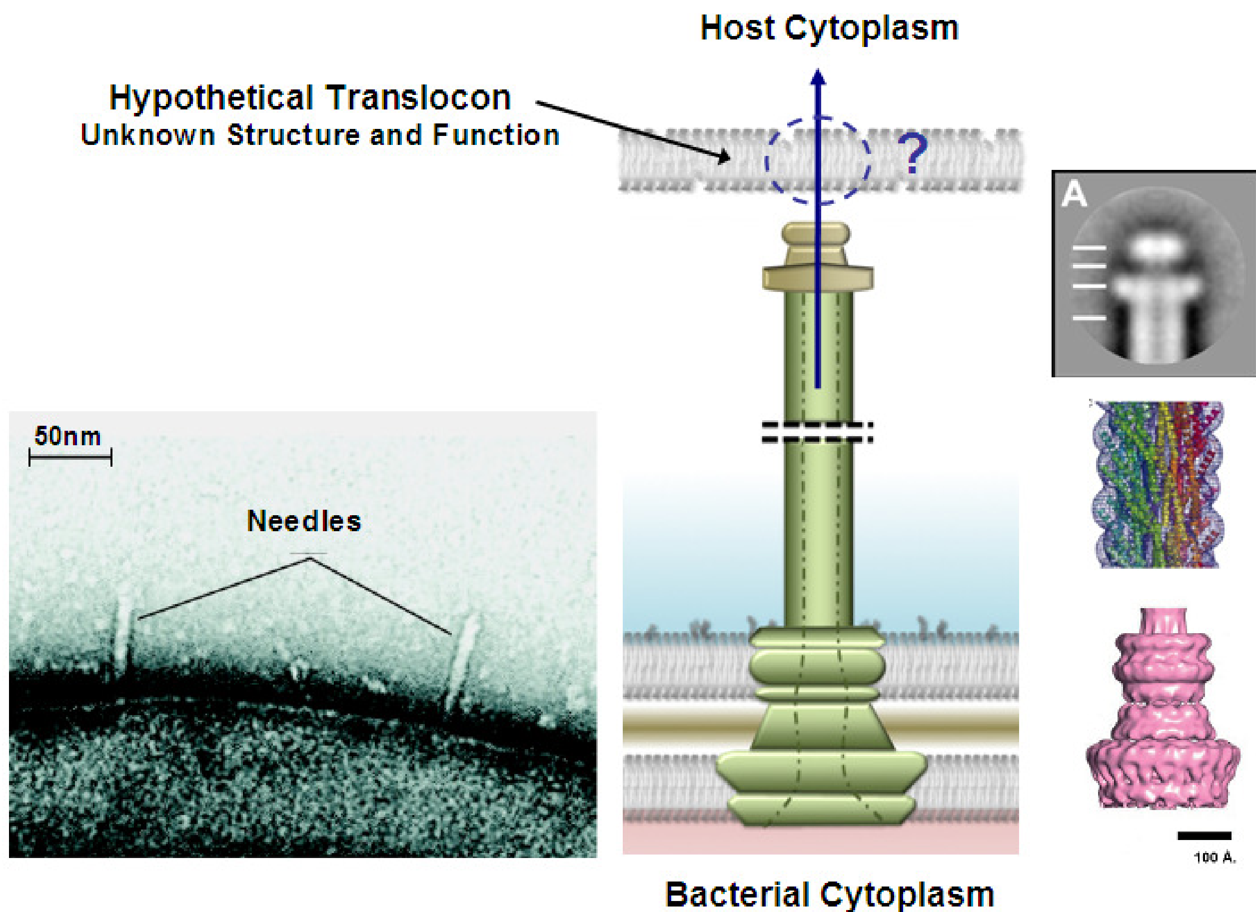

Bacterial colonization of human cells depends on their ability to secrete toxins into the extracellular fluid or to inject them directly into the cell cytoplasm. Pathogenic Gram-negative bacteria use a common and particularly efficient delivery mechanism, called the type III secretion (T3S) system. The T3S system acts as a syringe that injects toxic proteins from the bacterial cytoplasm directly into the human host cell. Two secreted T3S proteins insert into the target cell membrane to form a pore through which effectors are translocated. Despite recent advances on the characterization of these translocon proteins, their structure and mechanism of assembly remains unknown. At the Heuck lab we study these properties focusing on two translocon proteins from the *Pseudomonas aeruginosa* T3S system, PopB and PopD. The puzzle we aim to address is: Unlike known translocons which are co-translationally inserted into the membrane, which is the mechanism that allows PopB and PopD to spontaneously insert into a membrane and assemble a protein translocon? To tackle this question we use a biochemical/biophysical approach where environment sensitive fluorophores are incorporated into full length translocon proteins reconstituted into liposomal membranes. By combining a series of fluorescence spectroscopy techniques it is possible to obtain topological data of the membrane inserted translocon proteins. Using this approach we are starting to shed light into this exiting biochemical phenomenon.