ESRF	Ion Concentration Profile and Out-of-plane Structures of Bacterial Lipopoly-saccharides in the Presence of Divalent Cations and Antibacterial Peptides	**Experiment number**: SC- 2572
Beamline: ID10B	**Date of experiment**: from: 01.10.2008 to: 07.10.2008	**Date of report**: 18.03.2009
Shifts: 18	**Local contact(s)**: Dr. Amarjeet SINGH	*Received at ESRF:*

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The goal of our experiment was to investigate the concentration profile of monovalent and divalent cations near a monolayer of negatively charged mutant bacterial lipopolysaccharides (LPSs) at the air / water interface using X-ray fluorescence. The monolayer acts as an artificial model of the surface of Gram-negative bacteria, and was studied at room temperature and a lateral pressure of 20 mN/m. To prove the applicability of the technique, we focused on LPS molecules purified from Re mutants (LPS Re, see Fig. 1 left), since we had already characterized the electronic structure of LPS Re monolayers in our GISAXS (grazing-incidence small-angle x-ray scattering) experiments at ID10B beamtime. A sketch of the experimental setup is shown in Fig. 1 (right).

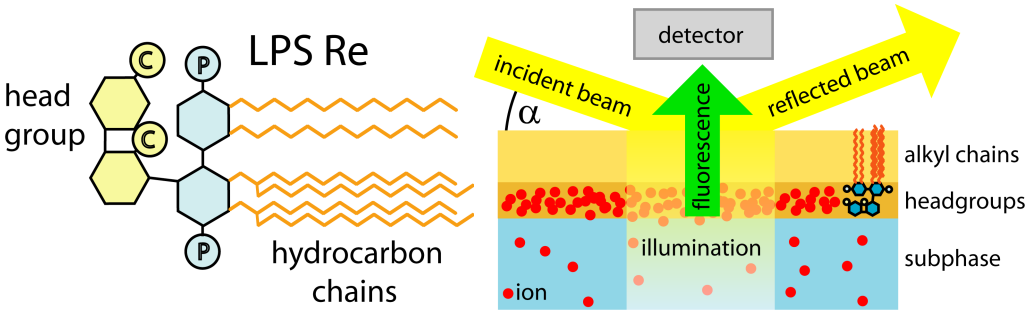

Figure 1: (left) Schematic structure of the studied molecule, LPS Re. (right) Sketch of the experimental setup for X-ray fluorescence.

In our previous GID (grazing-incidence x-ray diffraction) and GISAXS experiments, we demonstrated that divalent cations have a significant impact on the in-plane ordering of the alkyl chains and the out-of-plane conformation of saccharide head groups of mutant LPSs [1]. In fact, the observed conformational change of saccharide groups seems responsible for the survival of bacteria against protamine, a cationic antimicrobial peptide [2]. During our recent beamtime allocation we used a monochromatic beam of 8 keV to excite the fluorescence of K^+, Cl^-, and Ca^{2+} ions as a function of the incident angle α (see Fig. 1). Fig. 2 (left) shows two fluorescence spectra from an LPS Re monolayer on Ca^{2+}-loaded buffer, recorded below and above the critical angle of total

reflection with an energy-sensitive detector. The right panel shows the integrated K^+ fluorescence intensities from an LPS Re monolayer on Ca^{2+}-free buffer and from a blank buffer as a function of the vertical component of the scattering vector, which varies along with the angle of incidence.

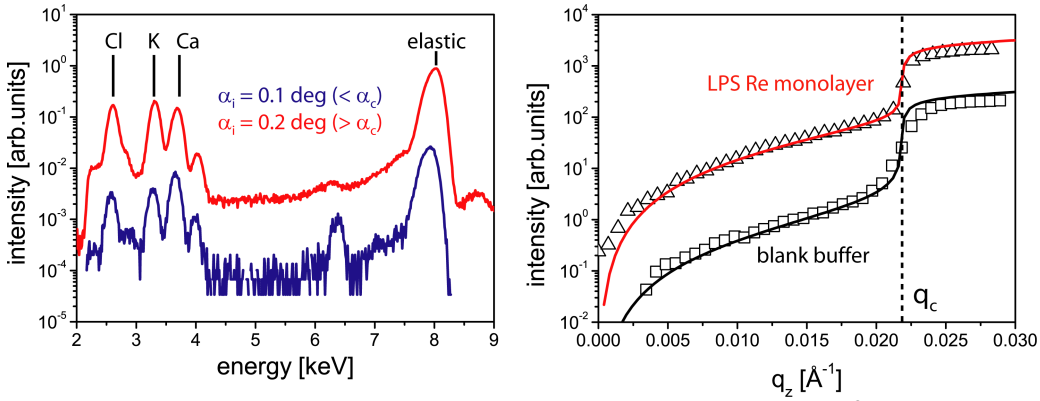

Figure 2: (left) Fluorescence spectra from LPS Re monolayer on Ca^{2+}-loaded buffer. (right) K^+ fluorescence intensity from LPS Re monolayer on Ca^{2+}-free buffer (triangles) and from blank buffer (squares) as a function of q_z.

To quantify the amount and the distribution of monovalent and divalent cations near the negatively charged LPS Re monolayers, the fluorescence signals in the presence of a monolayer were normalized by those from the blank buffers. This allows for a quantitative comparison with modeled fluorescence signals, which we simulated for variable ion distribution profiles under consideration of the electronic structure of the monolayer systems. This is illustrated in Fig. 3, where the buffer-normalized fluorescence intensities are presented for LPS Re monolayers on Ca^{2+}-free buffer (left panel) and on Ca^{2+}-loaded buffer (right panel). Open circles indicate K^+, while closed circles indicate Ca^{2+}. The data points are in good agreement with a model (dashed line) which assumes the peak of the K^+ concentration in the center of the LPS Re headgroups on Ca^{2+}-free buffer. On Ca^{2+}-loaded buffer the data points are well represented assuming the vast replacement of K^+ from the headgroup region, while the Ca^{2+} concentration peaks in the headgroup region. These results are in excellent agreement with coarse-grained Monte Carlo simulations of LPS monolayers [1].

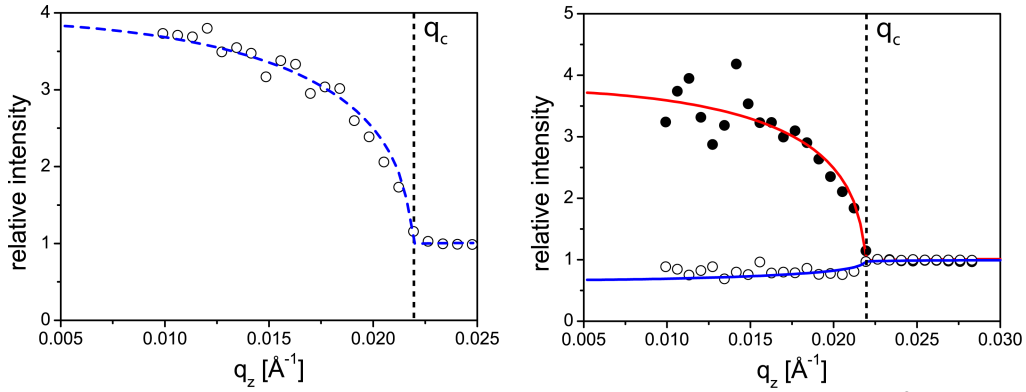

Figure 3: Buffer-normalized fluorescence signals from LPS Re monolayers on Ca^{2+}-free (left) and Ca^{2+}-loaded (right) buffer. Open circles: K^+, closed circles: Ca^{2+}, solid and dashed lines: modeled signals.

[1] R.G. Oliveira, E. Schneck, K. Brandenburg, U. Seydel, B. Quinn, O.V. Konovalov, T. Gill, D.A. Pink, M. Tanaka, *Comptes Rendus Chimie* **12**, 209-217 (2009)

[2] D. A. Pink, et al., Langmuir **19**, 8852 (2003)