Two-component pore formation by the novel CDCL proteins ALY short and ALY long from *Elizabethkingia anophelis*

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Cholesterol-dependent cytolysins (CDCs) are bacterial pore-forming toxins that are secreted as soluble monomers and oligomerise into large circular pre-pores on the surface of cholesterol-rich membranes. Various structural changes and transitions results in insertion of β-hairpins into the lipid bilayer, forming a large β-barrel pore that results in cell lysis [1]. We have identified a highly conserved structural motif of CDCs that plays a critical role in the prepore-to-pore transition [2]. Furthermore, this motif is also highly conserved in a large, diverse family of uncharacterised proteins from over 220 species, which we have designated the name "CDC-like" (CDCL) protein family [2]. One partner of the CDCL pair, termed CDCL long, consists of four domains: three similar to CDCs and a unique fourth domain. The other partner, CDCL short, possesses three domains, all similar to CDCs. One CDCL pair, referred to as ALY long (ALY^L) and ALY short (ALY^S), originate from the species *Elizabethkingia anophelis; an emerging and opportunistic pathogen of unknown virulence and transmission. X-ray crystallography revealed the structure of monomeric* ALY^L consists of characteristic CDC domain 1 – 3 structure despite only 22% identity with the archetype CDC perfringolysin O; however, domain 4 is completely different to the equivalent domain in CDCs that plays a role in sensing cholesterol. In the presence of lipids, ALY^L and ALY^S show pore-forming activity and analysis by electron microscopy reveals a large circular oligomeric complex reminiscent of CDC pore complexes. ALY^S also forms a non-lytic circular oligomer in the absence of ALY^L. Cross-linking mass spectrometry data reveals structural changes between the monomeric and protomeric states, giving insight to the mechanism of pore formation. To determine the atomic structure of ALY pores, cryo-EM single-particle analysis is currently being pursued In summary, we have shown that the ALY toxins share some structural resemblance to CDCs, but in contrast form a two-component pore complex. CDC-like proteins are present in a wide range of bacterial species and are suspected to play key roles in microbial survival and human disease. An understanding of pore formation by ALY may yield new knowledge of *Elizabethkingia anophelis virulence, in addition to providing a system that could be applied to biotechnological applications.* Our work on ALY provides the first functional and structural insights into this fascinating family of proteins.

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[2] Evans JC, Johnstone BA, Lawrence SL, Morton CJ, Christie MP, Parker MW and Tweten RK (2020), "A Key Motif in the Cholesterol-Dependent Cytolysins Reveals a Large Family of Related Proteins", *mBio*, 11(5): e02351-20

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