ESRF	**Experiment title:** High-Resolution Diffraction from Phospholipid Membranes with Pore Forming Antibacterial Peptides	**Experiment number:** SC-450
Beamline:	**Date of experiment:** from: 22-Apr-98 to: 27-Apr-98	**Date of report:**
Shifts:	**Local contact(s):** A. Souvorov	*Received at ESRF:* 1 - MAR 2000

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We have investigated fundamental structural aspects of the interaction between lipid membranes and the antimicrobial peptide magainin 2. This alphahelical peptide of 23 residue belongs to a large class of amphiphilic peptides common to the immune system of vertebrates. By direct interaction with the cell membrane, these peptides can lead to bacterial and microbial cell lysis [1]. Despite some recent advances, the microscopic mechanisms responsible for the function are presently not well understood. To elucidate the effect of the peptides on the membrane elasticity of the model lipid dimyristoyl phosphatidylcholine (DMPC) in its liquid $L\alpha$ phase, we have investigated highly aligned films of controlled peptide to lipid ratio P/L on Si substrates. For the x-ray experiment at BM5 (optics beamline) the films with a total thickness of about 10 μm were kept in a temperature and humidity controlled chamber.

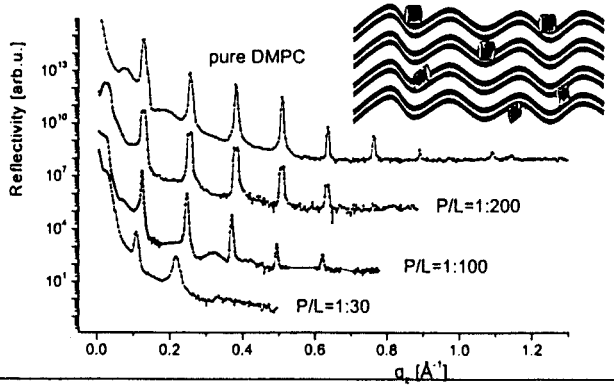

Fig.1: The reflectivity of the DMPC/Magainin membranes as a function of increasing peptide concentration. The decay of the higher orders and the overall reflectivity indicate increasing fluctuations and positional disorder with P/L. The schematic shows an undulating stack with short amphiphilic peptides.

For the first time reflectivity and diffuse scattering have been measured for a highly oriented stack of a lipid/peptide system. The mosaicity of the samples was below the resolution limit (≈0.01°). Despite long exposure times no radiation damage was observed at an x-ray energy of 20 keV due to the significantly reduced absorption in the samples. For the reciprocal space mappings of the diffuse (nonspecular) scattering measured both in and out-of- the plane of incidence the high brilliance of the source was particularly important.

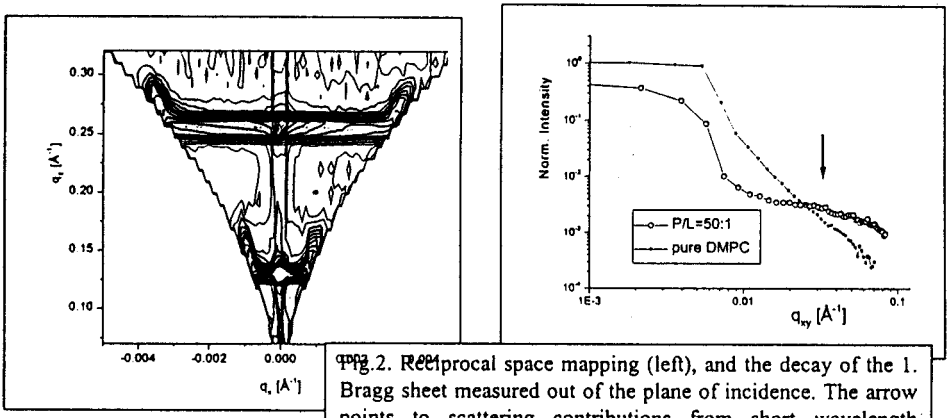

Fig.2. Reciprocal space mapping (left), and the decay of the 1. Bragg sheet measured out of the plane of incidence. The arrow points to scattering contributions from short wavelength distortions of the membrane induced by the peptides.

The results for the reflectivity curves show a drastical P/L dependence, see Fig.1. Two clear trends can be directly inferred from the data: (1) An increase in the d-spacing of the multilamellar stack which can be attributed to a swelling due to an increased charge density (4 charged lysine groups per peptide). This conclusion is presently supported by an ongoing reconstruction of the unit cell density profile (2). The ordering of the membranes decreases strongly with the number density of adsorbed peptides as is shown clearly by the vanishing of the higher order peaks and the overall intensity decay. To unambigiously determine the elastic constants B (compressional modulus) and K (bending modulus) we carried out extensive measurementsof the diffuse scattering. Fig. 2 displays a typical dataset of reciprocal space mapping in the plane of incidence for the case of P/L=1:200. The pronounced diffuse Bragg sheets indicate highly conformal thermal fluctuations which are vertically correlated over about 20 layers. Addtional scans were performed out of the plane of incidence. Fig.2 (right) illustrates the difference in the integrated intensity decay of the first Bragg sheet between a pure lipid and a lipid-peptide sample (ongoing analysis of the associated height-height correlation functions according to [2]).

In conclusion, we have been been able to apply for the first time both the specular and nonspecular reflectivity technique to lipid membranes with controlled peptide content. Dramatic changes in the fluctuations and hence in the elasticity constants occur already at a few molar percent of Magainin 2, which is believed to be in the physiologically relevant concentration range. The corresponding changes in elasticity could possibly be fundamental to the peptide function. They also provide valuable experimental data for comparison with current theoretical models of lipid-peptide interaction. After the completion of the ongoing data analysis future studies will concentrate on the role of the membrane charge as well as on the formation of stable pores as reported in litterature [1]. In a future study combining the out-of plane scattering geometry with an analyzer crystal at an undulator source it may become possible to detect directly the pair-pair correlation of the peptides.

[1] S.J. Ludtke et al., *Biochemistry* 35, 13723 (1996); R.A. Cruciani et al. *Proc.Natl.Acad.Sci. USA* 88, 3792 (1991); K. He et al, *Biochemistry* 34, 15614 (1995), and references therein.

[2] T. Salditt et al., *Europhys.Lett.* 32, 331 (1995).