Experimental report

10/02/2020

Proposal: 8-02-869 **Council:** 4/2019

Title: Dynamics of E. coli membrane mimics: influence of antimicrobial peptides on the flip-flop rate

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine
1,2-dimyristoyl-d54-sn-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt)
1,2-dimyristoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt)
1,2-dimyristoyl-d54-sn-glycero-3-phosphoethanolamine
C77H132N18O20 (peptide)
C106H140ClN33O14 (peptide)

Instrument	Requested days	Allocated days	From	To
D11	4	2	14/09/2019	16/09/2019
D22	4	0		

Abstract:

In this work we aim to further explore how antimicrobial peptides with different structures, incl. alphahelical, random coiled, beta sheets, affect the dynamics of model membrane systems mimicking E- coli using Time resolved SANS and contrast variation. The structural characterization and interactions with membranes have already been carried out using SAXS. By comparing the structural data and TR-SANS, we hope to clarify whether increased flip-flop is a common feature of such peptides and ultimately, conclude about the possible role in the mode of action of AMPs. Increased flip-flop is believed to be linked to increased ion transport through the membrane, causing disturbed osmotic regulation and cell death. The use of neutrons is crucial to be able to explore these systems as no other methods is able to probe both exchange and flip-flop kinetics without significant physical/chemical perturbation.

Dynamics of E. coli membrane mimics: influence of antimicrobial peptides on the flip-flop rate

D11 14-16 September 2019, Experiment number: 8-02-869

Antimicrobial peptides (AMPs) are a group of surface active molecules that are shown to have effect against a broad spectrum of pathogens. AMPs seem to be able to evade much of the bacterial resistance mechanisms and are therefore promising candidates for future antibiotics. The precise microscopic mechanism of AMPs has not fully been proven, however a physically perturbation of the cytoplasmic membrane has been suggested. Changes in lipid dynamics has been suggested as possible mechanisms of this perturbation. Increase in lipid flip-flop might explain how some peptides that are shown to not form pores in the membrane, like Indolicidin,(1, 2) still induce bacteria death. Increased flip-flop of lipids in the membrane caused by peptide insertion can be linked to an increase in the transport of ions across the membrane resulting in disturbed regulation of the osmotic balance ultimately leading to bacterial cell death.

In this experiment we used a previously developed hydrogen/deuterium (H/D) contrast variation technique based on TR-SANS as a "label-free" method to study molecular exchange processes.(3, 4) Contrary to other methods such as EPR, fluorescence and temperature-jump experiments, this method does not require chemical labelling or perturbation from equilibrium and was originally developed to investigate the dynamics of block copolymer micelles. However, it has been demonstrated that this method also can be used to study lipid flip-flop and inter-membrane exchange in lipid vesicles.(5)

We have earlier used the method to study the impact of various AMPs on the dynamics of lipid vesicles composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (DMPG).(6) However, even though DMPC is often used to mimic the zwitterionic lipids of the bacterial cell membrane the real membrane does not contain any PC lipids but rather the smaller phosphoethanolamine (PE) lipids. PE has a higher phase transition temperature than PC due to its ability to form hydrogen bonds. In this experiment we measured the dynamics of DMPE-DMPG vesicles with and without the addition of various natural AMPs in different concentrations. To be able to determine whether the significant acceleration effects of different peptides that we saw on DMPC-DMPG bilayers in the past would be affected by the change from PC to PE.

As seen in Figure 1 all the AMPs added to the lipids except Colistin (known to interact with LPS on the outer membrane and not the membrane itself) accelerate the lipid dynamics when comparing the R(t) plots to the pure lipids. The experiments presented in

Figure 1 are all done at 37 degrees C. While we also attempted to increase the temperature to 47 C to be above the phase transition temperature of DMPE, and to be able to see the temperature effects to extract the activation energy through Arrhenius equation, the increase of temperature resulted in destabilisation of the system with formation of multilamellar vesicles and eventual phase separation.

We are currently working on correlating these results with SAXS data on the peptide-lipid interaction using the same lipids (where we see that the peptide influences the volume of the lipid tail and headgroups indicating a breakage of hydrogen bonds and general disordering of the packing), and a manuscript is currently in progress.

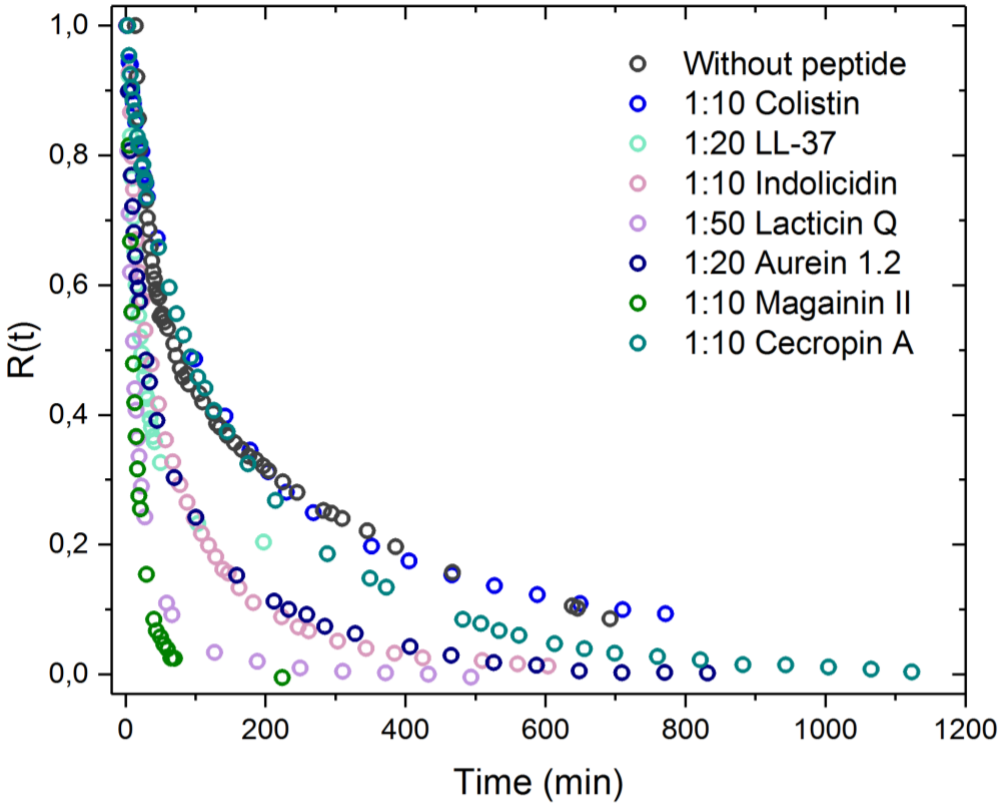

Figure 1. NR measurements of SLB before and after addition of 1 μM Indolicidin. A) Reflectivity profiles for the measurements of pure d54-DMPC bilayers before and after being exposed to peptide together with best fit and corresponding SLD profiles (B). C) Reflectivity profiles for the measurements of 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. Lund R., Willner L., Richter D. Kinetics of block copolymer micelles studied by small-angle scattering methods. Controlled Polymerization and Polymeric Structures: Springer; 2013. p. 51-158.
4. Lund R., Willner L., Stellbrink J., Lindner P., Richter D. 2006. Logarithmic chain-exchange kinetics of diblock copolymer micelles. Physical review letters. 96(6):068302.
5. Nakano M., Fukuda M., Kudo T., Endo H., Handa T. 2007. Determination of interbilayer and transbilayer lipid transfers by time-resolved small-angle neutron scattering. Phys Rev Lett. 98(23):238101.
6. 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).