TUTORIAL IN SMALL ANGLE

X-RAY SCATTERING ANALYSIS

at the

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Trieste, 15. May 2006
(14:30-17:15)

The Fluid Lamellar Phase of Phospholipids:
Membrane Structure and Its Temperature Dependence

Introduction

Phospholipids, the main constituents of the biological membrane-matrix, display a distinct polymorphism depending on thermodynamic parameters (e.g., *T, p, c*). One-dimensional repeat of bilayers in a stack (compare Fig. 1), two-dimensional ordered tubular structures or three dimensional cubic networks are only a few examples for the structural variety of the supra-molecular associates, wherein the fluid lamellar phase (L_α) is the biologically most relevant phase.
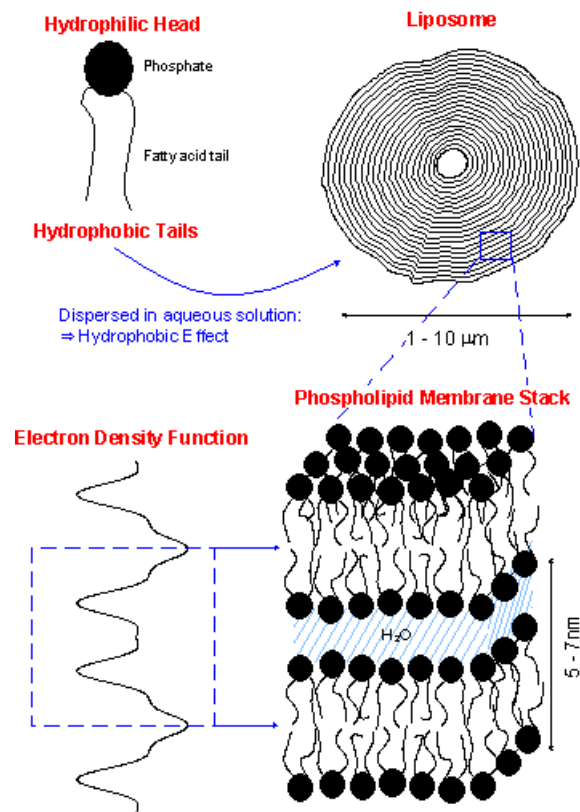

Fig. 1: *The formation of a phospholipid membrane. Phospholipids aggregate spontaneously into ordered supra-molecular structures in the presence of water. This can be explained in simple terms by the fact that phospholipids feature a hydrophilic headgroup (attracting water) and hydrophobic hydrocarbon-chains (expelled from water). The average 1-dimensional repeat distance d, i.e., bilayer plus water layer of the depicted fluid lamellar phase (L_α) is in the range of 5-7 nm. The electron density distribution of a bilayer (bottom left corner) has maxima in the headgroup regions and a minimum at the methyl terminus of the hydrocarbon-chains.*

Modeling of X-ray Patterns of Membranes [1]

From the viewpoint of a scattered X-ray wave, lipid bilayers in the L_α phase are two-dimensional fluids. There is a modulation of the electron density along the z-coordinate, which is normal to the bilayer plane. On the other hand, the electron density is practically constant in the x, y-plane of the bilayer at a certain z, i.e. a lipid bilayer may be considered to be ideally flat. Hence, in the case of a dispersion of multilamellar vesicles the scattered intensity is dependent on the radial scattering vector q only:
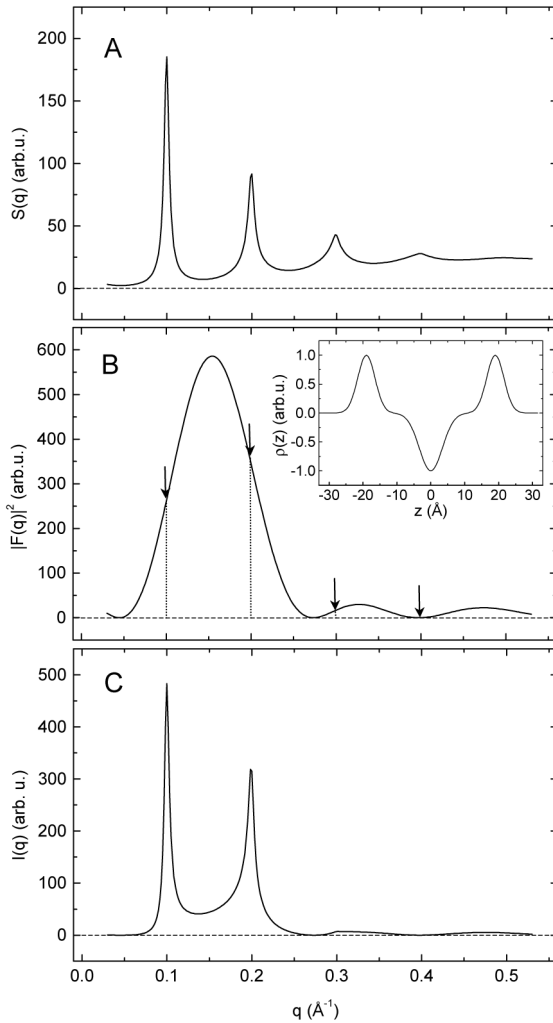
$$I(q) = \frac{S(q)|F(q)|^2}{q^2},$$ (1)

where $S(q)$ is the structure factor given by the Fourier transform of the crystalline lattice and $F(q)$ the form factor, which is the Fourier transform of the bilayer electron density distribution:

$$F(q) = \int_{-d/2}^{d/2} \rho(z)\exp(-i\,q\,z)dz.$$ (2)

Fig. 2: Effects of the structure factor (A) and the form factor (B) on the observed diffraction pattern (C) (Eq. 1). The form factor acts basically as a weighing function, decreasing in the present example the third order and extinguishing the fourth order quasi-Bragg reflection (arrows mark the peak positions).

**Exercise 1: The diffuse scattering arising from unilamellar vesicles
(file: Formfactor.opj) [1]**

The electron density profile of a fluid bilayer can be easily modeled by one Gaussian per head group and another for the methyl trough at the center of the bilayer (compare Fig. 3). This accounts for the general features of the electron density modulation along the z-direction and requires the adjustment of only four parameters. Its Fourier transform is given by

$$F(q) = \sqrt{2\pi}\left[2\sigma_H \exp\left(-\sigma_H^2 q^2 / 2\right)\cos(qz_H) - \sigma_C \rho_r \exp\left(-\sigma_C^2 q^2 / 2\right)\right], \tag{3}$$

where z_H and σ_H and are the center and the width of the headgroup Gaussian, σ_C and ρ_r (= ρ_C / ρ_H) the width and the relative amplitude of the hydrocarbon chain Gaussian (Fig. 3). Figure 2 (see above) gives an illustrative example of a form factor and how it affects the observed scattered intensity.
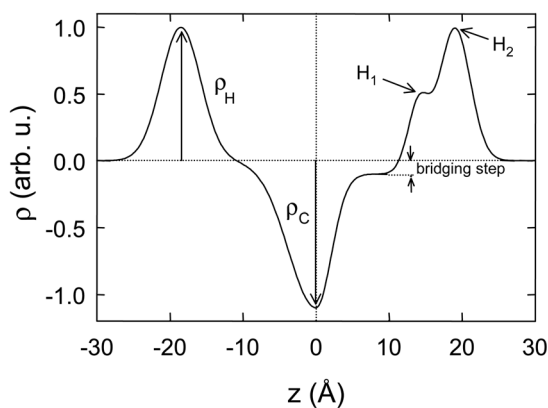

Fig. 3: *Comparison of the electron density profile models using 2 Gaussians (left-hand side) and 3 Gaussians, respectively (right-hand side).*

In the worksheet "raw data" the q-axis (q = $4\pi\sin(\theta)/\lambda$) and the scattered intensity of unilamellar lipid vesicles is given.

1.) How big is the influence of the structure factor on the scattered intensity?

2.) Fit the diffuse scattering using the 2-Gaussian model of equation (3)

3.) How thick is the bilayer, how big is the headgroup?

Exercise 2: Electron density map of DMPE @ 55 °C (file: Rho.opj) [2]

In the worksheet "raw.data" the s-axis ($s = 2\sin(\theta)/\lambda$) and the intensity data $I(s)$ of DMPE in the liquid crystalline phase are given. This phase has a one-dimensional lattice and the first four reflections $I(h)$ are included ($h = 1, 2, 3$ and 4). However, before the reflections can be analyzed, the data has to be corrected with the so-called Lorentz factor (this factor accounts for the underestimation of higher order reflections due to geometry). After the intensities of the reflections are determined and the repeat distance d of the lattice has been deduced, the electron density function can be calculated. For centro-symmetric structures as for membrane stacks the electron density function reduces to the Fourier summation of the cosine terms:

$$\rho(x) = \sum_{h=1}^{h\,max} \pm F_h \cos\left(\frac{2\pi xh}{d}\right),$$

(4)

wherein h is the order of the Bragg-reflection, x are distances in real-space, d gives the lattice constant, i.e. $d = 1/s_{h=1}$, and F_h are the form factors.

1.) Multiply $I(s)$ with the Lorentz factor s^2 and plot the data (compare eq. 1).

2.) Fit all four orders $I_h(s)*s^2$ with Lorentzian's and write down d and the area of the peaks.

3.) Create an x-axis for one unit cell in the worksheet "rho".

4.) Calculate and plot the electron density $\rho(x)$. The form factors F_h are given by the square-root of the fitted peak areas. The corresponding phases are -,-,+,-.

5.) Identify lipid headgroup, hydrocarbon and water regions in the electron density map.

Exercise 3: *d*-spacing of POPC as a function of temperature (file: Tscan.opj) [3]

The worksheet "tscan" includes the *s*-axis and 9 diffraction patterns at different temperatures - for all patterns POPC is in the lamellar liquid crystalline phase. The aim of this exercise is to determine the temperature dependence of the *d*-spacing.

1.) For each temperature find out the *d*-spacing by fitting the first order Bragg peak.

2.) Create a *T*-axis and plot *d* versus *T*.

Exercise 4: *d*-spacing of POPC during a temperature jump (file: Tjump.opj) [4]

The worksheet "tjump" includes the *s*-axis and 11 frames containing the diffraction patterns at different times during a *T*-jump experiment. The time-axis of the experiment and the measurement time per frame is given in the worksheet "time". The *T*-jump of about 15°C in 2 ms was induced with a IR-Laser (Fig. 4). The temperature before the laser trigger was set to 30°C.
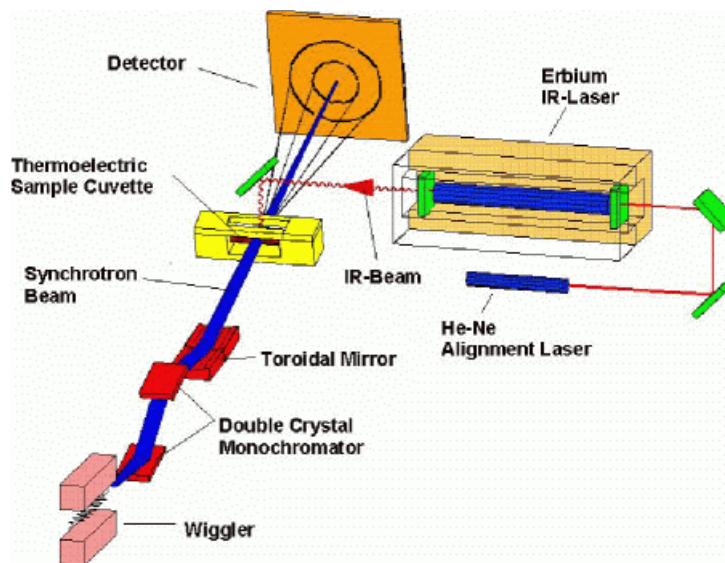

Fig. 4: *Sketch of the T-jump set-up at the SAXS-beamline (ELETTRA).*

1.) For each frame find out the d-spacing by fitting the first order Bragg peak.

2.) Plot *d*-spacing versus time.

3.) Compare the *T*-scan results with the *T*-jump results.

Literature:

Parts of this tutorial (pages 3-4) base on the book chapter:

[1] Rappolt, M., Laggner, P., and Pabst, G. (2004): Structure and elasticity of phospholipid bilayers in the Lalpha phase: A comparison of phosphatidylcholine and phosphatidylethanolamine membranes. In: Recent Res. Devel. Biophys. Vol 3, Part II, Transworld Research Network, editor S.G. Pandalai, Trivandrum, pp. 363-392.

For a deeper insight into the exercise 2, you may read:

[2] Rappolt, M., Hickel, A., Bringezu, F. and Lohner, K. (2003): Mechanism of the lamellar/ inverse hexagonal phase transition examined by high resolution X-ray diffraction. Biophys. J. 84: 3111-3122.

The data of exercises 3 and 4 are published in:

[3] Laggner, P., Amenitsch, H., Kriechbaum, M., Pabst, G. , and Rappolt, M. (1998): Trapping of Short-Lived Intermediates in Phospholipid Phase Transitions: The $L_{\alpha*}$ - Phase. Faraday Discuss. 111, 31-40.

[4] Pabst, G., Rappolt, M., Amenitsch, H., Bernstorff, S. , and Laggner, P. (2000): X-ray kinematography of temperature-jump relaxation probes the elastic properties of fluid bilayers. Langmuir 16, 8994-9001.