Another model system: monolayers at the air/water interface

Preparation: spreading of approx. 0.5 mM organic lipid solution on water subphase

Liquid in a Langmuir trough (teflon)

Monolayers are spontaneously formed, reducing the suface tension:

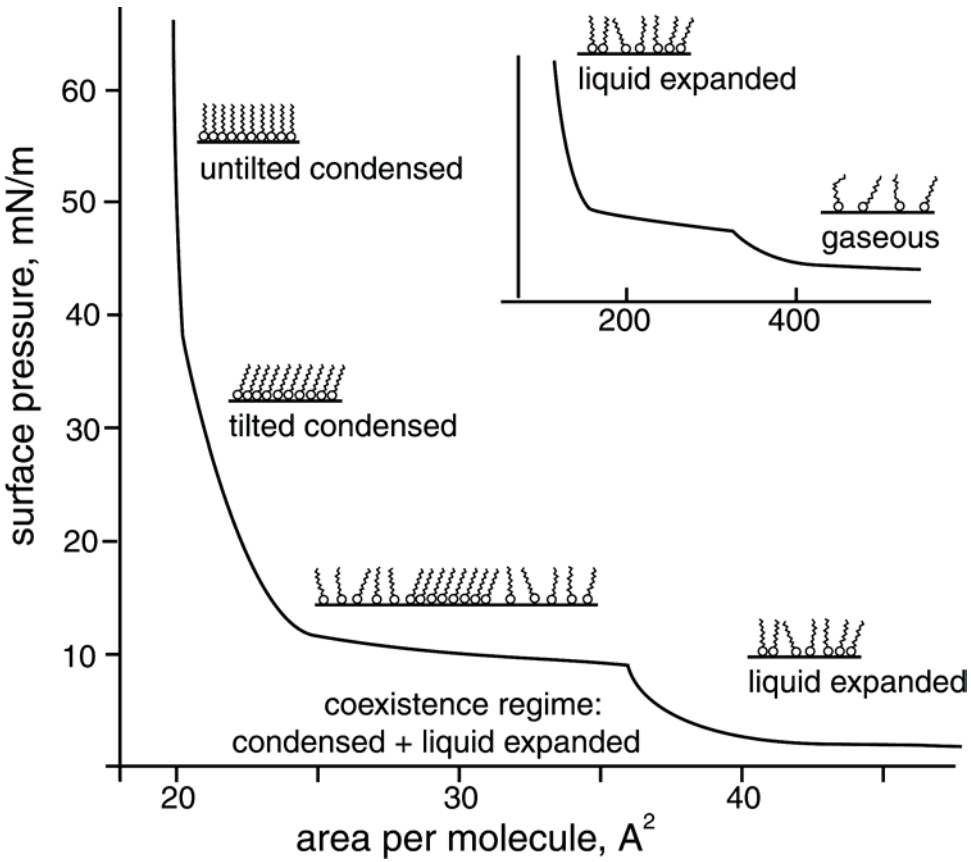
Definition of the surface tension, γ

$$\gamma = \left(\frac{\partial G}{\partial A} \right)_{p,T,n}$$

Lateral pressure, π
γ_0: surface tension clean surface
γ : with monolayer

$$\pi = \left| \gamma - \gamma_0 \right|$$

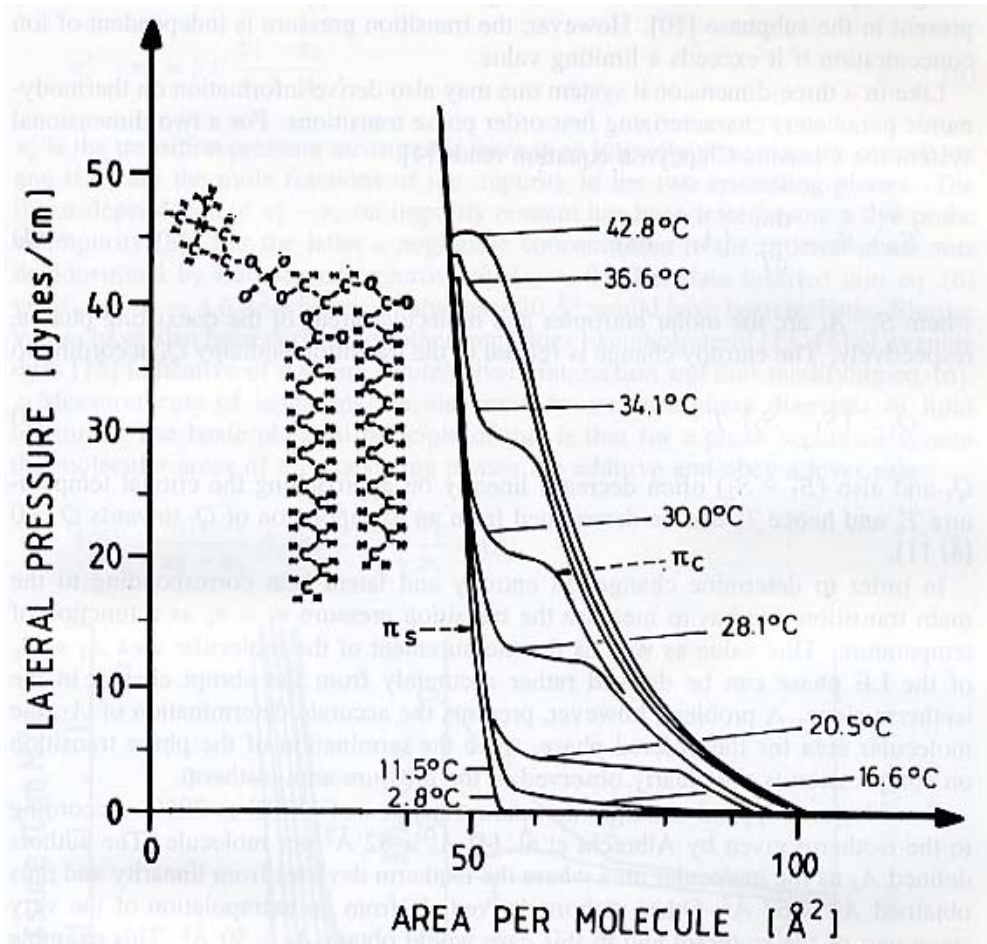
phase behavior of monolayers



Analogy to 3d phases!
Gas, liquid, solid

Condensed phases have all-trans hydrocarbon chains

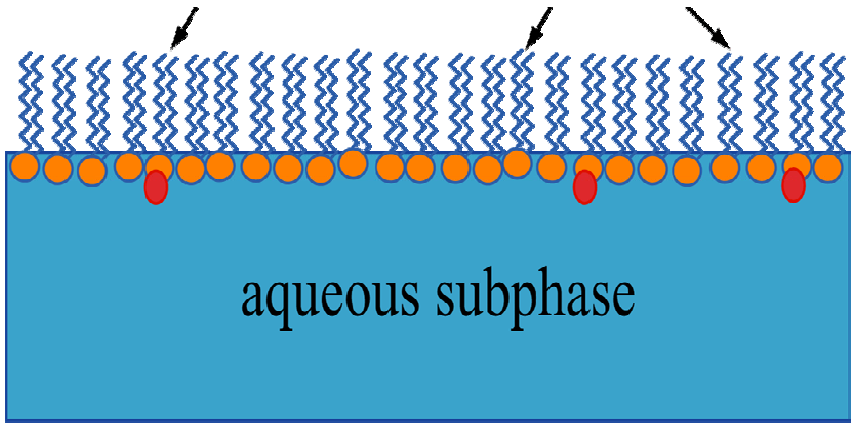
Dependence on temperature

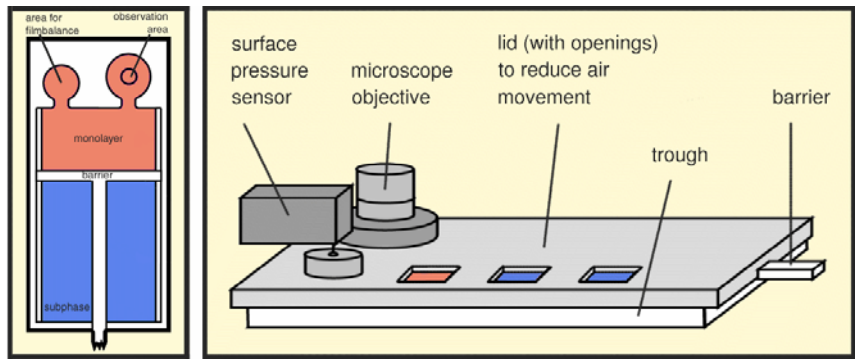


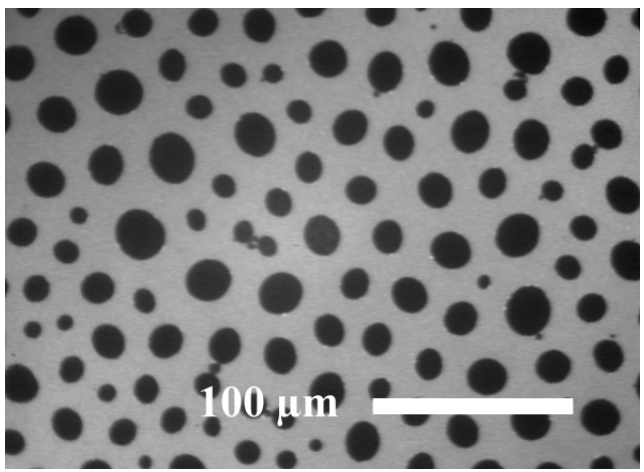
DPPC on water

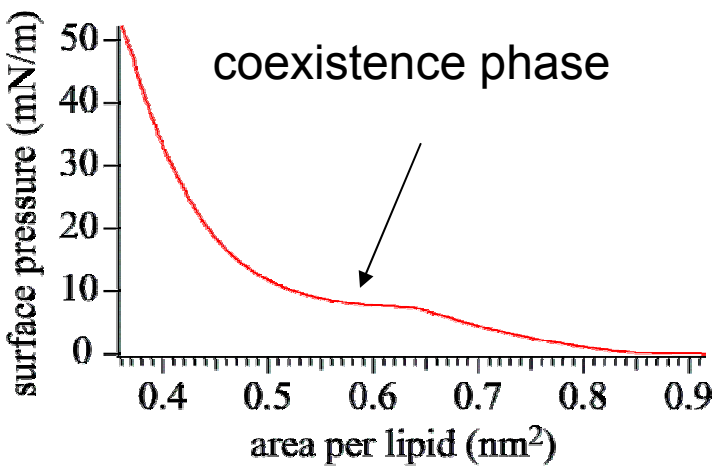
Below a temperature threshold, the liquid phase vanishes

Observation of coexistent phases









Monolayers on water

Thermodynamic measurements:

-Compressibility $$\mathrm{X} = -\frac{1}{A}\left(\frac{\partial A}{\partial \pi}\right)$$

-isobaric thermal expansivity $$\lambda = \frac{1}{A}\left(\frac{\partial A}{\partial T}\right)_{\pi}$$

both functions: derivatives of free energy
-phase transitions are *visible by changes in the slope*: first visible upon compression: liquid-expanded/liquid-condensed *(in DPPC):* first order transition,
Evidence extensive purification of the lipid lead to a slope of ~0,
-adding impurities changes the slope likewise!--- extrapolation: 0.2% impurities present in the DPPC shown

-further compression: at As, πs: discontinuous change, 2nd order transition; at lower temperatures: main transition is missing, at ps is maintained
$-\pi$s is not temperature dependent, can be dependent on counterion content in the subphase, until a threshold value is reached

Clausius-Clapeyron equation for a 2-dimensional system:

$$S_1 - S_2 = \frac{d\pi}{dT}(A_1 - A_2)$$

- S= entropy of phase, A area of phase
- The transition enthalpy is obtained by

$$Q_t = (S_1 - S_2)T$$

- Q_t and ΔS decrease often linearly on approaching T_c, so, T_c can be obtained by extrapolation of Q_t towards Q=0.

- Errors can be made in determining the area As…, not unambiguously to be defined.

- From Raoults law one can also derive A_1-A_2 : , π_c`: when there is an impurity present (dye), x_i are the mole fraction of the impurity in the two phases. Linear dependence on the dye content was tested, relatively small ΔA e.g. 6 Angstroms reflects large solvent solute IA

$$\pi_c' - \pi_c = kT\frac{x_1 - x_2}{A_1 - A_2}$$

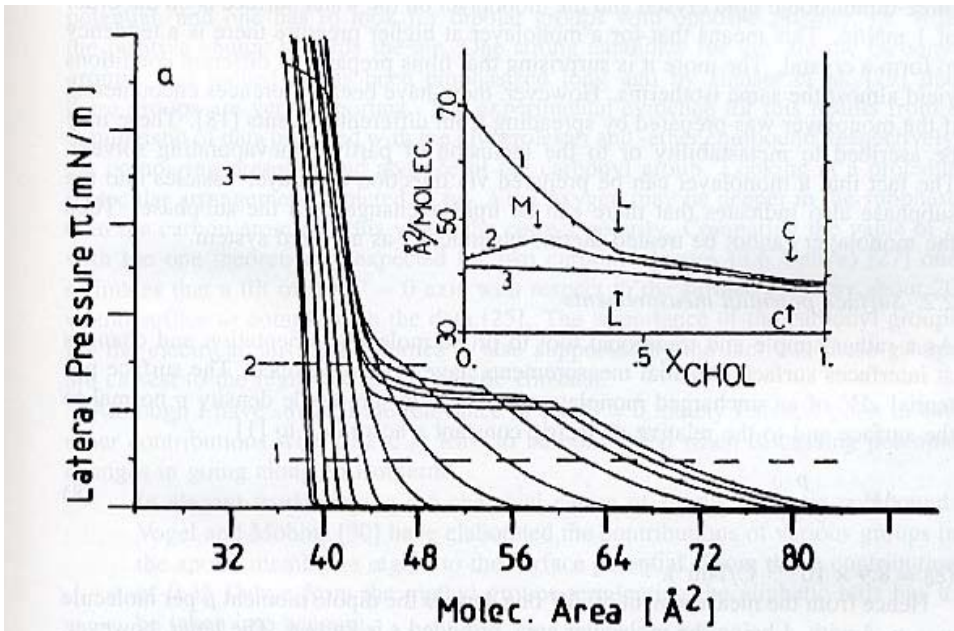
- Phase diagrams of lipid mixtures: principle: the areas of the both phases are additive and obey a lever rule

$$A(\pi) = \frac{x_1 + x_0}{x_2 - x_1} A_1(\pi) + \frac{x_0 - x_1}{x_2 + x_1} A_2(\pi)$$

- example DMPA/cholesterol phase diagram

- $x_{1,2}$ mole fractions of the one component in the 2 phases, x0 total content.
- A(pi) plot vs. T : extrapolation to x_1,x_2 is possible…composition of the two phases obtained
- At low pressure: two linear slopes
- X´at the change : composition of the mixture with one pure phase…**assumption** A(x) must be always linear
- Complication: PL monolayer form a metastable equilibrium pressure between a pc crystal and a monolayer at the air-water interface: 1mN/m!!!
- That means: At higher pressures: tendencs to form a crystal….interesting: isotherms ARE reproducible!!!
- Other point: different solvents used for spreading can give different isotherms
- Monolayers can also be prepared by injection of vesicles into the subphase! -> no thermodynamically closed system, since lipid exchange with the subphase is possible

DMPE/Cholesterol mixture isotherms



Surface potential measurements

Simple and traditional tool (can be sometimes hard to reproduce)

- Surface potential is related to the dipole density r normal to the interface and to the relative dielectric constant ε

$$\Delta V = \frac{\rho}{\varepsilon\varepsilon_0}$$

- the dipolar moment is obtained from

$$\mu = \rho A$$

- A complication arises since ε is not exactly known; (decrease from 80 to 1)

- Consequence: Quantitative interpretation is difficult.

- With charged monolayers: second contribution to ΔV
  - calculation of the possible contribuition (Gouy-Chapman-Stern)
  - measurement by respective sensitive dyes (if position is known)

- Properties of uncharged phospholipids:

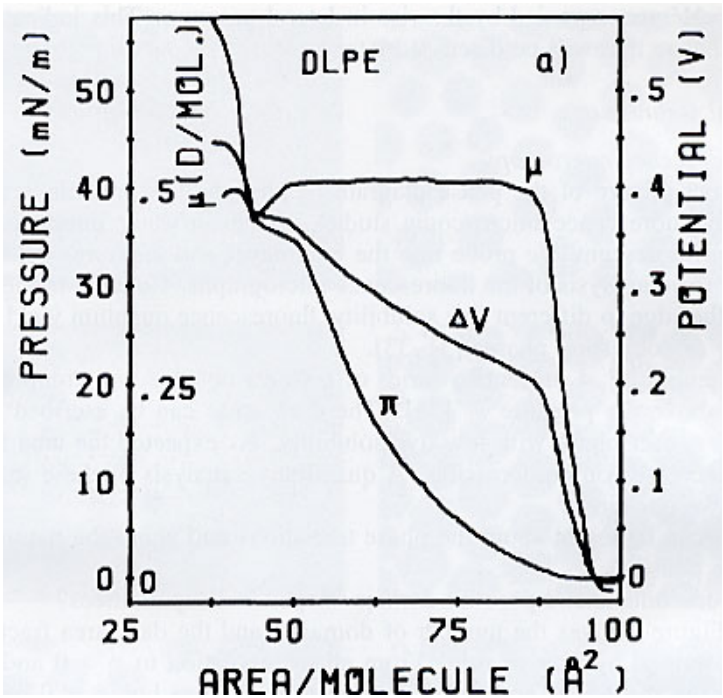
Expectation: *contribution of highly polar headgroups* dominate the dipolar moment and determine the potential

- Estimation of μ: ~15 D, but in measurements: only values < 1 D
- Reason: dielectric screening of the groups in water

- Dipolar headgroups may also be oriented approx. parallel to the surface, but cationic ammonium groups of PC and PE should be deeper in the subphase than the phosphate residue;

- Other possible contributions: -C=O groups
- -CH3 groups
- free water interface: water at the surface is oriented (can have up to 500 mV)

Correlation of pressure area isotherms with suface potential (from Miller, Helm, and Möhwald 1987)



Discussion DLPE

At π=0, meaningless potential, effect of fluctuations

In LE phase: μ constant, proportional to μ

Above π_c: linear slope, less extent, 2 coexisting phases, decrease in μ: due to changed orientation in water, reorientation of C=O groups possible
Vogel and Möbius 1988: Dipolar moment in lipids **predominantly caused by terminal CH3** groups (0.35 D), minor contribution of headgoups!
Use of CF3 groups in a carboxylic acid: on decreased tilt (in condensed phase) the surface potential changes likewise (more negative, with CH3, it becomes more positive on compression)
Above pis: possible other phase transition

In the same publication: use of a tip electrode (up to 100 V) 200 μm above the surface:

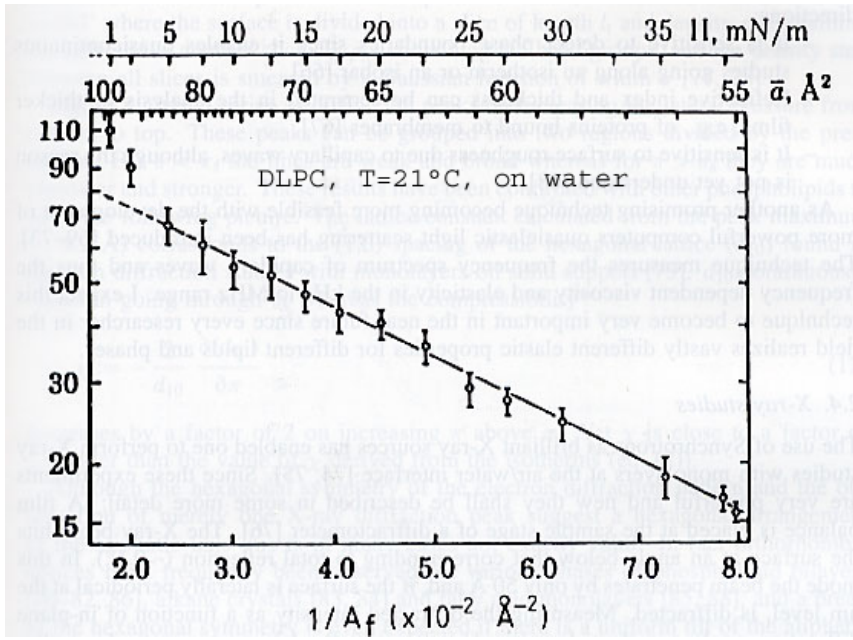
domain repulsion/attraction could be observes by a reverse fluorescence microscope;

repulsion/attraction potential was calculated to 6000 kT, using μ from the potential measurements

Dipolar property of condensed domains proven!

Fluorescence recovery after photobleaching

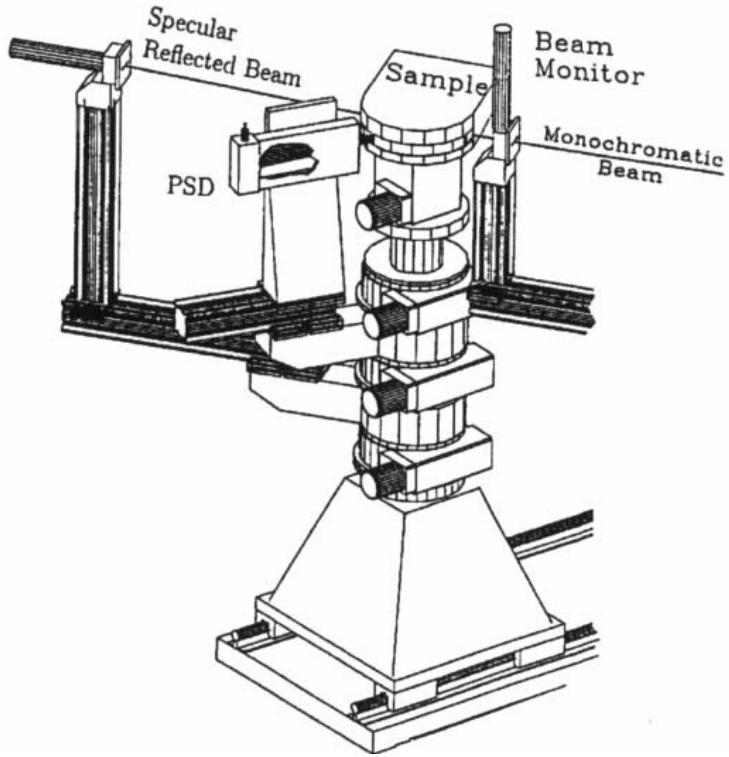
- Principle: photobleaching of a spot, measurement of the fluorescence intensity recovery
- emission recovery of the bleached spot or
  change in contrast
- Diffusion constants:
  LE phase: > 10-8 cm2/s
  LC phase: < 10-10 cm2/s
- Applying the free volume model for the motion of small molecules in the membrane (can change place due to free available volume)
- Excellent agreement with the model observed:



$$\ln D = \ln a - \frac{b}{A_f}$$

Synchrotron X-ray measurements

- Synchrotron radiation enabled to investigate monolayers by x-ray scattering methods: specular reflectivity and grazing incidence x-ray diffraction

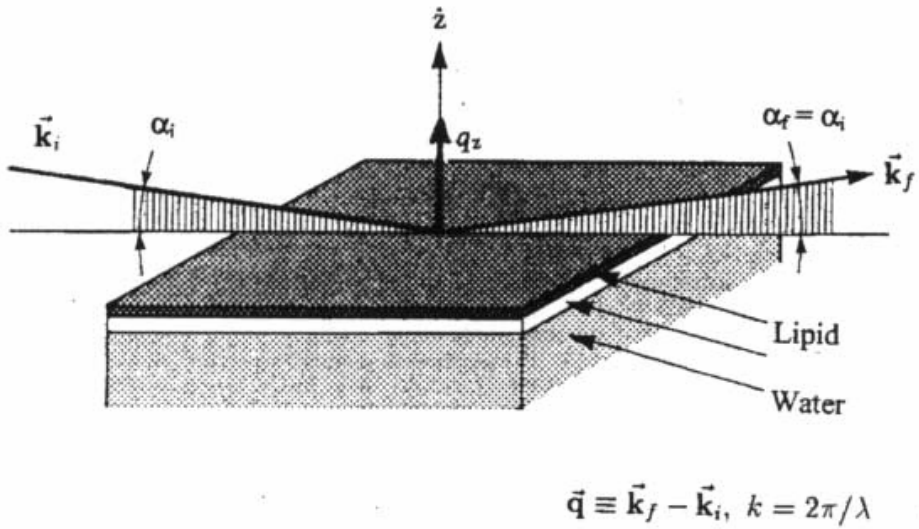


Triple axis spectrometer used for liquid surface scattering at the HASYLAB, DESY, HAMBURG

the electron density across the surface can be calvulated using the master formula for reflectivity.

The ***master formula*** shows only the modulus, not the phases for the function measured.

The reflectivity can be inverted t yield the laterally averaged electron density using a layered model. An electron density model can be build from a stack of slabs. Every slab is assigned to a particular fragment with specific electron density. So, some information about the structure of the lipid becomes availale

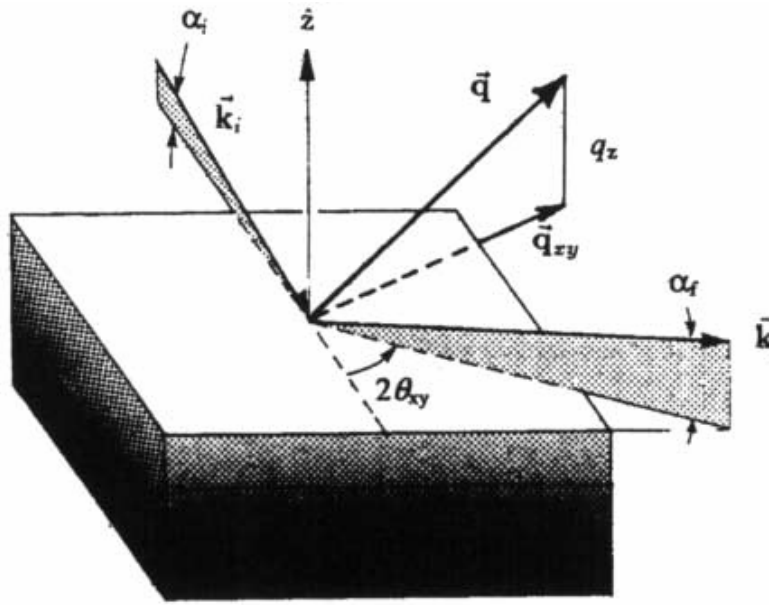


$$\frac{R}{R_F} = \left| \int \frac{d\rho}{dz} \exp(iQ_z) dz \right|^2 \frac{1}{\rho_w}$$

grazing incidence x-ray diffraction

is performed with =1.3 Å, the critical angle of total reflection ac= 0.13°, the incident angle ai=0.85ac, ba this only a 90 Å thick layer is illuminated by the evanescent wave. The direction of the x-rays are conveniently given by the wave vector $\left|\vec{k}_i\right| = \left|\vec{k}_f\right| = k = 2\pi / \lambda$ the scattering process is characterized by the scattering vector $\vec{q} = \vec{k}_f - \vec{k}_i$ which is separated in a horizontal and vertical component this is, together with the Miller indices used to determine the lattice parameters

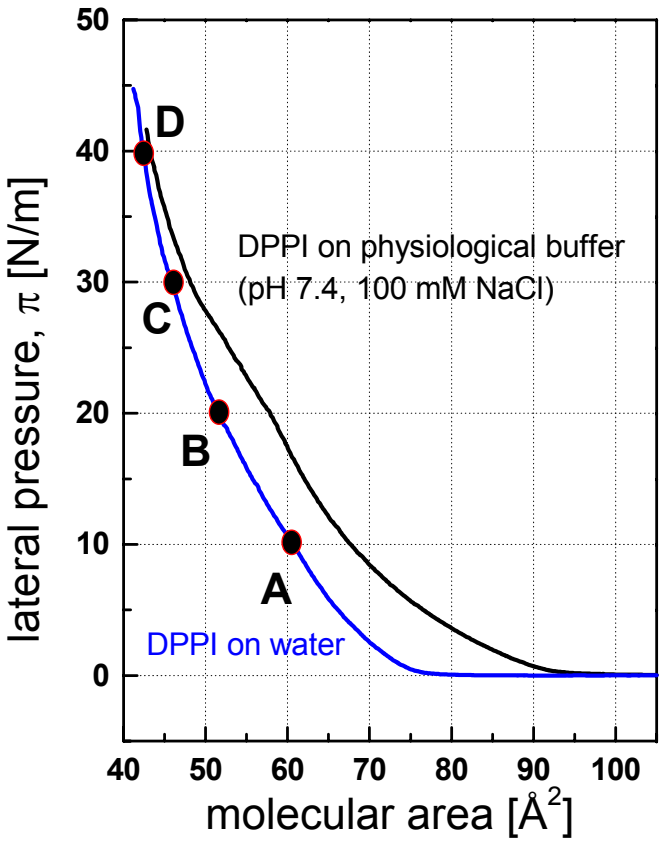
$$d = 2\pi / q_{xy}$$

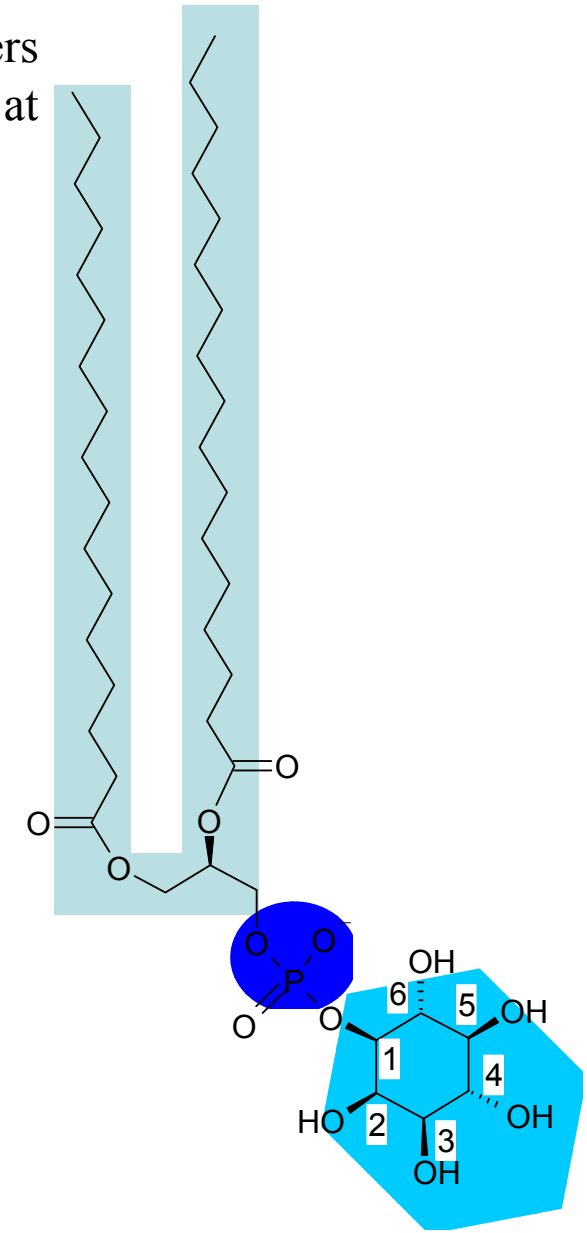


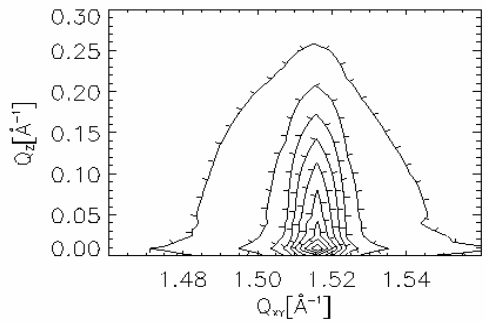
$$\vec{q}_z \approx (2\pi / \lambda)\sin\alpha_f$$

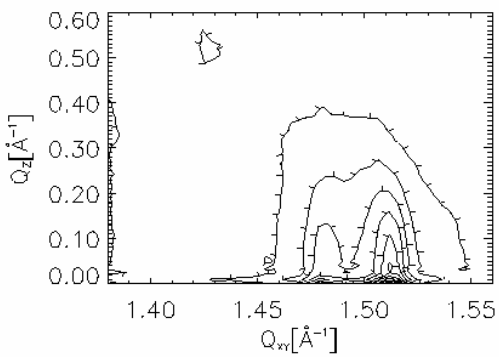
$$q_{xy} \approx 4\pi / \lambda \sin(2\Theta_{xy} / 2)$$

Pressure-area isotherms for DPPI monolayers on pure water and on a physiological buffer at 20 °C.





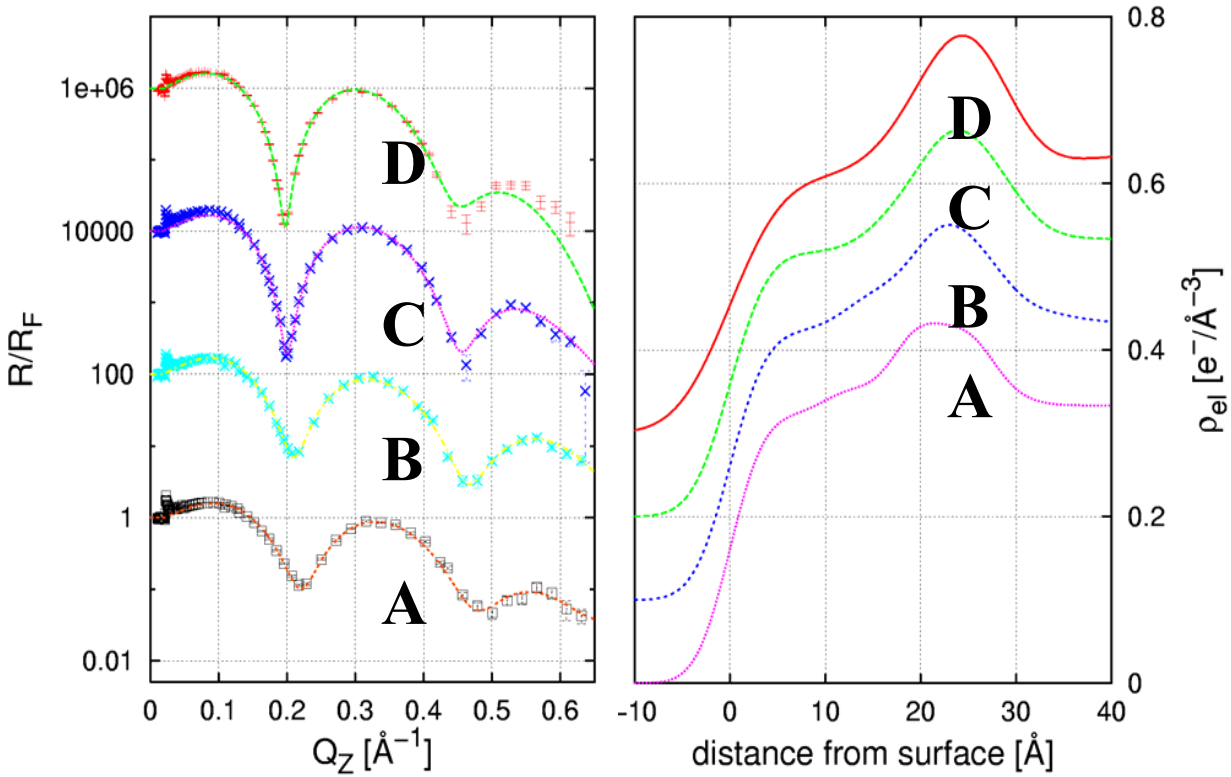




Examples of GIXD data of DPPI on water (1, upper) and on a physiologigal buffer (2, lower), both recorded at a lateral pressure of 40 mN/m.

The diffraction data give direct evidence for minimal molecular area and chain tilt. These parameters obtained by GIXD were complementally used for modelling of the XR data.

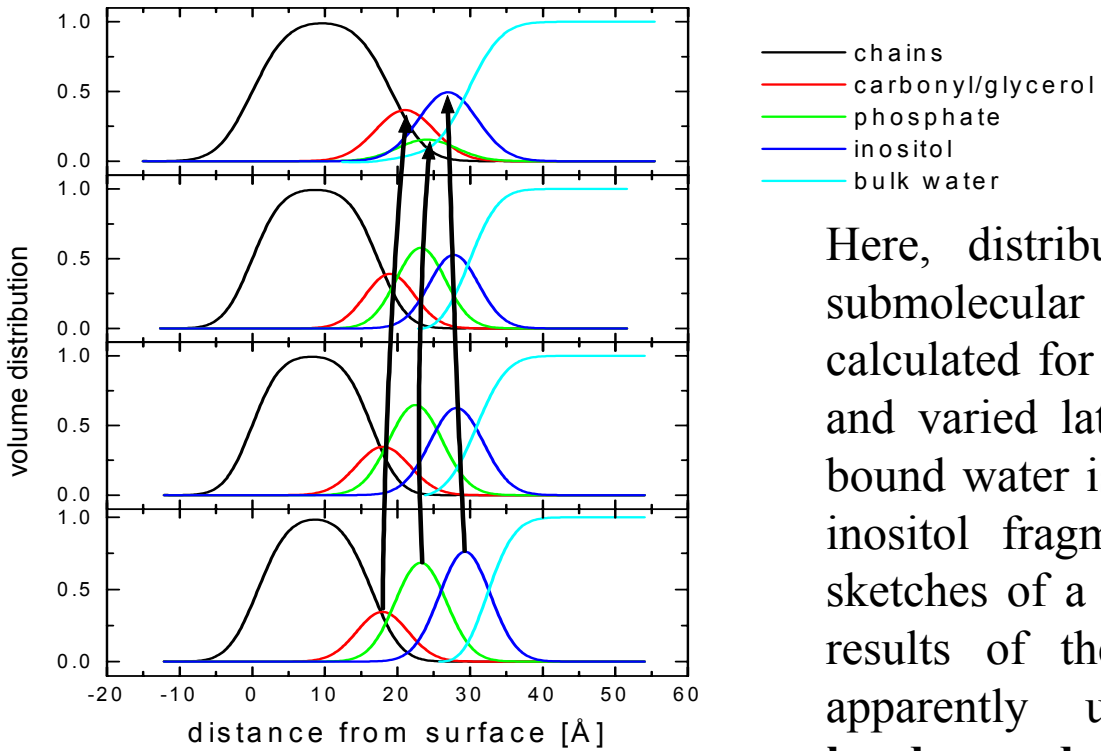
Note that in (2) two phases with different chain tilt are most likely present. At all lateral pressures studied, no homogenous condensed phase was observed for DPPI monolayers on the physiological buffer.



Left panel: XR data recorded for monolayers of DPPI on pure water at 20 °C at different lateral pressures (normalized to Fresnel reflectivity of water; A:10 mN/m, B: 20 mN/m, C: 30 mN/m, D: 40 mN/m). The curves recorded at higher lateral pressures are shifted for better visibility. Best fits obtained are superimposed as lines.

Right panel: calculated electron density profiles across the surface normal for the data shown in the left panel. Note the relatively large differences in the head group region in dependence on the lateral pressure in the range of ~18-30 Å distance from surface.

Series of volume distributions calculated for each molecular fragment of DPPI.



Here, distributions of the volumes assigned to submolecular fragments across the surface normal calculated for a DPPI monolayer on water at 20 °C and varied lateral pressure are depicted. Note that bound water is added to both the phosphate and the inositol fragment. The graphs correspond to the sketches of a structure of DPPI which visualize the results of the calculations. The DPPI molecule apparently undergoes **changes** in both the **headground** and the chain organisation when the lateral pressure is varied (on water).