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Multiple approaches towards understanding the type II secretion system

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Transport of folded proteins across membranes is a feat accomplished by few biomacromolecular machines. One of the machineries able to do so is the sophisticated type II secretion system (T2SS). It can translocate key virulence factors from the bacterial periplasm into the lumen of the gut of the human host. A prime example is the secretion of cholera toxin by Vibrio cholerae. The T2SS consists of ~12 different proteins, most of these present in multiple copies, organized into three subassemblies: (i) the Inner Membrane Platform; (ii) the Pseudopilus in the periplasm, which acts most likely as a piston pushing exoproteins through the outer membrane pore; (iii) the Outer Membrane Complex, allowing passage of ~100 kDa folded proteins. We have determined crystal structures from more than a dozen T2SS domains, yet, a full understanding of the architecture and mechanism of action of the T2SS remains a formidable challenge. Our approaches include the use of "assistant-multimers" to promote recalcitrant multimer formation and of nanobodies to overcome reluctant crystal formation. The Inner Membrane Platform is interacting with the secretion ATPase GspE which most likely needs to be hexameric for full activity. Full-length GspE co-crystallized with its major partner, the cytoplasmic domain of GspL, revealed a tremendous flexibility of this ATPase, and, most unexpectedly, also the organization of the same linear arrangement of cyto-GspL domains throughout three entirely different crystal forms. Two very different hexamers of GspE were elucidated by linking the GspE subunit to the subunit of Hcp1, which successfully acted as an "assistant hexamer", inducing hexamer formation by GspE. The dodecameric nature of the ~ 850 kDa GspD, the major component of the Outer Membrane Complex, evident in earlier electron microscopy studies, was observed in the dodecameric ring-like helix in crystals of its N-terminal domain. The contacts between GspD and the inner-membrane protein GspC will be discussed as well as the remarkably frequent occurrence of dimers of Inner Membrane Platform domains. How dimers are co-assembled with an ATPase hexamer with C6 symmetry and the Outer Membrane Complex dodecamer with C12 symmetry remains one of the many fascinating outstanding questions of the T2SS.

[1] *Lu et al (2013) Hexamers of the type II Secretion ATPase GspE from Vibrio cholerae with increased ATPase activity, Structure 21, 1707–1717,* ***[2]*** *Korotkov, K.V., Sandkvist, M. and Hol, W. G. J. (2012) The type II secretion system: biogenesis, molecular architecture and mechanism, Nature Reviews Microbiology 10, 336-351*

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