Single Model Biomembranes at Solid-Liquid Interface: Comparing Neutron and X-Ray Scattering.

(Do we really need neutrons for such studies?)

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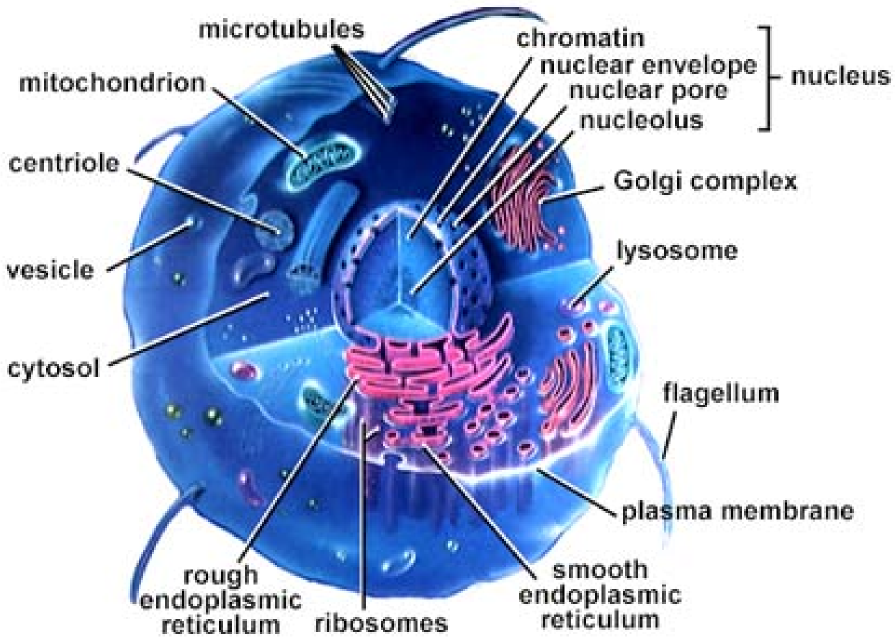
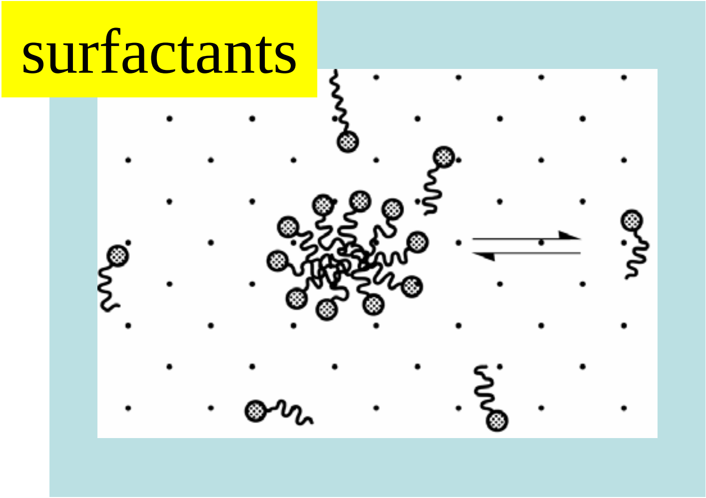
Dhaval Doshi, Erik Watkins - LANL

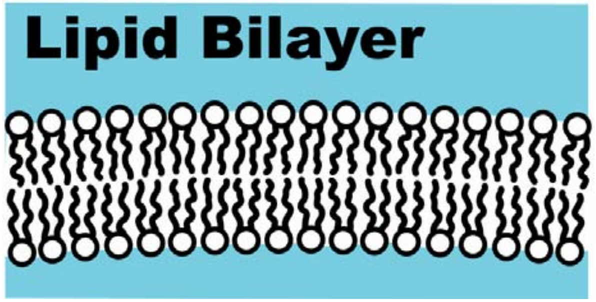
NSN User Meeting, October 13th, 2005

Biophysics of the Cell Membrane



Self-Assembly



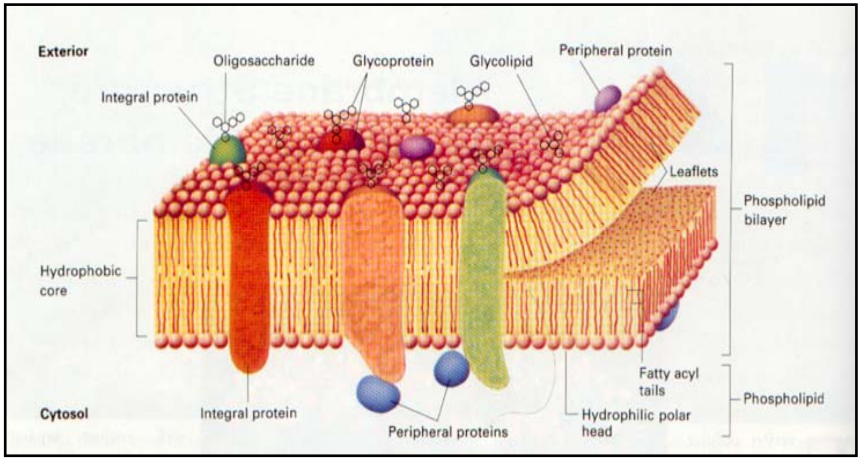


Membranes are where the ACTION takes place!

Outline

Lipid Bilayer Membrane Applications:

- Biosensor platforms
- Protein and receptor presentation
- Cell-surface interactions
- Fundamental membrane biophysics
- Membrane-protein interactions



X-ray/Neutron Reflectometry of Model Membranes at Solid-Liquid Interfaces

- Grazing Incidence X-ray Diffraction

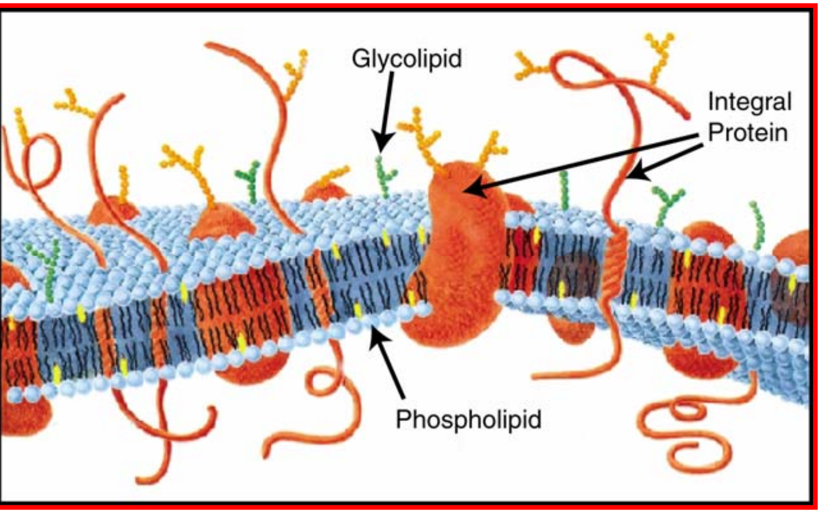
Examples: Neutron and X-ray Reflectivity

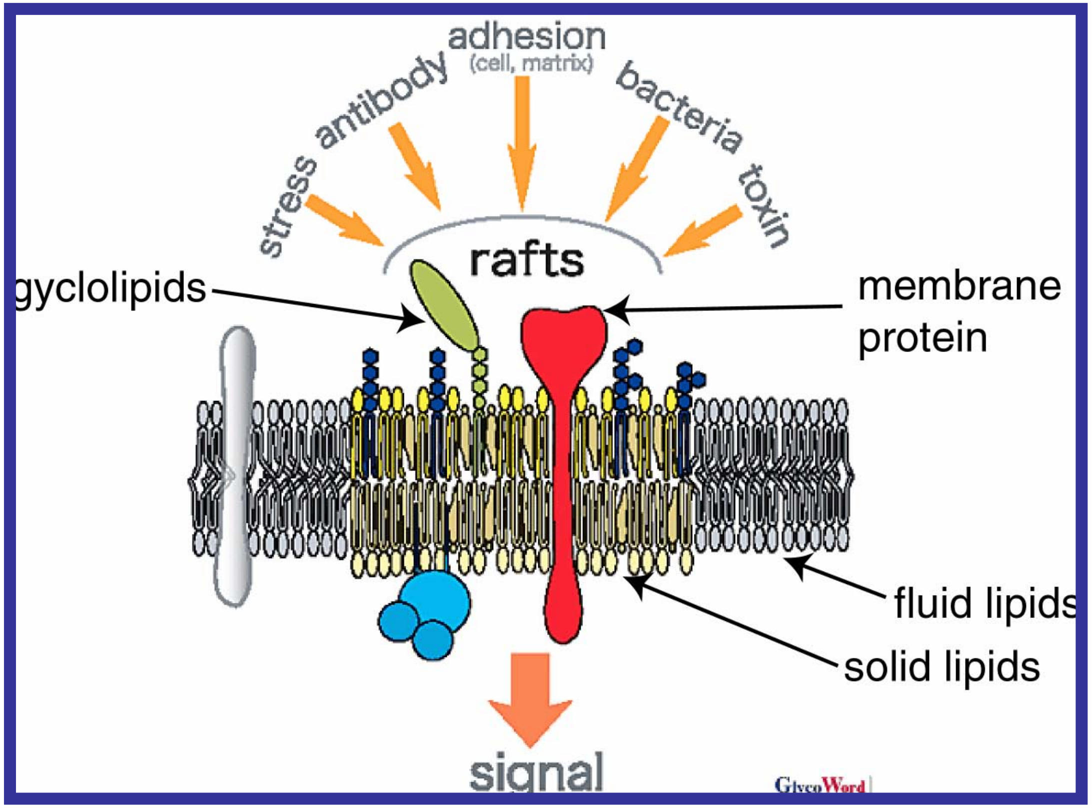
Summary

Homogeneous *vs.* Heterogeneous

Fluid mosaic model *vs.* Lipid domains – “rafts”

Since 2000, over 2500 articles



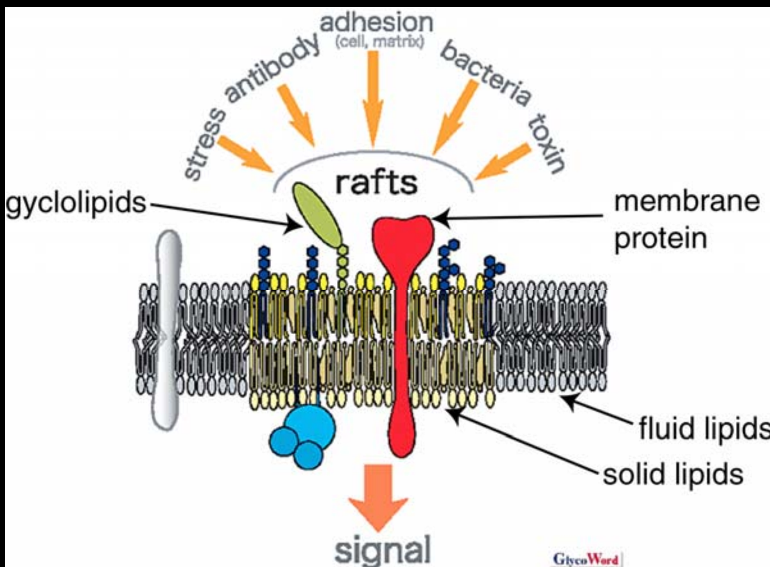


Cell membranes are no longer thought of as simply passive 2-D liquids.

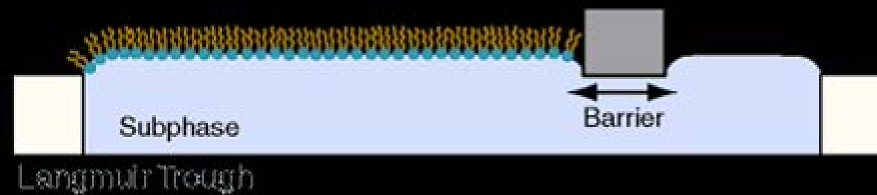
cell polarity, protein trafficking, **signal transduction**

Model Membranes

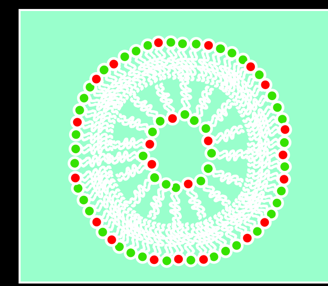
Cellular Membranes



monolayer (water-air interface)

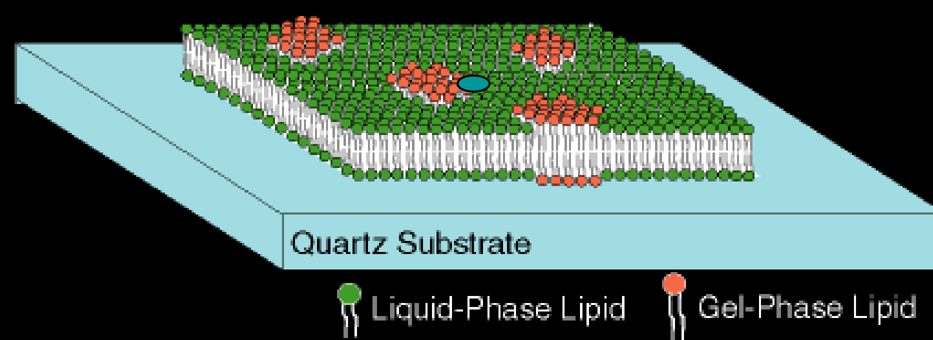


"Model Membranes"



vesicle

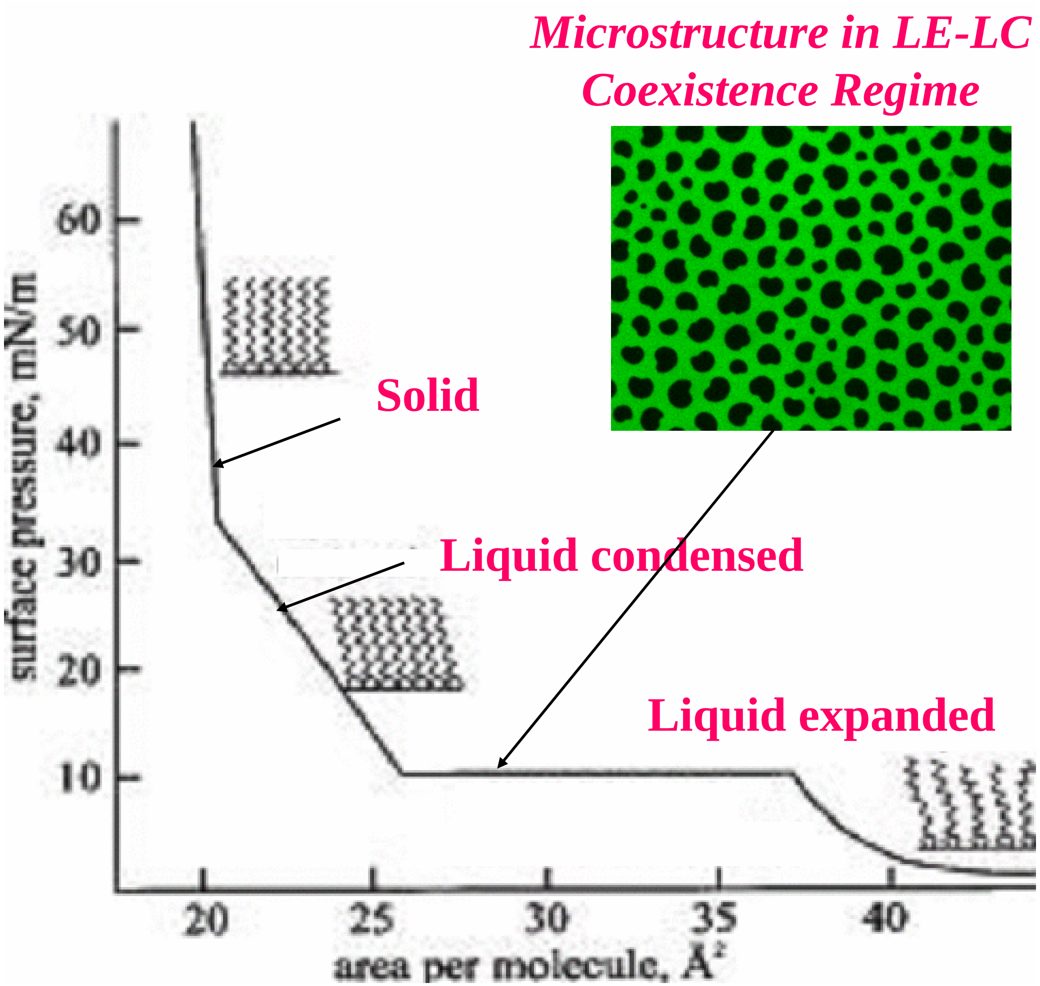
bilayer (solid-water interface)



80% of all Drugs interact with membrane proteins

BIOSENSORS

Liquid Expanded – Liquid Condensed Phase Coexistence



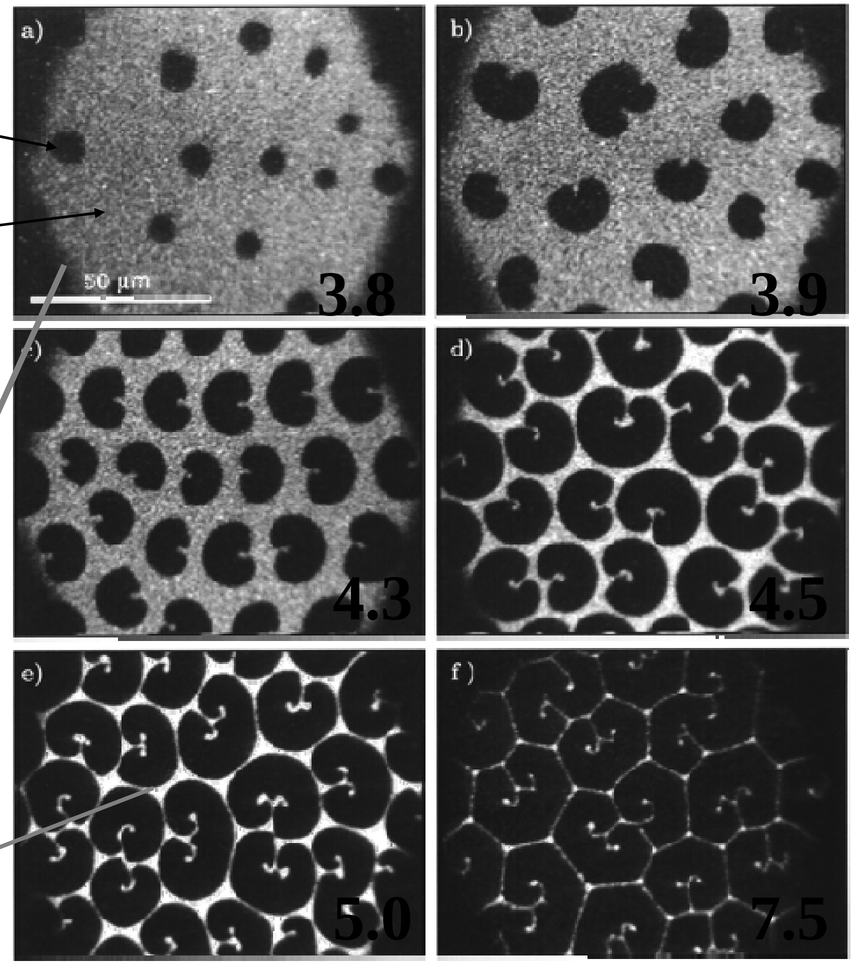
Lateral heterogeneity also involves liquid-liquid immiscibility in the membrane plane.

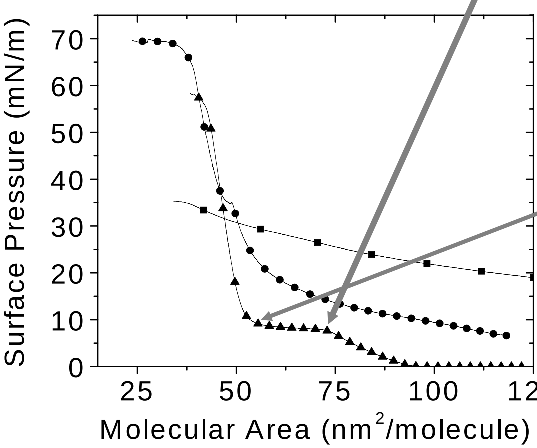
V. M. Kaganer, Review of Modern Physics,Vol 71,779-819 1999

Domain Shape in LE-LC Coexistence Regime

Liquid condensed lipid

Liquid expanded lipid





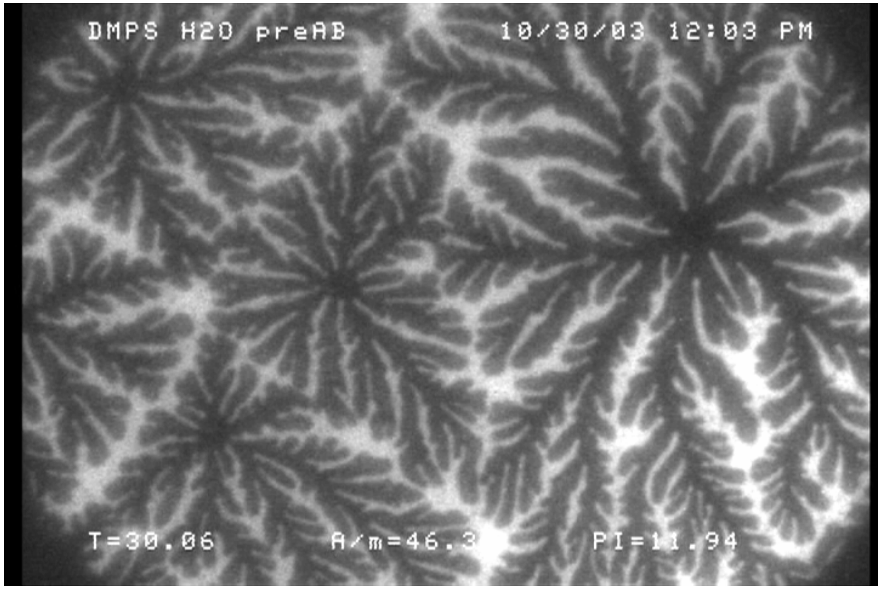
Di-16-PC = DPPC

Cary W. McConlogue

Langmuir 1999, 234-237

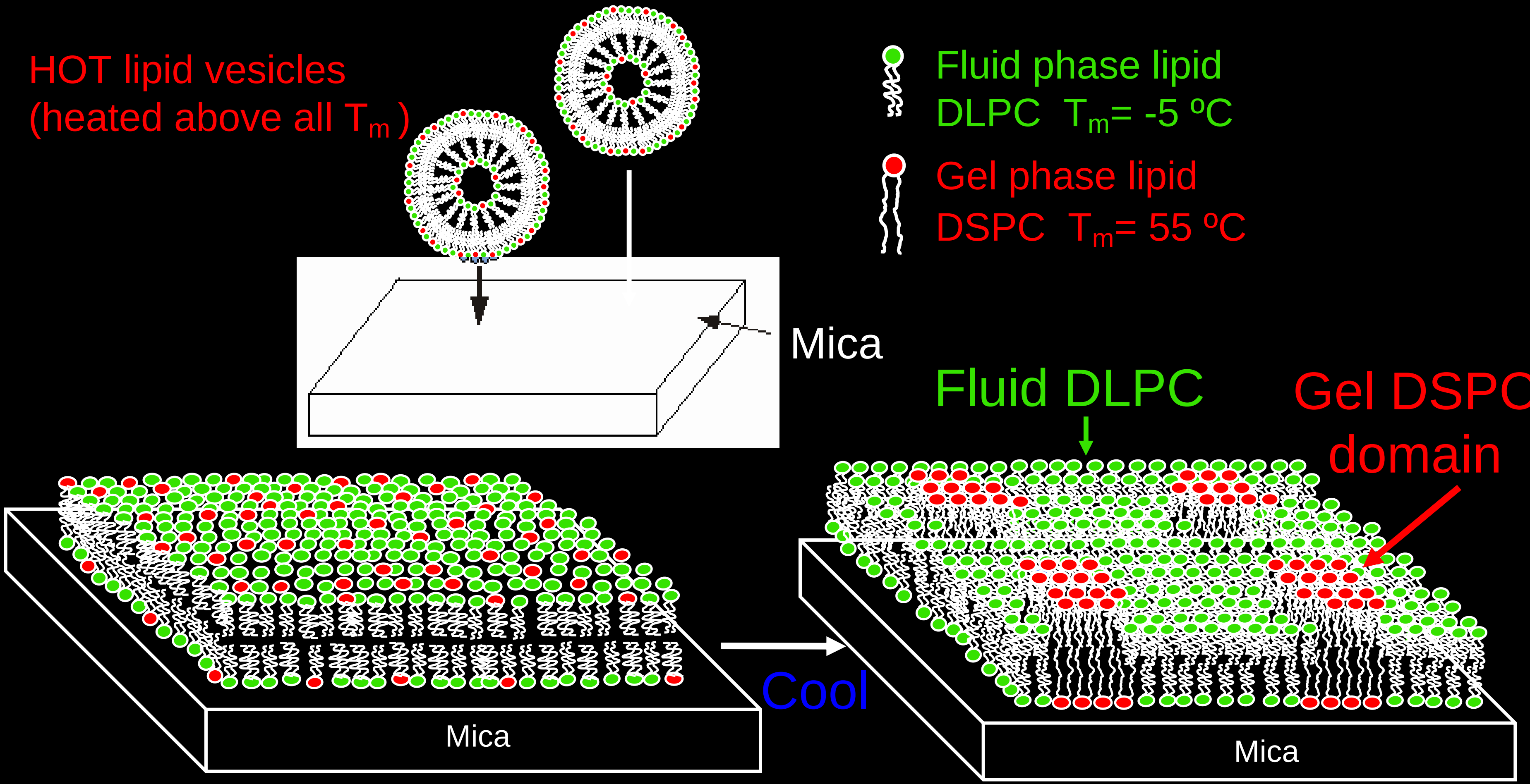
Fluorescence/Brewster Microscopy Image: DMPS lipids

Maximum resolution ~1 μm

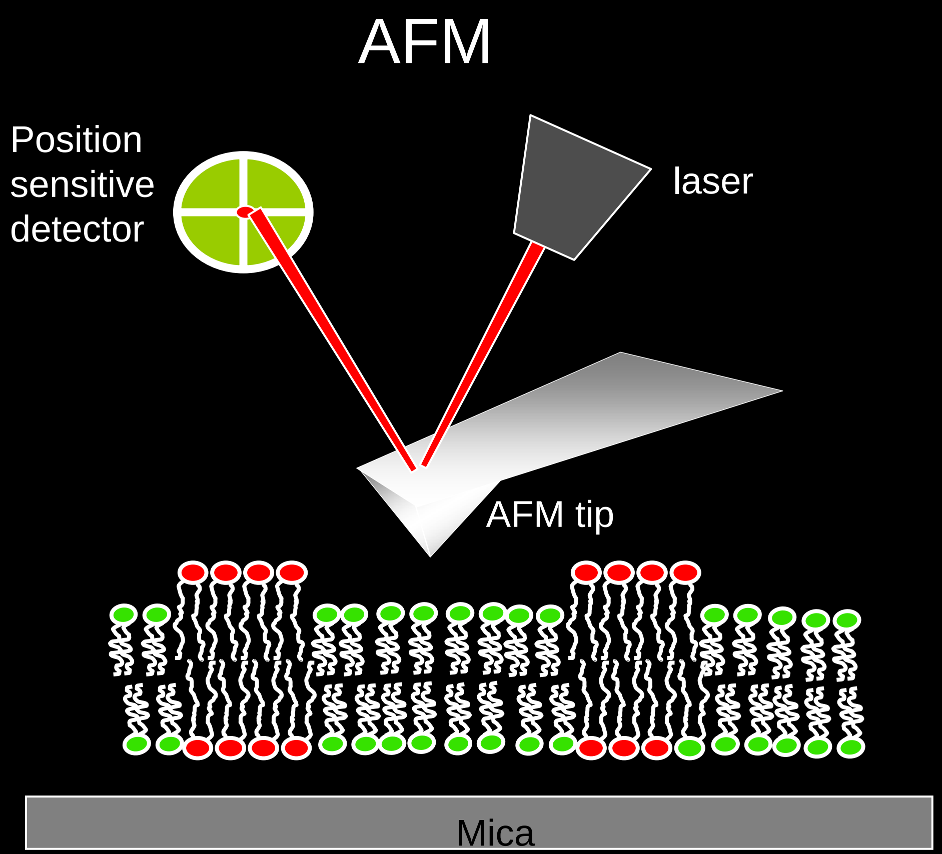


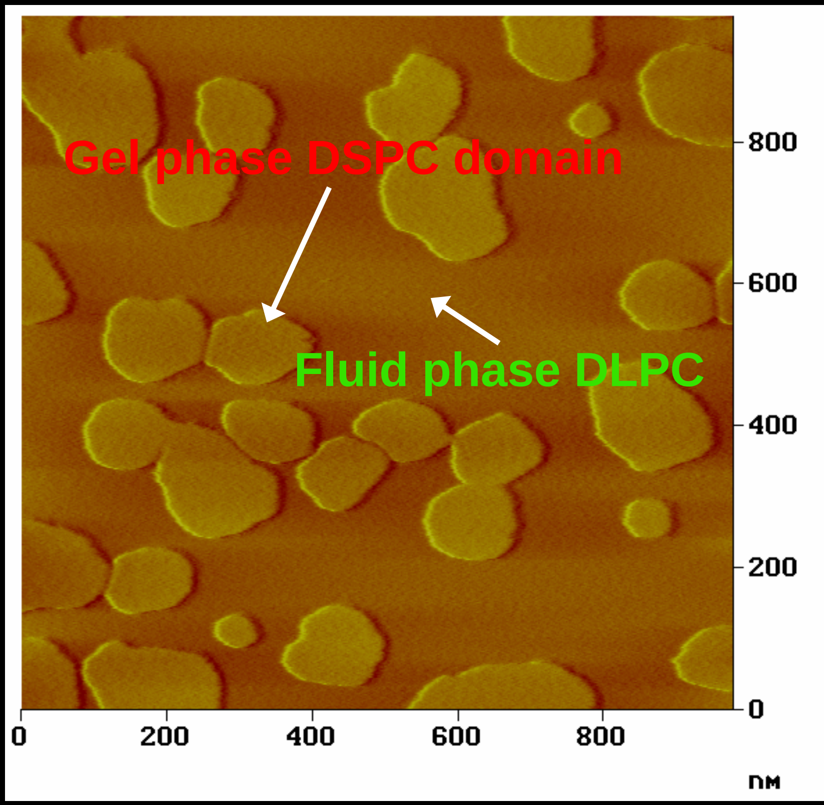
Methods for Studying Lateral Domains

Sample Prep - Quenched Vesicle Fusion



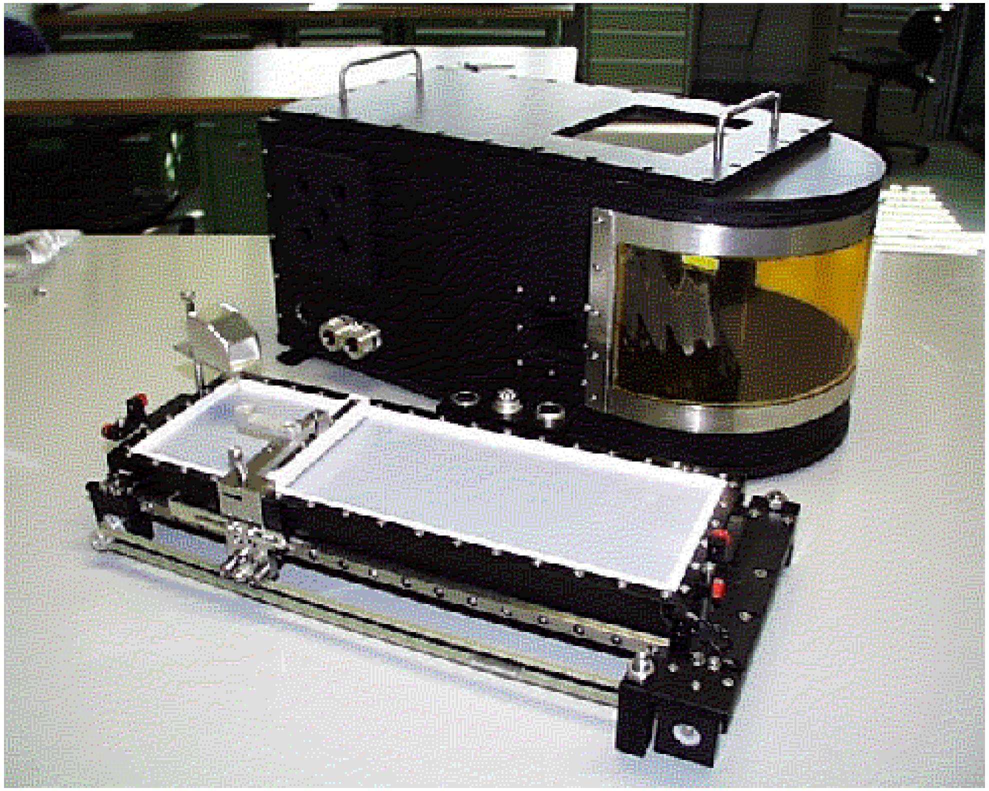
Characterizing Microstructure by AFM





"Obstructed Diffusion in Phase-Separated Supported Lipid Bilayers, A Combined AFM and FRAP Approach", *Biophysical Journal*, Ratto, T. V., Longo, M. L., 2002, 83:3380-3392

Langmuir trough for membranes at liquid-air interface.

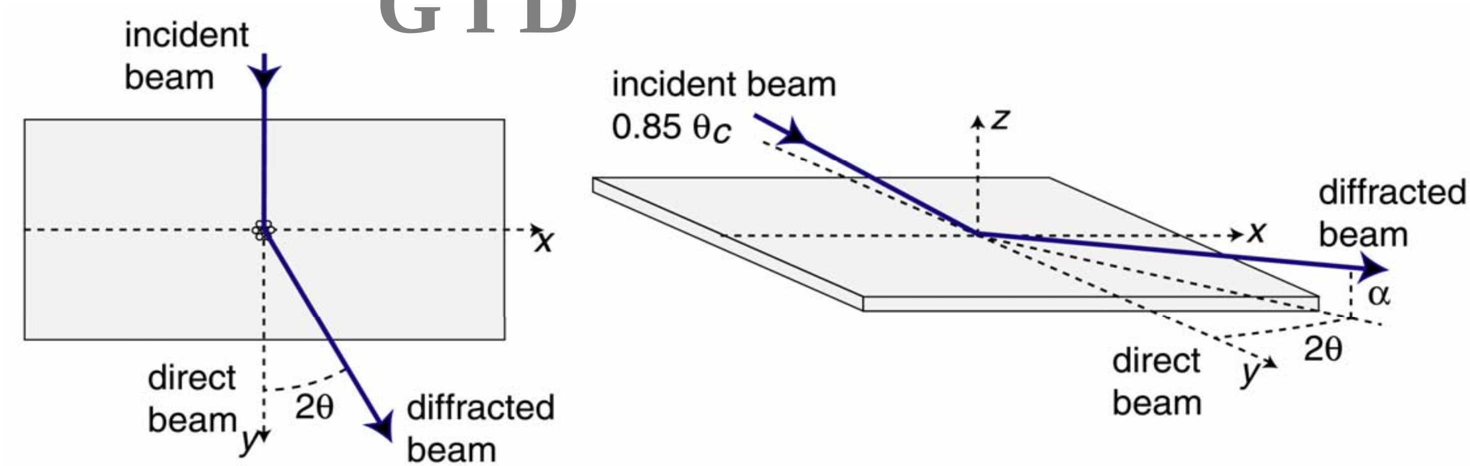


Can be used as independent unit under different enclosure!
Can automatically control pressure, area or temperature,
Oxygen, temperature, pressure (heated!)- sensors and controls,
High compression rate, low liquid subphase volume (<200 ml),
Can be used for NR, XR and GIXD (GIND?) or optical spectroscopy.

Air-Water Interface: Reflectivity and X-ray Grazing Incidence Diffraction

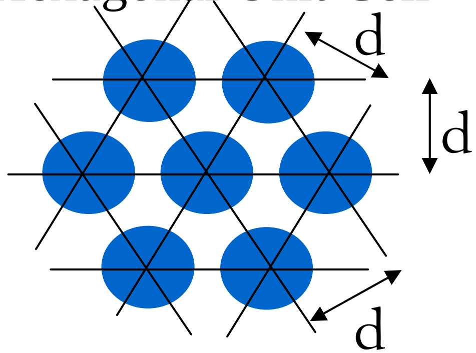
-Reflectivities up to 0.8Å^{-1}(~10^{-10})
- Possibility of measuring GID!

G I D

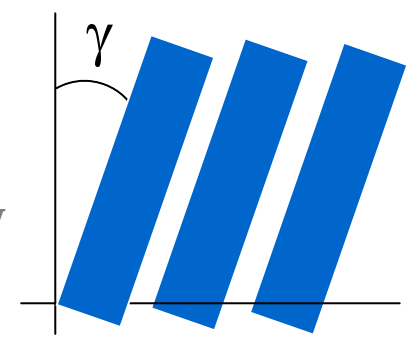


Hexagonal Unit Cell

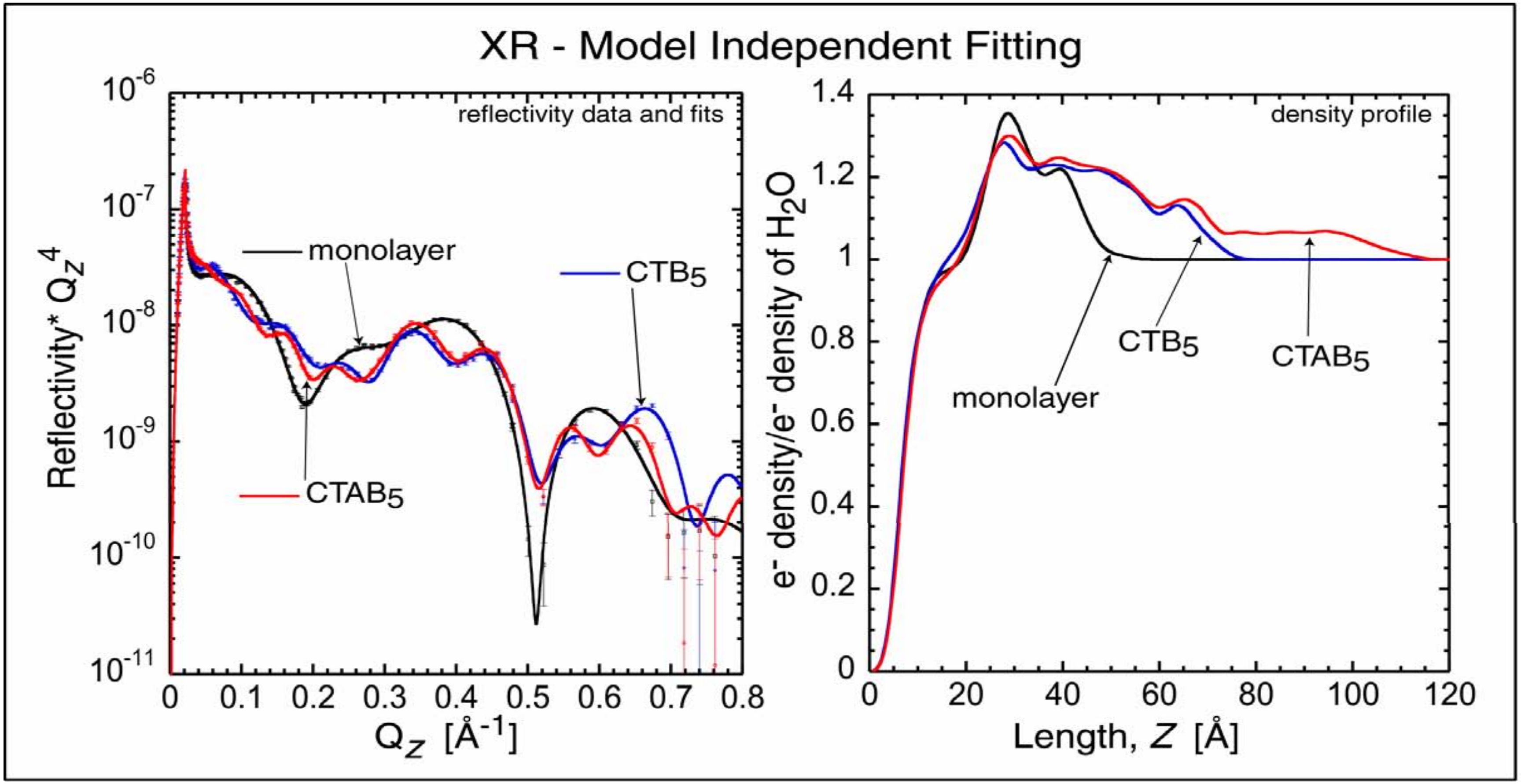
Top View



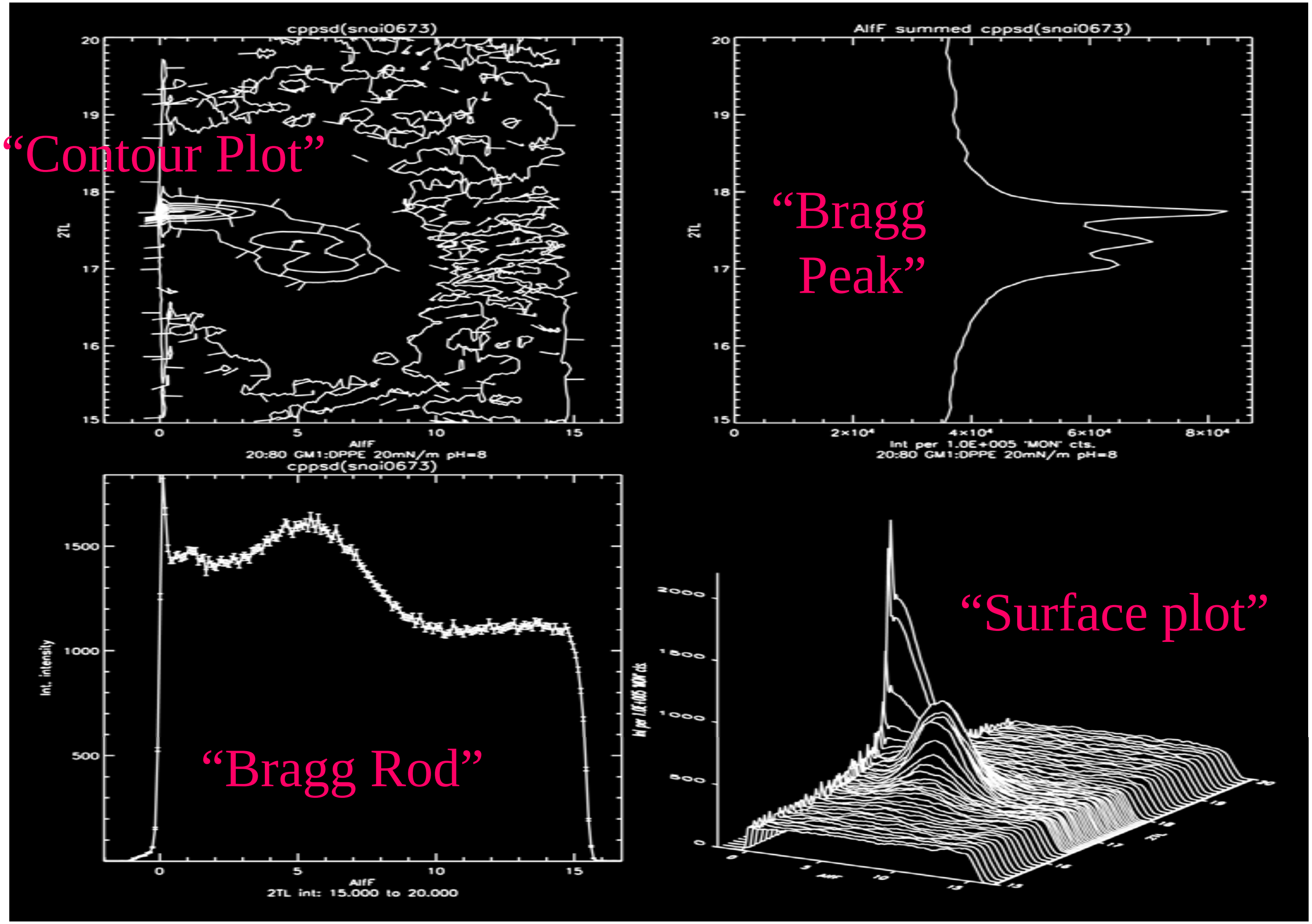
Side View



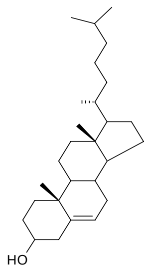
X-ray Reflectivity: Air-Liquid Interface

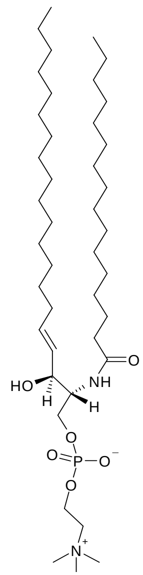


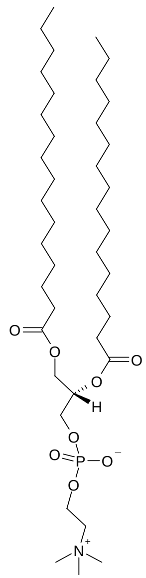
Typical GIXD Data:Air-Water Interface

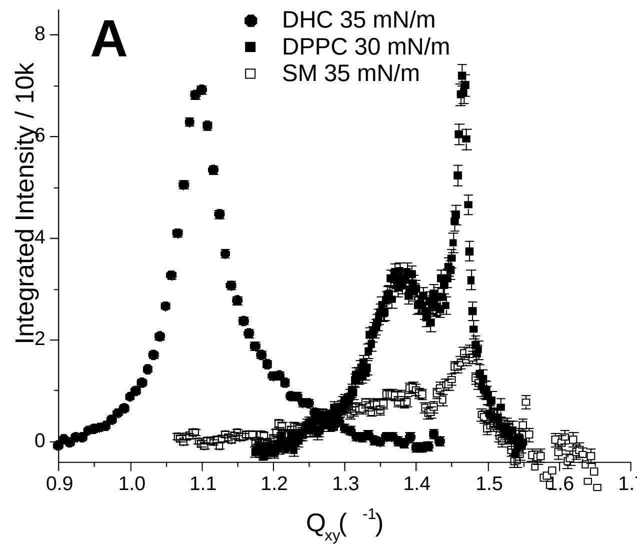


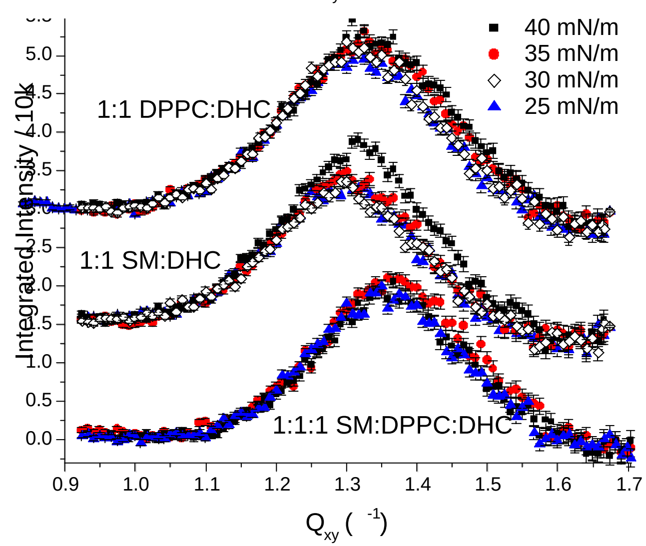
Molecular Rafts: DPPC/SM/Cholesterol







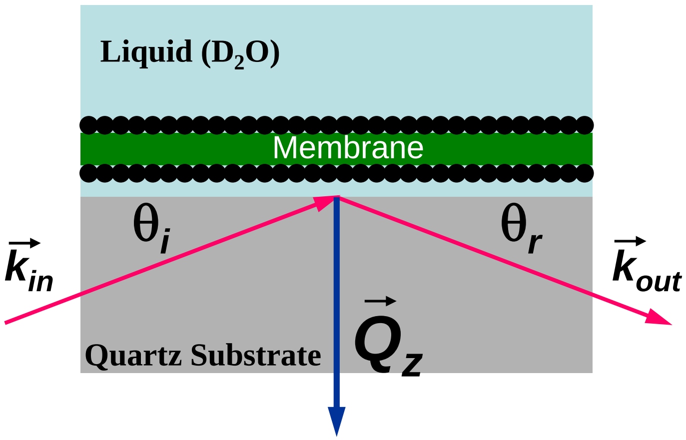




- coherence length ~ 22 Å
- d-spacing ≠ f (surface pressure)
- coherence length ≠ f (surface pressure)
- amt of scattering entities ≠ f (surface pressure)

Neutron Reflectivity

$\theta_i = \theta_r$



Optical analogue

$Q_z = k_{out} - k_{in} = 4\pi \sin\theta / \lambda$

$R = I_{out}/I_{in}$

$n = 1 - \lambda^2\beta/2\pi$

β - scattering length density (SLD) of material

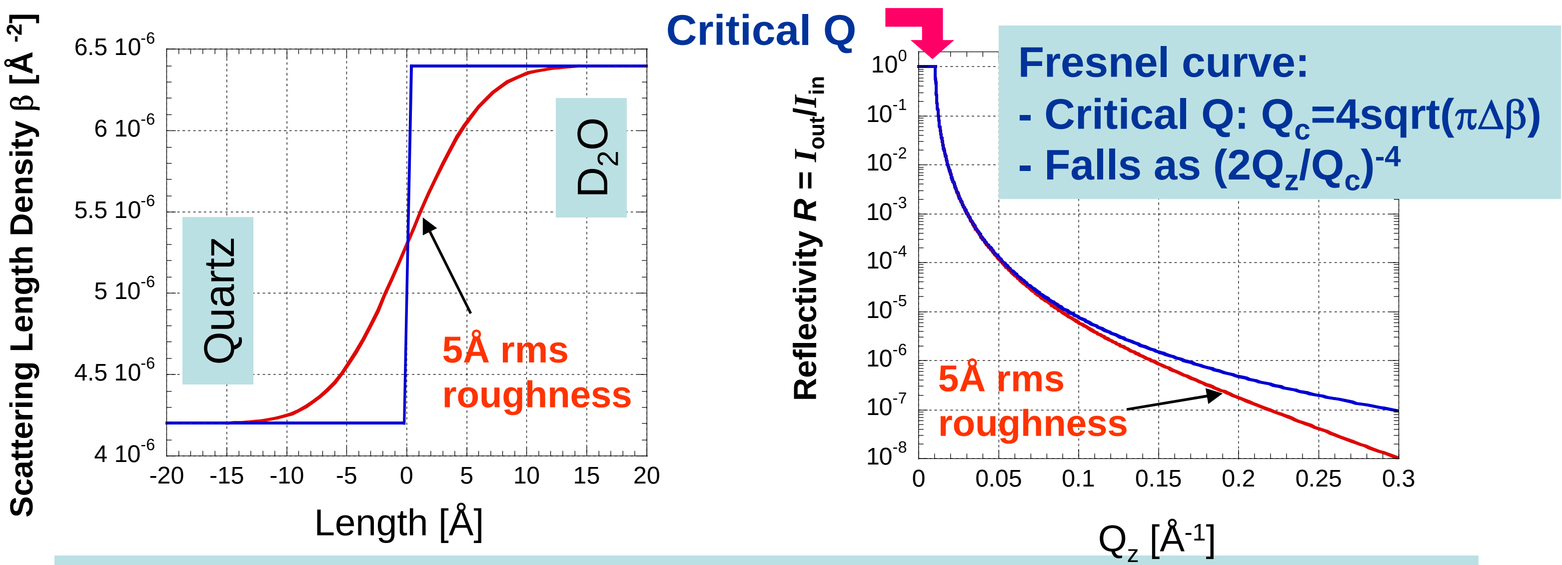
<u>Measures:</u>
average density structure
normal to the interface.
-thickness, density, roughness

<u>Advantages</u>:
Sensitivity to light elements
Deuteration ⇒ Contrast
Buried interfaces (low absorption)
Non-destructive

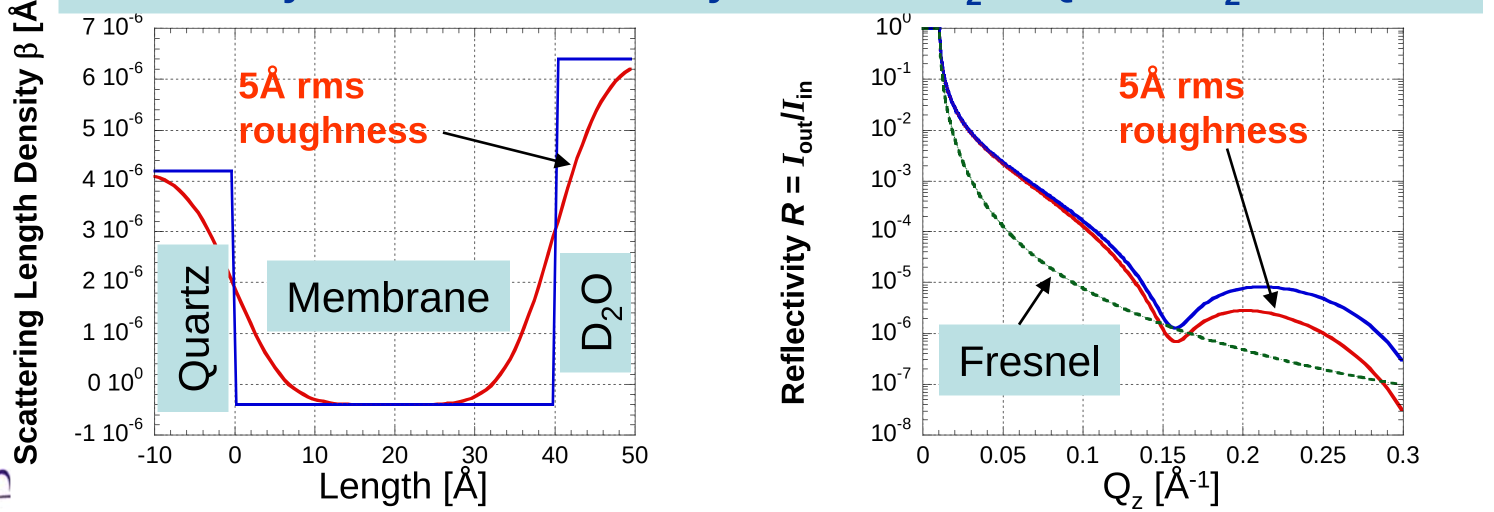
<u>Disadvantages</u>: requires big flat surfaces due to small neutron fluxes

Simple Examples (Neutrons)

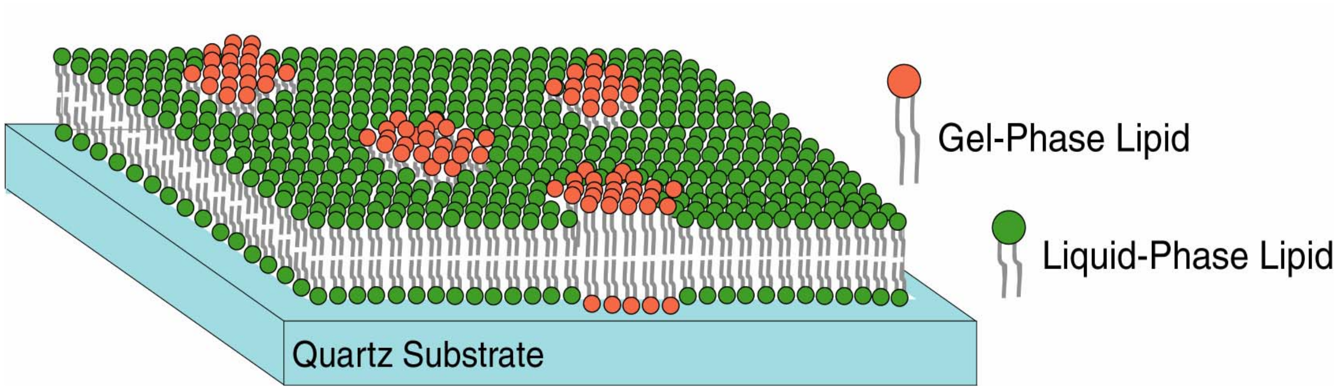
Reflectivity from Step-Like Interface (Quartz-D_2O)



Reflectivity from 40Å of Densely Packed CH_2 at Quartz-D_2O Interface

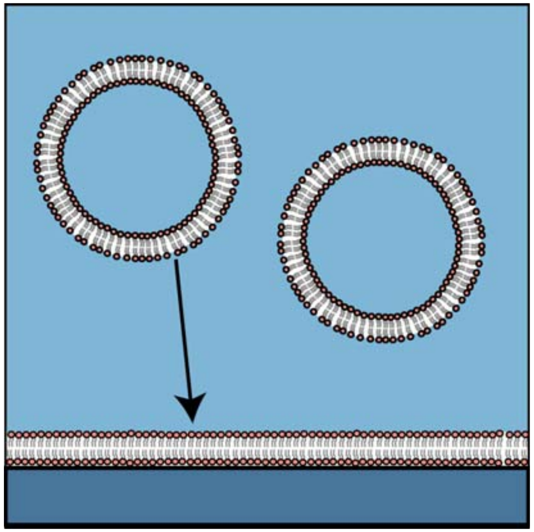


Lipid Bilayers at the Solid-Liquid Interface

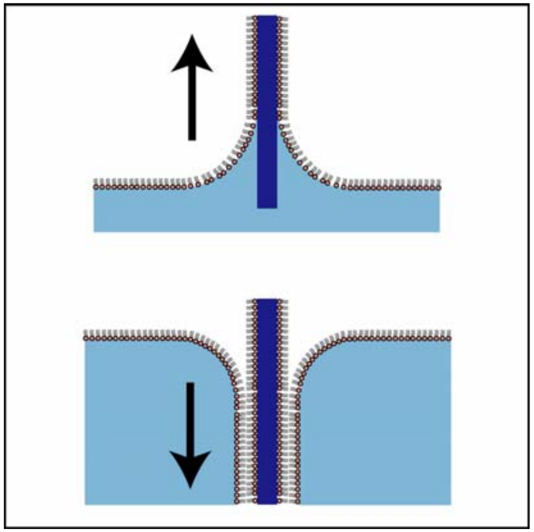


- Provide excellent models for studying membrane assembly (Boxer, 2000; Sackmann, 1996)

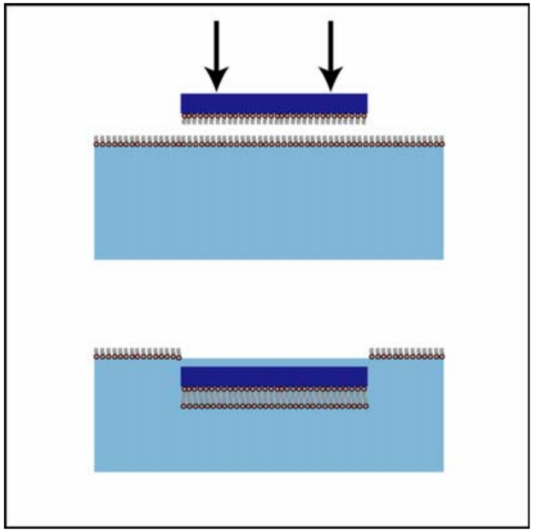
Vesicle Fusion



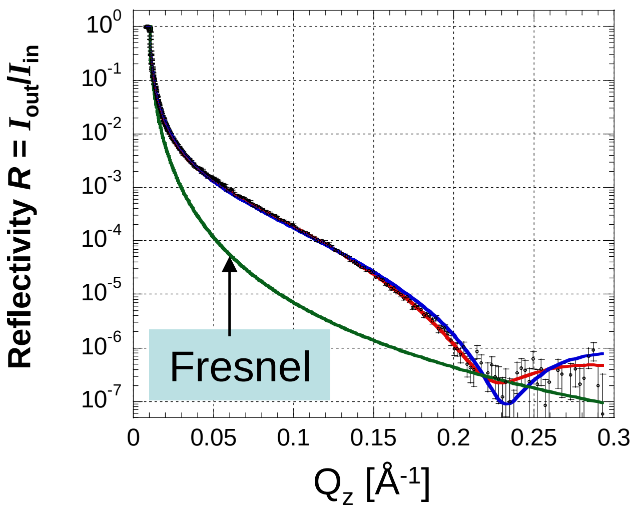
Langmuir-Blodgett (LB)

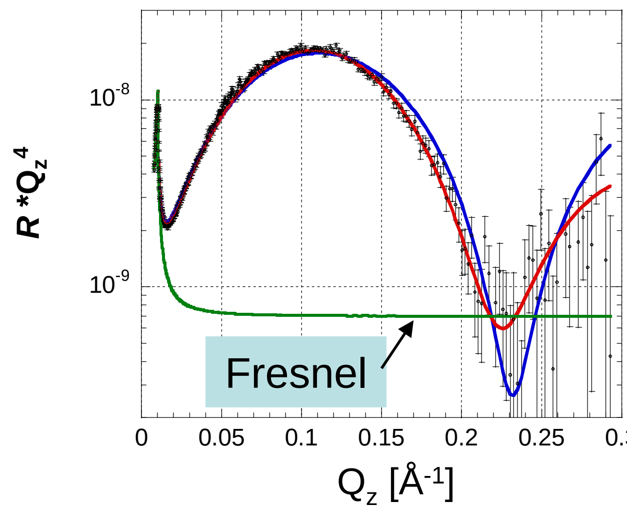


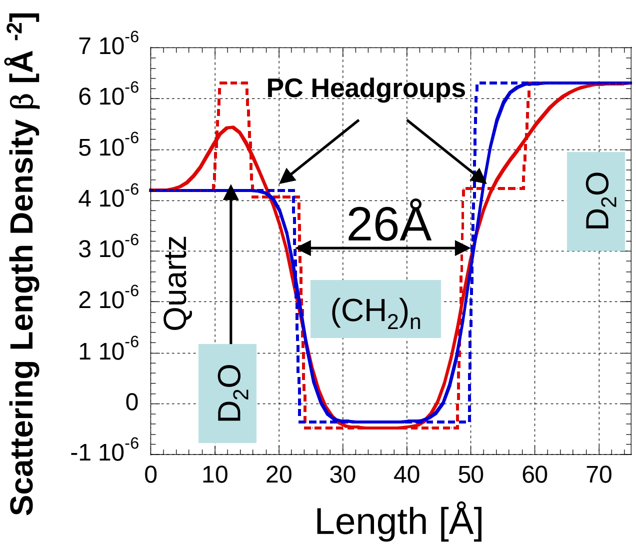
Langmuir-Schaffer (LS)



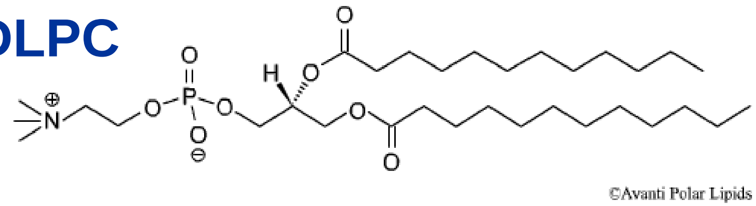
A Typical Neutron Reflectivity of a Model Membrane: DLPC on D_2O -Quartz Interface. Box Model Fits





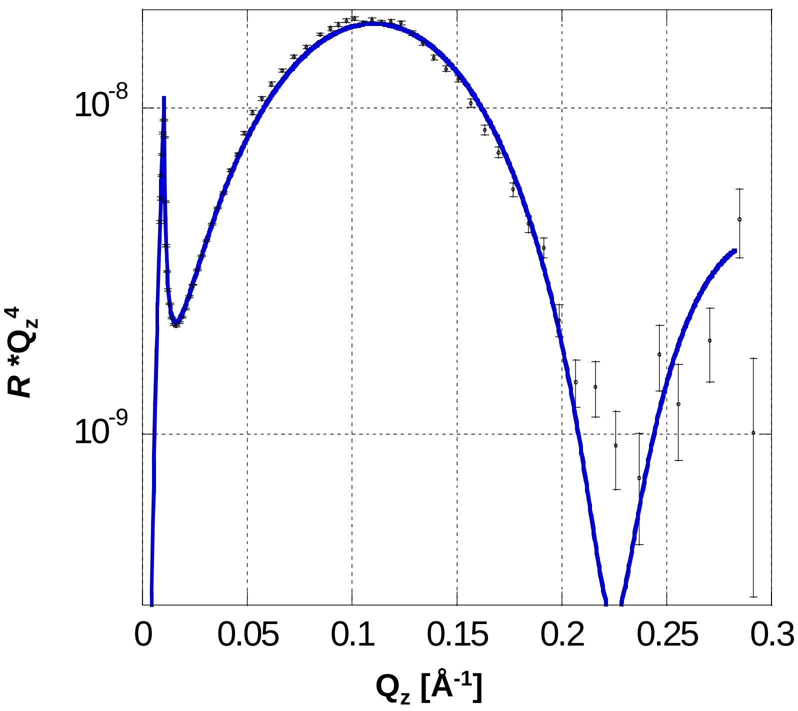


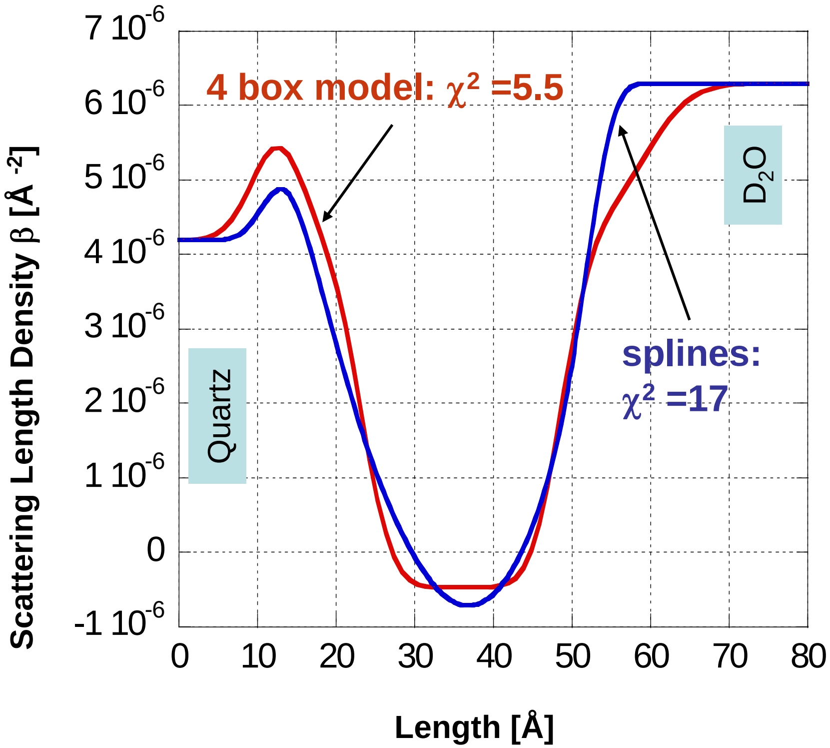
DLPC



Two models:
Simple one box for hydrocarbon tails: χ^2=7.8
Complicated (but structurally relevant)
4 box model: χ^2 =5.5

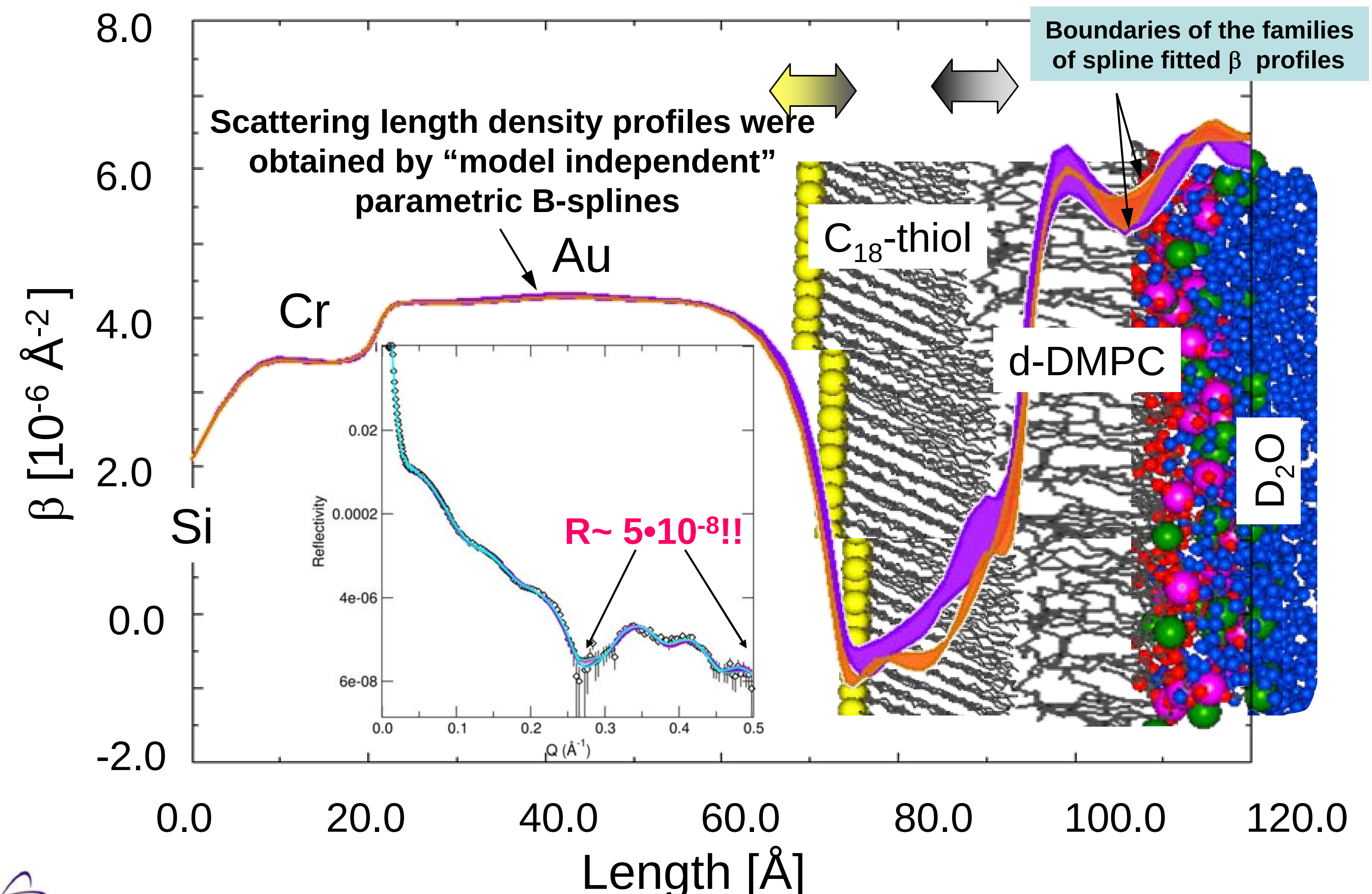
Model Membrane: DLPC on D_2O-Quartz Interface
Model Independent (Free Form) B-Spline* *vs*. Box Fit





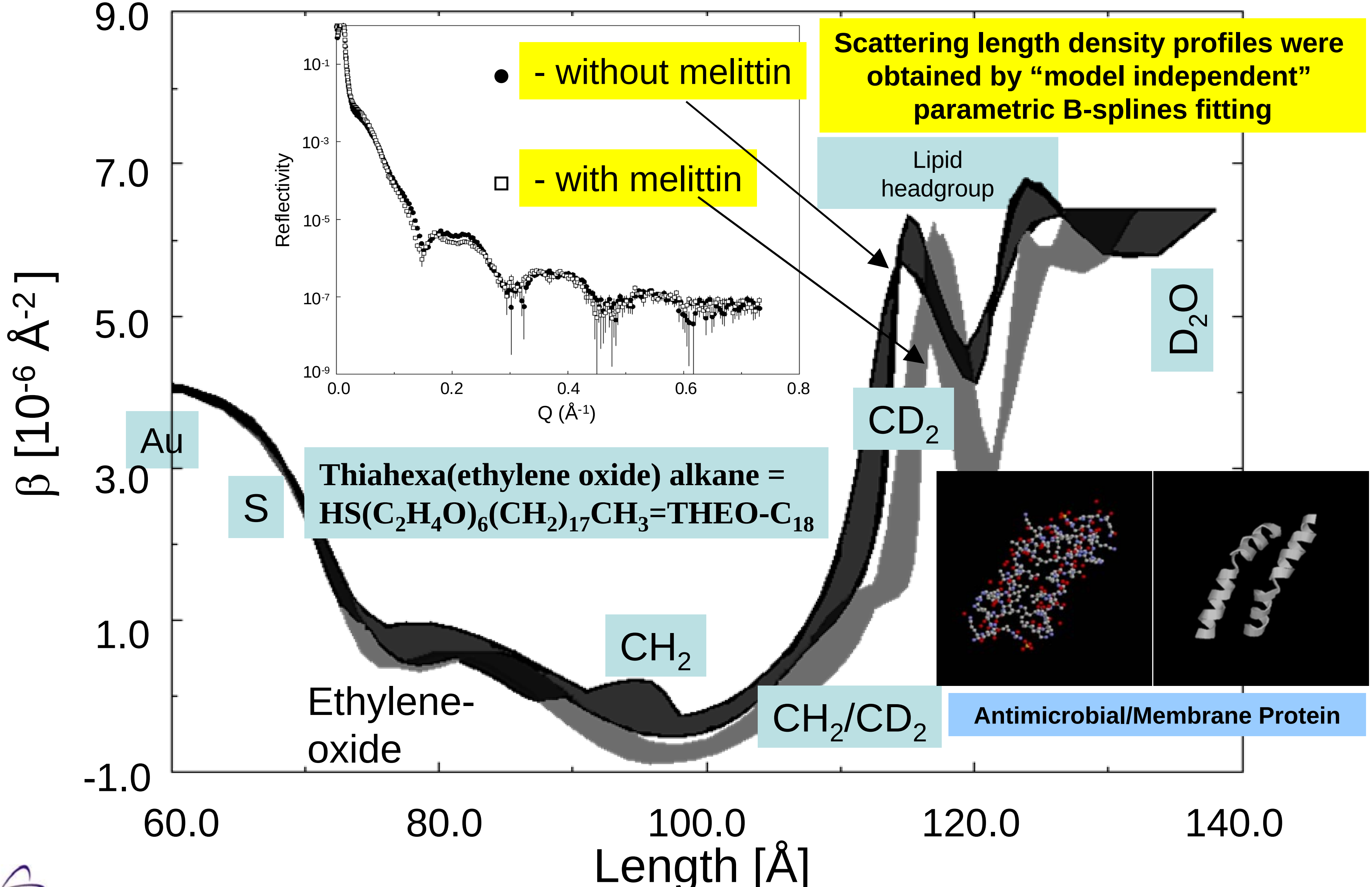
Cubic b-splines ftting:
- J.S. Pedersen and I. W. Hamley, *J. Appl. Crystallogr*. **1994**, *27*, 36.
- N. F. Berk and C.F. Majkrzak, *Phys. Rev. B.* **1995**, *51*, 11296.

Example: Neutron Reflectivity of Hybrid Bilayer Membrane (Octadecanethiol/d-DPPC) on Gold: S. Kruger *et al*, *Langmuir*, 2001, 17, 511-521.



Example: Neutron Reflectivity of Hybrid Bilayer Membrane (THEO-C_{18}/d-DMPC) on Gold Interacting with Melittin Protein : S. Kruger *et al*, *Langmuir*, 2001, 17, 511-521.

Scattering length density profiles were obtained by "model independent" parametric B-splines fitting

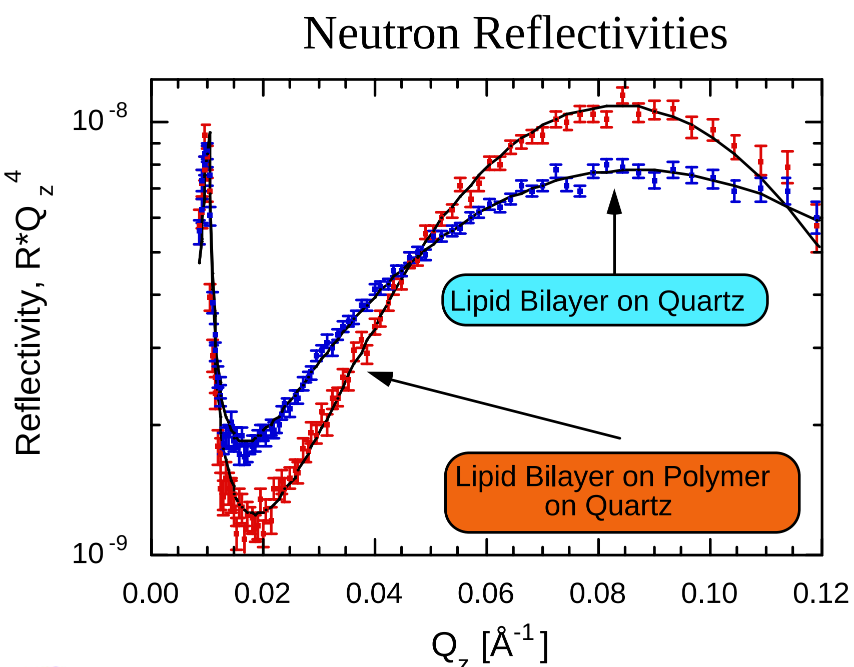


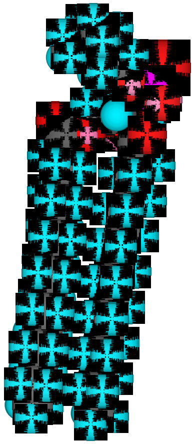
Antimicrobial/Membrane Protein

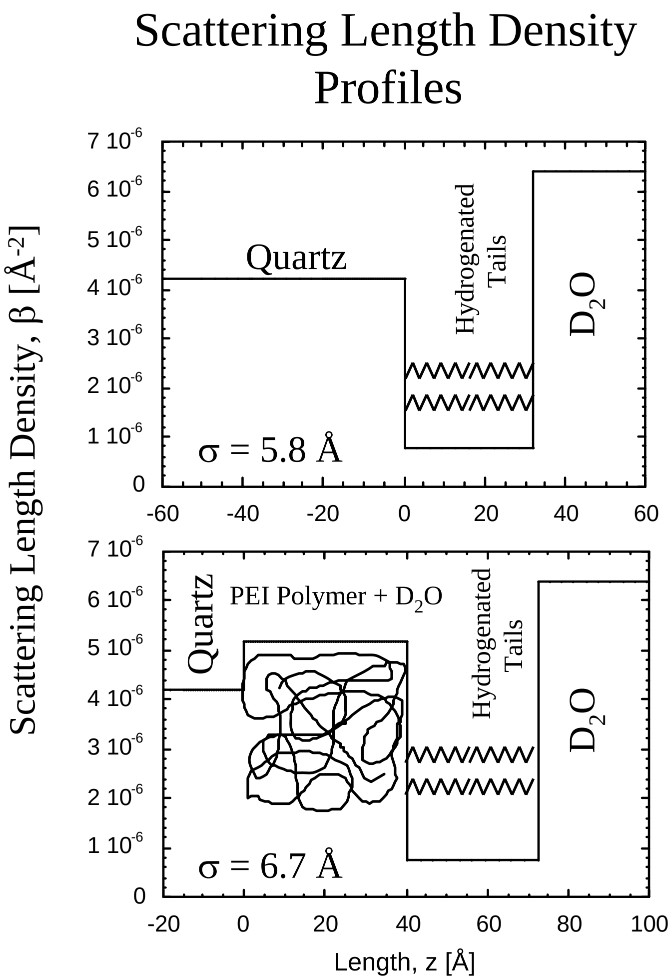
Example: Neutron Reflectometry of Model Bio-Membrane With and Without a Polymeric Cushion Layer

1. DMPC bilayer obtained by vesicle fusion on the pure, uncoated quartz block
2. PEI polymer was added after quartz was covered by the lipid bilayer
3 PEI appeared to diffuse under the membrane!

Even with v. limited Q_z range (0.12Å^{-1}) we can obtain:
- position, thickness and hydration of polymer
- roughness
- membrane thickness and coverage



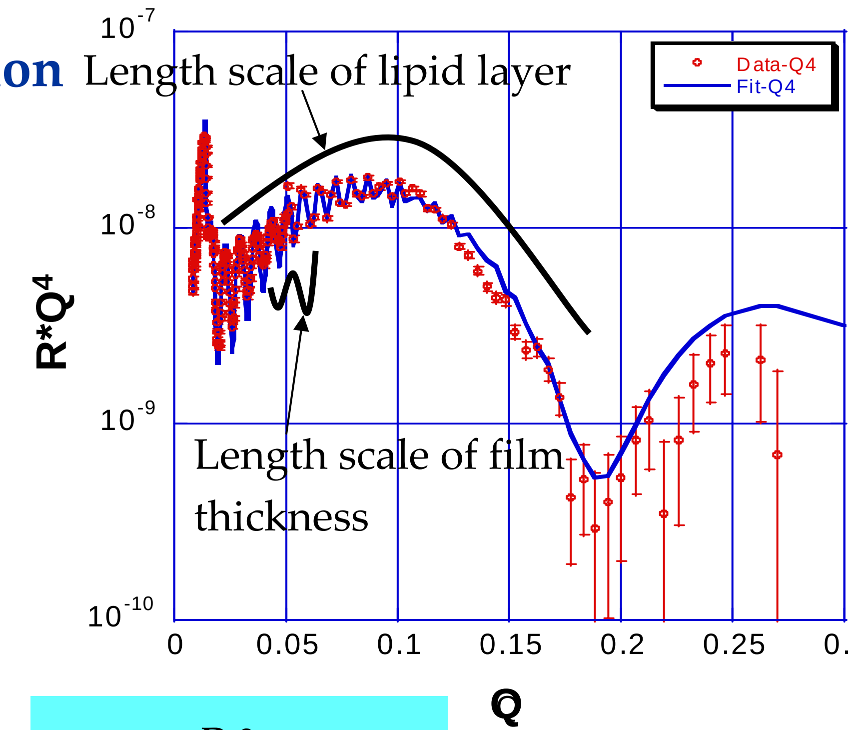


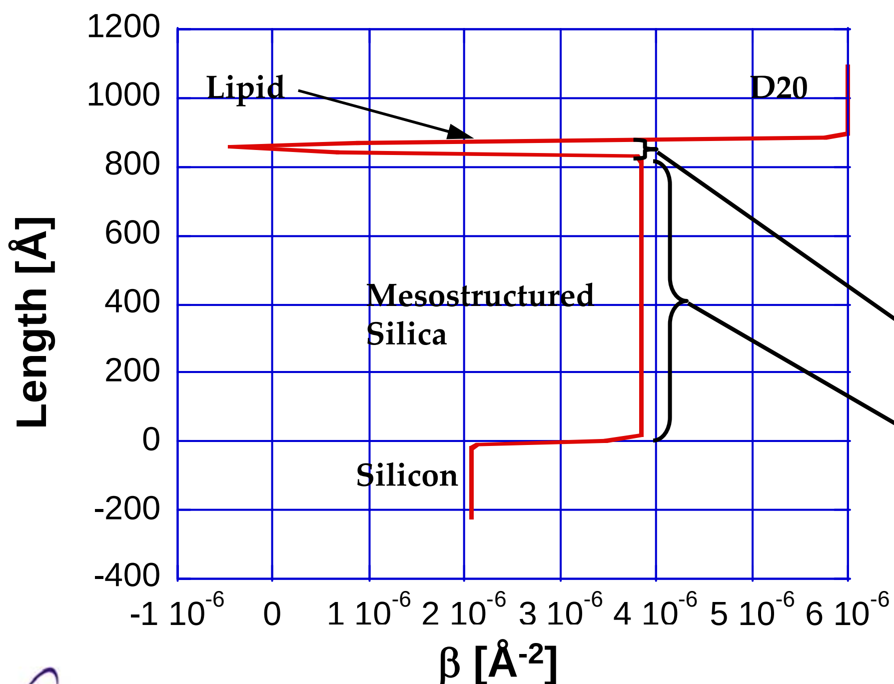


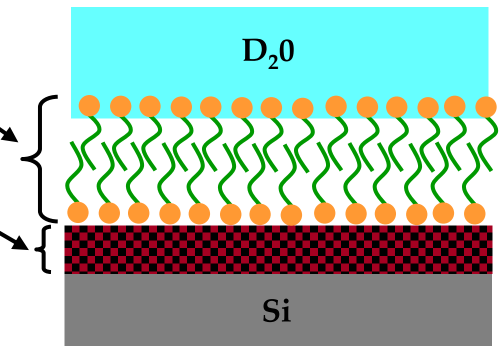
Example: Neutron Reflectivity of Single Lipid Membrane on Ordered Nano-Composite and Nanoporous Silica Thin Films

POPC membrane formed by vesicle fusion

Layers	Thickness (Å)	Rho ($Å^{-2}$)x10^6	Roughness (Å)
Silicon Substrate	N/A	2.070	N/A
Mesostructured Silica	841	3.840	5.0
Lipid Bilayer	33	-0.462	4.3
D_2O	N/A	5.995	5.0

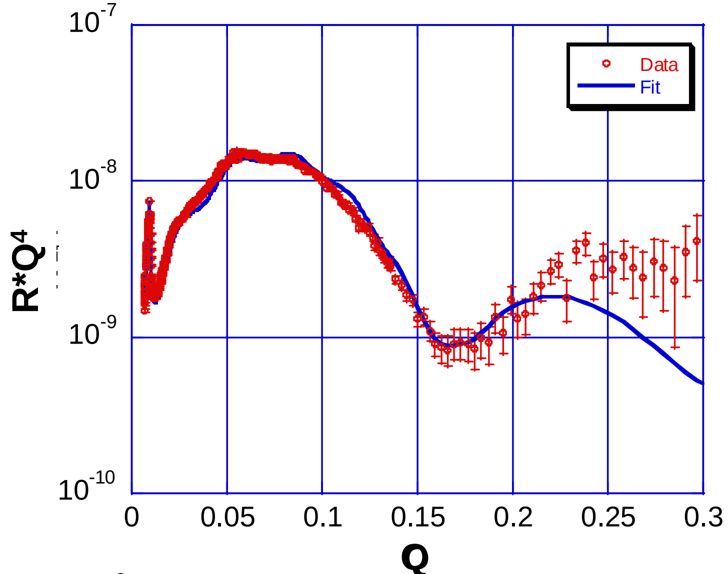






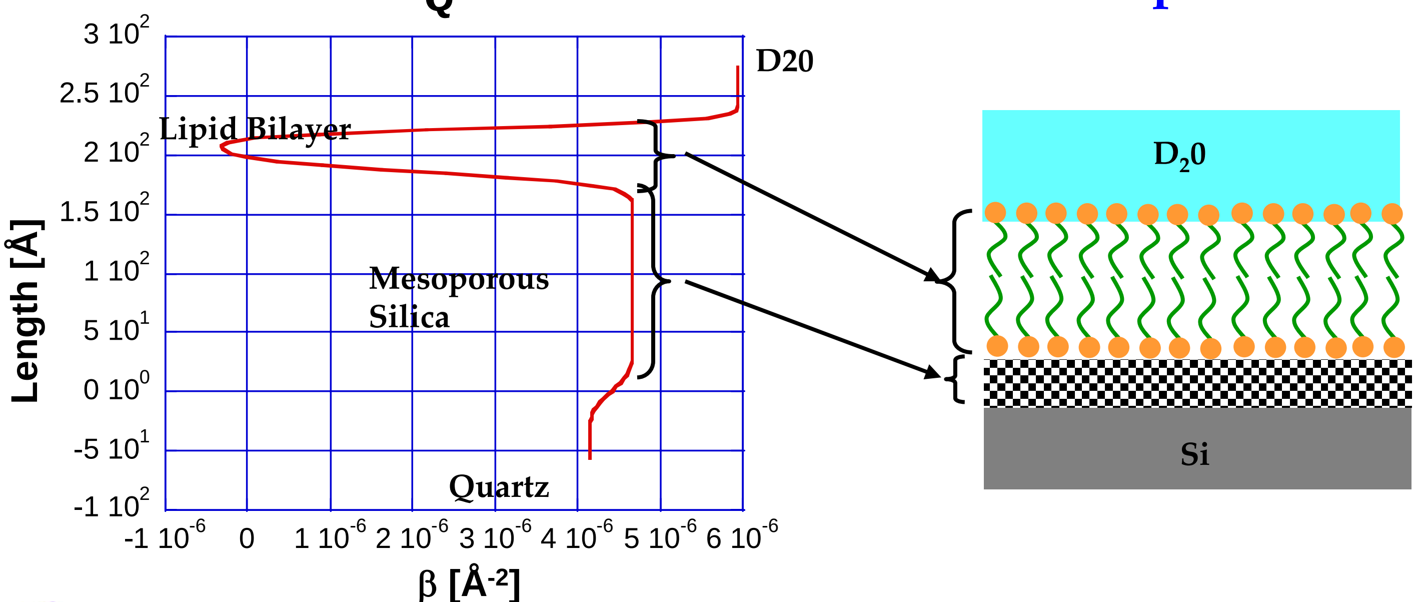
A smooth POPC lipid bilayer on meso-structured silica.

Example: Neutron Reflectivity of Single Lipid Membrane on Ordered Nano-Composite and Nanoporous Silica Thin Films



Layers	Thickness (Å)	Rho ($Å^{-2}$)x10^6	Roughness (Å)
Quartz Substrate	N/A	4.150	N/A
Mesoporous Silica	185	4.66	12
POPC Lipid Bilayer	37	-0.371	8.7
D_2O	N/A	5.93	5.5

A lipid bilayer on a thinner layer of mesoporous silica.



X-ray Scattering from Solid Supported Bilayers

Problems:

High photon energies have to be used (*in our case 18.008 keV or 0.69 Å*)

- very low angles of incidence ⇒ hard to control size of the beam footprint

- extremely small slit sizes made of highly absorbing materials (Mo, Ta, W, *etc*.) (hard to polish and make parallel)

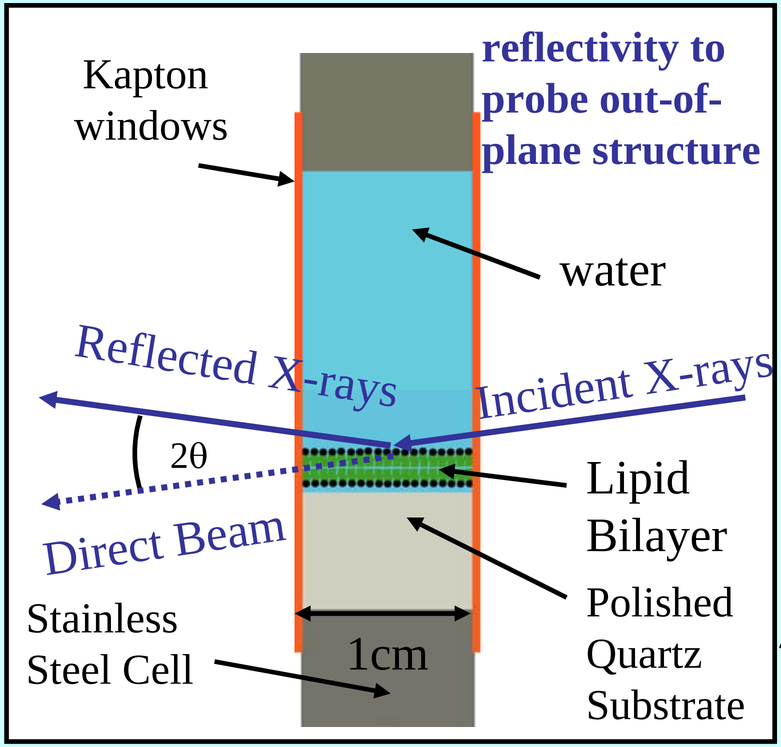
- very focused beams ⇒ beam damage

- low transmission through water (*40% for 10 mm path-length and 18keV*) ⇒ incoherent scattering from substrate

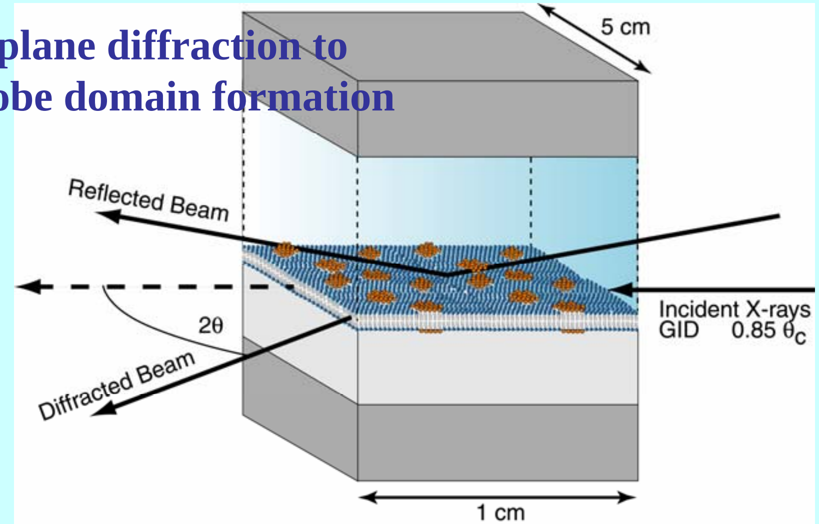
- small sample sizes along the direction of the beam

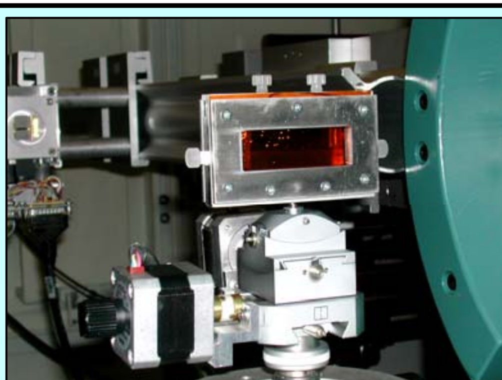
-renormalization of data due to differences of sample length *vs.* beam height

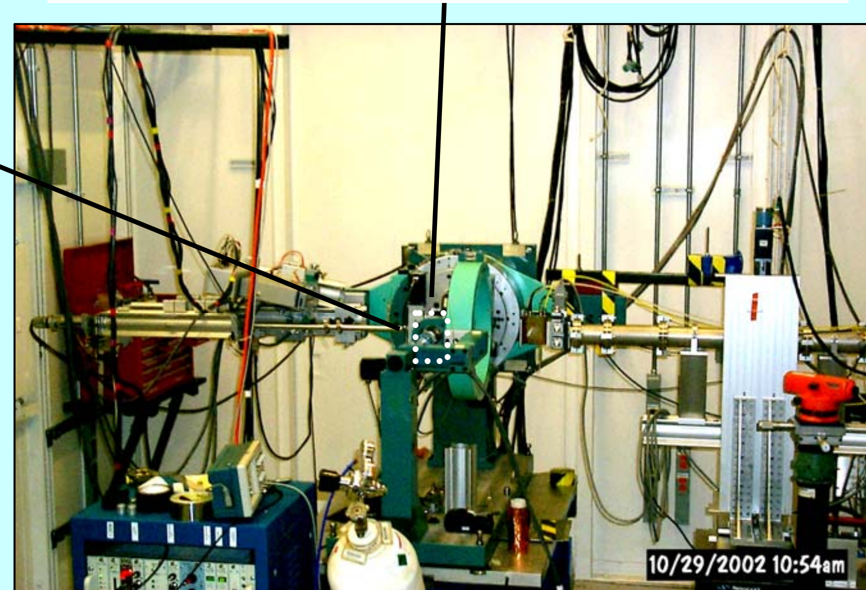
X-Ray Reflectivity at the Solid – Liquid Interface (CMC-CAT, APS)



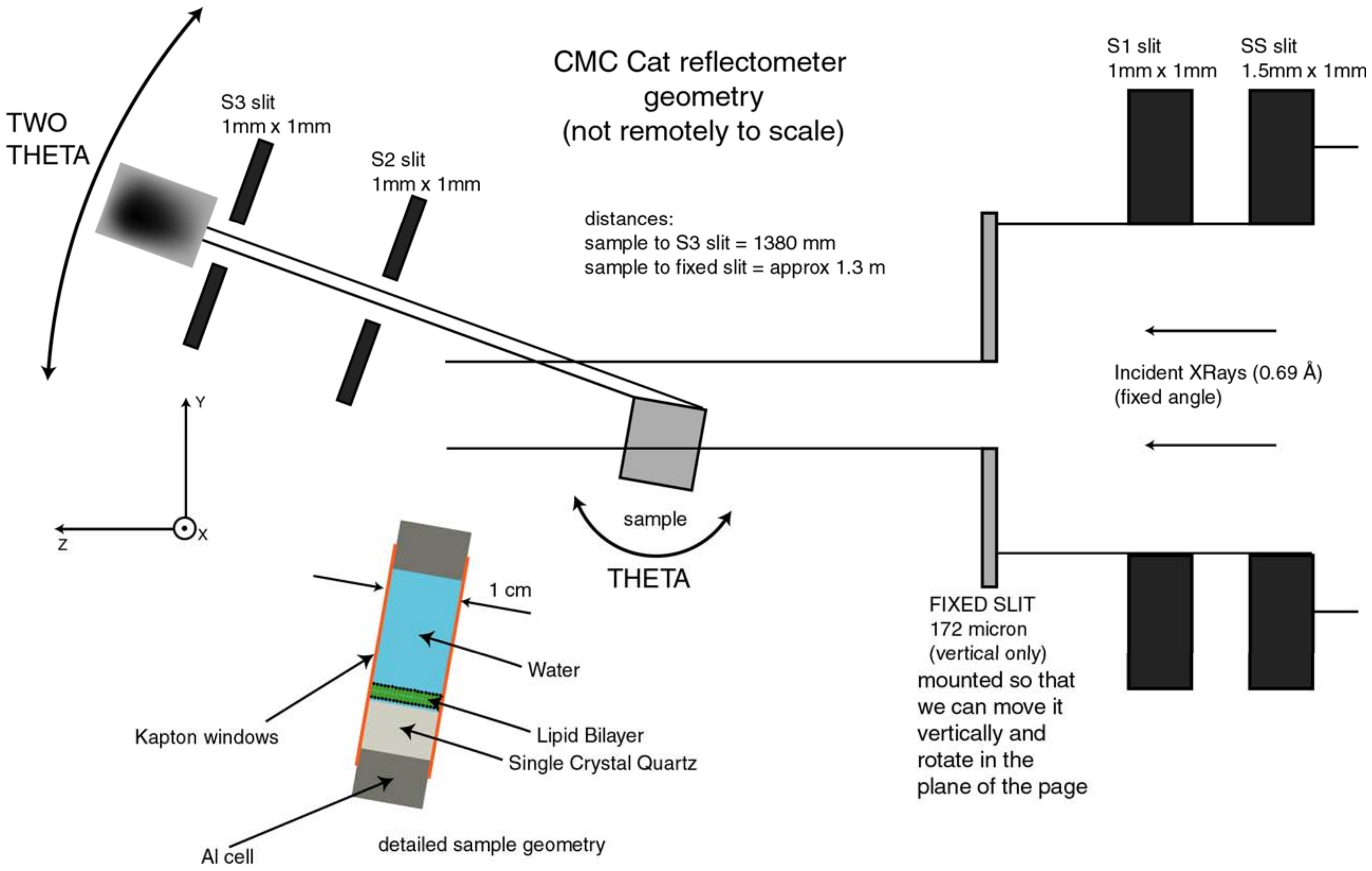
in-plane diffraction to probe domain formation



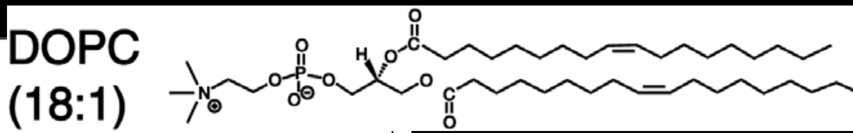


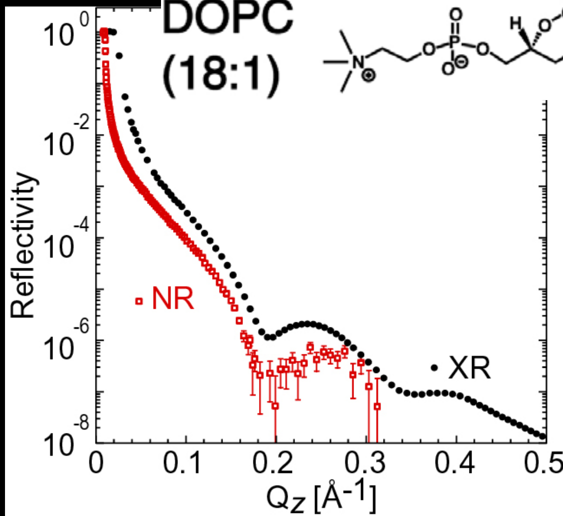


CMC Cat geometry (APS Argonne)

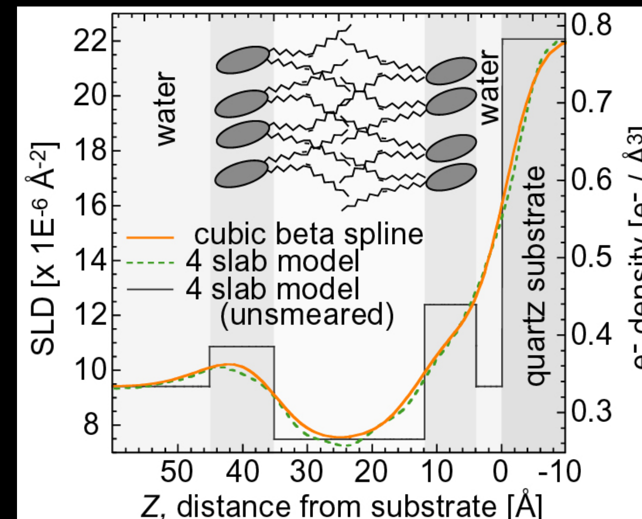


Comparison to Neutron Reflectivity



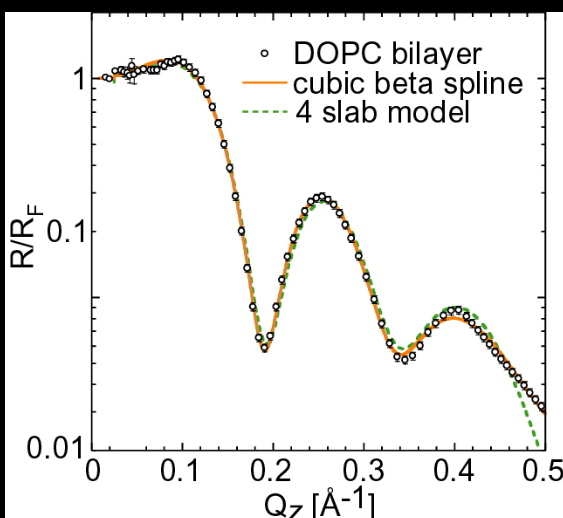


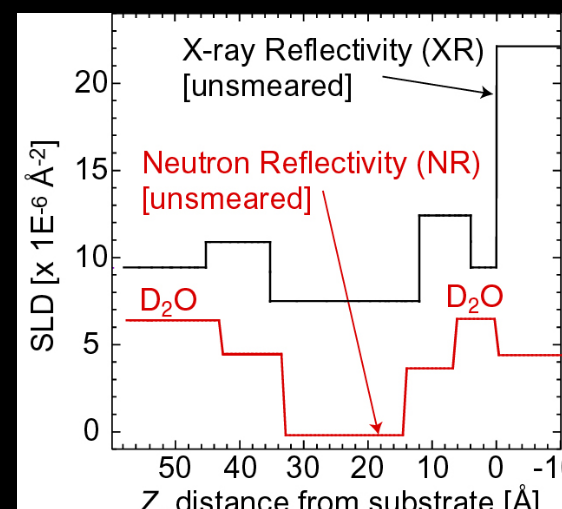
DOPC [18:1] bilayer
Formed by vesicle
Fusion on quartz.



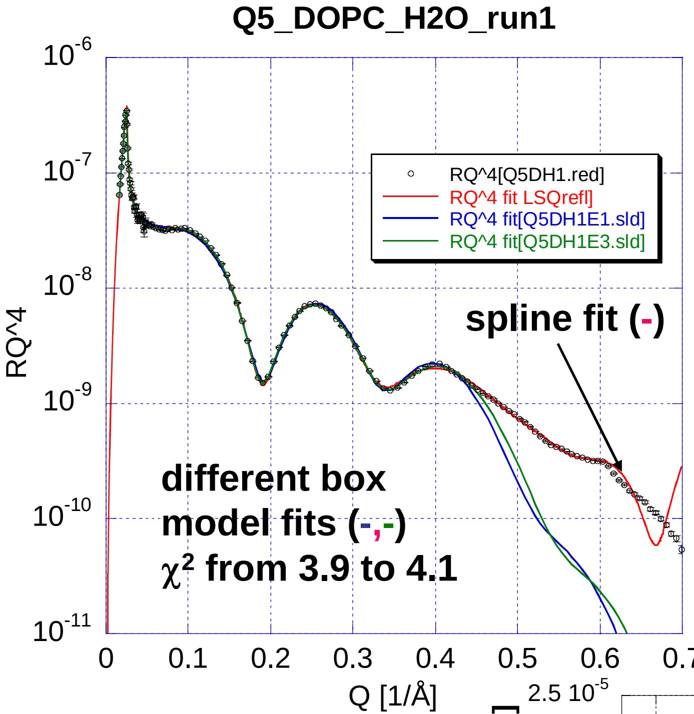
Tails
23.2 ± 0.5 Å
Full extension
23.4 Å per leaflet

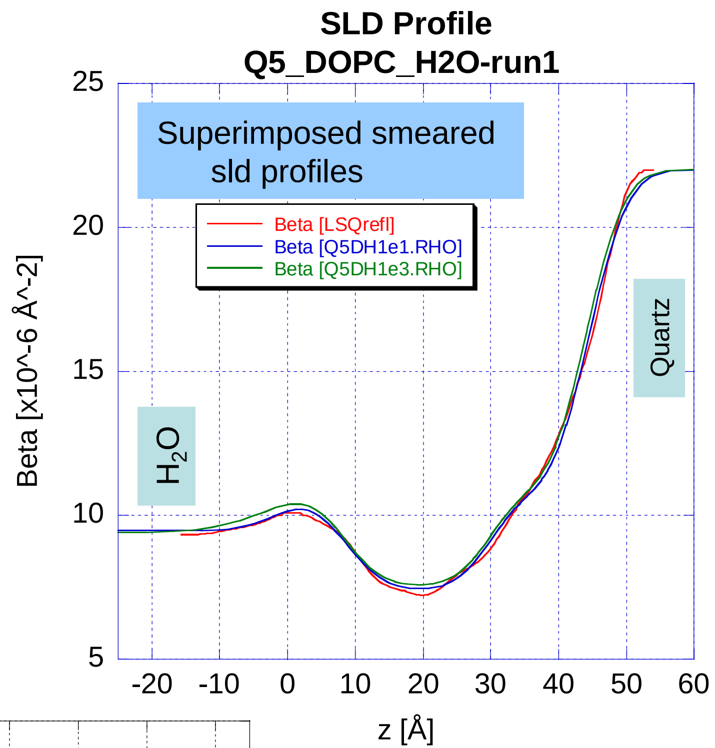
Water cushion
4 ± 0.5 Å

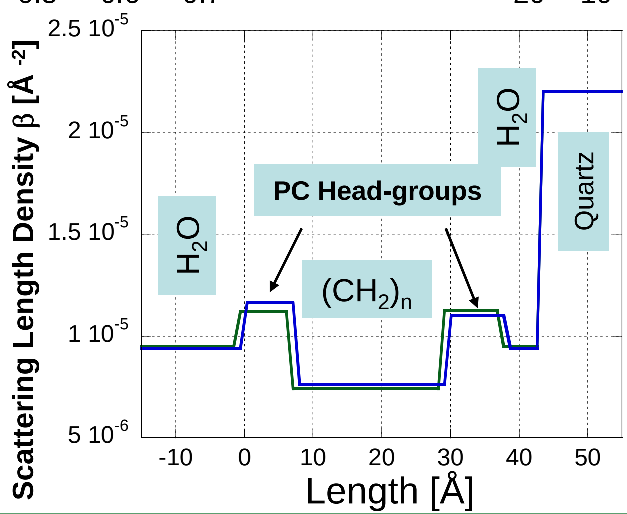




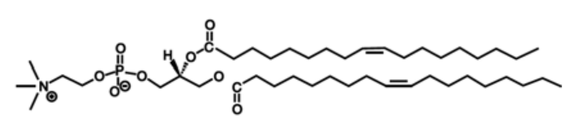
Next Example: X-Ray Reflectivity of DOPC Membrane at H_2O-Quartz Interface Formed by Vesicle Fusion. b-Spline* *vs*. Different Box Model Fits



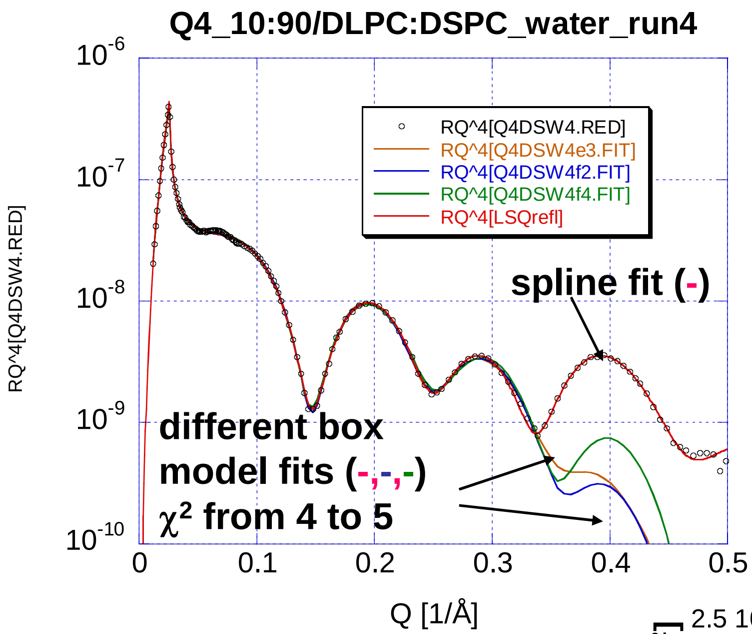


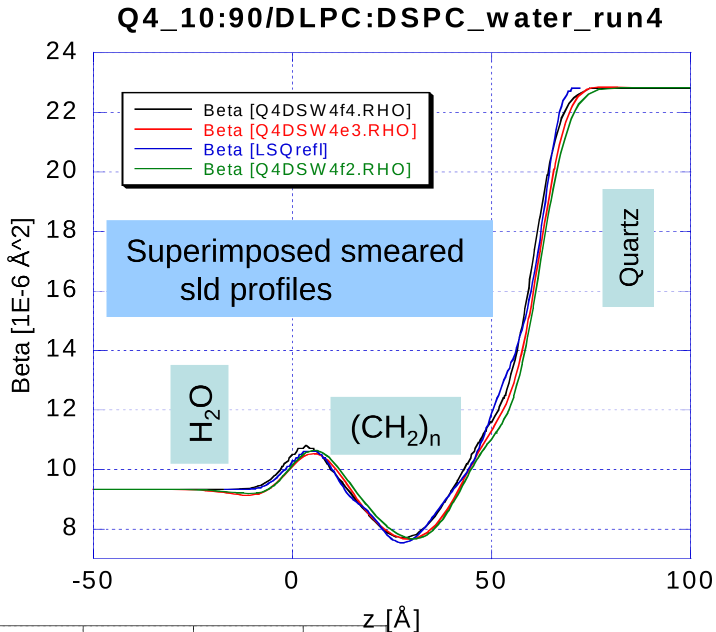


DOPC (18:1)



X-Ray Reflectivity of Model Membrane: 10:90/DLPC:DSPC at H_2O-Quartz Interface
b-Spline* *vs*. Different Box Model Fits

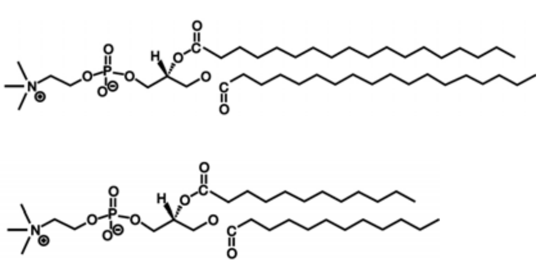


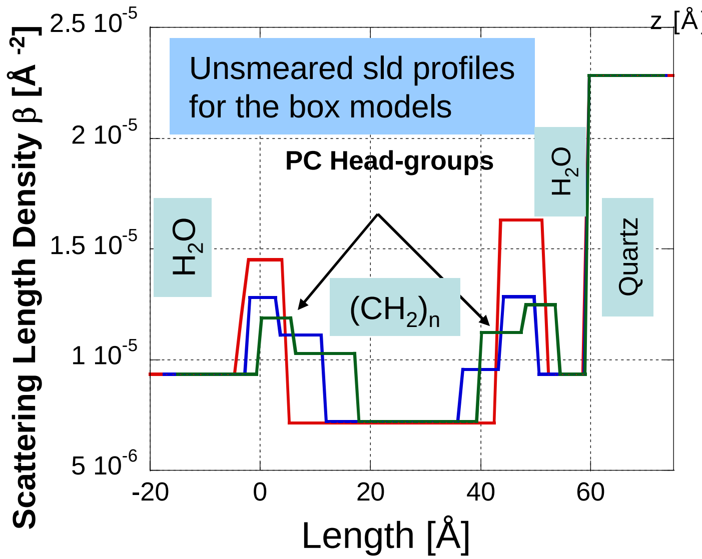


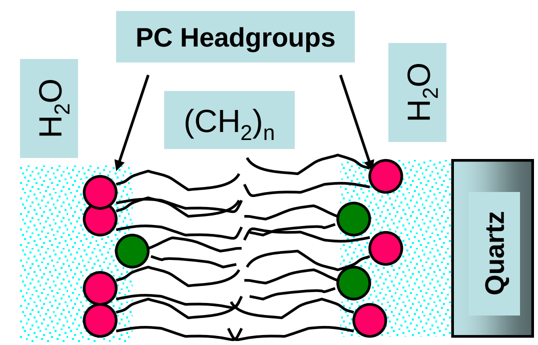
DSPC:DLPC membrane formed by vesicle fusion at 20°C

DSPC (18:0)

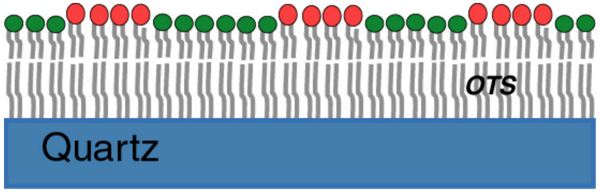
DLPC (12:0)



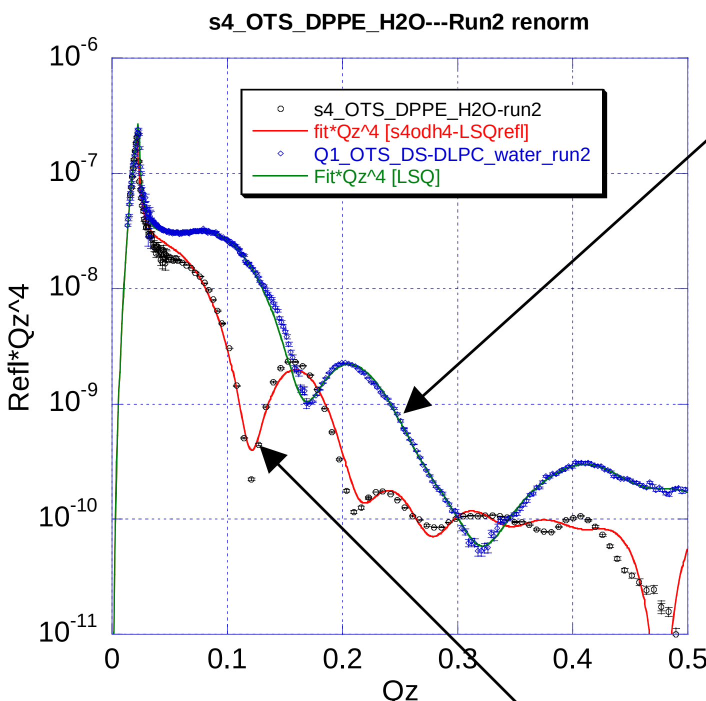


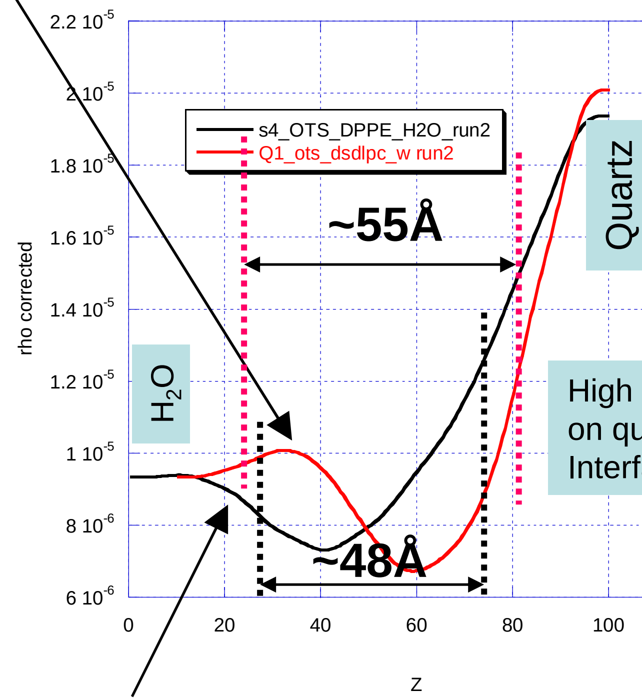


Next Example: X-Ray Reflectivity of OTS supported Phospholipid Monolayers at the H_2O-Quartz Interface. Formed by LB and Vesicle Fusion. b-Spline Fits



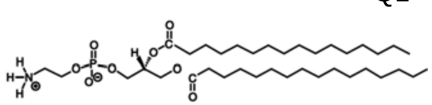
10:90-DLPC:DSPC vesicle adsorbed onto OTS





High roughness on quartz-OTS Interface!?

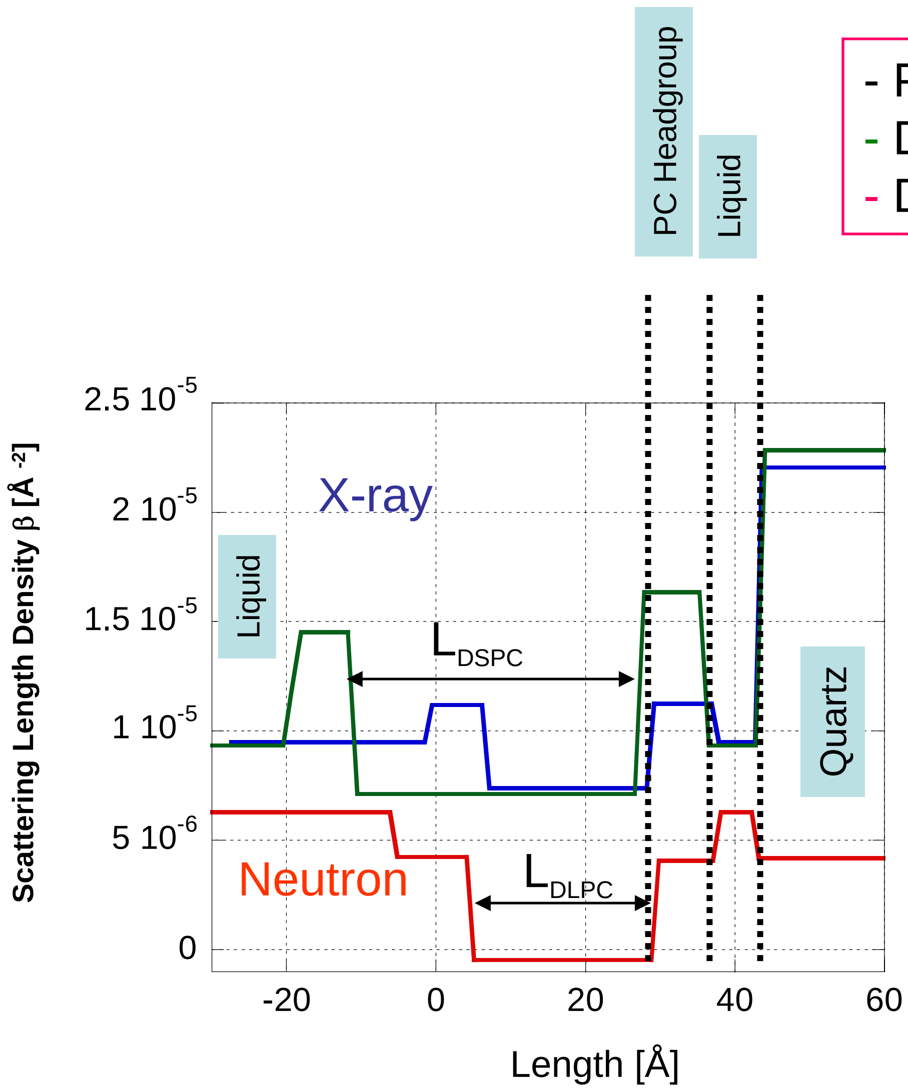
DPPE (16:0)



DPPE one was LB deposited on OTS: More sensitive for the beam damage!

Comparison Between X-Rays and Neutrons

- POPC – unsaturated $(CH_2)_{18}$ (X-ray ref.)
- DLPC/DSPC saturated $(CH_2)_{18}$ (X-ray ref.)
- DLPC saturated $(CH_2)_{12}$ (neutron ref.)



X-ray and neutrons give similar results:

- length of D_2O or H_2O liquid cushion
- length of the PC headgroups
- length of saturated $(CH_2)_n$ tails scales as ratio of *n*. 12/18 = $L_{DLPC} / L_{DSPC} \cong 0.66$

GIXD from Solid-Liquid Interfaces

It is possible to observe GID at the solid-liquid interface

For the first time local in-plane structure of a bilayer has been observed.

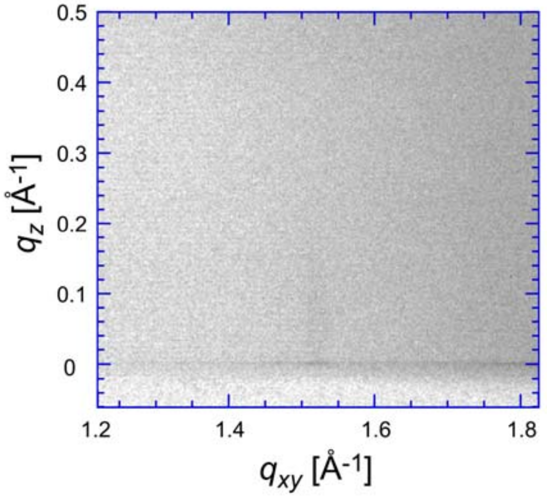
Useful Information:

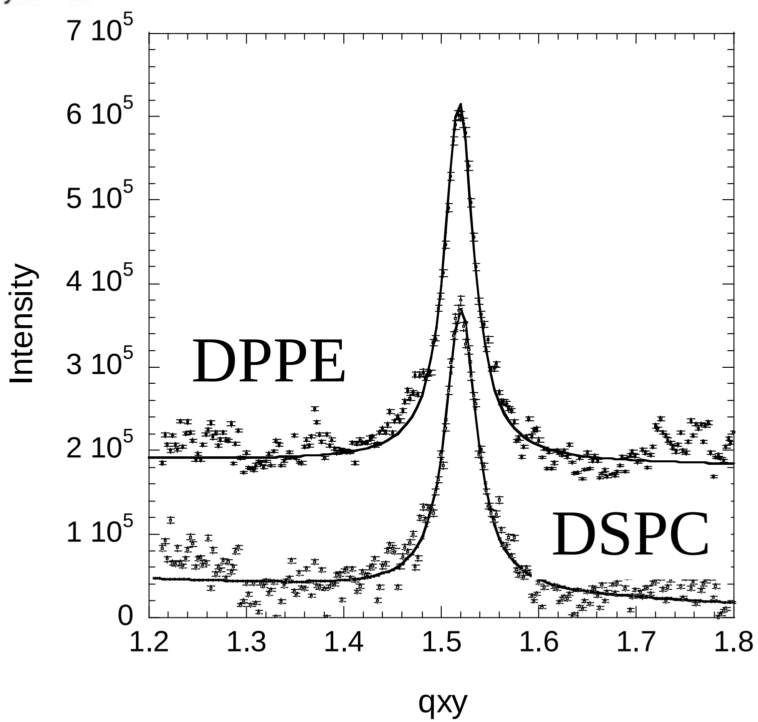
Are domains between leaflets coupled?

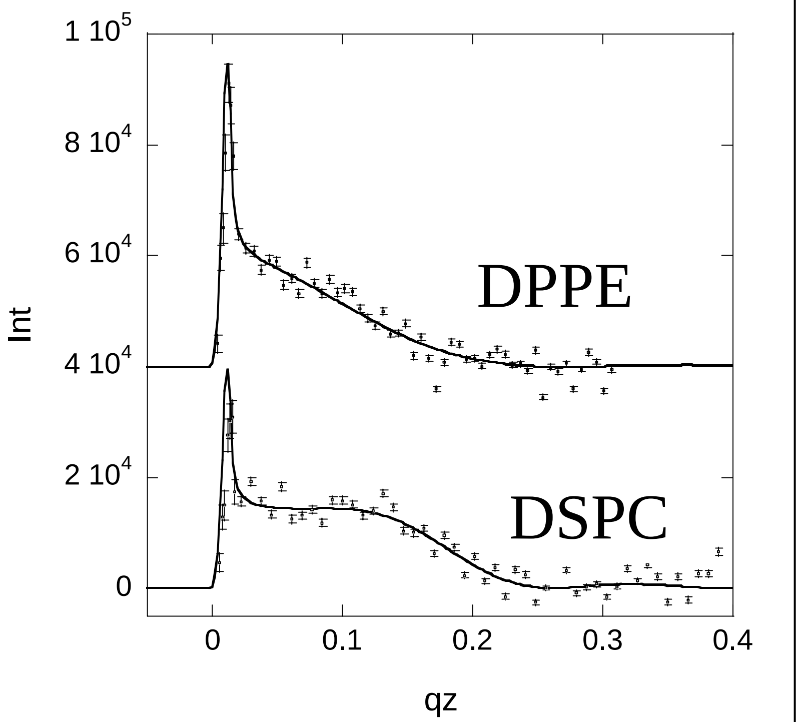
Are cell membranes crystalline in regions?

What are the sizes of these scattering domains?

GIXD Scattering from Lipid Bilayer at the Solid-Liquid Interface







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Summary

- X-ray reflectivity provides alternative way of determining out-of-plane structure of the model membranes at the solid-liquid interfaces. Experiments are short!

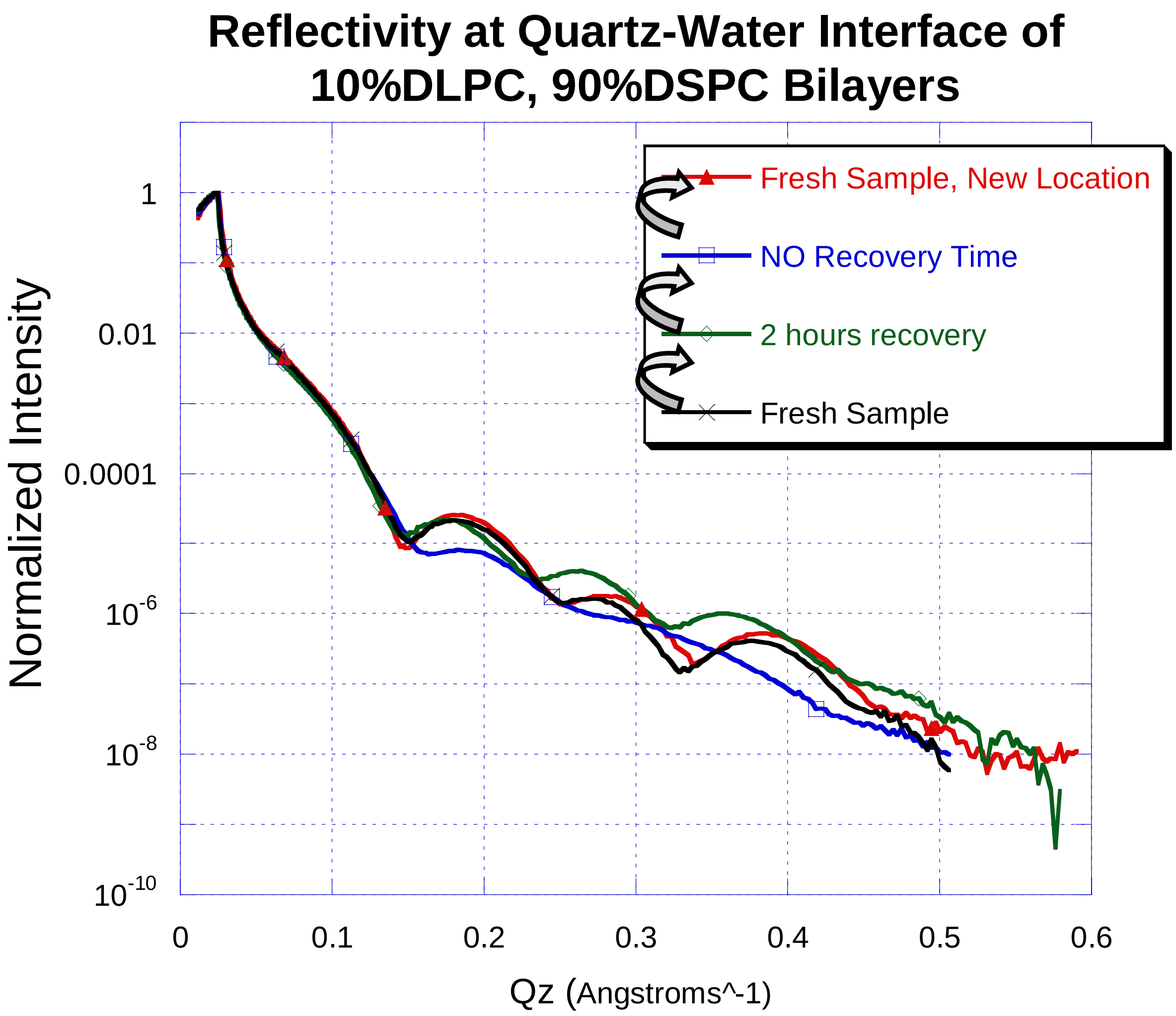
-With some effort X-ray reflectometry through the liquid should be possible using rotating anode/sealed tube X-ray sources with Mo or Ag targets!

-grazing incidence x-ray diffraction from the membrane at solid-liquid interface was multilayers!

-Beam damage is a very important factor: translation of sample during scans is required!!

- Higher momentum transfer vectors ($Q_z \sim 0.7$ Å^{-1}) and lower reflectivities ($\sim 10^{-9}$) can compensate for lower contrasts (as compared with neutrons) and can provide better resolution in the direct space and therefore better understanding of the membrane structure.

Effects of Beam Damage!!!



"Fresh Sample", "2 Hrs Recovery", and "No Recovery" were all performed on the same area of the sample.

Next, the sample was translated to a new location and measured. This result was very similar to the first "fresh sample".

NOTE: sample not translated during each scan. Possible beam damage in higher Q_z region.