Experimental report

10/11/2015

Proposal: 8-02-718 **Council:** 10/2014

Title: Biomimetic bilayers mimicking the outer membrane of Gram-negative bacteria and their interaction with antimicrobial peptides

Research area: Soft condensed matter

This proposal is a continuation of 8-02-669

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Samples: alamethicin
Lipopolysaccharides
polymyxin B
Phopholipids (SOPE, SOPG, CL)
plasticins
phosphate buffer saline

Instrument	Requested days	Allocated days	From	To
FIGARO User-supplied	5	3	12/05/2015	15/05/2015
D17	5	0		

Abstract:

We plan to prepare model membrane systems of the outer membrane of Gram negative bacteria and study the mechanisms regulating the penetration of antimicrobial peptides into the asymmetric outer membrane. Asymmetric phospholipids/lipopolysaccharide bilayers will be formed by Langmuir-Blodgett techniques to mimic the outer membrane of Gram-negative bacteria cell wall.The structural modifications of the bilayer due to interaction with selected antimicrobial peptides will be monitored with increasing peptide concentrations as well as different H2O/D2O media to allow a better accuracy in data analysis. These results combined with our QCM-D and x-ray diffraction measurements will constitute a body of evidence of the penetration mechanism of plasticins into the asymmetric outer membrane.

Experiment 8-02-718

Biomimetic bilayers mimicking the outer membrane of Gram-negative bacteria and their interaction with antimicrobial peptides

May 12-15th, 2015, Figaro, ILL, Grenoble

Participants

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Sample preparation and characterization

Neutron reflectometry is a technique that has proved valuable in determining the structure and composition of model membrane systems at the solid/liquid interface. Model bilayers will be prepared by using the Langmuir-Blodgett and Langmuir-Schaeffer techniques in the Soft Matter Laboratory facility at the ILL. Full asymmetric planar bilayers with a PE/PG/CL proximal leaflet and a LPS distal one will be built in order to mimic the lipid matrix of the outer membrane. Pure LPS molecules as well as the ternary PL mixture (SOPE/SOPG/CL) with the molar ratio (80/15/5) will be used.

Proposed experiment

This new proposal is in continuation of proposal 8-02-669. Neutron reflectometry measurements will be performed on asymmetric LPS/PL single bilayers as well on symmetric PL/PL single bilayers. Comparison of the neutron reflectometry spectra will provide structural information about the two leaflets, determine the asymmetrical character of the LPS/PL bilayer and the eventual modifications of leaflet composition due to flip-flop exchange. Further interaction of the two plasticins with these model bilayers will be monitored. Peptide penetration and the subsequent structural modifications of the bilayer will be examined for three different peptide concentrations (0.1, 1 and 25 µM close to the minimal inhibitory concentrations of the active plasticin). Hydrogenated and deuterated lipids will be helpful to discriminate the interaction of peptides with specific lipids. The use of three different media characterized by different H_2O/D_2O ratios (1:0, 0:1 and 0.62:0.38) will allow a better accuracy in the data analysis process. These results will complete the previous ones (proposal 8-02-669) and are expected to submit our publication.

Surfaces

We had 3 sapphire blocks. After a first cleaning with organic solvents that prevent the effective transfers of phospholipid monolayers, they were finally cleaned in piranha solution for 20 minutes, then thoroughly rinsed and kept in ultrapure water.

Media

Pure **H_2O**, pure **D_2O**, **1MW** and **3MW** have been used as contrast media for this study. T=22°C.

Results and discussion

Values of parameters displayed in *italic* are **fixed** values.

-Sapphire bare surfaces were first analysed by NR

The rugosity of sapphire surfaces was 5 ± 1 Å.

- The formation of the three first asymmetric bilayer systems on sapphire surfaces were not successful due to the fact that the sapphire blocks were first cleaned with organic solvents in sonication baths (chloroform, acetone, ethanol). But this procedure was not sufficient to obtain clean flat positive Al_2O_3 surfaces. We decided then to clean all the blocks in piranha solution for 20 minutes, then thoroughly rinsed and kept in ultrapure water.

- Formation of a first asymmetric PL/LPS Re bilayer by LB transfer of SOPE/SOPG/CL monolayer followed by a LS transfer of LPS Re 595 monolayer, both at 40 mN/m

Figure 1 shows the R(Q) versus Q plot and 5-slab model fit for this asymmetric bilayer:

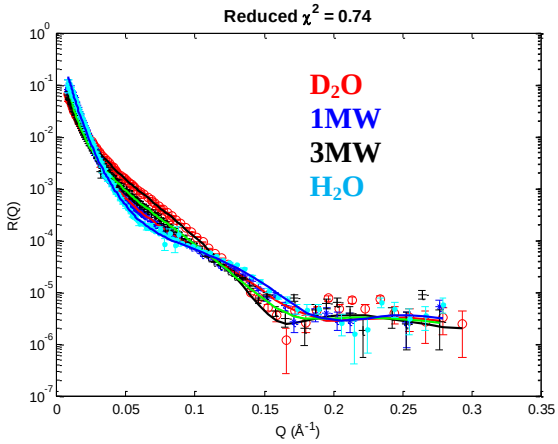

Figure 1: R(Q) vs Q plot and and 5-slab model fit for the LBLS5 bilayer.

The model is made of 5 slabs with a water layer between sapphire and bilayer.
Here is below the fit with a 5-slab model:

Layer	Thickness (Å)	SLD x10^{-6} (Å^{-2})	Water content (%)	Roughness (Å)
water	1.8	-0.56	100	5
Inner h-PE/PG/CL heads	8.9	1.37	43	2
h/d-PL Tails	14.4	0.8	2	2
h-LPS Tails	14.2	-0.1	6	2
Outer h-LPS heads	11.8	4.1	46	4

SLD media: D_2O: 5.77; 1MW: 1.82; 3MW: 3.35; H_2O: 0.37.

Reasonable values were found for the thicknesses, water content and roughness of both leaflets. The SLD value corresponding to the PL headgroups is close to the ones expected for PE headgroups (1.36x10^{-6}Å^{-2}). The SLD value corresponding to the PL mixed deuterated/hydrogenated chains is found lower than the ones expected (3.17x10^{-6}Å^{-2}). The SLD value corresponding to the LPS hydrogenated chains is found close to the ones expected (-0.3x10^{-6}Å^{-2}). Finally the SLD corresponding to the LPS headgroup is found in the range 4 to 5x10^{-6}Å^{-2}, consistent with the literature [1].
A homogeneous asymmetric PL/LPS Re 595 bilayer was formed on the sapphire surface.

- <u>Interaction with the cationic active peptide PTCDA1-KF at 25 µM concentration in deuterated buffer</u>
Figure 2 shows the R(Q) versus Q plot and 5-slab model fit for this asymmetric bilayer in interaction with PTCDA1-KF at 25 µM. No water layer was detected between the sapphire surface and the bilayer.

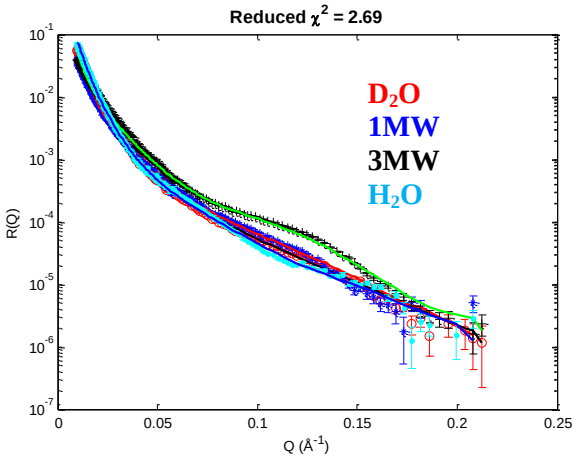

Figure 2: R(Q) vs Q plot and and 5-slab model fit for the LBLS5 bilayer in interaction with PTCDA1-KF at 25 µM.

Layer	Thickness (Å)	SLD x10^{-6} (Å^{-2})	Water content (%)	Roughness (Å)
Inner h-PE/PG/CL heads	9.8	**-0.56**	**15**	2
h/d-PL Tails	14.9	**1.02**	**11**	3.8
h-LPS Tails	15.2	**3.1**	**55**	4
Outer h-LPS heads	**45.8**	**2.52**	**80**	4

SLD media: D_2O: 6.35; 1MW: 2.18; 3MW: 3.1; H_2O: 1.39.

The interaction with the cationic active peptide PTCDA1-KF induced dramatic changes in the upper LPS leaflet, as expected from previous studies [2]: the thickness increased due to heavy adsorption of peptide oligomers onto the LPS headgroups. The water content showed that the integrity of the upper leaflet was lost. The hydrogenated LPS chains were also affected with a big increase of the water content and of their SLD values: the peptide penetrated into the LPS leaflet and disrupted it. Drastic changes also appeared in the lower leaflet that is adsorbed directly onto the sapphire surface: dehydration was observed at the level of the polar PL headgroups and the thickness and SLD value for the PL headgroups and mixed deuterated/hydrogenated chains increased: the peptide penetrated the apolar layer of the lower leaflet.

References:

[1] A.P. Le Brun, L. A. Clifton, C. E. Halbert, B. Lin, M. Meron, P. J. Holden, J. H. Lakey, and S. A. Holt, Biomacromolecules 14, 2014−2022 (2013).

[2]] J.P. Michel, Y. X. Wang, E. Dé, P. Fontaine, M. Goldmann and V. Rosilio., *Biochimica and Biophysica Acta Biomembranes 1848*, 2967-2979 (2015).