Experimental report

10/02/2026

Proposal: 9-13-1096 **Council:** 4/2023

Title: Determining the Effect of Antimicrobial Peptides on the Permeability of Water through Phospholipid Membranes.

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Vladimir KOYNAREV

Experimental team: Henrik TORJUSEN
Vladimir KOYNAREV
Reidar LUND
Thomas VOGELAAR

Local contacts: Lionel PORCAR

Samples: DSPC: C44H88NO8P
Indolicidin: C100H132N26O13
DSPG: C42H82O10PNa
DMPE-PEG2000: C125H251N2O55P
LL-37: C205H340N60O53
Cardiolipin: C81H156Na2O17P2

Instrument	Requested days	Allocated days	From	To
D22	3	2	02/11/2023	04/11/2023

Abstract:

The finetuned permeability of water through the cellular membrane is vital for all cells and allows for the creation of the electrochemical gradients necessary for life. We propose to investigate the permeability of water through model lipid membranes, and how this is affected by the antimicrobial peptides Indolicidin and LL-37. We propose to use small unilamellar vesicles with different lipid compositions that roughly mimic the charge composition of E.coli. With this system, and in collaboration with Dr. Porcar at D22, we will measure the water exchange kinetics utilizing a stopped-flow setup to mix H2O filled vesicles with D2O buffer and monitor the fall in scattering intensity corresponding to the exchange of H2O/D2O in the vesicle interior as water diffuses across the membrane. Sufficient counts are achieved through several repetitions per sample using the stopped-flow and the high flux of the D22 instrument. We propose to investigate 2 membranes with 2 peptides at 4 physiologically relevant concentrations, and at several temperatures in the gel phase which would allow us to accurately determine the kinetic parameters. This work is part of the PhD of the main proposer.

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Background

The separation of the cell from its surroundings is one of the most fundamental functions of the cellular membrane. It is essential in maintaining the electrochemical gradients necessary for the survival of the cell through carefully controlled active transport and finely tuned permeability. It is generally believed that antimicrobial peptides (AMPs), which are a group of surface-active molecules, derive their activity through a physical perturbation of the cytoplasmic membrane, and have emerged as a promising candidate for future antibiotics due to their broad activity and ability to evade much of the bacterial resistance mechanisms. Traditionally, a lot of emphasis is placed on the ability of AMPs to form pores in the membrane. However, this has been increasingly contested in more recent years, with more subtle disruption of the membrane emerging as more probable mechanisms at physiologically relevant concentrations. In this experiment we investigated the effect of AMPs on the water-permeability through model lipid-membranes.

Experimental setup

In this experiment we carried out a systematic study of model lipid membranes, in the form of small unilamellar vesicles (SUVs), comprised of a mixture of zwitterionic phosphatidylcholine (PC) and anionic phosphatidylglycerol (PG) lipids, mimicking the surface charge of *E.coli*. While keeping the membrane charge constant we systematically varied the lipid tail saturation and length. We investigated DSPC/PG lipids with 18 C tails, DPPC/PG with 16 C tails, and DMPC/PG with 14 C tails, as well as POPC/PG lipids which are monounsaturated. We also systematically investigated the interactions with two highly studied and clinically promising AMPs, LL-37 and indolicidin.

The vesicles were prepared in H_2O and either incubated with AMPs, or used directedly as a reference. These vesicles where mixed in a 1:3 volume ratio with 100 % D_2O in the stopped-flow mixing apparatus. Upon mixing there is initially a high scattering intensity arising from the significant contrast between the H_2O in the interior vesicle pocket and the 75 % D_2O in the external buffer. As water is exchanged across the membrane, the contrast and correspondingly intensity decreases until the buffer inside the vesicle matches the external. The rate of this relaxation in scattering intensity is directedly dependent on the water permeability through the membrane, and the relaxation rate can be expressed as:

$$R(t) = \sqrt{\frac{I(t) - I_\infty}{I_o - I_\infty}}$$

Where I_o and I_∞ are the initial and final intensities, respectively.

Results

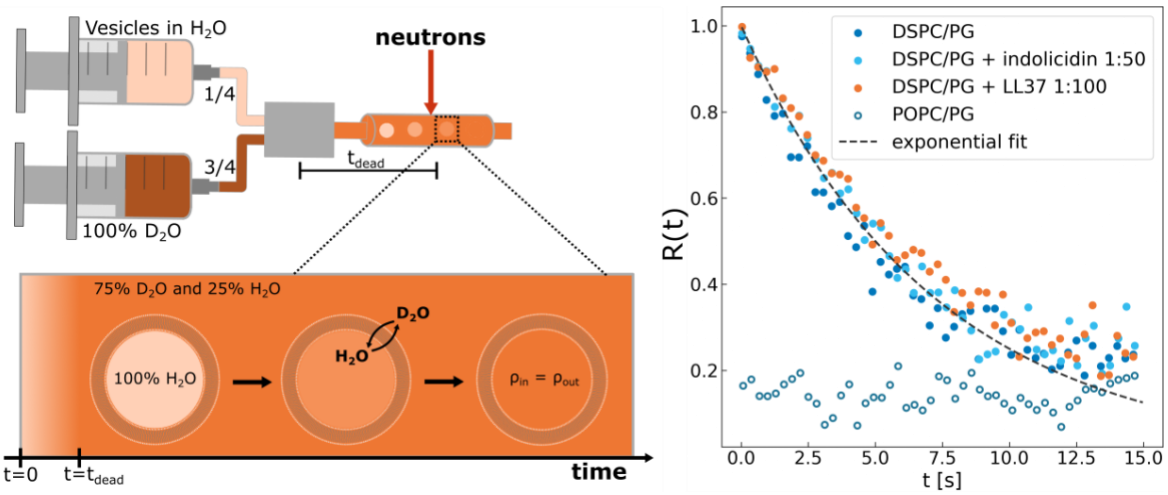

Figure 1: TR-SANS measurements, with a schematic illustration of the experimental setup to the Left and the resulting water-exchange rate, expressed in terms of the relaxation function R(t), to the Right.

The main findings of the experiment are presented in figure 1. In case of short (DMPC/PG and DPPC/PG) as well as unsaturated (POPC/PG) membrane the water exchange kinetics were too fast to probe, even with the high flux achievable at the D22 beamline. However, in case of the long and saturated DSPC/PG membrane, the water exchange kinetics were sufficiently slow and could be measured with. Interestingly, the peptides did not affect the rate of water exchange. This directedly contradicts the notion that AMPs form transmembrane pores, the kinetic SANS results obtained at D22 were instrumental in determining a novel mechanism of action where the AMPs permeabilize the membrane by inserting on the membrane surface and facilitating the formation of transient water channels associated with lipid flip-flop. These results were published in PNAS, https://doi.org/10.1073/pnas.2517944122, see below for full publication details. With the data obtained at ILL and complementary measurements, we could present a previously unknown mechanism of AMPs, which is expected to contribute towards targeted design and clinical realization of AMP-based antibiotics. Despite the short time since publication, this work has generated interest in the field and a commentary was recently published, also in PNAS (https://doi.org/10.1073/pnas.2529116122)

Publication of results

The results from this experiment are published in:

1. V.R. Koynarev, M.L. Nader, K.K.A. Borgos, H.M. Cezar, T. Narayanan, L. Porcar, M. Cascella and R. Lund, *Structural pores not required: Antimicrobial peptides induce ion permeabilization of lipid membranes through transient water channels*, PNAS, 2025. https://doi.org/10.1073/pnas.2517944122