Electron cryo-tomography of type I secretion complexes caught in the act of substrate transport.

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Bacteria have developed numerous systems to secrete proteins or DNA in order to modify their immediate surroundings or to obtain an advantage in a competitive and hostile environment. Because Gram-negative bacteria possess a cell wall in addition to inner (cytoplasmic) and outer membranes, the transport machines for protein secretion have the challenging task of circumventing two barriers to reach the exterior. **See Figure 1**

Almost all the Type I transport substrates are polypeptides, varying from the small *E. coli* peptide Colicin V, (10 kDa), to the impressive size of the *Pseudomonas fluorescens* cell adhesion protein LapA of 900 kDa. While these two examples reflect the range of the size of Type I transport substrates, one of the best characterized families of substrates are toxins of the RTX (repeats in toxin) family. For a review see [1]

The Type I secretion apparatus is composed of two inner membrane proteins, a so called membrane-fusion-protein (MFP) and an ATP Binding Cassette transporter, and one outer membrane protein extending into the periplasm to complete the continous tunnel to the exterior. In the model organism *E. coli* the paradigm for Type I secretion is the Haemolysin system with the corresponding proteins being HlyD (MFP), HlyB (ABC transporter) and TolC in the outer membrane. The respective transport substrate is the 110 kDa haemolytic toxin, Haemolysin A (HlyA). HlyA secretion is frequently associated with uropathogenic strains of *E. coli* in both animals and humans, and it constitutes a significant clinical and veterinary problem.

We therefore started an approach to trap the ternary complex of the Type I secretion system and subsequently study the structural and functional implications of substrate transport. The full length maltose binding protein was fused onto the c-terminal end of the the HlyA toxin in order to prevent the completion of transport which would lead to the dissociation of the complex. The HylA toxin is over expressed in the presence of constituitive expression of HylB and HylD. The expression of HylA leads to an altered appearance for the outer membrane with the trapped complexes appearing to cluster and form crevasses in the outer membrane. This is often accompanied by the appearance of vesicles occasionally seen still connected to the cytoplasmic membrane.

The expression is carried out in a strain of *E. coli* a mutation in the *min* locus [2] which results in unregulated cell division where cell division and genome replication are uncoupled. The result is production “mini” *E. coli* lacking the genome [3] but still capable of biochemical function [4] including expression of plasmids. The mini-cells are an ideal subject for tomography since one can select the appropriate (small) sized mini-cells. A marker free alignment method was developed for this system allowing for the use of gold conjugated antibodies to detect the presence and distribution of the trapped protein and channel components on the surface of the cells. We have successfully labeled the HylA toxin on the surface and reconstructed the labeled cells allowing the identification of the complex

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in 3D (**Figure 2)**. We currently are using sub-tomogram averaging of individual complexes to obtain a picture of the complex in action at moderate resolution.

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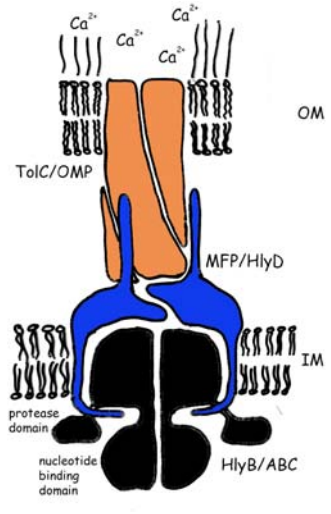

Figure 1

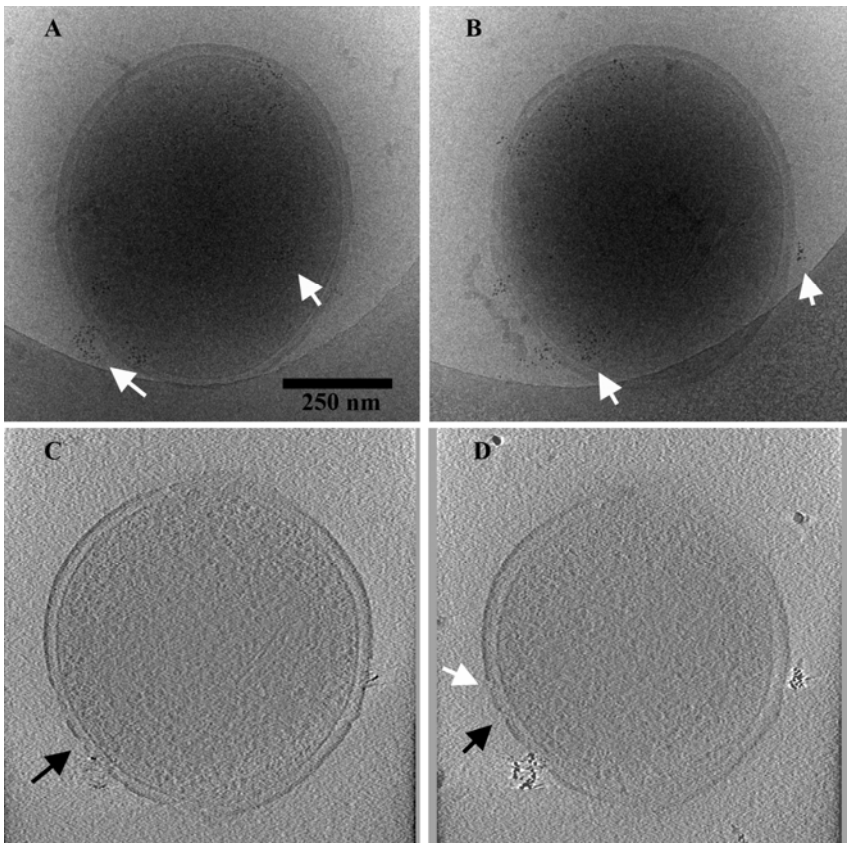

Figure 2

Figure 1 A schematic of the type secretion system.

Figure 2 Panels A and B are tilt images acquired from a mini-cell labeled with anti-HylA antibodies, then reacted with gold-conjugated secondary anti-body. The white arrows indicate clusters of gold label which are found near the surface of the cell. There is no gold in the background. Panels C and D are sections from the tomographic reconstruction. The arrows indicate the crevasses which are found in the outer membrane. The gold clusters can be seen in the sections from the reconstruction.

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