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Beamline: ID01	**Date of experiment**: from: May 11th , 2009 to: May 17th , 2009	**Date of report**: March 1st, 2010
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Report:

We have studied the rhombohedral (*R*) phase in the lipids DOPC, DPhPC, DOPC/Cholesterol mixtures (90:10, 80:20, 70:30) and mixtures of a brominated lipid (DSPC-Br) with DOPC and DPhPC. For DPhPC, the rhombohedral phase has been shown to consist of so-called *stalks* [1], which are regarded as intermediate structures during fusion of biological membranes. Goals of the experiment were to study (a) how stalk structures vary for different lipids and cholesterol content, (b) if and to what extent demixing of lipids with different spontaneous curvature can be observed and (c) how the phase transition from the lamellar (*L*) to the *R* phase proceeds. Data analysis is still in progress, below we present some preliminary results.

The rhombohedral phase can be observed in some phospholipids upon controlled dehydration, which is achieved by lowering the relative humidity (RH) of the air surrounding the sample. To this end, we used a home-built environmental chamber with precise RH control, which was interfaced with the beamline. The lyotropic (i.e. hydration-dependent) phase diagrams of the lipids were determined prior to the experiment at ID01 using a laboratory-scale GISAXS instrument described in [2].

At ID01, we used highly aligned stacks of phospholipid bilayers on Si substrates with a size of 15×10mm². Sample preparation is described e.g. in [4]. Measurements were carried out in grazing-incidence geometry at a photon energy of 17 keV. The beam was collimated to a size of 500×100µm², the ionization chamber indicated a primary intensity of typically 8.0×10^9 photons/s. Diffraction patterns as shown in Fig. 1 were recorded on the Princeton CCD detector (1340×1300 pixels) at a sample-to-detector distance of typically 50cm. For each sample, diffraction patterns where recorded at 5-6 different hydration levels (ΔRH=2%) in order to solve the crystallographic phase problem by the swelling method

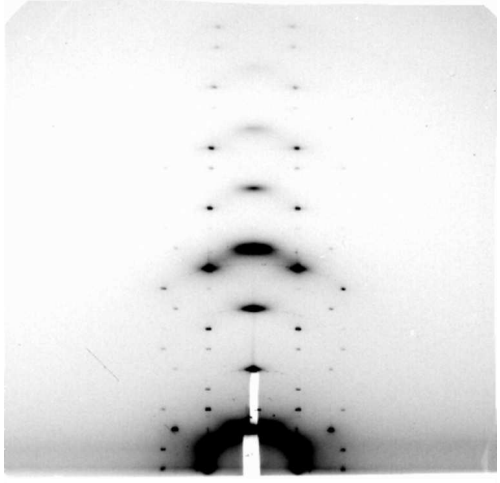

Fig. 1: Diffraction pattern of the rhombohedral phase of DOPC/cholesterol (70:30 mol:mol) recorded in grazing-incidence geometry at ID01. The image is the sum of several CCD images. Between subsequent exposures, the sample was shifted along the beam to avoid radiation damage.

[3]. Additional reflectivity scans required for correct determination of the form factor amplitudes $|F(\mathbf{q}=(0,0,q_z))|$ along the q_z axis were recorded after beamtime using a home-built instrument at our institute. Electron density maps as shown in Fig. 2 can then be reconstructed by Fourier synthesis. In further steps of the analysis, we will extract characteristic structural properties such as stalk width or thickness of the water layer as a function of lipid composition and attempt to adress the curvature characteristics of the individual lipid monolayers.

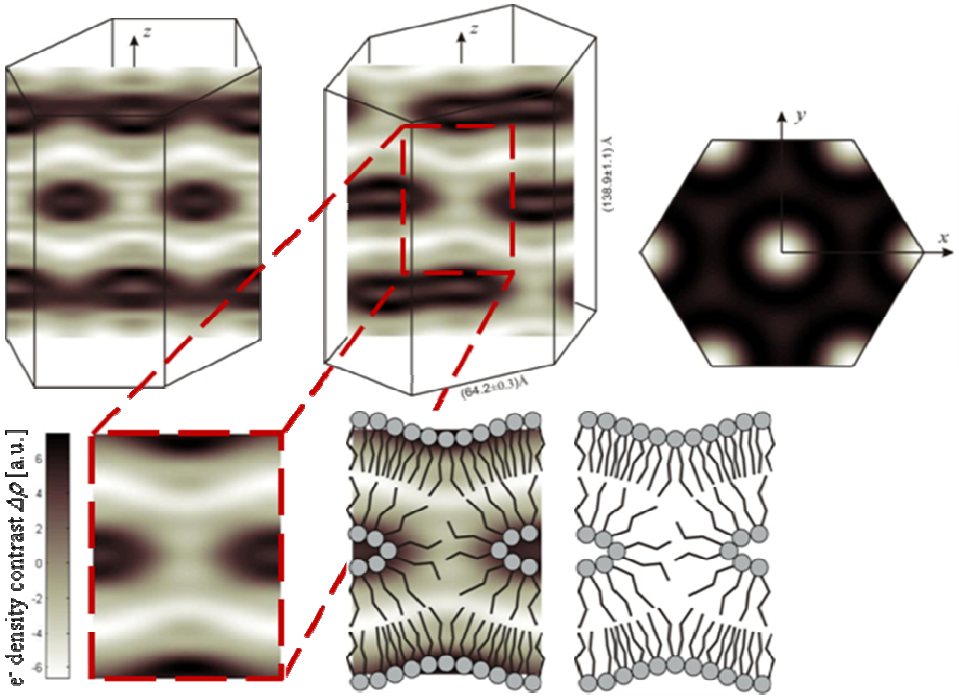

Fig. 2: *upper row:* Electron density maps in the *xy*, *yz*, and *xy* planes of the hexagonal unit cell of the *R* phase. White corresponds to low electron density regions (methyl trough region), high electron density (lipid head-groups) is indicated black. The sample consists of continuous lipid monolayers, adjacent lipid bilayers are connected by stalks. *lower row:* Magnification of the electron density of one stalk along with a sketch of possible lipid molecule positions.

A second objective of the experiment was to get further insight into how the phase transition from the lamellar to the rhombohedral phase proceeds. Our hypothesis is that stalks may form transiently and without long-range correlation when lipid bilayers in the lamellar phase are brought into sufficiently close contact. To test whether this is the case, we recorded GISAXS patterns as described above at RH values around the $L \rightarrow R$ phase transition for the lipids lipids DOPC (RH=50-40%) and DPhPC (RH=87-78%) in RH steps of 1%.

We could observe two apparently different scenarios: In DPhPC, coexistence of the *L* and *R* phases with clearly different *d*-spacings was observed, indicating a 1st order phase transition. In DOPC, in contrast, the phase transition proceeded in a rather continuous fashion. Prior to appearance of distinct off-axis peaks of the rhombohedral phase, pronounced diffuse scattering and changes in the shape of the Bragg sheets could be observed (Fig. 3). Both features were absent in case of DPhPC. Further analysis similar to [5] will involve modeling of the scattered intensity $I(q_{||},q_z)$ of single and correlated stalks.

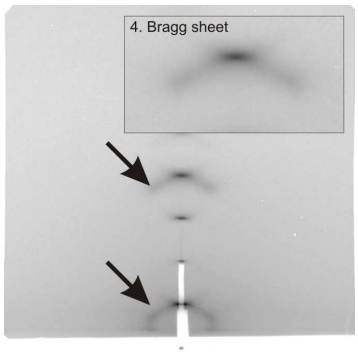

Fig. 3: Diffuse scattering at a hydration level slightly above the $L \rightarrow R$ phase boundary (DOPC, RH=43%).

References

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