Nano-crystal induced phase transitions in bio-membranes

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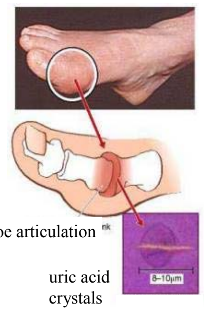
Introduction

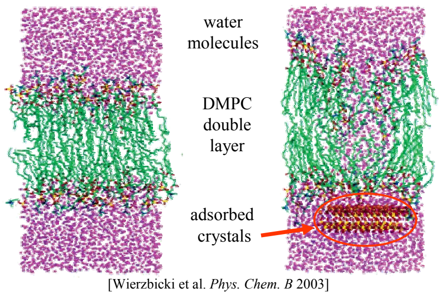
- charged crystal surfaces in the vicinity of a lipid membrane are supposed to cause membranolysis: the rupture of bio-membranes



- crystal-induced particular inflammation plays an important role in arthritic diseases like for instance gout

- in the case of gout the unhealthy crystals are uric acid crystals which typically first accumulate in the toe articulation and cause painful inflammations there

- recent molecular dynamic simulations (Wierzbicki et al.) gave for the first time an insight to the phenomenon of membranolysis on a molecular level



[Wierzbicki et al. *Phys. Chem. B* 2003]

- simulations included a lipid double layer (DMPC), representing the bio-membrane, surrounded by water

- in the vicinity of a crystal with charged surface, adsorbed beneath the outer layer of the membrane by electrostatic interaction, the lipid bi-layer loses its integrity

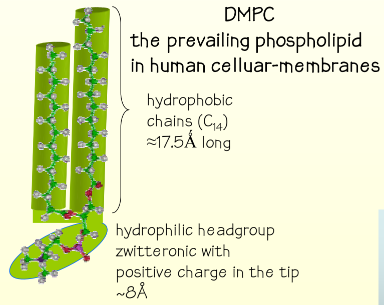
- the penetration by water molecules and the destruction of the membrane follows

- a reduced diffusion coefficient for the lipids in contact with the adsorbed crystal points towards demobilisation of these lipids being the driving force for the rupture of the membrane

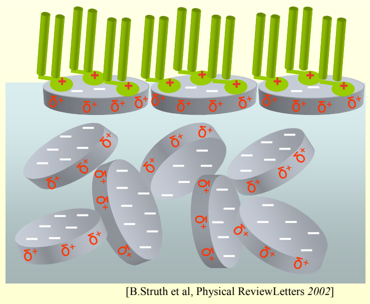
our work: experimental study of the thermodynamic and structural properties of DMPC Langmuir monolayers in interaction with charged crystal surfaces

The System

DMPC
the prevailing phospholipid in human celluar-membranes

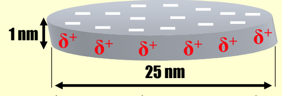


use of industrial fabricated nano-minerals with charged surfaces as a model system for unhealthy crystals



[B.Struth et al, Physical ReviewLetters 2002]

clay nanoparticles: Laponite



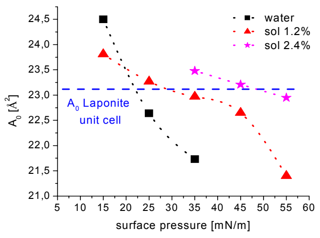
- synthetic monodisperse minerals
- charge deficiency of 0.7 per unit cell

electrostatic interaction →
- adsorbed mineral monolayer
- formation of a bulk network

complementary experiments

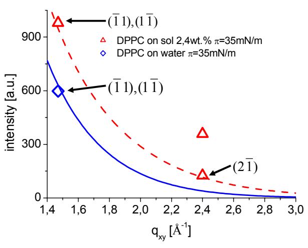
DPPC (same phospholipid as DMPC but with C_{16} chains) was used to study in general the influence of the crystallites on the structural properties of lipid films

→ lattice formed by the lipid chains is adapted to the internal atomic structure of the crystals:



→ crystals might favour nucleation and growth of the 2d chain lattice

→ the Debye-Waller factor for a lipid film in contact to the nano-crystals is reduced:

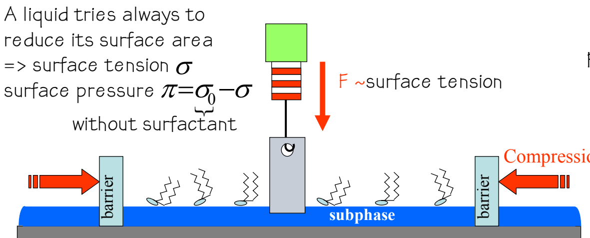


→ adsorbed crystals damp surface fluctuations and support the formation of an ordered surfactant layer

The Langmuir Technique

A liquid tries always to reduce its surface area => surface tension σ

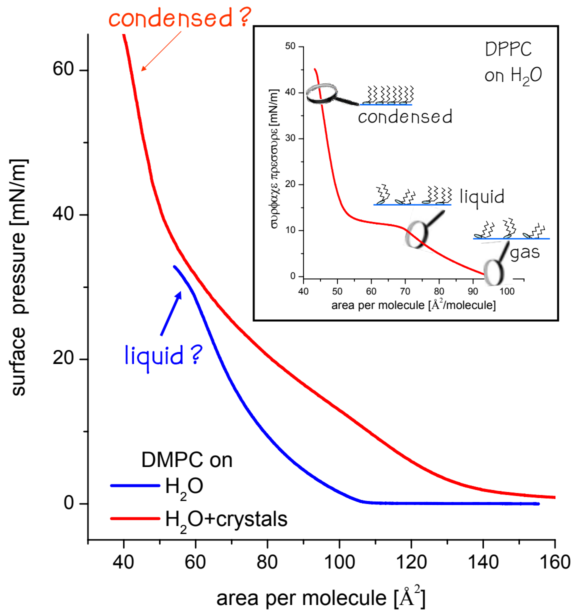
surface pressure $\pi = \sigma_0 - \sigma$ (without surfactant)

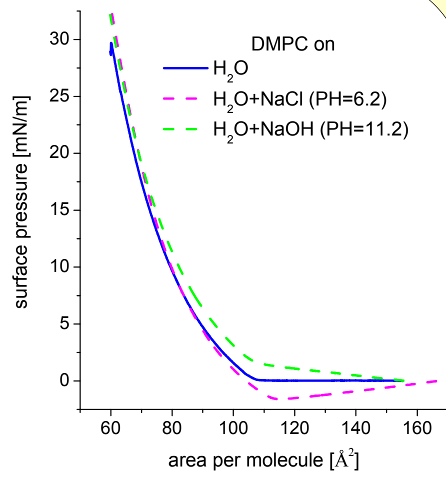


Pressure/Area Isotherms

(2d analog of the pV-diagramm for the bulk)

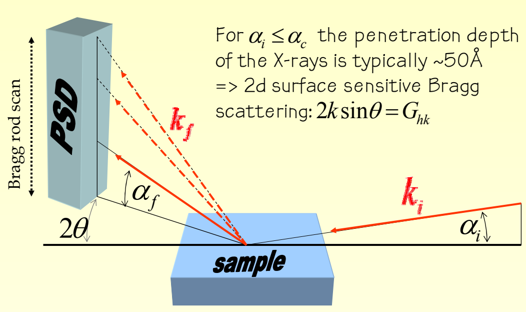
- DMPC on H_2O: the shape of the isotherm and the values for the area per molecule point towards the film being in a liquid state, even close to the collapse point
- DMPC on H_2O with crystals: the values of the area per molecule and the increasing slope of the isotherm give high evidence for the film reaching a condensed state with ordered alkane chains
- the distinct differences between DMPC isotherms on H_2O with and without crystals cannot be assigned to a modified pH value and Na^+ concentration in the subphase → real effect due to the adsorbed nano-crystals





during the dissolving process of the Laponite powder Na^+ ions are released into the subphase, modifying pH and ion concentration

Grazing Incidence Diffraction (GID)

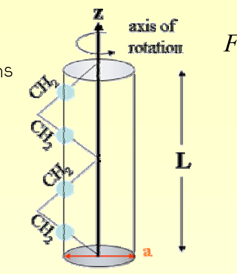


For $\alpha_i \leq \alpha_c$ the penetration depth of the X-rays is typically ~50Å => 2d surface sensitive Bragg scattering: $2k\sin\theta = G_{hk}$

cylinder model for lipid chains
- free rotation of the chains
- substitution of the electron density of the chains with the electron density of an oxygen atom
- smearing out of the electron density over a cylindrical shell

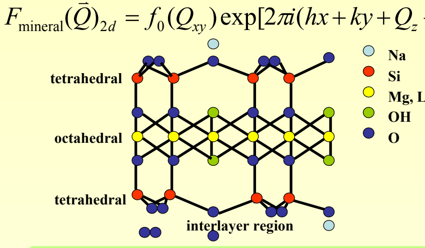
$$F_{mol}(Q) \propto f_0(Q_{xy}) \frac{\sin(\frac{L}{2}\cdot Q_z)}{Q_z \cdot L} \frac{J_1(\frac{a}{2}\cdot Q_{xy})}{\frac{a}{2}\cdot Q_{xy}}$$

[Als-Nielsen in *Phase Transitions in Soft-Condensed Matter*; Porod in *Small Angle X-ray Scattering*]



Bragg rod for the crystallites:

$$F_{\mathrm{mineral}}(\vec{Q})_{2d} = f_0(Q_{xy})\exp[2\pi i(hx+ky+Q_z\tfrac{c}{2\pi}z)]$$



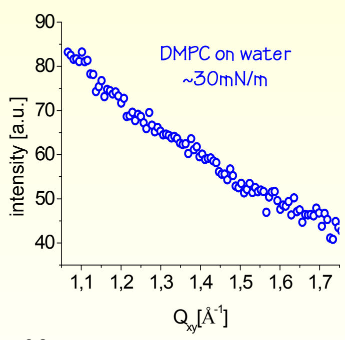
- the lattice parameters of the crystal and the lipid chain lattice are similar, i.e. their Bragg peaks will overlap
- Bragg rods contain information about the scatterer itself and make it possible to distinguish the different contributions

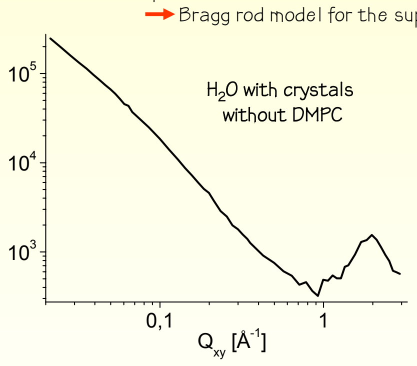
→ Bragg rod model for the superposition of lipid and crystal rods is needed:

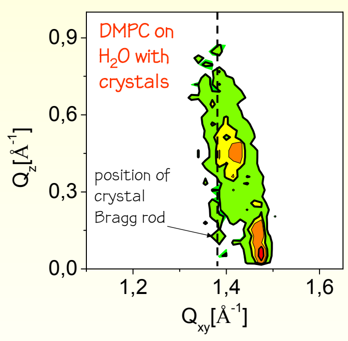
$$I(Q_z) = \left[|F_{\mathrm{mineral}}|^2 \cdot a \cdot A + |F_{\mathrm{mineral}}|^2 \cdot n\right] \cdot V^2 \cdot s + b$$

a: absorption factor
n: lipid chains/unit cell
s: scaling factor
A: crystal surface coverage
V: Vineyard peak
b: background

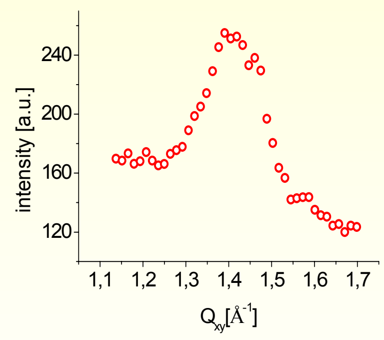
analysis of the in-plane Bragg peaks:
- no in-plane Bragg peaks for DMPC on H_2O
  → DMPC is an inherently non-ordered lipid at ambient temperature
- no in-plane Bragg peaks for H_2O with crystals but without DMPC
- in-plane Bragg peaks for DMPC on H_2O with crystals
  → perform Bragg rod analysis to distinguish between lipid and crystallite reflections

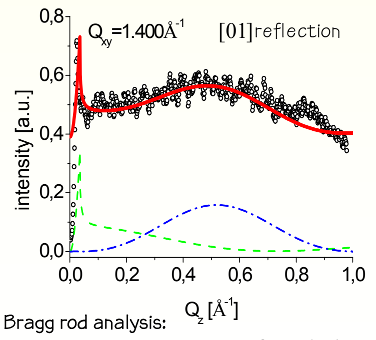


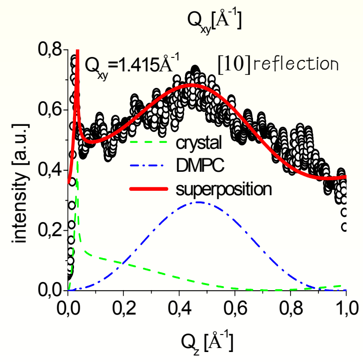


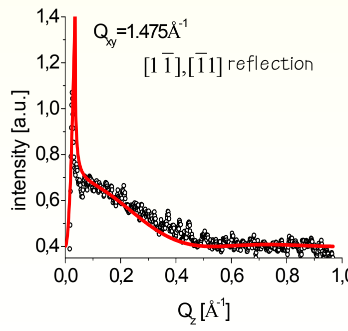


integration over Q_z









Bragg rod analysis:
- the measured Bragg rods of the [01] and [10] reflection for DMPC on H_2O in the vicinity of nano-crystals with charged surfaces contain contributions both from the adsorbed crystallite and a lipid chain lattice
- the measured Bragg rod of the [1 1̄],[1̄ 1] reflection can be modeled as a pure lipid rod

crystal induced ordering of the lipid alkane chains

Conclusions

in the vicinity of a charged crystal surface a nucleation process is induced, forcing the inherently non-ordered DMPC chains in an ordered liquid condensed phase

first direct observation of the crystal induced demobilisation of the lipids in a membrane

the strongly modified thermodynamic behaviour of the membrane gives a hint that transport mechanisms through the membrane might be hindered even without rupture, restricting its functionality

Outlook

screening of charges to inactivate crystal surfaces and ameliorate crystal induced arthritic conditions