Experimental report

09/02/2016

Proposal: 9-13-602 **Council:** 4/2015

Title: Distance-dependent Conformation ofSaccharides in Models of Interacting Bacteria Surfaces

Research area: Biology

This proposal is a new proposal

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Samples: D2O
bacterial lipopolysaccharides
Phospholipids (DSPC)
hydrophobized silicon blocks
PEGlipid (lipopolymer)
synthetic lipopolysaccharides

Instrument	Requested days	Allocated days	From	To
D17	4	4	29/10/2015	02/11/2015

Abstract:

Bacterial biofilms are involved in most microbial infections and also have important impacts on industrial processes. The outermost membrane surfaces of an important bacteria class are rendered with negatively charged lipid-anchored carbohydrates. These complex sugar layers govern bacterial interactions in biofilms and it was shown that the chemical structure of the carbohydrates influences structural and mechanical properties of biofilms. In order to gain molecular-scale structural insight into the interaction of bacteria membranes we propose the use of neutron reflectometry with model membranes displaying oligosaccharides.

EXPERIMENTAL REPORT

EXPERIMENT N° **9-13-602** INSTRUMENT D17

DATES OF EXPERIMENT 29/10/2015 to 02/11/2015

TITLE Distance-dependent Conformation of Saccharides in Models of Interacting Bacteria Surfaces

EXPERIMENTAL TEAM
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Date of report 06/02/2016

Bacterial biofilms are involved in most microbial infections and also have important impacts on industrial processes. The physical interaction between neighboring bacteria is crucial to biofilm formation and growth. The outermost membrane surfaces of Gram-negative bacteria, an important bacteria class, are rendered with lipid-anchored carbohydrates, known as lipopolysaccharides (LPSs, see Fig. 1). In essence, LPSs consist of a lipid anchor with a branched oligosaccharide headgroup ("core saccharides") to which a linear polysaccharide ("O-side chain") can be connected. Some of the saccharides in the core and in the O-side chain carry chemical groups with electric charges (mostly negative). These complex sugar layers govern to a large extent the interaction between the membranes of adjacent bacteria in a biofilm and it was shown that the chemical structure of LPSs influences structural and mechanical properties of biofilms [1]. However, little is still known about the interaction mechanisms between bacteria surfaces and the structural manifestations of these mechanisms such as distance-dependent molecular conformations.

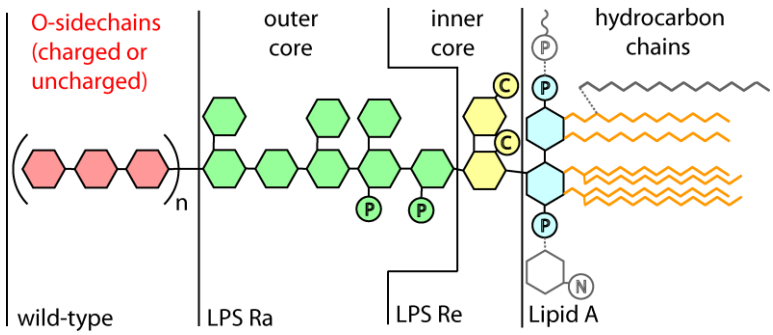

Figure 1: Schematic chemical structure of bacterial lipopolysaccharide LPS molecules.

During experiment 9-13-602 we studied realistic models of bacteria surfaces composed of LPS extracts of full structural complexity (i.e., including O-side chains) from wild-type E. coli bacteria. In the first step, single LPS surfaces, reconstituted at the solid/liquid interface on top of hydrophibized silicon substrates, were structurally characterized by specular neutron reflectometry (NR).

In the next step, a combination of Langmuir-Schaefer and Langmuir-Blodget transfers was used to create an architecture in which two LPS monolayers, realistically mimicking the surfaces of neighboring bacteria, are brought into contact. These architectures were then structurally characterized for various surface separations realized by the exertion of various dehydrating pressures using the powerful new D17 humidity chamber. Fast TOF acquisitions allowed reflectivity measurements in a wide range of relative humidities including extremely high humidities > 98% which can typically only be achieved transiently.

Fig. 2 (left) shows representative reflectivity curves obtained for low and high humidity conditions. The substantial shifts in the q_z positions of the Kiessig minima reflect the dramatic swelling of the aqueous region between the monolayers from ≈ 8nm to > 30 nm within the covered humidity range. Solid lines in the figure indicate the best-matching simultaneous fit to 5 H_2O hydration levels and 2 D_2O hydration levels.

The corresponding global model for all hydration levels and water contrasts comprising the volume fractions of silicon, solicon oxide, lipid hydrocarbon chains, core saccharides, O-side chains, and water is presented in Fig. 2 (right) exemplarily for the highest hydration level realized (> 30 nm thickness of the aqueous layer between the hydrocarbon chains). Data were modeled along the lines presented in [2, 3]. It is seen that NR not only confirmed the targeted sample architecture, but also yields detailed structural insight in the distance-dependent structure of the interacting LPS surfaces. The fit results obtained with single LPS surfaces at the solid/liquid interface (not shown here) and interacting LPS surfaces at controlled humidity (Fig. 2) are in remarkable agreement. The structural picture obtained by NR may contribute significantly to our insight into the physics of bacterial interactions in biofilms.

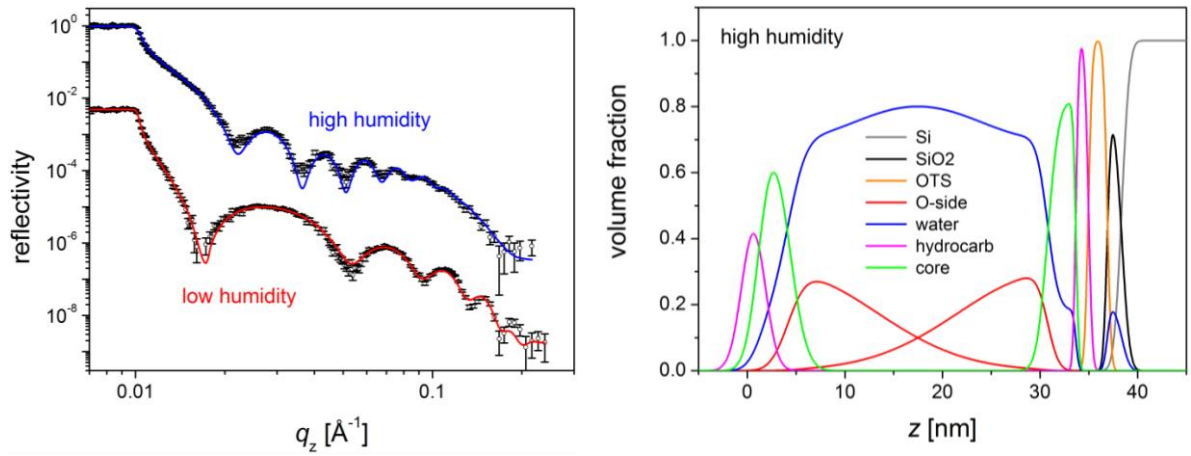

Figure 2: (left) Representative reflectivity curves from two interacting wild-type LPS monolayers at low and high humidity. Solid lines indicate the best simultaneous fit to 5 H_2O and 2 D_2O humidity levels. (right) The corresponding global model comprising volume fractions of silicon, solicon oxide, lipid hydrocarbon chains, core saccharides, O-side chains, and water.

[1] Lau et al., J. Bacteriol. 191, 6618 (2009)

[2] Schneck, Schollier, Halperin, Moulin, Haertlein, Sferrazza, Fragneto, *Langmuir* **29**, 14178 (2013)

[3] Schneck, Berts, Halperin, Daillant, Fragneto, *Biomaterials* **46**, 95 (2015)