ESRF	**Experiment title:** In-plane and out-of-plane strutures of bacterial lipopolysaccharides: Influence of divalent ions and protamine	**Experiment number**: SC1878
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In-plane and out-of-plane structures of bacterial lipopolysaccharide (R595 LPS from *Salmonella minnesota*, Fig. 1A) monolayers at the air/water interface have been studied by combination of grazing incidence X-eay diffraction (GID) and grazing incidence diffuse X-ray scattering out of the specular plane (GIXOS) at ID10B. Monochromatic beam (λ = 1.54 Å) from the synchrotron radiation illuminates the LPS monolayer on a Langmuir film balance slightly below the critical angle (α_i = 0.086 °), and intensity of the diffracted beam was collected with a position sensitive linear detector perpendicular to the surface normal at various azimuth angles. GID measures the diffraction from the quasi-2D crystalline Bragg rods at relatively wide angles and thus can reveal the inter-chain (or inter-sugar) distances, the degree of lattice distortion from the ideal hexagonal packing, and the tilt angle of chains to the surface normal. On the other hand, GIXOS measured near the specular line ($q_x \sim 0.029$ Å^{-1}) can be used to gain quasi-specular reflectivity. By using this technique, the vertical electron density profile can be reconstructed with a simplified model based on the Parrat algorithm within 1 ~ 2 min, which usually takes 2 – 3 h by a conventional specular reflectivity (θ-2θ scan).

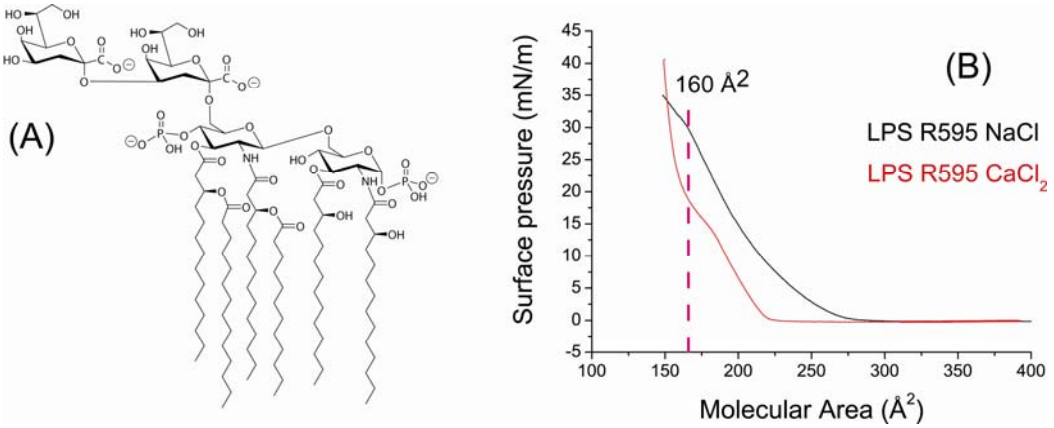

Fig. 1: Chemical structure (A) and the pressure-area isotherms of R595 LPS.

Here, we use two types of subphase: (i) "NaCl subphase" consists of 5 mM Hepes and 100 mM NaCl, and (ii) "$CaCl_2$ subphase" is 5 mM Hepes, 100 mM NaCl, and 50 mM $CaCl_2$ (Fig. 1B). When we compare the isotherms on NaCl and $CaCl_2$ subphase, the phase transition pressure of the monolayer on $CaCl_2$ subphase is lower than that on NaCl subphase, indicating

that the interaction between LPS molecules is more cooperative in the presence of $CaCl_2$. This result suggests that the hydrogen bonding network in the KDO layer is strengthened by multivalent ions.

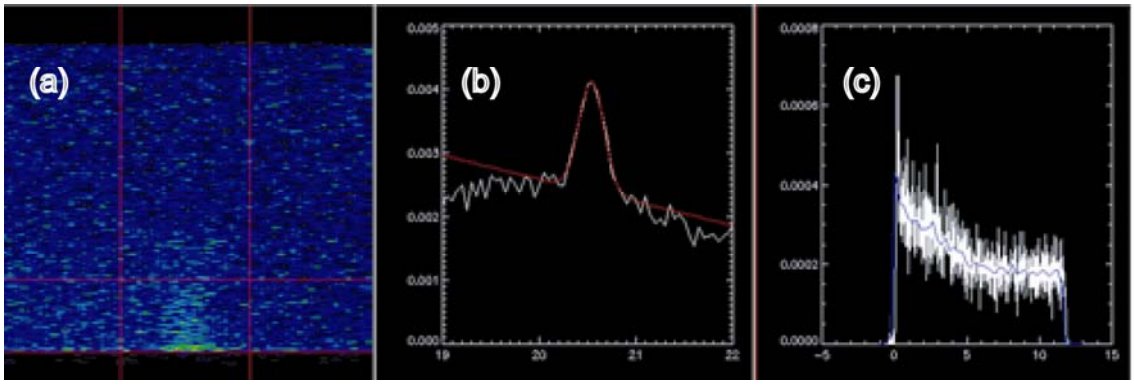

Fig. 2: (a) Reciprocal space map, (b) integrated intensity along q_z, and (c) integrated intensity along q_x (c) of R595 LPS on $CaCl_2$ subphase

To highlight the influence of Ca^{2+} ions, we measured GID and GIXOS of R595 LPS monolayers at the same area per molecule (160 $Å^2$, indicated by a broken line in Fig. 1B). Fig. 2 represents the reciprocal space map (a), integrated intensity along q_z (b), and integrated intensity along q_x (c) of R595 LPS monolayer on $CaCl_2$ subphase. From the global shape of the isotherm, the monolayer enters already in a liquid-condensed phase at π = 18 mN/m. In fact, the single peak at q_z = 0 $Å^{-1}$ indicates that hydrocarbon chains take almost upright conformation, whose lattice parameters are $a = b = 4.95$ Å and $\gamma = 120$ °. At the same area per molecule, the monolayer on NaCl subphase is in the phase coexistence despite of the higher surface pressure (28 mN/m), evidenced by a weaker GID signal from the ordered hydrocarbon chains. Moreover, the vertical electron density profiles obtained from GIXOS distinguished a clear change in saccharide head groups in the presence of Ca^{2+}.

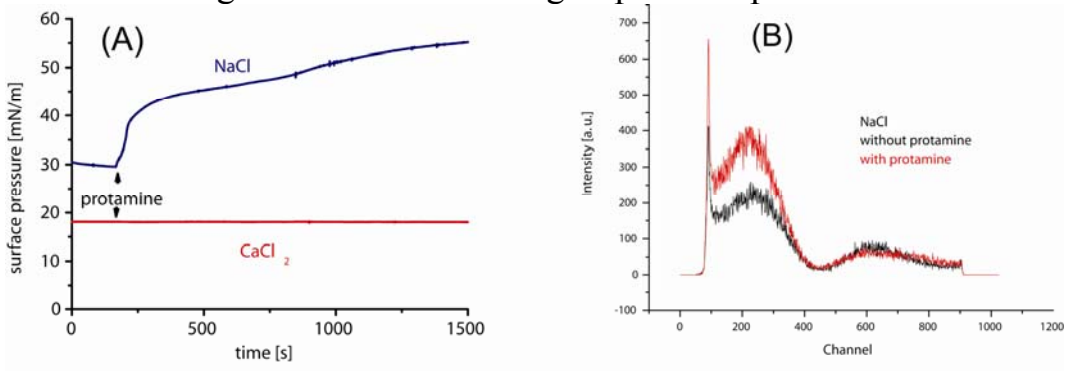

Fig. 3: (A) Changes in the surface pressure upon the injection of protamine. (B) GIXOS of R595 LPS on NaCl without (black) and with (red) protamine.

Upon the injection of protamines to the subphase, the LPS monolayer on NaCl led to a remarkable increase in the surface pressure, which amounts to $\Delta\pi > 25$ mN/m. This clearly indicates the intrusion of protamines even to the hydrophobic core of the monolayer. In fact, GID prooved that the chains were completely disordered, and GIXOS cannot be interpreted with the box model for the pure LPS monolayer. On the contrary, the monolayer on $CaCl_2$ subphase exhibited almost no increase in the surface pressure in the presence of protamines. GID indeed verified that the lateral chain ordering was not disturbed at all. Although the reconstruction of the electron density profile from GIXOS is still on-going, the obtained results successfully demonstrate that bacteria can survive against the invation of protamines in the presence of divalent cations by increasing intermolecular cooperativity.