Direct interaction of a chaperone-bound type three secretion substrate with the export gate

Dominic Gilzer[1], Madeleine Schreiner[1], Hartmut H. Niemann[1]

[1]Department of Chemistry, Bielefeld University, Bielefeld, Germany

dominic.gilzer@uni-bielefeld.de

Type III secretion systems (T3SS) are molecular assemblies employed by a variety of pathogenic gram-negative bacteria to inject effector proteins directly into host cells. The inner membrane-bound export gate protein SctV is a crucial regulator of protein secretion and recognizes substrate:chaperone complexes prior to their secretion [1]. Structurally, SctV family proteins contain a large cytoplasmatic domain that has been shown to form cyclic nonamers *in vivo*. SctX is one T3S substrate with an elusive function and unknown structure. Together with its cognate chaperone SctY, it binds to the export gate, an interaction essential for the assembly of a secretion-competent T3SS.

We co-crystallized the cytosolic domain of SctV together with SctX:SctY to obtain crystals that diffracted to roughly 4 Å. The complex crystallized with 18-fold symmetry by stacking two nonameric rings in the asymmetric unit. To enable structure solution, we followed a divide-and-conquer approach to generate higher resolution models of the subcomplexes. Here, we present the structure of both the SctX:SctY complex alone, and bound to the cytosolic domain of the export gate SctV. SctX entwines its chaperone by binding it at two distinct sites. The C-terminal helix of SctX is presented by this SctX:SctY complex and acts as major recognition interface for the export gate SctV. In the structure of the ternary complex, this helix directly interacts with SctV by inserting between neighboring subunits in the nonameric SctV ring. This structure represents the first instance of a direct interaction between a T3S substrate and the export gate, as previous structures of ternary flagellar export gate:substrate:chaperone complexes showed mediation by the chaperone [1]. Furthermore, the SctX binding sites on SctV have previously been reported as recognition sites for flagellar substrate:chaperone pairs or the ATPase stalk protein SctO [2] and are as such functionally compelling.

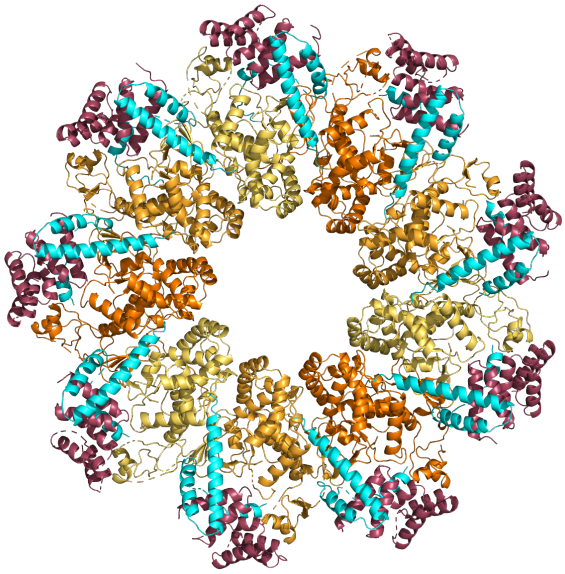

Fig. 1: Structure of the SctVXY complex viewed from the cytoplasmatic face.

[1] Xing Q, Shi K, Portaliou A, Rossi P, Economou A, Kalodimos C. Structures of chaperone-substrate complexes docked onto the export gate in a type III secretion system. Nat. Commun., 9, 1773 (2018)

[2] Jensen J L, Yamini S, Rietsch A, Spiller B W. The structure of the Type III secretion system export gate with CdsO, an ATPase lever arm. PLoS Pathog., 16(10): e1008923 (2020)