Nuclease colicins, *E.coli* bacteriocins, target susceptible cells through binding to a specific outer membrane receptor followed by translocation of their cytotoxic nuclease domain across the outer membrane, periplasm and finally the cytoplasmic membrane in order to exert their lethal effect through genomic DNA (DNases) or 16S rRNA (rRNases) cleavage. Currently little is known about the molecular processes underlying this final step: the interaction with and transport across the inner membrane.

Our objective was to use a reductionist approach to study colicin inner membrane translocation by using model membranes and purified nuclease cytotoxic domains. We have used large unilamellar vesicles (LUVs) composed of synthetic and *E.coli* lipids to characterize (a) the dynamics of the nuclease membrane interaction; (b) their insertion depth through acrylamide and brominated lipid quenching analysis; and (c) their effect on membrane integrity via vesicle leakage, aggregation and lipid mixing assays.

Our results show that in addition to a role for electrostatic steering in the initial membrane association, the average hydrophobicity of the nuclease domains is also an important factor in their effect on membrane integrity and their insertion depth much in the same way as has recently been shown for antimicrobial peptides. This study could help to explain the *in vivo* observed differences in cell killing efficiency of nuclease colicins and forms the basis of ongoing work to unravel the molecular mechanisms underlying their inner membrane translocation.