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Perfringolysin O pore formation dynamics: monomeric interactions, membrane tracking and membrane composition effects

Conall Mc Guinness[ab], James Walsh[ab], Michelle P. Christie[c], Michael W Parker[cd] and Till Böcking[ab]

[a] *EMBL Australia Node in Single Molecule Science, School of Medical Sciences, UNSW, Sydney, Australia.*
[b] *ARC Centre of Excellence in Advanced Molecular Imaging, UNSW, Sydney, Australia.*
[c] *Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria, Australia.*
[d] *St. Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia*

Perfringolysin O (PFO) is a cholesterol dependent cytolysin (CDC) secreted by *Clostridium perfringens*, which forms pores in cholesterol containing membranes (1). CDCs are virulence factors and could represent a novel drug target for infectious diseases in humans. CDCs are secreted as soluble hydrophilic monomers which oligomerise on lipid bilayers, ultimately forming bilayer spanning ring or arc-shaped β-barrel pores. Perfringolysin O (PFO) was the first CDC to have its crystallographic structure resolved in its soluble monomeric form and has since become the prototypical CDC for investigating pore-forming mechanism (2).

Previous studies on PFO have revealed a general outline of the steps involved in CDC pore formation; recognition of cholesterol and membrane binding, oligomerisation and ultimately membrane insertion to form large amphipathic pores. These steps have been elucidated using bulk assays and static imaging techniques such as electron microscopy or atomic force microscopy, however key mechanistic details remain uncharacterised due to the lack of time resolved data at a single pore level. Here we present an assay using total internal reflection microscopy to track PFO pore formation dynamics . Fluorescently labelled PFO and dye encapsulating liposomes and viral-like particles (VLPs) were employed in conjunction to measure the kinetics of PFO binding from solution, nucleation, and oligomerisation on the surface of cholesterol containing vesicles. By visualising fluorescent dye release from our liposomes, we were able to determine the number of molecules necessary for an oligomer to insert and form a bilayer spanning pore.

1. Christie MP, Johnstone BA, Tweten RK, Parker MW, Morton CJ. Cholesterol-dependent cytolysins: from water-soluble state to membrane pore. Biophys Rev. 2018;10(5):1337–48.
2. Rossjohn J, Feil SC, McKinstry WJ, Tweten RK, Parker MW. Structure of a cholesterol-binding, thiol-activated cytolysin and a model of its membrane form. Cell. 1997;89(5):685–92.