Elucidating interactions within bacterial tripartite drug-efflux pumps

October 2016

Project Description

A PhD studentship will be available in the group of Dr Vassiliy Bavro at the School of Biological Sciences, University of Essex, starting from October 2016 to study the mechanisms of recognition between the components of tripartite multidrug-efflux (MDR) pumps.

These pumps are key contributors to the rising global problem of multidrug resistance in Gram-negative bacteria and are composed of tree components spanning both the outer and inner membranes of the Gram-negative cell, namely the outer-membrane proteins (OMPs) [1], the energy-coupled inner-membrane proteins (IMPs) [2] and the periplasmic adapter proteins (PAPs) providing a link between the two [3, 4]. The OMPs belonging to the TolC family are central conduits for a number of efflux systems and hence an attractive drug target. Despite recent advances in structural characterization of pump proteins, the development of novel antibacterials is hindered by the lack of understanding of mechanics of pump assembly. Currently even such fundamental questions as pump stoichiometry and the energetics of opening of the OMP channel remain unclear [4, 5, 6] and inaccessible by standard structural biology techniques. Consequently, this PhD project seeks to address the gap in our current understanding of these clinically relevant systems by employing an integrative structural biology approach, combining a number of state-of-the-art technologies.

One line of investigation will pursue identification of inter-protein binding determinants and their effect on pump function with the view of disrupting and modulating its activity. Extensive mutagenesis and functional characterization of the complexes will be employed using variety of microbiological and biophysical assays. This work will pave the way for the design of novel classes of antibacterial therapeutics based on interference of protein-protein interactions.

A second line of investigation will aim to resolve the long-standing question of the stoichiometry of the pump and to differentiate between the currently proposed conflicting models [1, 4, 5] of binding modes of the PAP to OMP. This will be achieved by means of a radically new approach to the problem - namely using oxidative labelling of solvent-accessible protein residues combined with quantitative mass-spectrometry [6] which will be performed in collaboration with Dr Corie Ralston’s group at Lawrence Berkeley National Lab (USA). The protein-protein
interactions indentified will be used for creation of detailed structural models of the complete assembly, which will involve homology modelling and protein-docking simulations [1,4, 5].

A number of tripartite pump assemblies may be investigated, including the prototypical RND-transporter based tripartite pump AcrA/B-TolC from E.coli [1, 2,4,5], as well as homologous system from Neisseria gonorrhoeae MtrCDE [3] and the ABC-transporter based MacAB-TolC.

The project will involve large-scale recombinant protein production of membrane-proteins and in vitro reconstitution of the complexes, as well as in vivo functional and cross-linking assays.

The details of the project will be agreed between the supervisor and the PhD candidate. The candidate is expected to be fluent in basic biochemistry and molecular biology, and to be familiar with protein expression and purification. Previous experience of mass-spectrometric methods and/or functional microbiology assays would be a distinct advantage.

Successful candidate will benefit from learning a number of advanced biophysical methods, and will be expected to acquire high level of proficiency in mass-spectrometric approaches. Furthermore, the project will provide training in membrane protein production and handling [1,7].

In addition, the candidate will gain experience in structural biology including crystallography, as well as exposure to computational protocols exercised in the open and diverse research environment of the School of Biological Sciences at the University of Essex, as well as from collaborative contacts with the University of Birmingham, (UK), Jacobs University, (Bremen, Germany) and Lawrence Berkeley National Laboratory, (USA).

**Entry requirements and application procedures**

Applications should be submitted electronically by **Monday 4 July 2016** see here for details [https://www.essex.ac.uk/pgapply/enter.aspx](https://www.essex.ac.uk/pgapply/enter.aspx)

The candidates are expected to speak fluent English and meet our English Language requirements, if applicable, and to have at least 2.1 or equivalent degree.

Additional questions and queries about the studentship can be addressed to [ecrix@essex.ac.uk](mailto:ecrix@essex.ac.uk)

The target start date for this 3-year, fully-funded PhD studentship is 6th October 2016. This scholarship will be to the value of £12,500 per annum plus UK tuition fees.

**The University of Essex**

For general information about the School of Biological Sciences at the University please visit our webpages

[http://genomics.essex.ac.uk/](http://genomics.essex.ac.uk/)

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award for Outstanding Support for Students. Essex is a genuine global community. With more than 130 countries represented within our student body, and 40% of our students from overseas, we are one of the most internationally-diverse universities in the UK.

References:


