Membrane transport proteins of bacteria catalyse the energized uptake of nutrients and some are multidrug (Mdr) efflux proteins that contribute to emerging antibiotic resistance. We have cloned and expressed in *Escherichia coli* over 70 genes encoding secondary active membrane transport proteins from 13 species of Gram-positive and Gram-negative bacteria. In the *E. coli* host the transport activity of the heterologous protein is established using radioisotope-labelled substrates. By incorporation of a tag at the C-terminus, purification of many of these proteins has been accomplished by NiNTA affinity and size exclusion chromatography.

The structural integrity of the purified proteins is established by biophysical measurements, including mass spectrometry, circular dichroism, and FTIR spectroscopy. The preservation of transport activity and cation selectivity is confirmed by reconstitution into liposomes composed of *E. coli* lipids and measurements of counterflow of labeled substrates in different isotonic salts. The fluorescence of tryptophan residues in the protein is often altered by the binding of ligands, enabling calculation of dissociation constants for both the organic substrate and the co-cation. These assays are useful also for trials of stability and crystallisability of the purified proteins.

Crystallisation trials have yielded three proteins with unexpected similarity to LeuT of the Neurotransmitter-active transporters, and its 12-helix structural fold revealed the Nucleobase–Cation–Symporter-1 family of secondary active transport protein structures.

In combination, both structures suggest a novel gating mechanism for pentameric ligand-gated ion channels where channel opening proceeds by a change in the tilt of the pore-forming helices. Our study thus provides a first structural view at high resolution into how a pLGIC may open and selectively conduct ions.