



	Experiment title: Structure of the lyotropic mesophases of the macroscopically large planar phospholipid membranes	Experiment number: SC-4246
Beamline: ID31	Date of experiment: from: July 27, 2016 to: August 1, 2016	Date of report: September 9, 2016
Shifts: 15	Local contact(s): Veijo Honkimaki, Maria Blanco	<i>Received at ESRF:</i>
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Report:

During the course of the experiments at ID31 we obtained new data on lipid polymorphism for the model biological membrane. We used recently developed method for obtaining macroscopically large planar phospholipid bilayers on top of a liquid substrate surface [1]. We have collected a set of data on the ordering of DSPC phospholipid molecules at the surface of hydrosol substrates with 5-nm and 7-nm particles at various sodium concentrations. We also found very sharp peaks in the diffuse scattering from the multilayer of membranes that allows studying interlayer correlations in it. The usage of high energy beam at ID31 (photon energy ≈ 71 keV with wave-length $\lambda \approx 0.17$ Å) appeared to be very useful for the reduction of the radiation damage of the samples. Overall, ID31 offers outstanding capabilities to carry out studies of liquid surfaces.

The surface normal structure of the lipid bilayers we studied by the high-resolution x-ray reflectometry up to $q_z = (4\pi/\lambda)\sin\mu \sim 1.5$ Å⁻¹ (see Figure 1a and 1b). Their lateral structure was probed by both grazing-incidence x-ray diffraction and off-specular scattering methods (see Figure 1c and 1d). For the scattering measurements we used the detector scan at the fixed grazing angle, μ , of the probe beam. The scattering measurements were performed at 3 different grazing angles of the probe beam varying from 0.01 deg to 0.1 deg.

In the preliminary analysis we have applied free-form approach for reconstruction of the dielectric function $\varepsilon(z)$ [2, 3]. This is self-consistent approach for x-ray data analysis, which is based on special iterative procedure taking into account the roughness parameters (PSD-function) when reconstructing the dielectric constant profile $\varepsilon(z)$ and, vice versa, the function $\varepsilon(z)$ when determining the PSD-function. The example of the reconstruction for multilayer is shown in Figure 1b. In further analysis we plan to use scattering data to distinguish between contribution to scattering of the surface roughness and nanoparticles placed in the liquid body. Analysis of the scattering distribution will be performed in the frame of the perturbation theory.

The following information we are going to obtain from the collected data: (a) to reconstruct the depth-distribution of the dielectric constant $\varepsilon(z)$ in the near surface layers; (b) to determine in-plane lattice parameters of the bilayers (c) to determine the PSD-functions of the studied surfaces. These results will allow us: (d) to compare the near surface structure of membranes with existing biophysical models, (e) to obtain new quantitative information about lipid mesophases that is necessary for the better understanding of the physical and chemical properties of biological membranes.

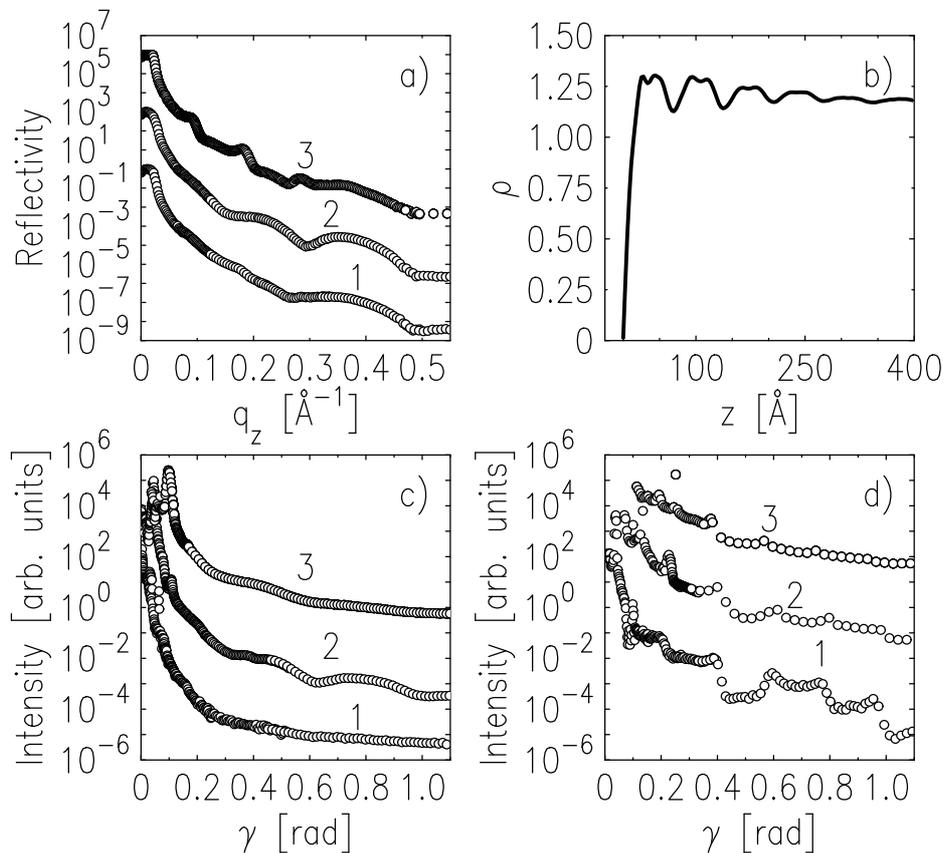


Figure 1. a) x-ray reflectivity as a function of q_z from the surface of silica hydrosol of 5-nm particles: 1 corresponds to monolayer; 2 corresponds to bilayer; 3 corresponds to multilayer; b) example of the reconstructed electron density profile of DSPC multilayer on top of 5-nm particle sol; c) x-ray diffuse scattering from lipid bilayer as a function of the out of plane angle, γ , at different incident angles: 0.01 deg (1), 0.018 deg (2) and 0.06 deg (3); d) x-ray diffuse scattering from the lipid multilayer as a function of the out of plane angle, γ , at different incident angles: 0.018 deg (1); 0.067 deg (2); 0.1 deg (3).

Preliminary results: The set of our data indicates that the total thickness of the adsorbed DSPC multilayer is defined by the Debye screening length $\sim \Lambda_D$ in the bulk of the hydrosol. The enrichment of the hydrosol substrate with NaOH results in a decrease by several times in the maximum thickness of the adsorbed lipid layer according to a decrease in Λ_D . The thickest (~ 450 Å) multilayer of six-eight DSPC bilayers is formed at the surface of the hydrosol of 7-nm particles at $\text{pH} \approx 9$ ($\Lambda_D \sim 300$ Å), the multilayer with a thickness of ~ 300 Å is formed at the surface of the hydrosol of 5-nm particles at $\text{pH} \approx 11$ ($\Lambda_D \sim 100$ Å) consists of three - four bilayers, and one bilayer appears at $\text{pH} \approx 11.5$ ($\Lambda_D \sim 50$ Å). In those cases, the oriented packing of molecules inside each bilayer corresponds to two-dimensional phospholipid crystals. We also found that at $\text{pH} > 11.5$ phospholipid molecules arrange at the hydrosol's surface into a monolayer.

To summarize, electron density profiles reconstructed within the model-independent approach demonstrate that the thickness of the DSPC film adsorbed on the surface of the hydrosol coincides in order of magnitude with the Debye screening length in the substrate. At the volume concentration of NaOH ~ 0.5 mol/L and $\text{pH} \approx 11.5$, a macroscopically flat phospholipid membrane with a thickness of ~ 60 Å and with the value for area per molecule ~ 45 Å². Sharp peaks in the diffuse scattering from the multilayer of bilayers are the evidence for organic crystal high degree of perfection at the hydrosol's surface.

References

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