ESRF	**Experiment title:** Grazing incidence diffraction of highly oriented multilamelar lipide-peptide systems	**Experiment number:** SC-718
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Report:

We used grazing incident diffraction (GID) to investigate the structure of lipid membranes and the influence of membrane-active (antibiotic) peptides by mapping a wide range of the reciprocal space with a 2-dimensional CCD-camera.

A high orientation of the membranes is necessary to also get information about the lateral structure of the membranes [1,2]. We achieved this by spreading lipid or lipid-peptide solutions on silicon wafer and rotating them at frequencies of about 200 rpm on a spincoater ('slowspinning'), the samples then consisted of several 100 membranes.

For the experiment which was carried out at an energy of 20 keV we used a Princeton CCD-camera with a resolution of 1242 x 1152 pixels. By this we were able to map a large part of the reciprocal space by moving the camera to some positions and taking a set of 5 pictures, each picture with an exposure time of 100 s and 300 s. The angle of incidence was α_{in} = 1° for the picture with the Bragg sheets and for the others it was set to α_{in} = 0.5° to optimize scattering intensity.

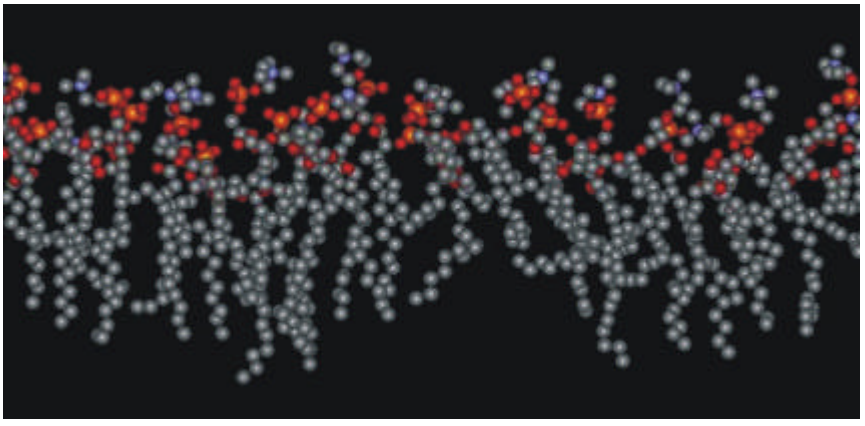

Fig. 1: A sketch of lipids in the L_a phase taken from a molecular dynamics simulation of Heller et al. [3].

All the samples were measured at a temperature of 45.0°C in a humidity chamber, thus the lipids were in the fluid (L_α-) phase (Fig. 1).

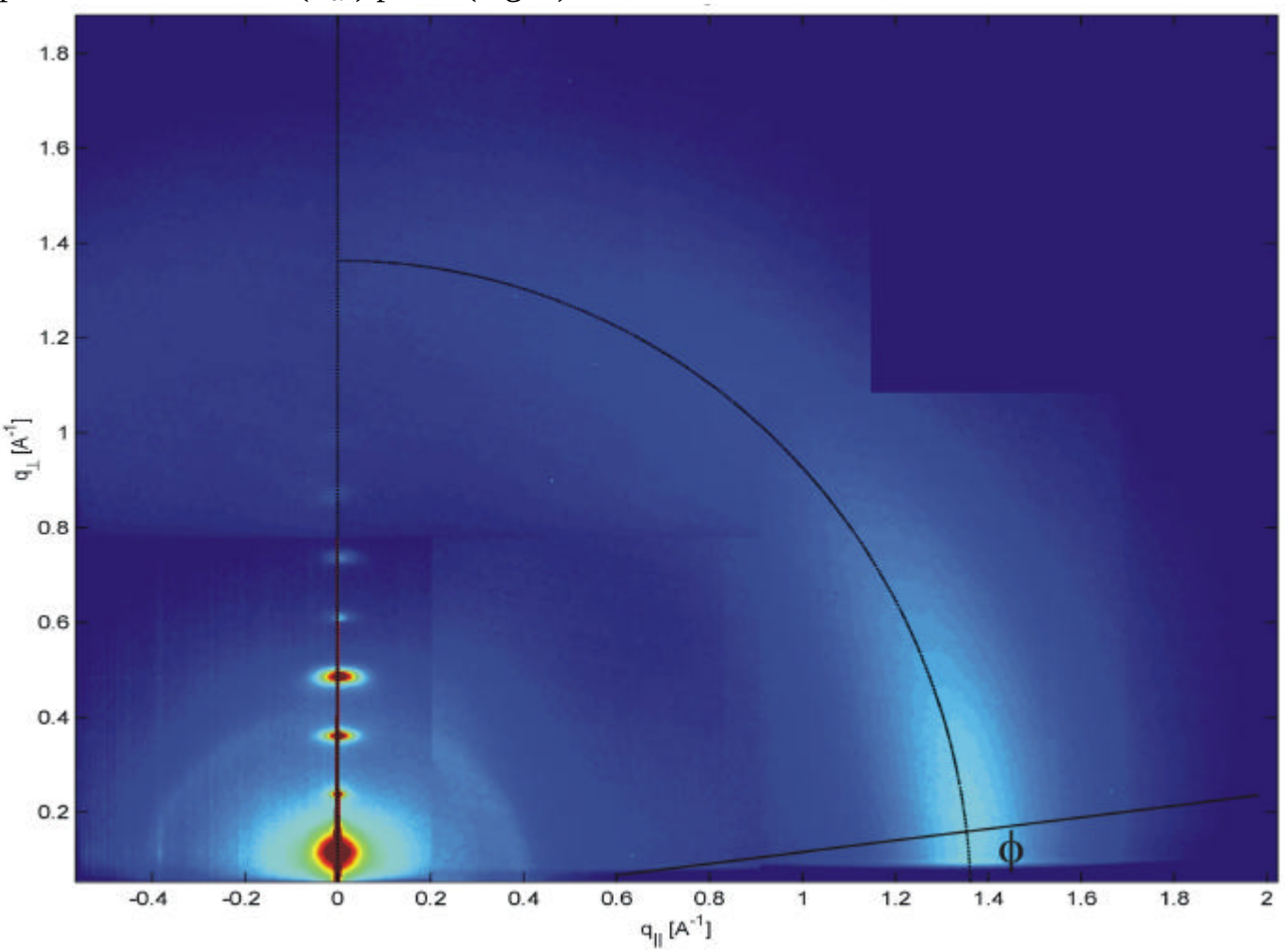

Fig. 2: The reciprocal space mapping (RSM) for pure OPPC with a radial slice at an angle ϕ to the $q_{||}$-axis. The has been computed by combination of different CCD exposures.

For the evaluation we used the MATLAB package and first wrote a script to combine the different exposures. Figure 2 shows the resulting map for a sample of pure OPPC.

One can clearly observe the Bragg sheets along the vertical q-axis (the red line is due to detector saturation) and the chain correlation peak at $q_{||}$ = 1.38 A^{-1}. The ring around the primary beam at q = 0.4 A^{-1} stems from the Kapton windows. Note that the primary beam itself is located below the image and therefore not visable.

In the analysis we concentrated mainly on the chain correlation peak by evaluating slices (from q = 0.6 … 2 A^{-1}) at angles of 5° to 90° to the $q_{||}$-axis. The lower limit of 5° is dictated by the sample horizon at q_z = 0.09 A^{-1}.

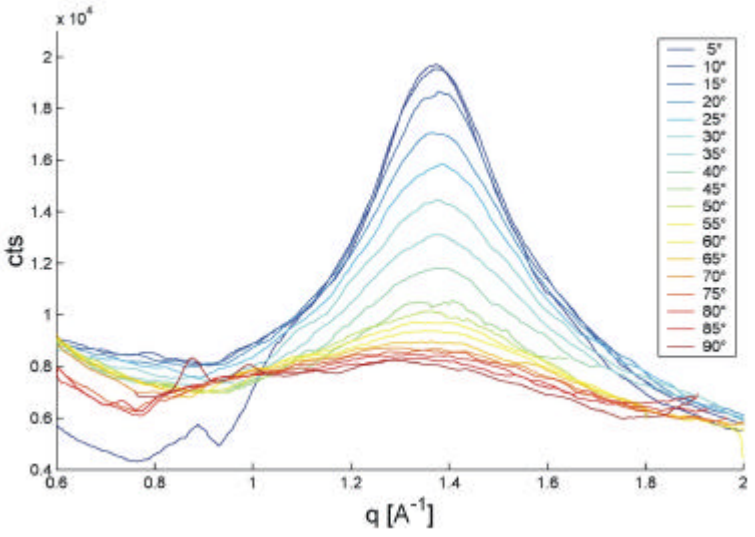

Fig. 3: The decrease of the chain correlation peak with increasing angle ƒ.

The decrease of the chain correlation peak with increasing angle ϕ is displayed in figure 3.

The peak lineshape is a Lorentzian and can be fitted automatically for all angles. These fits were evaluated with regard to the peak position, peak width at half maximum (FWHM), and t integrated intensity.
The position of the maximum for the different lipids DLPC, DMPC and OPPC is at about 1.38 $Å^{-1}$, independent of ϕ. This value corresponds to an average chain-chain-distance of 4.5 Å. From the FWHM of the peak, the chain correlation length can be calculated. In pure lipids it decreases with increasing ϕ [2]. The integrated intensity of the chain correlation peak then gives information about the fraction of the lipids which are inclined at an angle ϕ to the surface normal. In pure lipids in the fluid phase has its maximum at 0°, because most chains are perpendicular to the membrane, and decreases for larger ϕ.

Apart from pure lipids, samples containing membrane-aktive peptides (Magainin2, Alamethicin) were investigated to deduce peptide orientation and conformation at the molecular scale. In the corresponding RSMs, the helix peak reflecting the helical pitch of a transmembrane α-helix is observed as a dominant feature at large peptide cocentrations, as known from transmembrane proteins [5]. At the same time, the short range order of the chains decreases significantly. Further data analysis in currently in progress. Additional insight will be derived by comparison to MS results, see Fig.1. The corresponding molecular coordinates can be Fourier transformed and directly compared directly to the experimental results [6].

References:

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