Early-stage researchers – ITN Network Translocation

The network associated with the ITN Project "Translocation" is composed of 10 partners (7 academic institutions and 3 small and medium enterprises, SMEs). Additionally, three Pharmaceutical companies (Basilea Pharmaceutica International, GlaxoSmithKline, and Astrazeneca) will be Associate Members of the project. The Pharmaceutical companies will provide training courses and host secondments, which will complement the comprehensive research, scientific and personal development opportunities offered by the SME and Academic Members. The network is coordinated by the Jacobs University in Bremen (Germany) and seeks to recruit 12 postgraduate students for PhD positions which will last up to 3 years. The network will address the problem of the bacterial resistance by combining several scientific and technology platforms, ranging from electrophysiology, mass spectroscopy, biological assay development and molecular modelling. These platforms are well established within the consortia and most of the members have longstanding common research activities and collaborative programs already in place. Our goal is to better understand the molecular determinants of the rate limiting steps involved in antibiotic transport into bacterial pathogens as well as active compound clearance mechanisms used by bacteria. Addressing these problems is of critical importance in helping to develop new strategies to overcome multi-drug resistance and create better antibiotic treatments in the future.

The recruitment process will be centralised and applicants are invited to apply for one or more of the projects detailed below. Applicants should submit their CV and cover letter to Paolo Ruggerone (paolo.ruggerone@dsf.unica.it). The first closing date for receipt of applications will be October 31st, 2013. However, we will consider applications until the posts are filled. Applicants should familiarise themselves with the eligibility rules associated with the Marie Curie ITN programs, particularly with regard to certain restrictions on candidates applying for positions in countries where they have recently resided. Positions will start on January 1st, 2014.

The available positions are the following

	Project title	Host institution	Responsible	Description
1	Antibiotic translocation	Jacobs University, Bremen (D)	Mathias Winterhalter	Characterization of the influx of antibiotics or efflux pump blockers through porins using electrophysiology and complementary techniques.
2	Screening antibiotics on the chip	Nanion Technologies (D) - SME	Nils Fertig	Screening of antibiotic permeation through porins towards low throughput <100recordings/day. Nanion developed a unique planar patch clamp chip used for the electrophysiological analysis of mammalian cells. Planar bilayer recordings are attractive for investigations of membrane proteins not accessible to patch clamp analysis, like e.g. proteins from organelles or bacteria. This technique offers substantial advantages as compared to traditional patch clamp and BLM recording, in terms of facile handling and improved sensitivity. Particular the enhanced sensitivity will improve the time resolution of the measurements.
3	Pathway modeling	University of Cagliari (I)	Matteo Ceccarelli	The group combined MD simulations with an acceleration scheme to follow the translocation of antibiotics through porins at atomic scale. Multi-scale algorithms extend the simulation time to the range to milliseconds, making it possible to obtain the reactive pathway that antibiotics follow

				during passive diffusion. Accelerated MD simulations have revealed a putative translocation pathway for penicillins and fluoroquinolone through OmpF and porins extracted from resistant strains. Through the associated free energy surface (FES) of this process, affinity sites and activation barriers can be identified.
4	Crystallisation porins 1	University of Newcastle (UK)	Bert Van der Berg	Our goal is to clone, express and purify OmpU and OmpT, the two key OM uptake channels of *Vibrio cholerae* to give a better overall picture of the structure of a diverse set of porin proteins. In addition we will attempt to co-crystallize these OM channels with substrates, giving a rich body of structural information to help inform computation simulations.
5	Crystallisation porins 2	University of St. Andrews (UK)	Jim Naismith	The group uses x-ray crystallography to study the structures of membrane proteins. We are particularly interested in channels which conduct ions and polar molecules. For example, the crystal structure of OmpC mutants revealed that changes in antibiotic transport were more likely due to changes in the transverse electrostatic field. The group is also pioneering the use of PELDOR spectroscopy to monitor conformational change in integral membrane proteins.
6	Crystallization efflux pumps	Goethe University, Frankfurt am Main (D)	Klaas M. Pos	The RND component AcrB has been intensively studied using biochemical and structural methods. High-resolution structures of wild-type AcrB and several of its single-site variants have been determined via X-ray crystallography. Latest structural data revealed multiple binding sites for drugs simultaneously within the loose and tight protomer. Insights into the drug binding sites are key to further studies on how to develop inhibitors of the RND component and are important sources for forthcoming computational analysis on the drug efflux mechanisms.
7	Modelling the assembly of efflux pumps	Jacobs University, Bremen (D)	Ulrich Kleinekathoefer	The computational group has expertise in molecular dynamics simulations applied to ion and substrate transport through porins but more importantly in modelling TolC and AcrB (in cooperation with the groups from UCA and GUF). It is still an open question how and why TolC and its homologoues assume open conformations upon assembling of the tripartite complex. The group will complement ongoing experimental work using all-atom MD studies. Molecular-level hypotheses by the experimental partners can be tested and new experiments can be suggested. Attention will be devoted to the assembly of the different efflux pump components beyond simple static docking models.
8	Key residues in RND transporter	University of Cagliari (I)	Paolo Ruggerone	Concerning efflux systems, still unclear are several issues, such as to what extent the functional rotation, i.e., the specific series of sequential conformational changes, is essential for the drug extrusion and whether cooperativity effects are also involved. Thus, molecular details of the mechanism, recognition and uptake for AcrB and MexB require further investigations that will be

				performed by UCA in collaboration with JUB and GUF. Inhibitors, used in combination with antibiotics, expand the spectrum of antibacterial activity, reverse resistance and dramatically reduce the rates of resistance development, but the molecular details of their action are still elusive. The 'non-specificity' of the transporters asks for the role of the pump's putative affinity in resistance and inhibition. Additionally, insights on possible allosteric sites in the efflux pumps will be gained by extended MD simulations and indicate sites to be targeted by inhibitors.
9	Screening for drug-like compounds which modulate Porin function	European ScreeningPort GmbH, Hamburg (D) - SME	Phil Gribbon	Our overall aim to identify novel compounds capable of modulating antibiotic transport activity in a beneficial manner, therefore creating an opportunity to help better define future adjuvant strategies. Our principle approach is to improve the availability of antibiotics at their site of action by selectively blocking transport protein function. We will quantify isolated efflux systems using the Iongate (Surf2er) and Ionovation (Compact) in- vitro cell free electrophysiology technologies, by characterizing activation via capacitance-based readouts. A screening process, to monitor the ability of compounds to modulate the function of transporter proteins, will be defined and validated. The aim is to develop industrial quality Primary electrophysiological assays with the capacity to process 100's of compounds per week. Hit compound selection will be supported by high content secondary assays and classical fluorescence efflux measurements in cell based systems. We will work with molecular modelling teams to understand the putative mechanism of interaction of Hit compounds with key candidate proteins.
10	Envelope permeability during infection	University of Basel (CH)	Dirk Bumann	Current whole-cell assays for screening antimicrobials rely on standardized, well-accepted in vitro conditions. Although useful, such conditions may not fully reproduce relevant conditions that pathogens encounter in infected host tissues. Gram-negative bacteria such as Pseudomonas readily adapt to different conditions by comprehensively remodelling their cell envelope properties such as differential expression of one or more of the some 30 porins, induction of one or more of their ~20 efflux pumps, or modifications to the lipopolysaccharide. This envelope remodelling can substantially affect envelope penetration of antimicrobials. As a consequence, antimicrobials with promising activity under standard in vitro conditions might fail under relevant in vivo conditions because of insufficient penetration or increased expulsion/degradation in that environment.
11	Regulation of porins	University of Marseille-Aix (F)	Jean-Marie Pages	The second part of this WP focuses on genetic regulation of drug transport. Several specific regulators (e.g. RamA and RamR) play a key role in modulating the membrane permeability via the porin/efflux pump expression and contribute to MDR. In

				addition, various compounds such as salicylate, imipenem or chloramphenicol are able to induce or select the MDR response. This phenomenon has been observed in vitro by adding drugs to bacterial cultures as well as in clinical settings during antibiotic treatment of infected patients. Regulation of membrane permeability directly affects the intracellular accumulation of antibiotics. Our results showed the role of local (acrR, OmpX) or global regulators (MarR, RamR) involved in the emergence of MDR in Enterobacter. AMU currently performs an antibiotic susceptibilities analysis on their collections of clinically important Enterobacteriaceae. Moreover, AMU has recently developed a platform allowing the high throughput determination of the activity of large number of antibacterial agents e.g. last generation ß-lactams, fluoroquinolones, etc on several strains at the same time. We will identify which porins are expressed in resistant and susceptible strains, respectively. The relationships between porins and antibiotics efficiencies will be further explored by a rate-killing approach. In such experiments, the activity of antibiotics is measured as a function of incubation-time on an E. coli strain expressing a selected porin. AMU has developed a gene-fusion assay (omp-lacZ) to follow the expression of outer membrane porins in the presence of various chemicals. This genetic approach will be used to investigate the expression of Enterobacter porin genes. This monitors the kinetics of porin regulation during external stresses. While previous studies prove the control of membrane permeability in large populations of bacterial cells, they miss the response kinetic and the link in the regulation. Here we characterize the dynamics of gene expression. The analysis of regulators under external stresses allowed us to select appropriate regulators and determine its structure and the effect of mutations or chemical effector on their functional structure.
12	TRIC inhibitor	BioVersysAG, Basel (CH) -SME	M. Gitzinger	BioVersys follows the approach of inhibiting global or local transcriptional regulators of resistance gene expression. Our TRIC (Transcriptional Regulator Inhibiting Compounds) technology platform allows for the identification of target specific, non-cytotoxic and non-antibacterial small molecules that potentiate the activity of antibiotics. The combinatorial application of BioVersys` TRIC adjuvant compounds with existing antibiotics has been shown to allow for killing of even extensively resistant pathogens at clinically relevant doses of the antibiotic. One of our most advanced projects is specifically focusing on inhibition of efflux-mediated resistance via targeting the transcriptional regulator of the respective efflux-gene resistance cluster.