ESRF	Conformation of Lipopolysaccharides in Models of Bacteria Surfaces: A Standing-Wave X-Ray Fluorescence (SWXF) Study	**Experiment number**: **SC- 4024**
Beamline: ID10	**Date of experiment**: from: 11.12.2014 to: 14.12.2014	**Date of report**: 03.10.2016
Shifts: 9	**Local contact(s)**: Dr. Oleg KONOVALOV	*Received at ESRF:*

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The initial goal of this beamtime was the determination of lipopolysaccharide (LPS) conformations in models of bacteria surfaces using standing-wave x-ray fluorescence (SWXF) targeting the LPSs phosphorus (P) atoms in the presence and absence of divalent cations. Such specific structural information would be valuable to understand the role of divalent cations in the well-documented rigidification of LPS surfaces [1, 2].
The SWXF technique is based on the standing wave created at the solid/liquid interface by interference of the incident electromagnetic wave with the wave reflected from the substrate under Bragg reflection conditions generated with dedicated nanometric multilayers [3-5]. During a scan of the angle of incidence θ around the Bragg condition the maxima of the standing wave move along the surface normal (z). The standing wave is used to excite the specific fluorescence of the chemical elements of interest. From the angle-dependent fluorescence intensity the element distribution can then be reconstructed. Earlier SWXF studies have dealt with the localization of heavy chemical elements, often used as labels for biological systems [3, 4]. During beamtime SC-3802 on ID03 we extended the SWXF approach to the comparatively light yet biologically very important chemical elements S and P, which opens up new possibilities for the label-free application of SWXF to biomolecular samples, yielding element-specific density profiles at atom scale resolution [5].

During beamtime SC-4024 on ID10 we carried out SWXF measurements on mutant LPS surfaces composed of LPS Rd and LPS Ra at the solid/liquid interface, targeting their P distributions in the presence and absence of divalent calcium ions. However, at that point we were still struggling to prepare the samples with a reproducible water layer thickness confined between the solid substrate and a thin polymer foil. While those issues have since then been resolved, the results obtained with LPSs during beamtime SC-4024 remained without satisfactory conclusion. Nonetheless other measurements carried out during the same beamtime, on interacting models of lipid membranes, were successful and will be described in the following.

Surface interactions involving biomembranes, such as cell-cell interactions or membrane contacts inside cells play important roles in numerous biological processes. Structural insight into the interacting surfaces is a prerequisite to understand the interaction characteristics as well as the underlying physical mechanisms [6]. During beamtime SC-4024, we worked with simplified planar experimental models of membrane surfaces, composed of lipids and lipopolymers (see Fig. 1). Their interaction was studied at controlled dehydrating (interaction) pressures using a humidity chamber. For selected pressures, their internal structure was investigated by SWXF, yielding the density profiles of P and S belonging to lipid headgroups and polymer chains, as well as counter-ion profiles for charged surfaces.

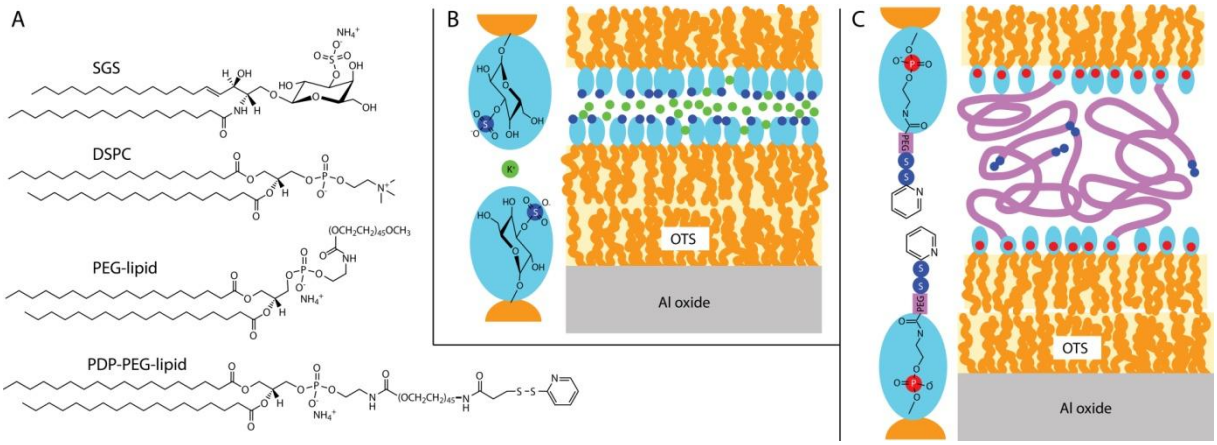

Figure 1: (A) Chemical structures of the amphiphilic molecules SGS (3-O-sulfo-D-galactosyl-ß1-1'-N-heptadecanoyl-D-erythro-sphingosine), DSPC (1,2-distearoyl-*sn*-glycero-3-phosphocholine), PEG-lipid (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]), and PDP-PEG-lipid (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[PDP(polyethylene glycol)-2000]). (B and C) Schematic illustration of the double-monolayer samples investigated in the present study: interacting SGS surfaces (B) and interacting lipid-anchored PEG brushes (C). The solid surfaces are hydrophobically functionalized with octadecyltrichlorosilane (OTS).

Fig. 2 exemplarily shows the results obtained with two interacting, negatively charged sulfoglycolipid (SGS) monolayers with K^+ as counterions. It is seen that S and K distributions virtually overlap. Moreover the water uptake at higher humidity clearly manifests as a simultaneous change in the characteristic angle-dependent S and K fluorescence intensities. The corresponding element profiles are illustrated in panel C. These results were recently submitted as an article to a peer-reviewed journal [7].

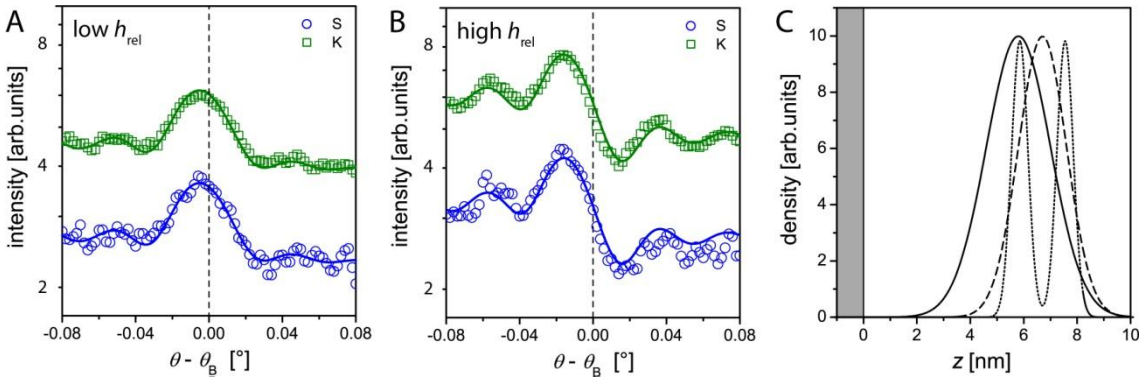

Figure 2: (A) Angle-dependent S and K fluorescence (symbols) from interacting SGS surfaces (see Fig. 1 B) at low humidity. (B) S and K fluorescence from the same sample but at high humidity. Solid lines in (A and B) indicate calculated theoretical intensities corresponding to the best-matching model parameters for the S distributions at low and high humidity. (C) S distributions at low humidity (solid line) and at high humidity when assuming unimodal shape (dashed line). The dotted line indicates a bimodal distribution that would result in approximately the same fluorescence signals (see text). The K distributions at low and high humidity are undistinguishable from the respective S distributions, so that the curves in panel C apply to both S and K.

References

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