



	<b>Experiment title:</b> Kinetics of the formation and thermotropic phase transition in the phospholipid multilayers at the hydrosol substrate	<b>Experiment number:</b> SC-4845
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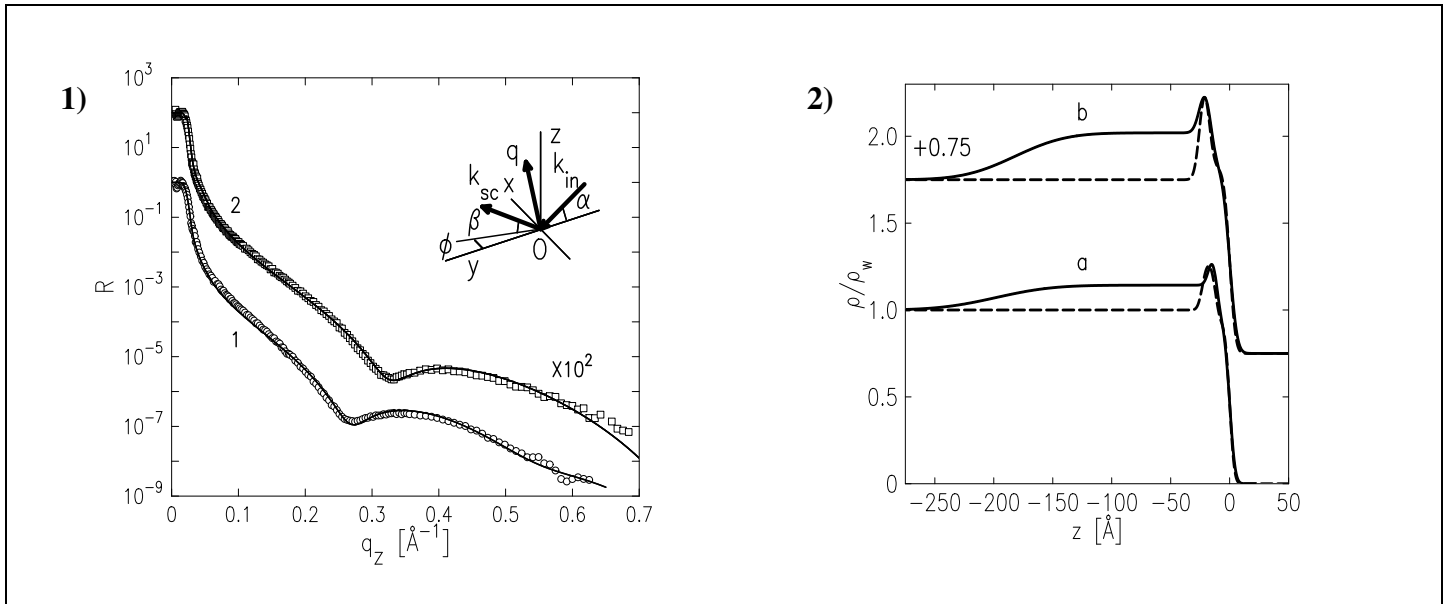
**Report:** During this round of experiments we have collected publishable sets of data on the reversible thermotropic phase transitions in planar multilayers of DMPS (1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine) and DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) phospholipid molecules at the surface of hydrosols substrates with particles 7-nm in diameter particles of SiO<sub>2</sub>. We used developed by us method for obtaining macroscopically planar phospholipid multilayers on top of the amorphous silica sol's surface [1,2]. For the description of three-dimensional surface structures from X-ray scattering data with high spatial resolution we used both a self-consistent free-form approach and a model approach in the distorted wave Born approximation (DWBA). Our preliminary analysis of the data obtained demonstrated the unique sensitivity of the ID31 equipment for the study of the phase transitions in the phospholipid layers.

The lateral structures of the multilayers were probed by both off-specular scattering and grazing-incidence X-ray diffraction methods. We carried out measurements of diffuse scattering background for the above mentioned systems in a wide range of the detector angle,  $\gamma$ , that is from 0 to  $\sim 1$  deg. For the scattering measurements we used the detector scans at the fixed grazing angle,  $\mu$ , of the probe beam (at 0.012 deg or 0.02 deg). We observed sharp peaks for both phospholipid liquid-crystalline multilayers. Finally, according to the experimental data as temperature rises the phospholipid multilayers undergo a reversible phase transition from solid to a liquid state. We applied grazing incidence diffraction method to study in-plane correlations in the multilayers to trace the phase transition in them from the hexagonal (liquid-crystalline) phase to a liquid phase that is related to the melting of hydrocarbon chains of phospholipid molecules.

According to our new data, the phase transition is reversible in temperature and it is the first order one. We reproduced the results of the previous experiment on the kinetics of the ordering of multilayer structures of DMPS and DPPC and obtained new more detailed results on the temperature behavior of these multilayer structures. We are now in the process of preparing a manuscript, so publication will take place shortly.

Further, the effect of the adsorption of a polypeptide on the lateral interaction of dimyristoylphosphatidylserine molecules in different phase states on the surface of a 10 mM KCl aqueous solution has been studied as well. In this part, to study the effect of the adsorption of polypeptide on the lateral interaction of lipid molecules in different phase states, we use dimyristoylphosphatidylserine (DMPS), which is saturated analog of phosphatidylserine. We collected experimental data indicating structural changes in the DMPS monolayer in both the liquid expanded (LE) and the liquid condensed phase (LC) state in the presence of various poly-D-lysine hydrobromide molecules at its water interface. These data were obtained when studying both the compressibility of the lipid monolayer using the Langmuir monolayer technique and from X-ray reflectometry with synchrotron radiation. These results are partially published in [5].

Figure I presents dependences of reflectivity  $R$  as a function of  $q_z$  for the DMPS monolayer on the surface of water at an area of  $72 \text{ \AA}^2$  for the lipid monolayer (circles) without and (squares) with the adsorption polymer layer. Lines 1 and 2 are the respective model calculations.



**Figure 1.** Dependence  $R(q_z)$  for the DMPS monolayer on the surface of water at an area of  $72 \text{ \AA}^2$  for the lipid monolayer (circles) without and (squares) with the adsorption polymer layer. Lines 1 and 2 are the respective model calculations. The inset shows the kinematics of surface scattering. **Figure 2.** Electron density profiles in units of the electron density in water under normal condition for the monolayer (dashed lines) without and (solid lines) with the polymer. The point  $z = 0$  is chosen at the interface between lipid molecules with air. Lines *a* and *b* correspond to the liquid (LE) and condensed (LC) states of the monolayer. For convenient comparison, lines *b* are shifted along the *y* axis by 0.75.

**Preliminary results:** Figure 2 presents the electron density profiles perpendicular to the surface of the aqueous subphase those have been reconstructed from reflectometry data within a model approach to the structure of an interface with two and three layers. These profiles indicate the existence of a wide diffuse polymer layer ( $150 \pm 40$ )  $\text{\AA}$  in width at the interface of the monolayer in both the liquid expanded and liquid condensed states. A decrease in the area per molecule in the monolayer by a factor of 2 results in the doubling of the surface density of the macromolecule film. The adsorption of the polymer also affects the integral density of the layer of polar phospholipid groups, which decreases by a factor of  $\approx 2$  in the liquid expanded phase and by  $\sim 30\%$  in the liquid condensed phase (LC).

To summarize the above results, we note that the choice of the polypeptide, its density, and a sufficiently large molecular mass ensures the maximum expected filling of the surface by macromolecules. Changes in the reflectivity curves are the most significant in the region where the monolayer behaves as a two-dimensional liquid. The structural parameters of the interface estimated within the two-layer model are in good agreement with the geometrical characteristics of the amphiphilic lipid molecule. We believe that the described methods for the study of complex polymer-lipid systems at the water–air interface are useful for various charged macromolecules having biomedical applications. Corresponding experimental results will allow confirming or rejecting molecular structures actively analyzed by molecular dynamics methods. Overall, ID31 offers outstanding capabilities to carry out studies of such planar lipid-polymer structures. The usage of high energy beam at ID31 appeared to be very useful for the reduction of the radiation damage to the samples.

## References

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