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Bastian Bräuning

Structural and Biochemical Characterization of the YaxAB Pore-forming Toxin from *Yersinia Enterocolitica*

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Structural and Biochemical Characterization of the YaxAB Pore-forming Toxin from *Yersinia Enterocolitica*

Doctoral Thesis accepted by
Technische Universität München, Garching,
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Supervisor's Foreword

Pore-forming toxins (PFTs) are among the most common bacterial toxins. They are produced by a variety of pathogens, which infect a wide range of organisms including plants, insects and humans. Bacteria utilize PFTs to gain access to nutrients, modulate their environment and suppress host immune responses. Expressed as soluble monomers, PFTs insert into cell membranes by forming an oligomeric pore complex. Subsequently, homeostasis of infected cells is impaired, ultimately resulting in necrosis and apoptosis.

The maturation of these particles and the structural changes required for pore formation are still poorly understood for many PFT families. The challenge of Dr. Bastian Bräuning's Ph.D. thesis was to decode the lytic mechanism of an α-helical pore-forming toxin composed of two different subunits. To this end, the YaxA and YaxB proteins from *Yersinia enterocolitica* were cloned, heterologously expressed in *Escherichia coli* and purified. Crystallization experiments were initially difficult, but eventually yielded diffracting crystals. Data collection was performed at a third-generation synchrotron facility at the Paul-Scherrer Institute in Villingen (Switzerland), which led to X-ray structures of YaxA, YaxB as well as the orthologue PaxB from the insect pathogen *Photorhabdus luminescens*. Next, the YaxAB pore complex was reconstituted in erythrocyte membranes as well as by detergent treatment. Together with Eva Bertosin and Prof. Dr. Hendrik Dietz at the Technical University of Munich, the oligomeric complex was subjected to single-particle cryo-electron microscopy analysis. The individual crystallographic models were placed into the sub-nanometer resolution electron density map of the assembled pore. The structure of YaxAB allowed identification of critical residues for pore formation, which were confirmed by a series of mutants together with haemolytic and membrane-binding activity assays. Using a diverse panel of biochemical and structural techniques, Dr. Bräuning and colleagues succeeded in dissecting the mechanistic contributions of the two toxin components and elucidated the lytic state of the pore complex.

The results of this Ph.D. thesis on the YaxAB system are applicable to orthologues from agriculturally relevant insect pathogens like *Xenorhabdus nematophila* (XaxAB) and *P. luminescens* (PaxAB). Promising insecticidal properties of YaxAB

orthologues have already been identified and future bioengineering efforts certainly will take advantage of the here presented structural and mechanistic insights into PFTs.

Garching, Germany
August 2019

Prof. Michael Groll

Abstract

Many pathogenic bacteria have acquired an arsenal of proteinaceous virulence factors, to counteract and highjack host immune response systems. Among these factors, pore-forming toxins (PFTs) are some of the most conserved and potent. A hallmark of PFTs is their ability to transform from soluble to membrane-bound states, a transition often accompanied by formation of large transmembrane oligomers. The vast majority of structurally elucidated PFTs form homooligomeric assemblies and produce β-barrel type pores (β-PFT). YaxAB from *Yersinia enterocolitica*, along with its orthologues from insect and plant pathogens, are two-component PFTs predicted to be entirely α-helical. As a putative virulence factor, obtaining mechanistic insights into its binary mode of action would inform future efforts to target the proteins therapeutically as well as to exploit them for biotechnological purposes. In this thesis, structures of the soluble components YaxA and YaxB could be obtained by X-ray crystallography, along with a cryo-electron microscopy structure of the reconstituted YaxAB pore. Together with structure-guided mutagenesis and biochemical assays, a plausible pathway of pore formation could be proposed. Comparison with the ClyA pore revealed great compositional diversity amongst members from the wider family of ClyA-like α-PFTs, which includes homomeric, binary and tripartite assemblies.

Parts of this thesis have been published in the following journal articles:

"Structure & mechanism of the two-component α-helical pore-forming toxin YaxAB"
Bastian Bräuning, Eva Bertosin, Florian Praetorius, Christian Ihling, Alexandra Schatt, Agnes Adler, Klaus Richter, Andrea Sinz, Hendrik Dietz, Michael Groll
Nature Communications **9** (1), 1806 (2018); https://doi.org/10.1038/s41467-018-04139-2

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