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In Situ Investigations of the Bacterial Type II Secretion System

Zhili Yu[1], Tong Huo[1], Muyuan Chen[1], Xiaodong Shi[2], Steven Ludtke[1] and Zhao Wang[1]

[1]Baylor College of Medicine, Houston, Texas, United States, [2]Xuzhou Medical University, Xuzhou, Jiangsu, China (People's Republic)

Secretion systems, which are cell envelope located protein complexes, are used by bacteria to secrete various virulence factor proteins that are important for bacteria's survival and pathogenicity[1]. One of the secretion systems is the type II secretion system (T2SS), which is widely used by gram-negative bacteria to secrete virulence-related proteins that can intoxicate target cells, absorb nutrients, help adhesion to host cells, and so on, causing various diseases[2]. The T2SS is made up of 12-15 protein components, including the outer membrane channel protein GspD (the secretin), which constitutes the last step of substrate transportation of T2SS. The *in vitro* structure of the GspD secretin has previously been revealed by cryo-EM single particle analysis (SPA), which provided its detailed architecture information[3]. However, its *in situ* structural information under the original physiological and biochemical conditions is still lacking, and macromolecules may require their native biological environment to achieve their functional state, through interactions with its surroundings such as the cell membrane and the peptidoglycan cell wall. In this study, we investigate the *in situ* structure of the GspD secretin through cryo-electron tomography (cryo-ET) and subtomogram averaging. We present that, when overexpressed in *E. coli* cells, the GspD secretin locates to the bacterial inner membrane where it can be clearly recognized from the tomogram. After subtomogram averaging, we solved a subnanometer resolution structure of the *in situ* GspD. This result identified the central gate region, the N3 constriction site and the N0 domain which is missing in the previous high-resolution *in vitro* structure. Also, our structure shows the transmembrane region of the GspD secretin *in situ*, which reveals the interaction of GspD with the cell membrane. As the first *in situ* high-resolution structure of the GspD secretin, these results provide new insights for future studies about the functioning and biogenesis process of the GspD secretin.

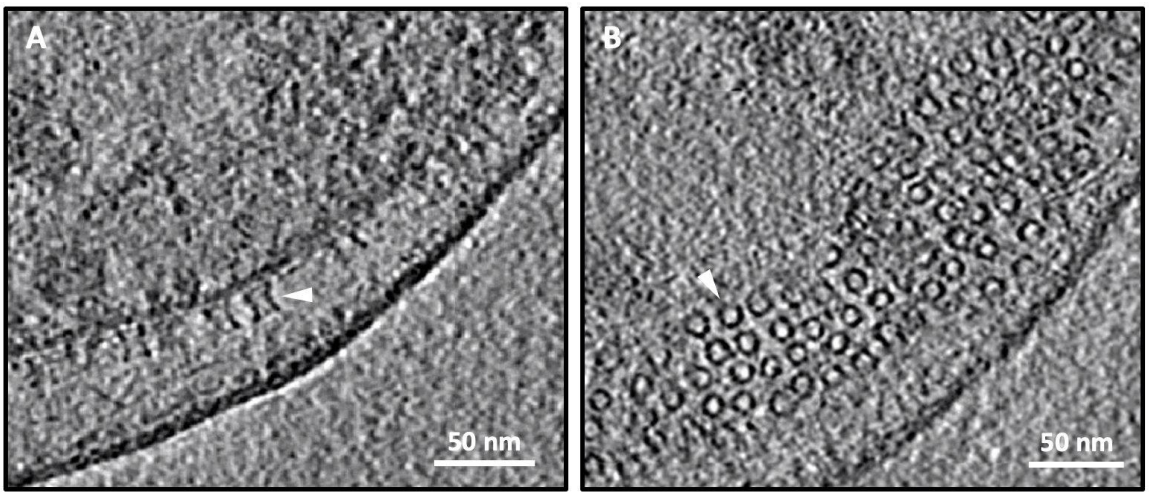

Figure 1. Cryo-ET of *E. coli* cell expressing the GspD secretin. (A) and (B) are showing slice views of the reconstructed tomogram at different z levels, with (A) showing side view particles and (B) showing top view particles. The white arrowheads point to one side view (A) and one top view (B).

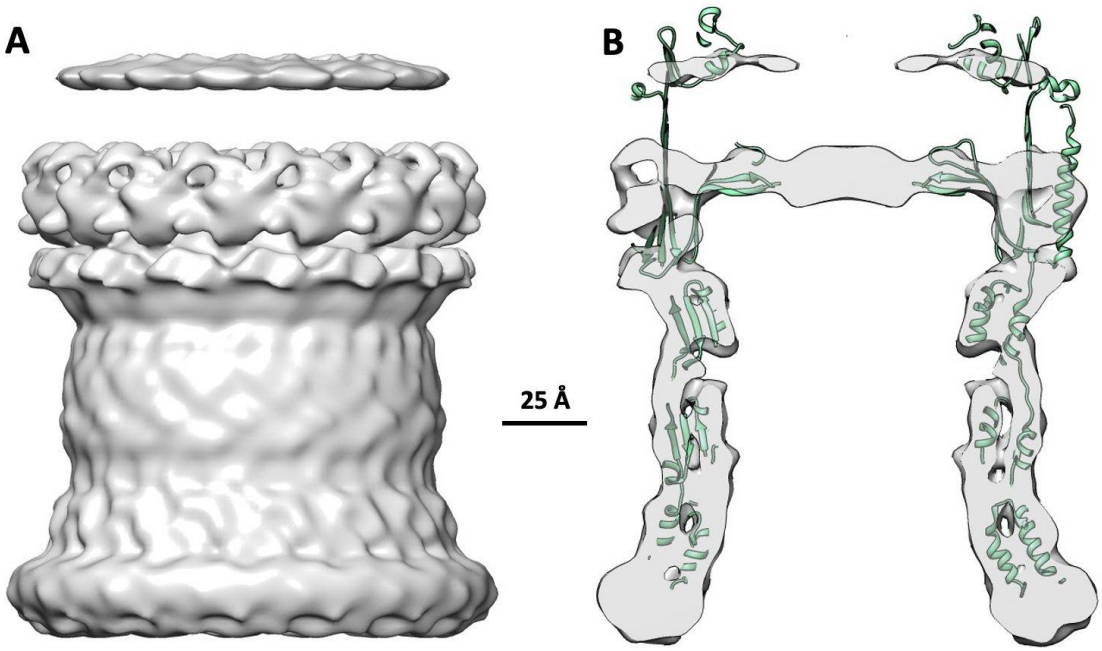

Figure 2. *In situ* structure of the GspD secretin. (A) Side view of the GspD secretin density map. (B) Central slice view of the GspD secretin density map with the *in vitro* structure (PDB: 5wq7) fitted in.

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