supported Bilayers

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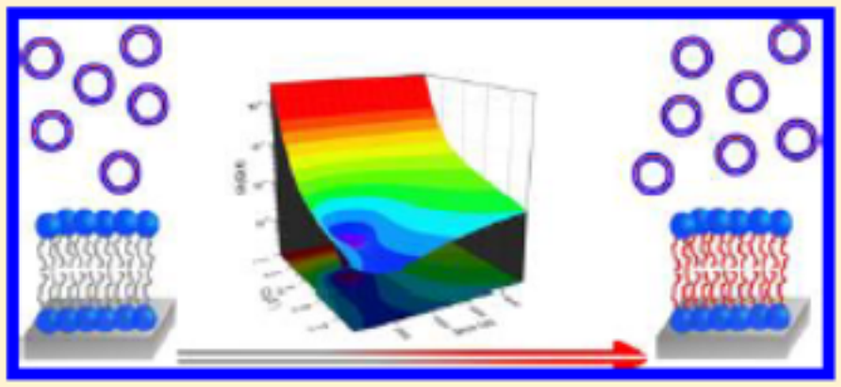
Supporting Information

ABSTRACT: Inter- and intrabilayer transfer of phospholipid molecules was investigated by neutron reflectometry. The structure of solid supported lipid bilayers exposed to a solution of isotopically labeled vesicles was monitored as a function of temperature, time, and vesicle concentration. Lipid interbilayer exchange was shown to be the time limiting process, while lipid intrabilayer movement, the so-called flip-flop, was too fast to be visualized within the experimental acquisition time. The exchange process was characterized by an Arrhenius-like behavior and the activation energy of the process was concentration-independent. The results are discussed and compared extensively with the literature available on the topic.



Model membranes: new analysis tool for floating bilayer (in preparation)

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Journal of Colloid and Interface Science 00 (2014) 1–20

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Investigation of DPPC bilayers internal structure with specular neutron reflectometry☆

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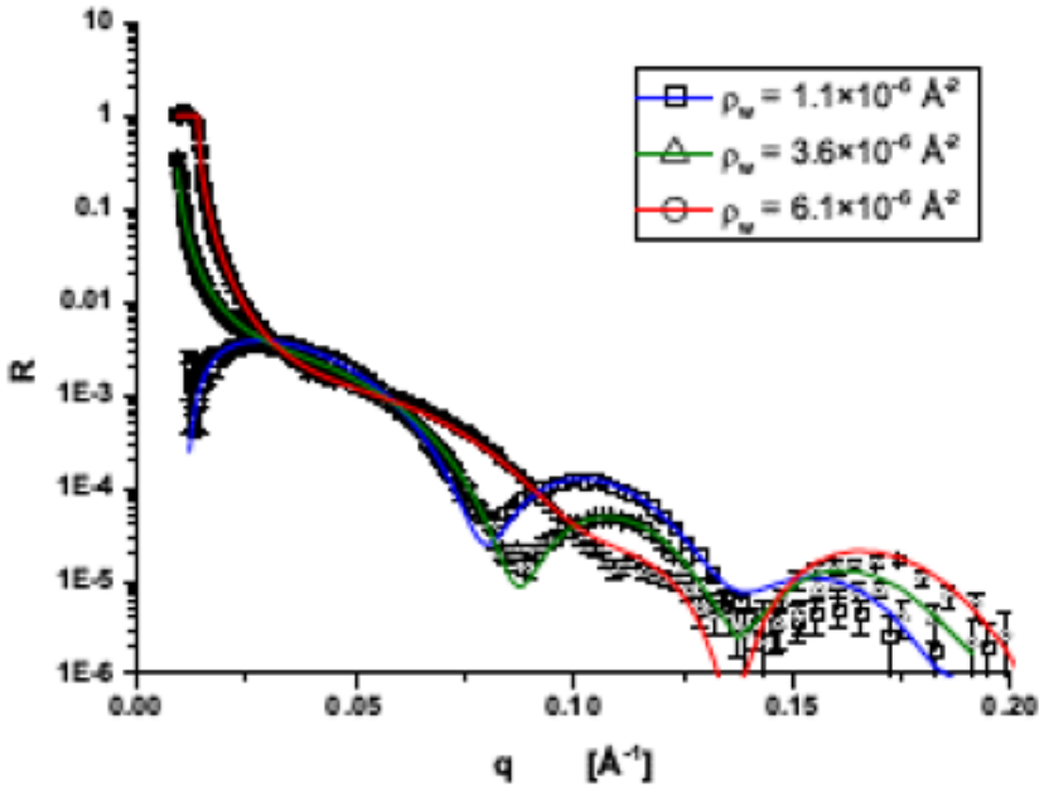
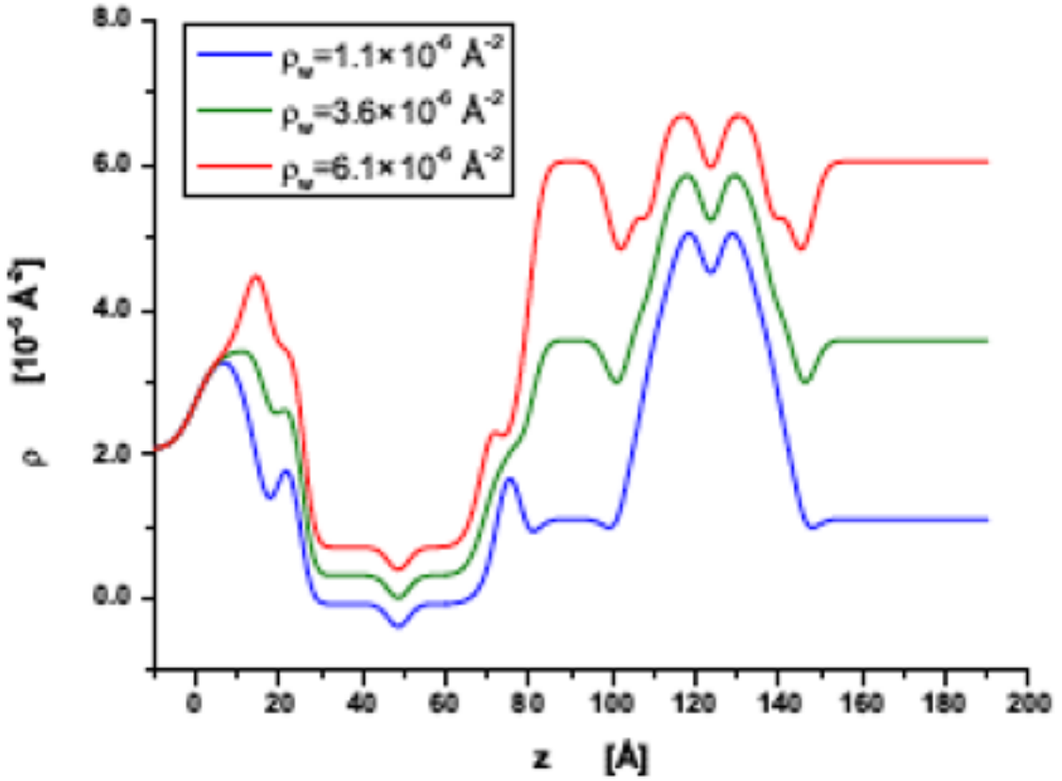
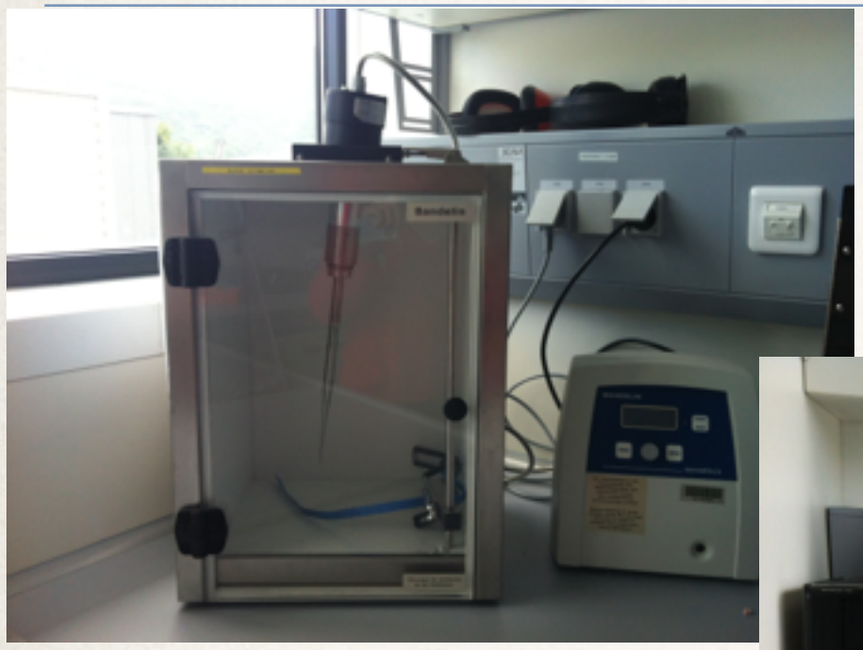
Abstract

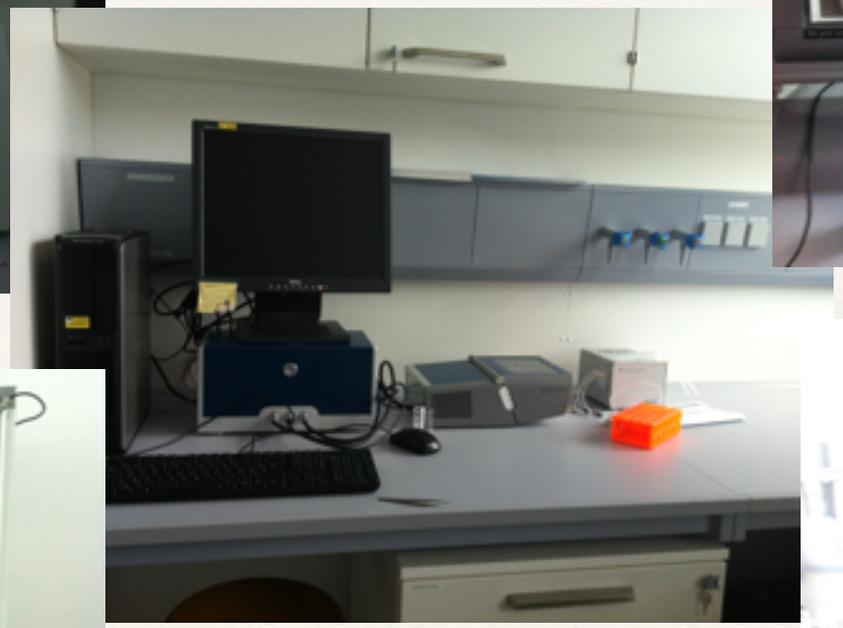
Specular neutron reflectivity was measured on the system of a floating bilayer consisting of 1,2-dipalmitoyl-d62-*sn*-glycero-3-phosphocholine (d62-diC16:0PC) over a 1,2-dibehenoyl-*sn*-glycero-3-phosphocholine (diC22:0PC) bilayer at 25 and 55 °C. The component model of a bilayer was applied for the scattering length density profile and model reflectivity curves were obtained by Parratt's formalism. The model was successfully applied to the supported bilayer in the gel phase and to the floating bilayer in the liquid-crystalline phase. The reflectivity data from the supported bilayer were evaluated individually and were used as an input to the system of a floating bilayer. The obtained structure of the floating d62-diC16:0PC bilayer displays high resemblance to the bilayer structure in the form of unilamellar vesicles, however, simultaneously it shows higher rate of fluctuations in comparison to unilamellar vesicles bilayers.

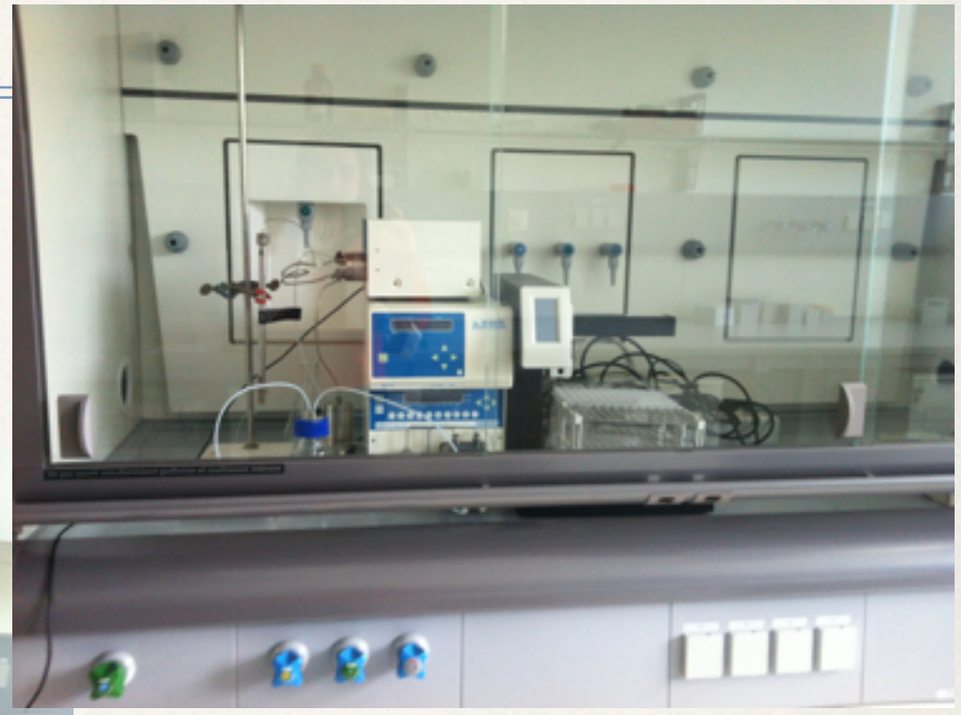


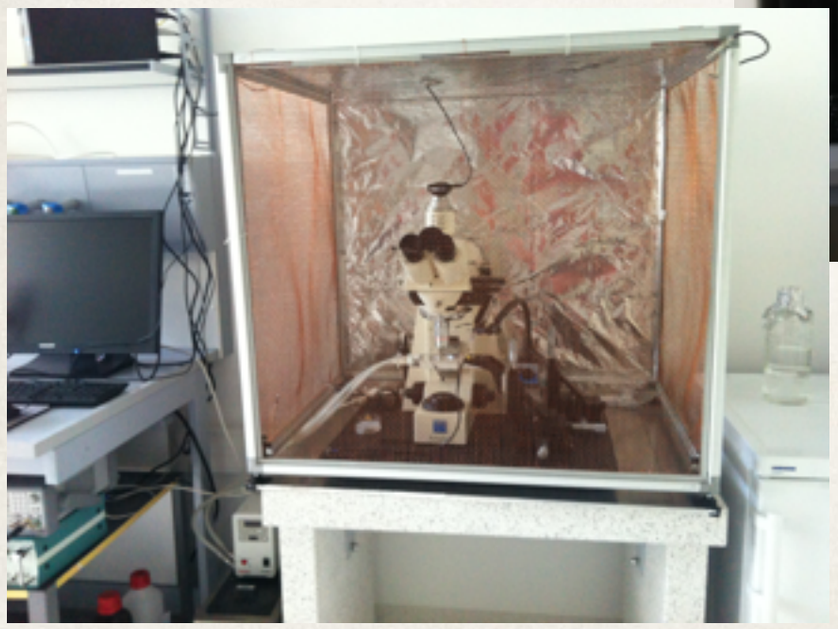


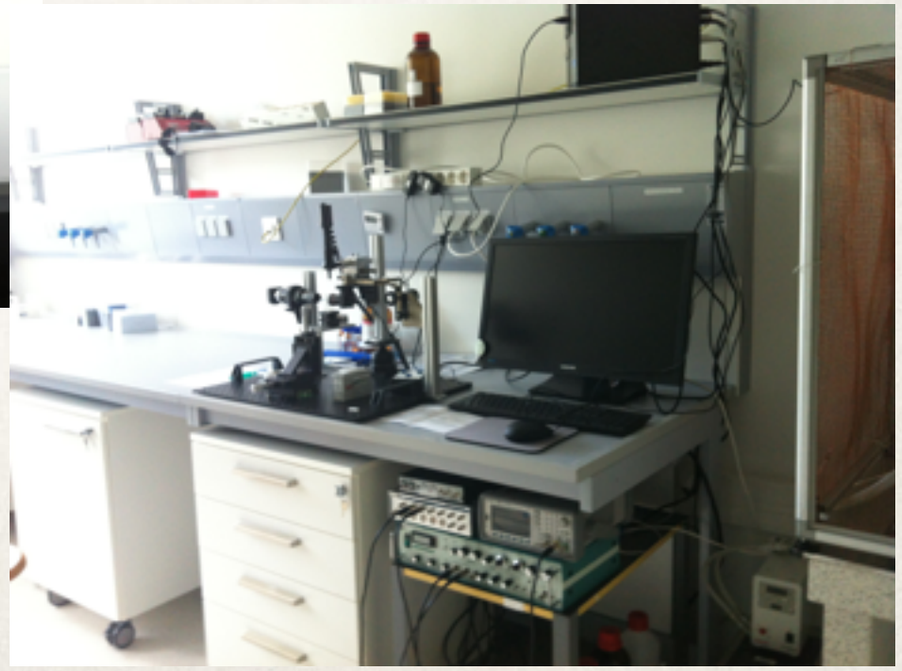
Lab 215 - Lipid Extraction/ characterisation











PSCM
Partnership for Soft Condensed Matter

Clean room 220 - Model Membrane Preparation by LB-LS/tensiometer