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Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2023019

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Experiment title	Ion Transport Through Lipid Membranes and the Effect of Antimicrobial Peptides	
MRF Instrument	**Cryogenic Electron Microscopy**	**Days requested:** 3
Access Route	Direct Access	**Previous GP Number:** NO
Science Areas	Biology and Bio-materials	**DOI:** -
Sponsored Grant	None	**Sponsor:** -
Grant Title	-	**Grant Number:** -
Start Date	-	**Finish Date:** -
Similar Submission?	-	
Industrial Links	-	

Non-Technical Abstract AMPs are a promising option to combat the growing bacterial resistance to existing drugs, and they have in many cases been shown to work by perturbing the cell membrane, potentially by allowing the transport of ions which is normally restricted, and thereby disrupting normal cell function. The full picture of how the AMPs work, however, is not yet clear. To study the effect of AMPs on the ion-transport, we propose to use unilamellar vesicles (ULVs) with a charge composition roughly mimicking that of E. coli as model membranes. By increasing the concentration of salt (NaCl) on the outside of the vesicles, they will be subjected to an osmotic shock causing them to deform, the experiments will be repeated with different, and physiologically relevant, concentrations of the human AMP LL-37 added to the vesicles prior to the osmotic shock.

Publications Bjørnestad, V. A et al (2023) Journal of Colloid and Interface Science (Vol. 641, pp. 553–567). doi.org/10.1016/j.jcis.2023.03.037

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IM@IT E-platform: No

Instruments	**Days Requested:**
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Sample record sheet

Principal contact Professor Reidar Lund, University of Oslo, NORWAY
MRF Instrument **Cryogenic Electron Microscopy** **Days Requested:** 3
Special requirements:

SAMPLE

Material	1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (DMPG), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (DMPE-PEG200), LL-37 (sequence: LLGDFFRKSKEKIGKEFKRIVQRIKD FLRNLVPRTES)	-	-
Formula	C36H72NO8P,C34H66O10PNa,C125H251N2O55P, C205H340N60O53	-	-
Forms	Liquid		
Volume	2 ml		
Weight	20 mg		
Container or substrate	-	-	-
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	Cryogenic conditions - K	-	-
Pressure Range	1000 - 1000 mbar	-	-
Magnetic field range	0 - 0 T	-	-
Standard equipment	None	-	-
Special equipment	NA	-	-

SAFETY

Prep lab needed	No	-	-
Sample Prep Hazards	NO	-	-
Special equip. reqs	-	-	-
Sensitivity to air	No	-	-
Sensitivity to vapour	No	-	-
Experiment Hazards	NO	-	-
Equipment Hazards	-	-	-
Biological hazards	NO	-	-
Radioactive Hazards	NO	-	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Disposed of by *IS*	-	-

Title: Ion Transport Through Lipid Membranes and the Effect of Antimicrobial Peptides

Scientific Importance

One of the critical roles of the cellular membrane is to maintain the electrochemical gradients that enable essential cellular functions. This is achieved through controlled active transport and finely tuned permeability of water and ions. 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 stable 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[1]. The exact mode of action of AMPs remains poorly understood and the effect they have on the permeability of ions through the membrane, especially in the absence of pores, has so far not been thoroughly investigated.

Preliminary work

The interaction between a wide range of antimicrobial peptides and model lipid membranes has been extensively investigated by our group using Small Angle X-ray (SAXS) and Neutron (SANS) Scattering.[2,3] Particularly focusing on the antimicrobial peptides Indolicidin and LL-37, we have shown that they increase the transverse lipid diffusion or “flip-flop”, without the formation of pores. From preliminary SAXS experiments where we have used the same model membranes of Small Unilamellar Vesicles (SUVs) with a charge composition roughly mimicking that of *E.coli*, we see that increasing osmotic pressure results in substantial deformation of the vesicles. (Fig. 1A). However, in the presence of the antimicrobial peptides, a much less severe deformation is observed (Fig. 1B), suggesting that the peptides facilitate transport of the salt ions through the membrane, thereby alleviating the osmotic pressure, despite the apparent lack of pore formation.

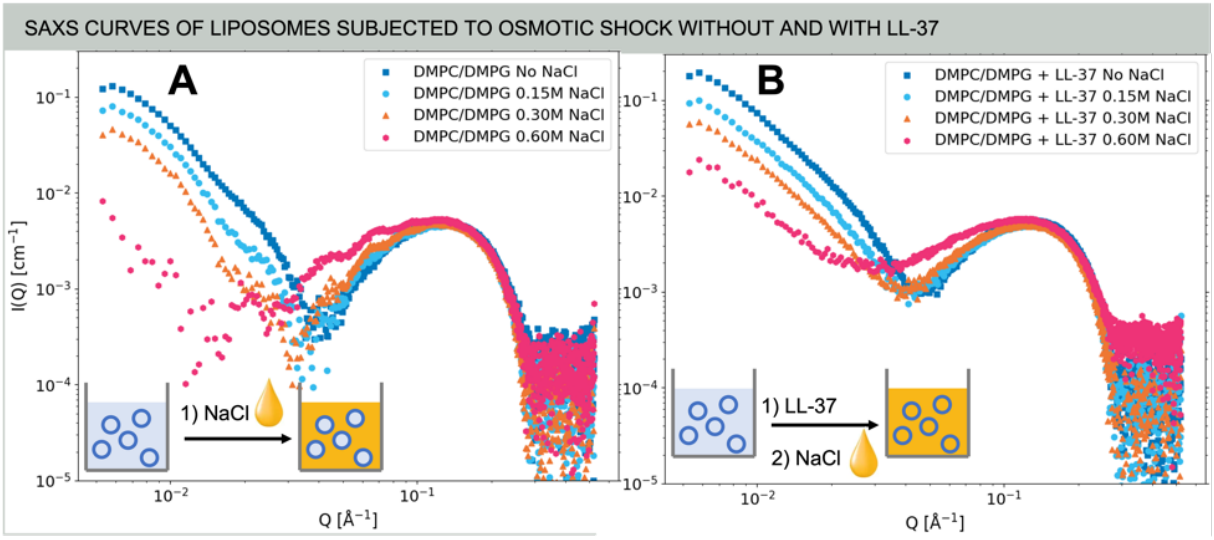

Figure 1: By increasing the concentration of NaCl in the ambient buffer, the liposomes were subjected to increasing osmotic pressures. For the liposomes without peptide (A), dramatic structural changes were observed, while liposomes incubated with peptide (B) prior to addition of NaCl showed significantly less deformation.

Using the same vesicles, we have performed initial Cryo-TEM experiments on vesicles with no added NaCl (Fig. 2A) and with addition of NaCl in both 0.15M (Fig. 2B) and 0.60M (Fig. 2C) concentrations. Addition of salt appears, in concurrence with the scattering data, to cause changes in both the shape and size of the vesicles and demonstrates the feasibility of the experimental setup. For the proposed work we direct our investigation towards the effect of antimicrobial peptides, in particular LL-37, and if its addition to the vesicle will negate the osmotic shock through the facilitation of ion-transport through the membrane.

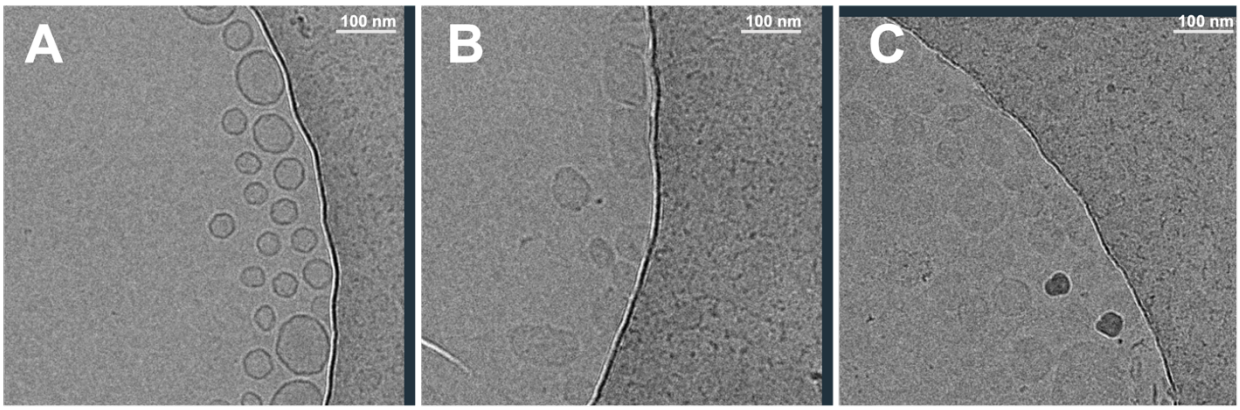

Figure 2: Cryo-EM micrographs of liposomes with no added salt (A), liposomes with 0.15M external NaCl concentration (B) and liposomes with 0.60M external NaCl concentration (C).

Experimental plan

From the preliminary SAXS data (Fig. 1) and Cryo-TEM experiments (Fig. 2) we see large deformations taking place because of the osmotic shock. These deformations appear to be much less dramatic when the AMPs are added. In the effort of understanding the deformations, resulting structure and the effect of AMPs, we believe Cryo-TEM in combination with SAXS will be of great use, based on previous success with the combination of imaging and scattering.[4] In the proposed experiment, to be carried out in collaboration with Prof. Marco Laurati, we want to study the effect of osmotic shock on SUVs with the external addition of NaCl in three different concentrations, 0, 0.15M and 0.6M, without added peptide and with 2 different and physiologically relevant concentrations of the AMP LL-37 (1:100 and 1:50 peptide:lipid ratio).

*For a total of 12 samples, (4 liposome reference at different salt concentrations and (2*4) liposome + peptide samples at the corresponding salt concentrations), we estimate that 3 days worth of beamtime is required.*

References

1. W. C. Wimley, Describing the mechanism of antimicrobial peptide action with the interfacial activity model, *ACS Chemical Biology,* **2010**, 5, 905–917.

2.Nielsen, J. E.; Resolving the structural interactions between antimicrobial peptides and lipid membranes using small-angle scattering methods: the case of indolicidin. *Soft Matter* **2018,** *14* (43), 8750-8763.

3. Eilsø Nielsen, J, *et al.* Beyond structural models for the mode of action: How natural antimicrobial peptides affect lipid transport.

4. Bjørnestad, V. A., Soto-Bustamante, F., Tria, G., Laurati, M., & Lund, R. (2023). Beyond the standard model of solubilization: Non-ionic surfactants induce collapse of lipid vesicles into rippled bilamellar nanodiscs. In Journal of Colloid and Interface Science (Vol. 641, pp. 553–567). Elsevier BV.