

Elucidating interactions within bacterial tripartite drug-efflux pumps

Project Description

A PhD studentship will be available in the group of **Dr Vassiliy Bavro** at the School of Biological Sciences, University of Essex, 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 three 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 identified 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 5th February 2018** see here for details <https://www.essex.ac.uk/pgapply/enter.aspx>

Shortlisting is expected to be completed by 16th February 2018 with interviews planned during first and second week of March 2018.

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 vb16181@essex.ac.uk

This scholarship will be to the value of £12,500 per annum plus UK tuition fees.

Please note: International (non-EU) students need to have additional funding to cover the difference in tuition fees which is £11,250.00. Evidence will be requested that you have these additional funds.

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://www.essex.ac.uk/bs/staff/profile.aspx?ID=4967>

In the recent Research Excellence Framework 77% of research at the University of Essex research is 'world leading' or 'internationally excellent' (REF 2014). We offer world-class

supervision and training opportunities and our research students work at the heart of an internationally-acknowledged and well-connected research community. In the 2013 Postgraduate Research Experience Survey, 84% of respondents said that they were satisfied with the quality of their research degree. At Essex we win awards for our pioneering student support schemes. We are the most recent winners of the prestigious *Times Higher Education* 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:

1. Bavro VN, Pietras Z, Furnham N, Pérez-Cano L, Fernández-Recio J, Pei XY, Misra R, Luisi BF. Assembly and channel opening in a bacterial drug efflux machine. *Molecular Cell*. 2008 Apr 11;30(1):114-21.
2. Blair JM, Bavro VN, Ricci V, Modi N, Cacciotto P, Kleinekathöfer U, Ruggerone P, Vargiu AV, Baylay AJ, Smith HE, Brandon Y, Galloway D, Piddock LJ. AcrB drug-binding pocket substitution confers clinically relevant resistance and altered substrate specificity. *Proc Natl Acad Sci U S A*. 2015 Mar 17;112(11):3511-6.
3. Janganan TK, Bavro VN, Zhang L, Borges-Walmsley MI, Walmsley AR. Tripartite efflux pumps: energy is required for dissociation, but not assembly or opening of the outer membrane channel of the pump. *Mol Microbiol*. 2013 May;88(3):590-602
4. Symmons MF, Bokma E, Koronakis E, Hughes C, Koronakis V. The assembled structure of a complete tripartite bacterial multidrug efflux pump. *Proc Natl Acad Sci U S A*. 2009 Apr 28;106(17):7173-8
5. Du D, Wang Z, James NR, Voss JE, Klimont E, Ohene-Agyei T, Venter H, Chiu W, Luisi BF. Structure of the AcrAB-TolC multidrug efflux pump. *Nature*. 2014 May 22;509(7501):512-5.
6. Gupta S, Bavro VN, D'Mello R, Tucker SJ, Vénien-Bryan C, Chance MR. Conformational changes during the gating of a potassium channel revealed by structural mass spectrometry. *Structure (Cell Press)*. 2010 Jul 14;18(7):839-46.
7. Bavro VN, De Zorzi R, Schmidt M, Muniz JR, Zubcevic L, Sansom M, Vénien-Bryan C, Tucker SJ. Structure of a KirBac Potassium Channel with an Open Bundle-Crossing Indicates a Mechanism of Channel Gating. *Nature Structural and Molecular Biology*. 2012 Jan 08, 19, 158-163