A structural perspective on pore formation and regulation of *Bacteroides fragilis* toxins

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Bacteroides fragilis is a gut commensal that can become pathogenic under dysbiotic conditions. They utilise a novel family of pore-forming toxins, Cholesterol-Dependent Cytolysin-Like proteins (CDCLs), to outcompete neighbouring microbes (1,2). Despite their emerging significance, the mechanisms by which these bacteria form pores and avoid self-damage remain poorly understood (3).

This study focuses on the proteins involved in pore formation and regulation by *Bacteroides fragilis*, including two CDCLs, Bf long and Bf short, as well as BcdI, a surface lipoprotein that provides immunity to the producing strain. The amazing conformational changes of these proteins when they go from the monomer to the pore state are being studied using an integrated approach combining X-ray crystallography, SAXS, and cryo-EM.

I will be presenting SAXS solution structures of these proteins in their soluble monomeric state, the first-ever crystal structures of Bf short and BcdI, and negative-stain images and cryo-EM models of prepore-like and inserted pore states of these proteins. These structural studies will also be complemented by discussions on strategies to reconstitute CDCL pores on liposomes and visualise them by cryo-EM, as well as functional assays including liposome rupture and pull-down experiments. These findings provide new insight into the structural aspects of bacterial toxins and the elegant mechanism used by *B. fragilis* to deploy them while avoiding self-harm.

References:

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