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Cryo-EM analysis of pore form of Mitilysin

Viridans streptococci (VGS) are a group of closely related Streptococci, includes pneumococcus that belong to normal human upper respiratory tract and oral flora. *Streptococcus mitis* is an opportunistic pathogen that secretes cholesterol-dependent cytolyin (CDC) Mitilysin, which forms pores in lipid membranes and contributes to host cell death. Presence of pore-forming toxins is generally beneficial to microorganisms and CDCs are one of the most important classes of pathogen virulence factors. Pore formation by CDCs involves sequential steps of membrane cholesterol-binding by toxin monomers, oligomerization to form pre-pores, membrane insertion and functional pore generation (transmembrane β -hairpin formation). This model of pore formation involving a pre-pore intermediate structure is ascribed to a majority of CDCs, wherein the pre-pore-to-pore transition is proposed to be facilitated by π stacking of aromatic residues between adjacent monomers. However, the detailed mechanisms of Mitilysin pore formation, particularly the amino acid residues involved in conformational transitions, have not been defined. We have optimized the Mitilysin expression and purification protocol and confirmed pore formation in red blood cells and liposomes. This project aims to extract Mitilysin pores from liposomes using detergents for Cryo-EM analysis and 3D reconstruction. The long-term goal is to identify key residues responsible for driving the conformational changes from pre-pore to pore formation at atomic level using cryo-EM.

The Sundborger-Lunna lab uses cryo-electron microscopy (cryo-EM) and functional biochemical and biophysical methods to elucidate the molecular mechanisms underlying critical cellular membrane remodeling and cell death processes.

Aims of the project

1. Expression and purification of Mitilysin in *E.coli*
2. Mitilysin pore formation
3. Cryo-EM sample preparation and analysis
4. 3D reconstruction and model building of Mitilysin pores

Time frame:

Aims 1 and 2 – 1 month

Aim 3. 1 month

Aim 4. 1+ months

Ref:

1. <https://elifesciences.org/articles/23644#s2>
2. <https://journals.plos.org/plospathogens/article/peerReview?id=10.1371/journal.pp.at.1009016>
3. <https://www.nature.com/articles/s41467-018-07653-5>
4. <https://www.nature.com/articles/s41467-021-26366-w#Sec2>
5. <https://www.nature.com/articles/s41467-020-19482-6#Sec2>