



<b>Experiment title:</b> Structure and correlations of alamethicin pores in lipid bilayers	<b>Experiment number:</b> SC1375	
<b>Beamline:</b> ID10A	<b>Date of experiment:</b> from: 28/04/04                      to: 03/05/04	<b>Date of report:</b> July 13, 2004
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## Report:

Continuing an investigation started during experiment SC1136 [1] (some results were already published in [2]) we studied the scattering signal from lipid bilayer stacks containing the voltage-gated antimicrobial peptide alamethicin (produced by the fungus *Trichoderma viride*), as a function of peptide/lipid concentration, under applied osmotic pressure.

We first tried to perform the measurements at an energy of about 13.5 keV, due to the experiment taking place concurrently with SC1376, which required this energy range. Unfortunately, our samples suffered from beam damage, so we had to increase the energy to 18.6 keV. The lack of the ID01 Princeton CCD detector posed another experimental problem, the replacement detector being less sensitive; as such, we could not observe the correlation peaks of the pores in the 2D images and we had to rely exclusively on detector line scans.

The experiment was performed in specially designed chambers with kapton windows; the beam path through the hydrating solution was 1 cm, in order to minimize absorption. The temperature was fixed by a Julabo heat bath at  $\sim 30^\circ\text{C}$  for all measurements. The samples (consisting of about 3000 bilayers of DMPC with dissolved alamethicin in a mol/mol ratio indicated by P/L) were hydrated by a solution of PEG 20000 at different concentrations. Some sample loss probably occurred by hydration, but it could not be quantified. All polymer solutions were prepared in 100 mM NaCl brine. We measured five samples under 31% PEG solution, at different P/L values, and another two samples at P/L=1/12.5 at lower PEG concentration (5.8 and 12.1%).

For each sample, we measured the specular reflectivity and a longitudinal scan; analysis of this data should yield the changes in electronic density profile, most notably the bilayer thickness, which appears to vary with peptide concentration [3].

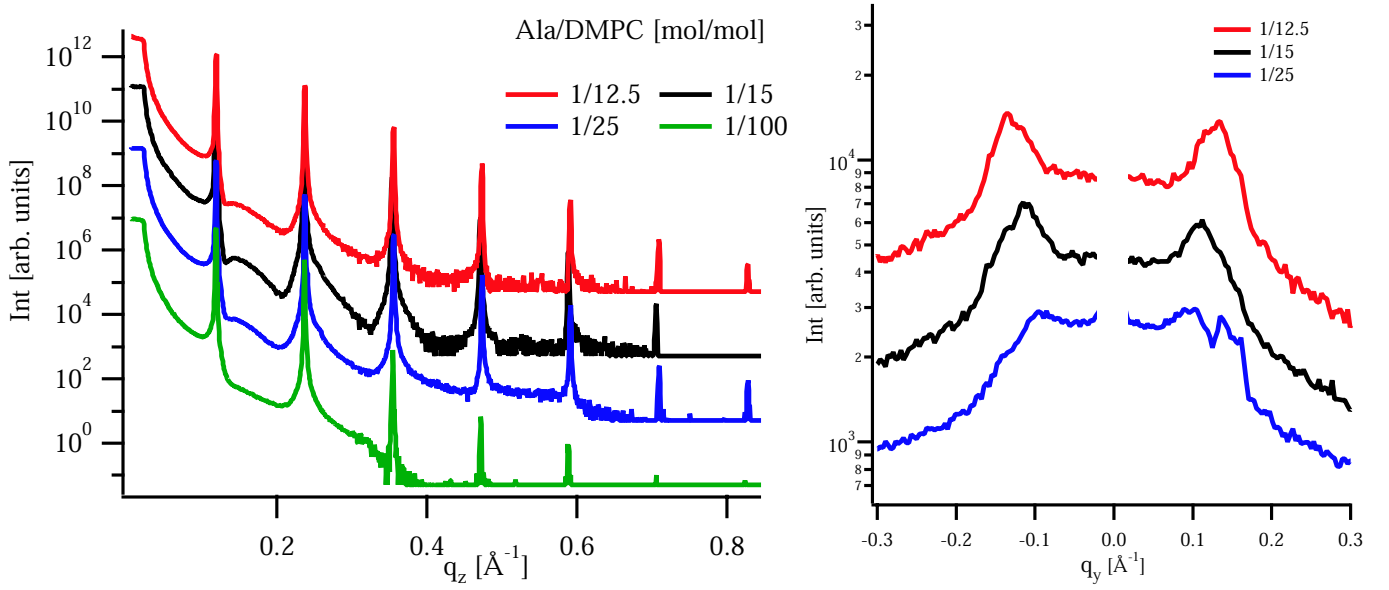


Fig. 1 : A) Specular reflectivity scans for different  $P/L$  concentrations in 31% PEG solution. Curves vertically shifted for clarity. B) Out-of-plane scans through the first pair of satellites for the same  $P/L$  values (except for 1/100, where no satellites can be detected). The central part, with a sharp specular component, was hidden. Curves vertically shifted for clarity.

We also scanned the  $q_y$  and  $q_z$  width of the 1<sup>st</sup> and 2<sup>nd</sup> satellites of the pore-pore structure factor. The "0<sup>th</sup> order" satellite, at  $q_z = 0$ , which was previously investigated [4], was not accessible in our case due to the scattering geometry [1]. Analysis of this data will be performed in the framework put forward by H. W. Huang *et al.* [5] and should yield information about the intra- and inter-bilayer pore-pore interaction. More precisely, the  $q_z$  scans through the satellites quantify the pore-pore correlation in the transverse direction (from one bilayer to the next), while the  $q_y$  scans (Fig. 1B) hold information on the correlation in the plane of the bilayers.

- [1] T. Salditt, D. Constantin and C. Ollinger, ESRF experimental report 25552\_A.
- [2] C. Li, D. Constantin, and T. Salditt, *J. Phys. Cond. Mat.*, **16** S2439–S2453 (2004).
- [3] F.-Y. Chen M.-T. Lee, and H. W. Huang, *Biophys. J.*, **84** 3751–3758 (2003).
- [4] K. He *et al.*, *Biophys. J.*, **70** 2659–2666 (1996).
- [5] L. Yang *et al.*, *Biophys. J.*, **75** 641–645 (1998) and **77** 2648–2656 (1999).