Chapter 2
Objectives

Orthologues of the YaxAB PFT are implicated as virulence factors both in insect pathogens of the genera *Xenorhabdus* and *Photorhabdus*, as well as the human pathogen *Y. enterocolitica*. Structural data is missing entirely on this class of PFTs, precluding a detailed mechanistic understanding of the assembly principle and lytic mode of action. As a two-component PFT and with its subunits predicted to contain only α-helices, elucidating the architecture of the YaxAB PFT would furthermore provide the first structural view on a heteromultimeric α-PFT.

The first objective was to obtain crystal structures of the monomeric forms of YaxA and YaxB. Here, orthologous proteins from several organisms should be tested for recombinant expression levels, solubility and achievable diffraction limits once crystallized. Obtaining reasonable yields of protein in the milligram scale was also crucial in order to perform structure-guided mutagenesis experiments to validate the mechanistic model of toxin action.

With the soluble components at hand, the next important step was to establish a reconstitution protocol for the pore complex. The stoichiometry and overall shape of the pore should be estimated using a combination of analytical ultracentrifugation and negative-stain transmission electron microscopy (TEM). Finally, a structure of the complex at a resolution sufficient for unambiguous assignment of secondary structure was sought: here, the aim was to obtain a cryo-TEM map at high resolution to allow visualization of the subunit conformational changes accompanying pore formation.

Based on both monomeric and pore complex structures, together with a series of biochemical mutagenesis studies, the major goal was to suggest a plausible mechanistic model for YaxAB pore formation, which rationalizes the peculiar properties of this binary PFT.

B. Bräuning, *Structural and Biochemical Characterization of the YaxAB Pore-forming Toxin from* Yersinia Enterocolitica, Springer Theses,
https://doi.org/10.1007/978-3-030-29439-7_2