Mechanosensitive Ion Channels as Membrane Tension Sensors in Bacteria.

An Alternative Mechanism for AMP Action?

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Introduction

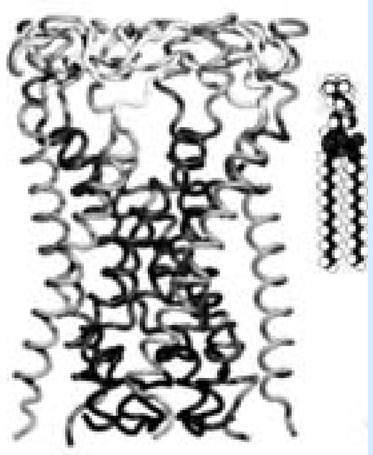
Antimicrobial peptides (AMPs) are an alternative to traditional antibiotics. We use a range of experimental approaches to investigate the interaction of AMPs with bacterial membrane mimics in order to understand their biological activity.

AMPs insert into bacterial membranes resulting in cell death.

- The canonical model is that they form pore structures within the bacterial membranes, however recent in vivo research suggests that may not be necessary.[1]
- Insertion of AMPs into the membrane may trigger the opening of pre-existent pores such as the Mechanosensitive ion channel of large conductance (MscL).[2]

The mechanosensitive ion channel of large conductance (MscL) is a highly conserved membrane protein due to its ability to save the bacteria from osmotic shock.

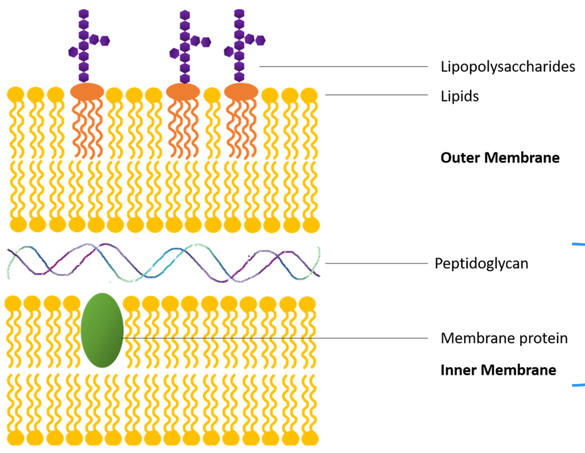
- When open the pore measures 30 Å in diameter.
- The Pore opens:
  - In response to an increase in membrane tension due to an increase in osmotic pressure.
  - In response to an increase in membrane curvature.
  - In response to an applied voltage.



Closed structure of MscL

Model Membranes

- Model membranes of bacterial cells are key for investigating the mechanisms of AMPs.

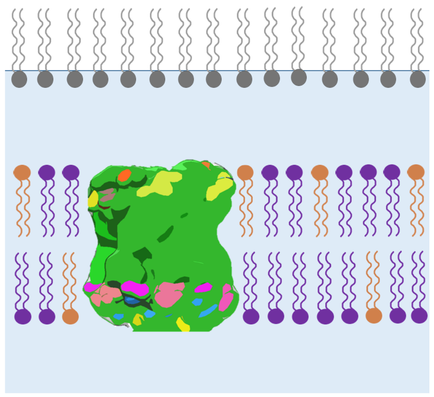


We will be mimicking the inner membrane of this structure, incorporating the MscL protein in the membrane.

Diagram of gram-negative bacteria membrane.

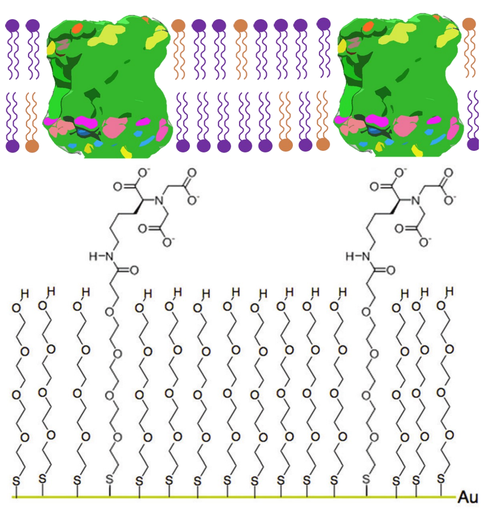
Two membrane mimics will be exploited: that of a novel suspended bilayer and that of a tethered bilayer.

Suspended bilayer mimic



- Cationic surfactant layer formed at air-water interface.
- 3:1 POPC:POPG protein containing vesicles injected into subphase.
- Vesicles rupture at interface to form a bilayer containing the protein of interest.
- Bilayer free of solid-support: potential to measure membrane tension.

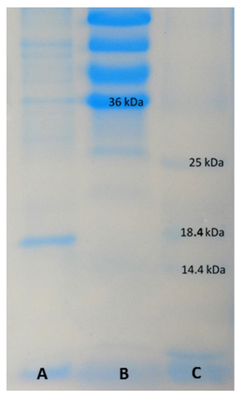
Tethered bilayer mimic



- PEG-thiol and NTA-PEG-thiol (the tether) are assembled on a gold interface.
- The PEG layer prevents non-specific binding of the protein to the solid surface.[3]
- Proteoliposomes are then injected into buffer solution above the gold surface.
- The his-tag on protein enables binding to the NTA-tether via Ni^{2+} chelation.

Results

MscL Expression into Proteoliposomes



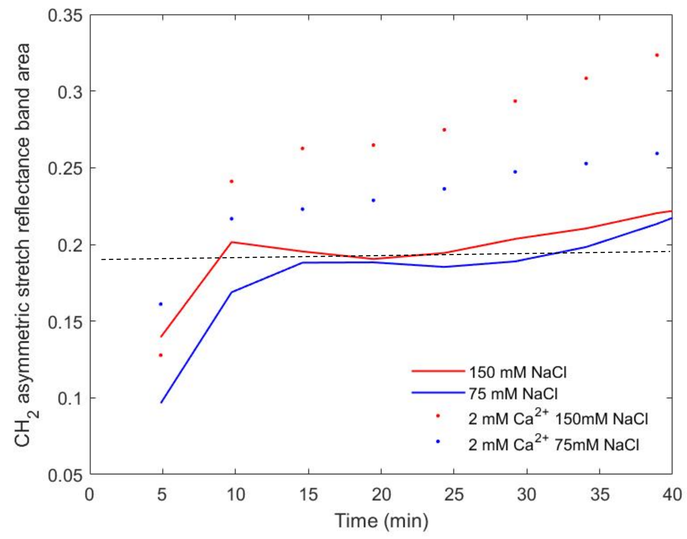
- The MscL protein has been successfully expressed by the cell-free protein expression method.[4]
- MscL was expressed directly into 200 nm diameter, 3:1 POPC:POPG vesicles (no detergents were required).
- Gel electrophoresis confirmed that the protein of expected weight 14.9 kDa (A) had been expressed.

FT-IR analysis of bilayer formation

Fourier Transform-Infrared Spectroscopy (FT-IR) can be used to analyse the formation of lipid bilayers by measuring the area under the CH_2 stretch bands over time.

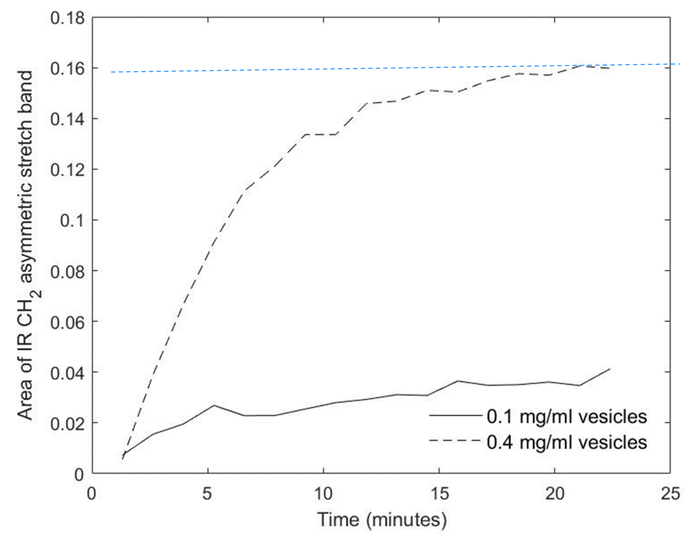
In the formation of the suspended bilayer:

- Successful formation of a lipid bilayer underneath a surfactant monolayer was observed by FT-IR.
- A bilayer amount of material formed at interface within 15 minutes (dashed line on right figure).
- Rate of bilayer formation increased with higher salt concentration.
- In the presence of Ca^{2+} ions: Amount of lipid at interface continues to increase immediately after bilayer formation.



When depositing vesicles at a solid Interface:

- High concentration of vesicles required (0.4 mg/ml) in order for vesicle adsorption and rupture to occur.
- Bilayer formation takes about 20 mins. Time frame agrees with Quartz-crystal microbalance with dissipation (QCM-D) results.



Future Work

Determine conditions for vesicle rupture onto PEGylated gold surfaces. This will be done using QCM-D and plasmon resonance.

Characterisation of proteoliposomes by SAXS: is there clustering of the protein channels?

Both membrane mimic systems will be analysed by neutron reflectivity (on INTER). The reflectivity data will determine:

- Whether lipid bilayers have been formed.
- The behaviour of the MscL channel on insertion of AMPs into the membrane.

Thanks and References

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[1] Wenzel et al., PNAS III (2014) 1409 [2] Martinac., Nature 348 (1990) 261
[3] Jagalski, et al., Soft Matter 11 (2015) 7707 [4] Abdine et al., J. Mag. Res. 204 (2010) 155
[5] Kloda et al., Biophys J. (2006) 1992 [6] Vanegas et al., PloS 12 (2014) 113947