Methodology strategies for the purification and biophysical characterization of bacterial ATP-binding cassette transporters

M.P. Ween, V.G. Lewis, J.C. Paton and C.A. McDevitt, Research Centre for Infectious Diseases, School of Molecular Life Sciences, University of Adelaide, SA 5000, Australia.

Bacterial membrane proteins are essential for bacterial pathogenesis and survival. They have a range of functions including toxin release, drug resistance, cell adhesion, and nutrient acquisition. *Pseudomonas (P.) aeruginosa* is a clinically important bacterial pathogen which predominantly infects the immuno-compromised (*e.g.* AIDS, cancer) and burns patients. It also remains the leading cause of morbidity and mortality in cystic fibrosis patients. ATP binding cassette (ABC) transporters in *P. aeruginosa* have essential roles in drug resistance and nutrient uptake and, consequently, are ideal antimicrobial targets.

We have developed a strategy for the isolation of *P. aeruginosa* ABC transporters, with the objective of further studying their biochemical, biophysical and structural properties. To this end, we have developed a ligation independent cloning system to enable rapid screening of His-tag polarity and signal sequence modification in *Escherichia (E.) coli*. Once the optimal protein expression construct has been identified, expression can be optimised by screening various *E. coli* strains under different induction parameters. The remaining bottlenecks of membrane protein solubilization and purification are addressed by screening non-ionic and zwitterionic detergents for their membrane protein extraction, assessed by western blot. The His-tag protein is then purified by immobilized metal affinity chromatography and analysed by gel permeation chromatography for monodispersity.

One example of this methodology's success is in isolating an integral membrane transporter from *P. aeruginosa*. The manganese ABC permease from *P. aeruginosa* has important roles in growth and surviving oxidative stress, under iron restriction. We have shown that the Mn ABC permease is successfully solubilized by lysophospholipid analog detergents preferentially to non-ionic maltoside or glucoside detergents. This protein has been successfully purified to greater than 95% homogeneity and is amenable to future biophysical and structural investigations.