ESRF	**Experiment title:** Electric field – dependent fluctuations and instabilities in lipid bilayers	**Experiment number:** SC1136
Beamline: ID01	**Date of experiment:** from: 30/04/03 to: 08/05/03	**Date of report:** 04/08/03
Shifts: 21	**Local contact(s):** A. Mazuelas	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

T. Salditt*, D. Constantin*, C. Ollinger*

Institut für Röntgenphysik
Geiststraße 11, 37073, Göttingen, GERMANY
Phone : (49) 551 39 9427
Fax : (49) 551 39 9430
Email : tsaldit@gwdg.de

Report:

We have carried out an x-ray diffraction study of highly-oriented and solid-supported stacks of lipid bilayers under transverse electric field. It is known that, under the influence of the field, the bilayers peel off the substrate and form vesicles in solution (electroformation). This leads to a loss of scattering volume from the lipid film, making quantitative comparison between the scattering signal with and without field very difficult. In order to overcome this difficulty, we applied an osmotic pressure on the stack by filling the chamber with a 31 wt. % solution of PEG.

This strategy proved successful, as we had no sample loss during the experiments (performed at an applied field of 10 Vpp, the highest we ever used). The reflectivity scans before and after turning on the field are practically identical; in particular, there is no discernable shift of the Bragg peaks, so the smectic repeat distance is constant.

We also studied the effect of the electric field on the diffuse signal, recorded using the Princeton CCD detector, with an exposure time of 300 s. The image in Fig. 1 a) shows the difference between the signal with and without applied field.

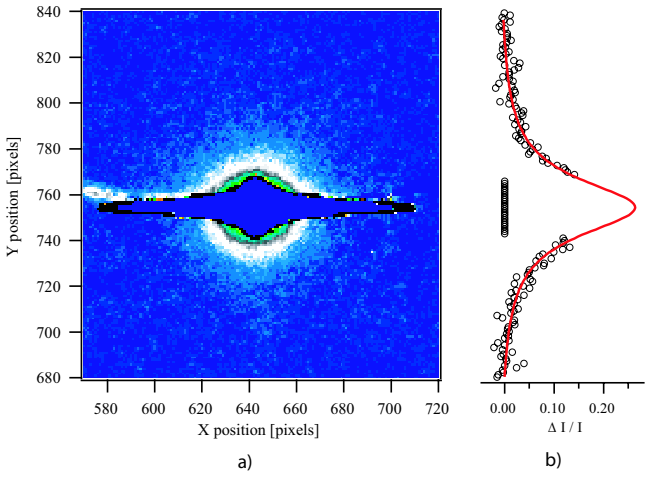

Fig. 1 : a) Difference between the scattering with and without electric field, in the region of the first Bragg sheet. b) Cut through the image in a), showing the relative intensity variation $\Delta I/I$. Solid line is a lorentzian fit. The gap corresponds to the saturated area.

One can notice an increase of the scattered intensity in the area of the Bragg sheets. In Fig. 1 b) we plot a section through the image in a), normalized to the initial intensity. The ratio $\Delta I/I$ is of about 20 %. The additional intensity might be due to an increased fluctuation amplitude of the bilayers, in all or only part of the sample. Detailed comparison with the point detector scans is required before reaching a conclusion. Combined with our previous results on the kinetics of unbinding, this result provides us with a global view of the electroformation phenomenon [1].

Besides their intrinsic physical interest, our results establish the feasibility of a stable experimental configuration, where a relatively high electric field can be applied to a stack of lipid bilayers in excess solvent for very long times (on the order of a few hours), without any detectable sample loss. We plan to use such a setup to study the influence of the electric field on biologically active molecules soluble in the lipid membranes, such as ion channels or antimicrobial peptides.

As a first step towards investigating the behavior of membrane-inserted peptides under electric field, we studied the scattering signal from lipid bilayer stacks containing the voltage-gated antimicrobial peptide Alamethicin [3] (produced by the fungus *Trichoderma viride*), as a function of temperature and concentration, under applied osmotic pressure, but without an electric field (for the time being).

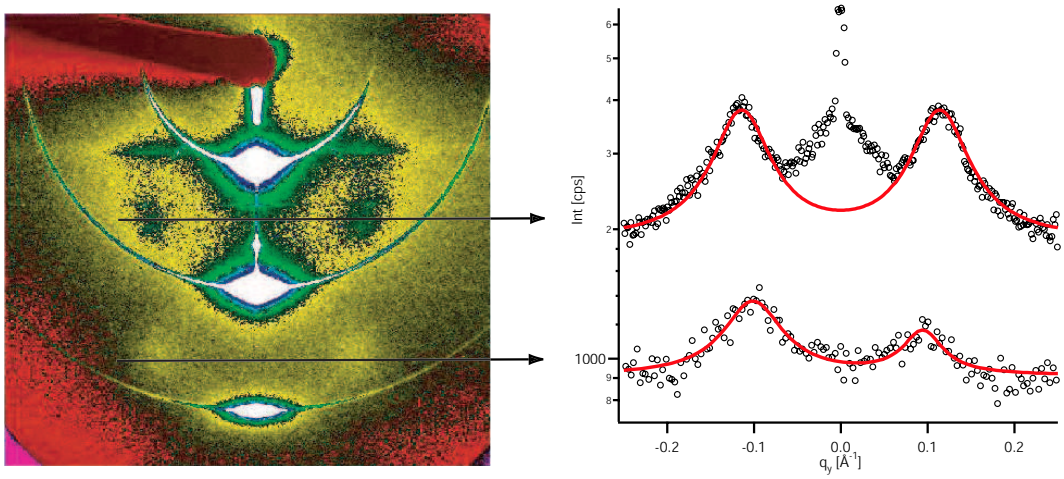

Fig. 2 : CCD image and point detector scans of the peptide correlation peaks for a peptide concentration of 50 mM.

It is generally accepted that antimicrobial peptides assemble into ring-like formation, creating pores into the membranes. Under certain conditions, these pores can crystallize in a 2D ordered structure (as shown experimentally for magainin and protegrin [2]). We observe that Alamethicin never crystallizes (even close to the main transition of the lipid), although it exhibits clearly visible correlation peaks (see Fig. 2). We conclude that the pores are in a liquid state at all the investigated concentrations, from 50 to 100 mM (at lower concentration, the peptide molecules do not form pores). Work is in progress on characterizing the intra- and inter- bilayer correlations of the pores [4].

[1] C. Ollinger *et al.* in preparation.
[2] L. Yang *et al.*, *Biophysical Journal*, **79** 2002-2009 (2000).
[3] B. Bechinger, *Journal of Membrane Biology*, **156** 197-211 (1997).
[4] D. Constantin *et al.* in preparation.