Structural and functional characterization of the membrane antibiotic transporter SbmA

Maria del Carmen Lopez1, Giulia Runti2, Marco Scocchi2, Natasha Cant3, Richard Collins3, Robert Ford3 and Konstantinos Beis1

1Imperial College London, Harwell Didcot, United Kingdom
2University of Trieste, Trieste, Italy
3University of Manchester, Manchester, United Kingdom

In *Escherichia coli*, the inner membrane protein SbmA is required for the internalisation of the class I microcins MccB17 and MccJ25 and for the uptake of thiazole ring containing antibiotics, such as bleomycin and the peptide Bac7.

In order to characterize SbmA and its homologue in *Rhizobium meliloti* BacA at structural and functional level, the recombinant proteins were expressed in *E.coli* fused to C terminal GFP and His tags to allow its detection and purification via affinity chromatography upon extraction in DDM. Subsequently, isolated SbmA was tested for its ability to bind bleomycin using Synchrotron Radiation Circular Dichroism. The results obtained with this technique confirm the formation of the complex by increased stability of the protein in presence of the ligand.

On the other hand, *in-vivo* functional studies were carried out by monitoring the uptake of fluorescent labelled Bac7 in whole cells expressing SbmA. The results from these kinetics assays point to a $K_m$ value of 17.67 mM and $V_{max} = 0.16$ mM/min for the internalization of Bac7 via SbmA. The effect of different inhibitors tested suggest that the transport is proton driven and does not require ATP. For structural analysis, purified SbmA and BacA were reconstituted in liposomes and analysed by Electron Microscopy. Projection maps of the two-dimensional crystals suggest a dimeric arrangement for both transporters.