The import of a single enzymatic colicin molecule into a bacterial cell is sufficient to induce cell death. This makes a single-molecule approach to investigating the mechanism used by a colicin molecule to cross the cell envelope biologically relevant. We have employed a combination of single-particle tracking, total internal reflection fluorescence microscopy, and fluorescence recovery after photo-bleaching microscopy to follow colicin E9 cell entry into individual cells. Fluorescent colicin E9 variants have been engineered which allow translocation to be halted at distinct steps along the cell entry pathway. Colicin E9-BtuB complexes alone, or interacting with Omp/TolB, have been tracked in the outer membrane. This work has revealed new information about receptor diffusion in the outer membrane. The inner membrane Tol complex is absolutely required for cell killing by colicin E9. The N-terminus of colicin E9 is known to capture TolB and facilitate the formation of a specific interaction between TolB and TolA. This implies that the colicin E9 translocon complex spans the periplasmic space prior to outer membrane translocation. Our efforts to track diffusion of individual Tol complexes in the inner membrane and follow formation of the translocon complex will be discussed.