Experimental report

10/02/2020

Proposal: 9-13-809 **Council:** 10/2018

Title: Comparing Antimicrobial Peptides and Peptoids: interactions with model lipid membranes mimicking bacteria and mammalian cells.

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Cholesterol
1,2-dimyristoyl-d54-sn-glycero-3-phosphocholine
1,2-dimyristoyl-d54-sn-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt)
Indolicidin
Indolicidin peptoid

Instrument	Requested days	Allocated days	From	To
FIGARO	4	4	24/06/2019	28/06/2019
D17	4	0		

Abstract:

We aim to provide a fundamental understanding of how the peptoid strategy for stabilization of antimicrobial peptides (AMPs) affect their interaction with bacterial membranes. In particular, we will compare Indolicidin and the synthesised peptoid version of Indolicidin. In this way, we hope to clarify whether this group has potential in the design of new antibiotics. In order to achieve this, we plan to explore the peptoid interaction with supported lipid bilayers (SLBs) made of lipids with and without cholesterol to shed light on the role of cholesterol and whether cholesterol play an significant role in protecting mammalian cells from lysis of AMPs as our preliminary SAXS data suggest. By using Neutron Reflectometry and contrast variation we will be able to highlight the structural interaction with higher resolution than with SAXS alone.

Comparing Antimicrobial Peptides and Peptoids: interactions with model lipid membranes mimicking bacteria and mammalian cells.

Figaro 24-28 June 2019, Experiment number: 9-13-809

Bacterial resistance towards conventional antibiotics represents a major setback of modern medicine and desperately calls for innovation of new compounds and strategies. Antimicrobial peptides (AMPs) are able to evade much of the bacterial resistance mechanisms because they often do not seem to block specific biochemical pathways, but rather act physically on the cytoplasmic membrane itself. However, there is no clear general consensus for the molecular basis by which AMPs act.

We have earlier showed how two natural occurring cathelicidins Indolicidin(1, 2) (originated from bovine) and LL-37(3) (originated from humans) interacts with model membranes mimicking bacterial cell membranes with a negative charge using a combination of model analysis of SAXS and Neutron reflectometry data. In this work we used the same peptides and looked at their interaction with membranes made to mimic mammalian cells. A major problem with AMPs as future medicine is the lack of selectivity towards bacteria resulting in hemolytic properties of some AMPs. The major difference between bacterial and mammalian cells is that the bacterial membrane includes negatively charged lipids while mammalian cell membranes include cholesterol. We therefor focused this experiment on the effect of cholesterol on the peptide-membrane interaction.

After characterization of the clean silica surface in D2O and H2O, supported lipid bilayers of d54-DMPC and perduterated cholesterol (0, 20 and 40%) (obtained from the ILL deuteration lab facility) were deposited in situ by vesicle fusion at the FIGARO beamline. The bilayers were measured at 37 °C in different bulk contrast environments (tris-H2O, tris-D2O and a tris-CMsi). The reflection profiles including best fits for the membranes can be found in figure 1 A and B, respectively. The membranes were fitted using a symmetric three-layer model as typically done for lipid bilayers.

After successful bilayer formation, LL-37 and Indolicidin were introduced to the membrane to follow the interaction behaviour. The data for Indolicidin upon DMPC cholesterol 0 and 40 % bilayers are shown in figure 1. It is clear from the raw data that the peptides induce changes in the bilayer structure in both cases, however the effect on the bilayer with cholesterol seems less pronounced. This is in compliance with SAXS results obtained at the ESRF where we saw that higher amounts of cholesterol can have a protective effect upon peptide addition. We are still working on analysis of the data and

are planning additional SAXS experiments (the past ones were done on POPC-cholesterol mixes so we also want to study the difference between the cholesterol effect on saturated versus unsaturated lipids due to the difference in melting temperature). We are currently working on a manuscript where we combine these data with SAXS data and in vitro cell data (with cells of different cholesterol levels).

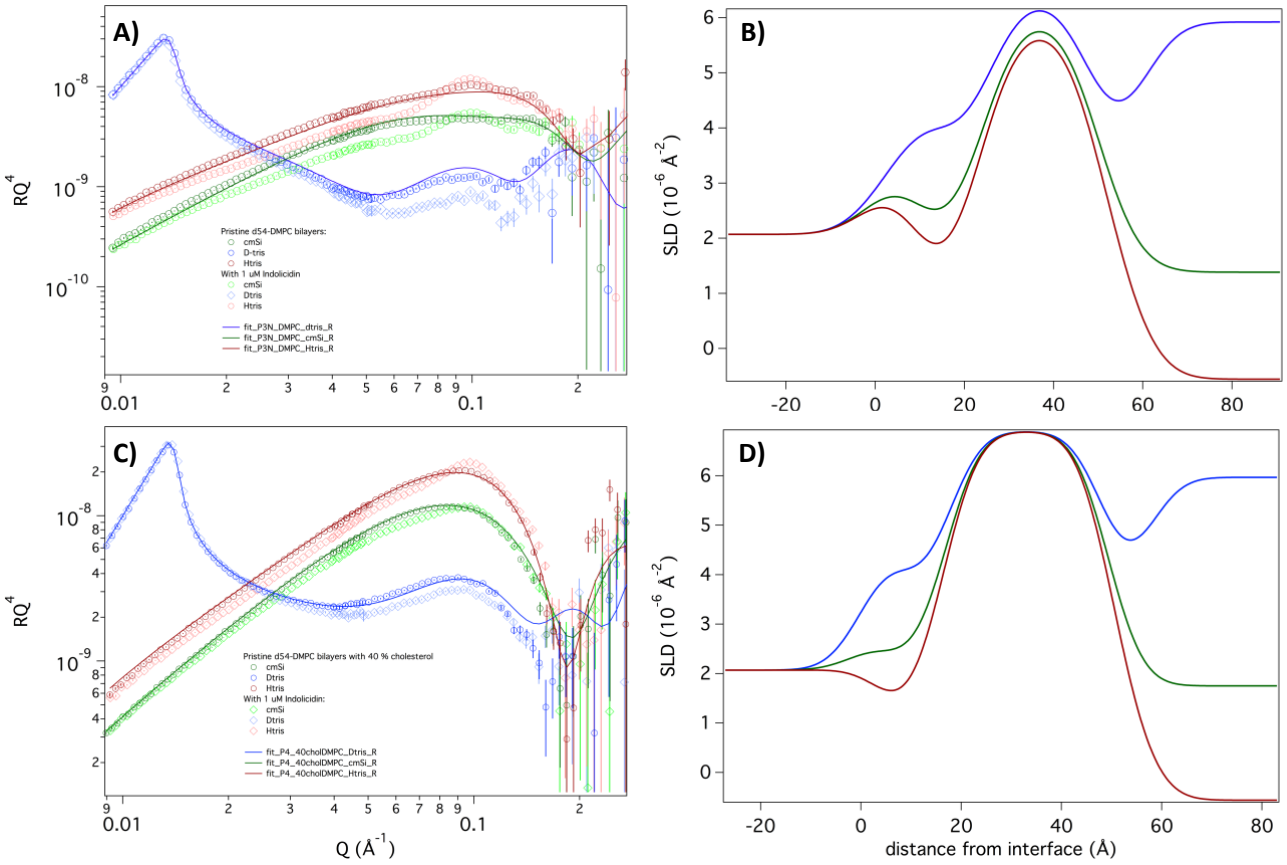

Figure 1. NR measurements of SLB before and after addition of 1 μM Indolicidin. A) Reflectivity profiles for the measurements of pure d54-DMPC bilayers before and after being exposed to peptide together with best fit and corresponding SLD profiles (B). C) Reflectivity profiles for the measurements of d54-DMPC bilayers with 40 % Cholesterol exposed to Indolicidin together with best fit and corresponding SLD profiles (D).

1. Nielsen J. E., Lind T. K., Lone A., Gerelli Y., Hansen P. R., Jenssen H., Cárdenas M., Lund R. 2019. A biophysical study of the interactions between the antimicrobial peptide indolicidin and lipid model systems. Biochim Biophys Acta, Biomembr. 1861(7):1355-64.
2. Nielsen J. E., Bjørnestad V. A., Lund R. 2018. Resolving the Structural Interactions between Antimicrobial Peptides and Lipid Membranes using Small-angle Scattering Methods: the case of Indolicidin. Soft Matter. 14:8750-63.
3. Nielsen J. E., Bjørnestad V. A., Pipich V., Jenssen H., Lund R. Beyond Structural Models for the Mode of Action: How Natural Antimicrobial Peptides Disrupts Lipid Membranes (to be submitted).