

In Situ Structures of Secretins from Bacterial Type II Secretion System Reveal Their Membrane Interactions And Translocation Process

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The type II secretion system (T2SS), which is a protein complex spanning the Gram-negative bacterial cell envelope, secretes diverse effector proteins or toxins that cause severe diseases such as diarrhea and cholera. The outer membrane component of T2SS, the secretin GspD, needs to translocate from the inner to the outer membrane to exert its function, and understanding this process is essential for developing new antimicrobial strategies. Here, we respectively reconstitute two secretins homologs, GspD α and GspD β , in *Escherichia coli* cells to determine their in situ structures by electron cryotomography subtomogram averaging. GspD α on the inner membrane exists in an unstable and flexible conformation, forming loose connections with the membrane. Enlarging the pore size of peptidoglycan by D-methionine could localize GspD α to the outer membrane, where it adopts a more stable conformation and membrane connection. GspD β locates on the inner membrane and forms a visible structure when its chaperone GspS is knocked out. In wild-type *E. coli*, GspD β locates on the outer membrane and exists in a complex with GspS, while a small amount of GspD β is still visible on the inner membrane. Based on these results, we propose different models for the membrane translocation of GspD α and GspD β , providing a more complete perspective on the inner to outer membrane biogenesis of T2SS secretins.