Swiss National Centre of Competence in Research

Newsletter
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NCCR TransCure
Excellence in Membrane Transport Research

Dear readers and colleagues,

The National Centre of Competence in Research (NCCR) TransCure, supported by the Swiss National Science Foundation (SNSF), has just started its 7th year and is moving towards the conclusion of the second phase (2014-2018). In this issue, we are pleased to update you on the activities and scientific progress of the network.

Halfway between milestones reached and future perspectives, this 7th year is very important for the network. Preparations are underway for the drawing up of the pre-proposal for the third and last phase of the NCCR (2018-2022). The strategic plan for the years to come was a focus of the last site visit of the SNSF Review Panel (24-25 October 2016). During the visit, the panel appreciated the enthusiasm and developing maturity of the fellows, and provided valuable suggestions for the future of the network.

Several network events took place over the last few months. The yearly NCCR Symposium, held in Bern on 16 September 2016, focussed on drug design. This event welcomed four internationally renowned female speakers from academia and industry, and dedicated a special session to gender equality (see article on p. 4). The 3rd Endocannabinoid Pharmacology Meeting (13-14 October 2016), also held in Bern, was very well attended. It updated the participants on the latest breaking news from the field.

Among its commitments, the NCCR TransCure provides high-quality training to its fellows. Specialised courses were organised by NCCR TransCure PIs in electron crystallography and in animal models for preclinical research. Moreover, a group of fellows attended a drug discovery and development simulation course offered by Novartis Basel. This was an excellent opportunity to learn about the many steps required in developing a drug and to get a glimpse of possible careers in the pharmaceutical industry. In addition to this specialised training, students also profited from soft skills courses, such as a CV writing workshop recently organised in collaboration with other NCCRs and led by a professional trainer.

The lively educational and network activities complement the rich scientific output of the NCCR TransCure. The publication highlights section (p. 7) features results on the AdiC transport protein and its involvement in bacterial acid resistance, as well as new insights into the relationship between the sodium/hydrogen exchanger NHA2 and glucose intolerance. The former topic is also the focus of the main research article of this issue (p. 2).

Events and news are regularly communicated online within and outside the network through the NCCR TransCure website and social media. The website was relaunched in summer 2016, with a new design and improved functionalities. We hope that this new version improves the information flow and usage—we are open to any feedback! General information about the NCCR TransCure is now also available in a newly designed leaflet. This print media serves a broad public and quickly provides an overview of the network as a whole.

The NCCR TransCure sends everybody season's greetings and best wishes for a joyful new year!

H. Abriel and J.-L. Reymond,
NCCR TransCure Directorate

From molecular working mechanisms towards drugs

Resolving the structure of transporters helps us to understand their working mechanisms. This in turn may lead to the discovery of new therapeutic approaches. Dimitrios Fotiadis, NCCR TransCure PI, outlines one of his recent studies in this field.

Specific transporters provide cells with amino acids, the building blocks of proteins and the nitrogen source for the biosynthesis of important metabolites. These amino acid transporters also play a crucial role in extreme acid resistance systems of bacteria, i.e., in responses that allow cell survival under extreme acidic conditions. The best characterised of these systems is that of the enterobacterium *Escherichia coli* (*E. coli*). Certain strains of *E. coli* have been reported to cause life-threatening foodborne gastrointestinal infections. A recent serious outbreak reported in the media was the *E. coli* O104:H4 EHEC strain outbreak of 2011 in Germany. An interesting research direction is the investigation of the key players of the bacterial extreme acid resistance system that allows for survival of pathogenic enterobacteria during the transition through the strongly acidic human stomach on their way to the gut.

The L-arginine/agmatine exchanger AdiC of *E. coli* is a key player in the extreme acid resistance system of this bacterium. To lower the intracellular proton concentration, and thus raise the pH, the acid-activated arginine-decarboxylase AdiA converts the amino acid L-arginine to agmatine, thereby consuming one proton (Figure 1). This proton ends up as a C-H bond in the product agmatine. Agmatine is then removed from the cell through the AdiC transporter, which exchanges one agmatine from the inside for one L-arginine molecule from the outside, e.g., from the gastric juice. Structural information is essential to understanding the working mechanism of AdiC at the molecular level.

A recent publication by our group illustrates the high-resolution crystal structures of AdiC in the presence and absence of the substrate agmatine [1]. The former allowed for the description of the protein-substrate interactions at the molecular level (Figure 2), while the latter made the identification of functionally relevant water molecules in the substrate binding pocket possible. The existence of bound water molecules in the substrate binding pocket of AdiC and their functional role was supported by molecular dynamics simulations. These were performed at human body core temperature, the temperature at which pathogenic bacteria act.

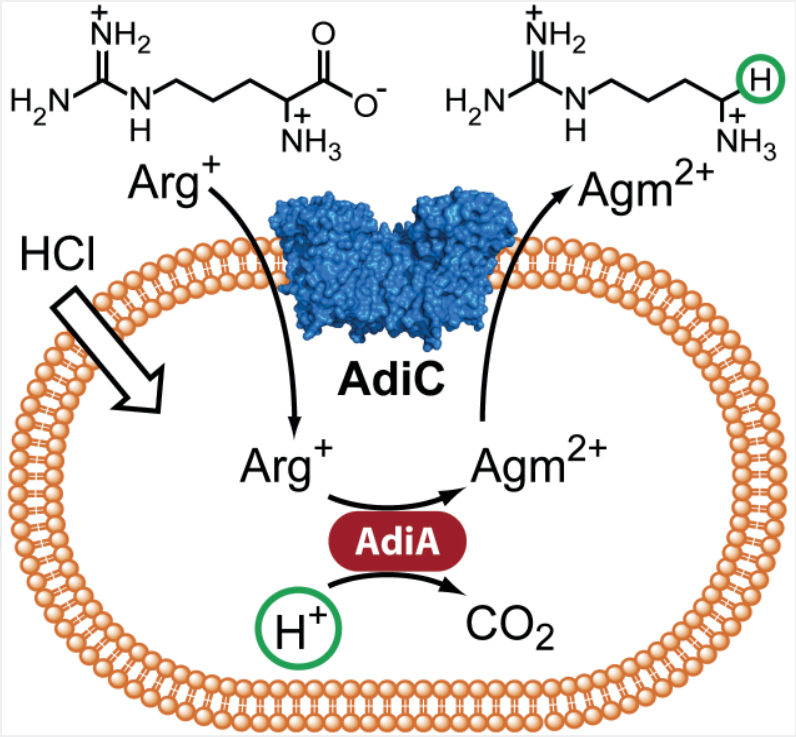


Figure 1: Schematic illustration of the arginine-dependent acid resistance system of *E. coli*. The consumed intracellular proton is circled in green. Dimeric AdiC embedded in the membrane is coloured blue. (Figure adapted from Ilgü et al. [1].)

In addition to the valuable insights into the molecular basis of substrate binding and specificity of AdiC obtained from the high-resolution structures, these structures also provide a solid basis for homology modelling studies of other transporters from the APC (amino acids/polyamines/organic cations) family, in order to understand their working mechanisms. Members from the APC family are closely involved in human physiology in health and disease.

In the framework of the NCCR TransCure project, we are particularly interested in the human APC transporter LAT1, which is overexpressed in a variety of cancer cells, and for which high-affinity inhibitors would be highly desirable in order to inhibit cancer cell proliferation. In a recent collaboration with homology modelling and structure-based drug design experts, our project team is testing hits obtained from virtual compound screening, using a human LAT1 homology model that was built based on our AdiC structures.

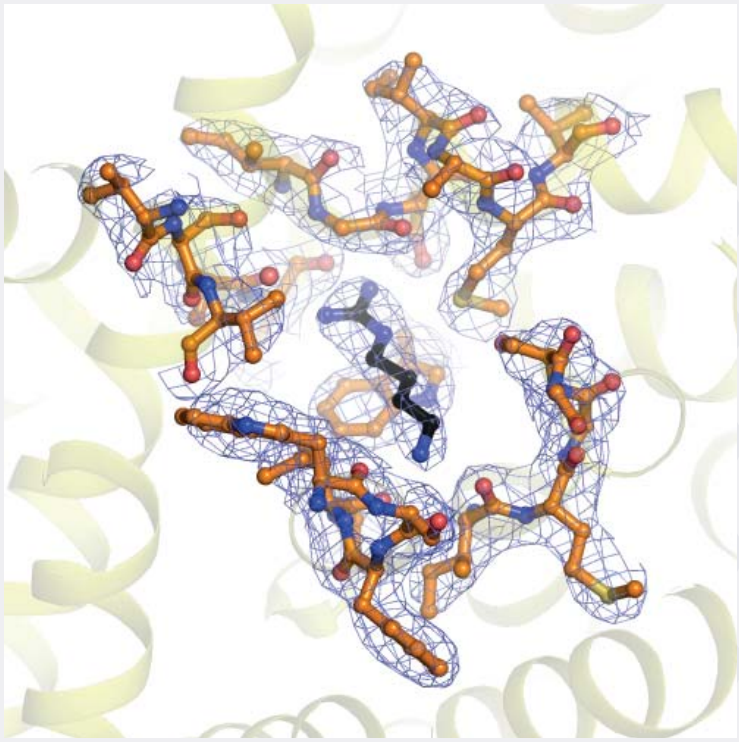


Figure 2: View into the substrate binding pocket of the agmatine-bound AdiC crystal structure. Amino acid residues involved in substrate binding are displayed as gold sticks. The bound agmatine molecule is in black. The AdiC structure is represented as a yellow ribbon and the electron density map in the substrate binding pocket as a blue mesh.

Our hope is to identify strong inhibitors that can eventually be developed into drugs and serve as new therapeutic approaches to fight cancer.

Dimitrios Fotiadis,
NCCR TransCure PI

References

[1] Ilgü H., Jeckelmann J.-M., Gapsys V., Ucurum Z., de Groot B.L. and Fotiadis D. Insights into the molecular basis for substrate binding and specificity of the wild-type L-arginine/agmatine antiporter AdiC. Proc. Natl. Acad. Sci. USA (2016) 113, 10358-10363.

How it works: Ion channels

Every cell of the human body is separated from its external milieu by a membrane that is made up of lipids and proteins. Some of these membrane proteins allow the passage of small electrically charged molecules called ions. These proteins are named ion channels since they form small openings in the cell membrane. Typical ions in the body are sodium, potassium, calcium, and chloride. They are essential for life since they permit, for example, transmission of information in neurons and contraction of muscle cells. Any disturbances in the opening and closing processes of ion channels can lead to disease. Many drugs that are used to treat neurological and cardiovascular disorders such as chronic pain, epilepsy, cardiac arrhythmias and hypertension are ion channel blockers. Current research is also focussing on the possibility of treating certain cancers by using ion channel blockers. However, too many "old" drugs that block ion channels have severe side effects. For this reason, scientists are looking for new ion channel blocking drugs to treat diseases more safely and efficiently.

Hugues Abriel,
NCCR TransCure PI and Director

NCCR TransCure Symposia: Events to promote excellent science and equal opportunities

One thematic focus, international experts and great talks but definitely not a YAMMM*: The last NCCR TransCure Symposium had female speakers only and included discussions on gender equality.

** Yet Another Mostly Male Meeting*

Every year, the NCCR TransCure organises a one-day symposium that focuses on a specific scientific topic. A distinctive feature of these symposia is the special attention given to the promotion of women in science. All of the speakers are female scientists and part of the event is reserved for talks and discussions on gender equality themes. This year's symposium was dedicated to drug design. It took place in Bern on 16 September 2016 and was coordinated by Prof. Karl-Heinz Altmann (ETH Zurich) and Prof. Jean-Louis Reymond (University of Bern).

The morning session was reserved for scientific talks and welcomed four international speakers from academia and industry. The first talk, given by Prof. Katherine L. Seley-Radtke (Department of Chemistry and Biochemistry, University of Maryland), focussed on flexible nucleosomes and flexible nucelobases, dubbed "flexomers" and "flex-bases", respectively. Prof. Seley-Radtke compared the mechanism of action of these new therapeutics to a chameleon that is able to adapt to its environment. Flexible inhibitors provide an important advantage in facing the widespread resistance against current therapeutics in coronaviruses and HIV, among others. Microorganisms defend themselves against the host immune system or therapeutic agents using so-called "escape" mutations. These are based on modifications of the phenotype that enable immune evasion or resistance against inhibitors (e.g., reverse-transcriptase inhibitors) by changing the structure of an antigen or enzyme binding pocket, respectively. By allowing inhibitors that target the binding pockets to be flexible, the therapeutics are able to successfully target a wider range of mutants. Flexible drugs are also promising as anticancer therapeutics, where preclinical trials have already been completed.

Dr. Dominique Douguet enlightens the audience on the statistics emerging from the FDA-approved small-molecule pharmacopeia.

The next talk was given by Prof. Christa E. Müller (Pharmaceutical Institute, University of Bonn) on the medicinal chemistry of orphan G protein-coupled receptors (GPCRs). This large family of transmembrane protein receptors is targeted by a high percentage of pharmaceuticals currently on the market. However, not all endogenous ligands (molecules binding to the GPCR on the outside of the cell) have been identified, leaving some G protein-coupled receptors orphaned. Prof. Müller's work focusses on a subset of these receptors, namely purine and pyrimidine activated receptors. Her group develops radioligands, fluorescent ligands and small molecule inhibitors to study the function of orphan GPCRs and their therapeutic target potential. Furthermore, she manages a compound library and a high-throughput screening facility at the University of Bonn, where a subset of the library focusses on GPCRs.

The third speaker, Dr. Dominique Douguet (Université Nice Sophia Antipolis), gave an overview of an FDA-approved small-molecule pharmacopeia and provided insights into the related statistics. The latter were generated from a virtual library containing the structures of 1822 FDA-approved drugs and active metabolites, annotated with pharmacokinetic, pharmacodynamic and registration data. Among others, the virtual library showed that most approved drugs are generic and that many pharmaceutical companies have never released new drugs. As an example, only 31 new drugs were released by 29 companies in 2015, 18 modified peptides have been approved in total and most drugs do not have chiral atoms. The database, called "e-Drug3D", also yields information about the structural diversity of approved drugs,

A successful academic career for a female scientist is compatible with family life: The panel discussion, moderated by Prof. C. Peinelt, focussed on the factors that make this possible.

showing that more than 40% of approved drugs are substructures of other drugs. This inspired the implementation of a genetic algorithm for *de novo* drug design using fragments.

The session closed with a talk by Dr. Karin Briner (Vice President and Head of Global Discovery Chemistry, Novartis Institutes for Biomedical Research) on the development of new medicines and the role of chemistry in this process. In the first part of her talk, Dr. Briner described the approach to the creation of new medicines, introducing synthesis-driven innovation in drug discovery, new chemical methods for protein functionalisation and the paradigm shift from empirical to pathogenesis-driven cancer research, among other current topics such as computer-aided drug discovery. She then presented some ongoing projects at Novartis. One project focusses on the protein ABL1, a kinase and known proto-oncogene. Another project is involved in the search for an oral VEGFR (vascular endothelial growth factor receptor) inhibitor. VEGFRs are a class of receptors for the vascular endothelial growth factor protein that induce the formation or reformation of blood vessels. Her goal is to develop a therapy to treat wet age-related macular degeneration, which leads to blood vessel growth behind the retina and consequent impairment of central vision. Both projects involve researchers from a variety of fields, showing the need as well as the importance of chemistry in interfacing with multiple disciplines.

Gender equality in science was the main subject of the afternoon session. Prof. Christine Peinelt (NCCR TransCure PI) moderated a panel discussion with the four speakers from the morning session, the organising PIs and the NCCR TransCure Director, Prof. Hugues Abriel. The debate dealt with reconciling work and family life in the career path of a female scientist wishing to become a professor. The discussion was inspired by the personal experiences of the speakers and their successful strategies to manage a family and a scientific career at the same time. Among the key factors mentioned were having a supportive and understanding family. This has been shown to be extremely important in tackling everyday life problems and in making decisions. At the personal level, the main suggestion was to maintain self-confidence. Female scientists should believe in their ability to both reach their desired goals and be competitive on the job market and in the search for funding.

Gender disparities are still a reality in many environments. However, society's awareness of equal opportunity issues is generally growing and many institutions are putting effort into applying new measures. As an example, Prof. Seley-Radtke mentioned scholarships for women in science provided by the IS3NA Chu Family Foundation, where she is involved as a member in addition to her research. The NCCR TransCure supports the promotion of women in science by encouraging job applications from female scientists and by offering mentoring programs for women who want to continue their career in academia. A concrete example of these measures in the last two years is the recruitment of two female PIs to the NCCR TransCure network: Prof. C. Peinelt and Prof. M. Bochud.

The podium discussion addressed how the trend in managing gender equality issues in science is changing at the institutional and political level. The impression is that progress is being made and measures to decrease gender disparity in science are being taken. Whether this progress is sustainable or whether more effort is necessary has yet to be seen.

Daniel Probst and Marion Poirier,
NCCR TransCure PhD students
(Reymond Group)

Meet the NCCR TransCure Fellows

Hüseyin Ilgü

After my Bachelor studies and Master's thesis in chemistry and biochemistry at the Izmir Institute of Technology in Turkey, I joined the laboratory of Prof. Dimitrios Fotiadis at the University of Bern as a PhD student. In January 2016, I successfully completed my PhD thesis, which mainly focused on the biochemistry, function and structure of transporters. Currently, I am a postdoctoral fellow in the same laboratory and a member of the NCCR TransCure network. My main focus is on the structural and functional analysis of amino acid transporters. In my research, I have characterised selected transporters, including the L-arginine/agmatine antiporter AdiC from *E. coli* using biochemical and biophysical methods. This study provided the required information for the successful crystallisation of wild-type AdiC in the absence and, for the first time, in the presence of the substrate agmatine. Both structures were solved at high-resolution. They provide a solid basis for homology modelling of human SLC7 family members in order to understand their working mechanisms and for structure-based drug design.

Romina Cabra

I am currently a PhD student in the bone biology group of Prof. Willy Hofstetter at the University of Bern. Our laboratory studies the biology of osteoblast and osteoclast lineage cells, and their molecular transport mechanisms. Within the NCCR TransCure, we focus on iron transport through the divalent metal transporter SLC11A2 (DMT1). My project has two main goals: Firstly, we are trying to understand the role of iron in the development and activity of osteoclasts by modulating iron uptake *in vitro* using SLC11A2 inhibitors, generated and screened by the groups of Reymond and Hediger, respectively. Secondly, we study the role of iron in bone metabolism by generating and analysing transgenic mouse models with SLC11A2 osteoclast-specific knockout, using the Cre/loxP recombination system. We aim to characterise the phenotype and role of SLC11A2 in osteoclasts. The collaboration between groups within the NCCR TransCure allows us to share and complement our knowledge and ideas in physiology, structural biology and chemistry.

Scott Jackson

I started a Marie Curie postdoctoral fellowship in Prof. Kaspar Locher's laboratory at ETH Zurich in January 2014. The goal of my research is to understand the structural basis of substrate transport and inhibition by human ABCG2, a multidrug transporter that limits the oral bioavailability of drugs and extrudes anti-tumour compounds in cancer cells. I am developing functional assays for purified, reconstituted ABCG2. These aim to identify modulators of the transporter as well as conformational antibody fragments that may be beneficial for structural studies or provide insight into the modulation/inhibition mechanism. Synthetic efforts, in collaboration with Prof. Karl-Heinz Altmann, are centred on derivatives of the fumitremorgin-C-derived ABCG2 inhibitor Ko143. The functional assays probe the structure-activity relationship of Ko143 inhibitors, antibody fragments and nanobodies, and assess their suitability to lock certain states/conformations of ABCG2.

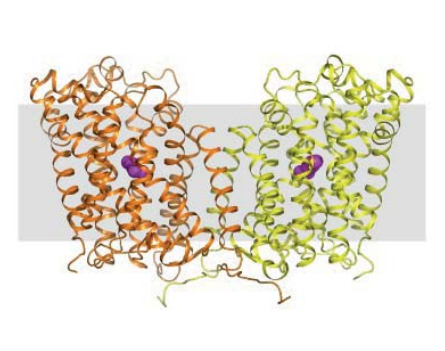
NCCR TransCure Alumni

Mohamed-Yassine Amarouch

I joined the laboratory of Prof. Hugues Abriel at the University of Bern as a postdoctoral fellow in September 2011. Then in January 2013, I received a Marie Curie fellowship in the "International Fellowship Program TransCure". Currently, as an Assistant Professor at the Multidisciplinary Faculty of Taza, University of Sidi Mohamed Ben Abdellah of Fez, Morocco, I am dividing my working time between teaching and research. I am giving physiology lectures and conducting research that aims to evaluate the pharmacological effects of natural compounds in the setting of cardiac channelopathies. During my postdoctoral training, the NCCR TransCure provided me educational, technical and financial support to conduct my research. This period was fruitful in terms of outstanding publications and was a turning point in my scientific life as it enabled me to work with scientists who are at the cutting edge of their speciality.

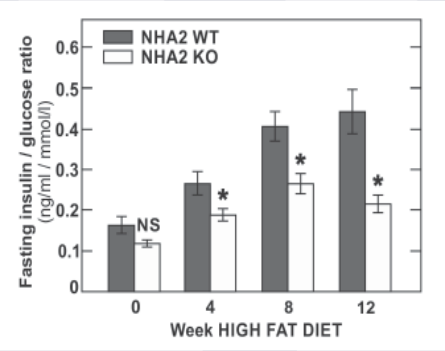
Publication highlights

Ilgü et al, Insights into the molecular basis for substrate binding and specificity of the wild-type L-arginine/agmatine antiporter AdiC, PNAS, 29 July 2016, doi: 10.1073/pnas.1605442113.



Dimitrios Fotiadis and his team solved the structures of the wild-type L-arginine/agmatine antiporter AdiC in the presence and absence of the substrate agmatine at high resolution. The AdiC transporter is involved in one of the mechanisms that allow pathogenic enteric bacteria to survive the extremely acidic environment of the human stomach. The study unveiled crucial water molecules in the substrate-binding pocket of AdiC and elucidated their functional role as well as defining the molecular basis for the binding of the substrate agmatine. These results significantly improve our understanding of the working mechanism of this transporter.

Deisl et al, Loss of Sodium/Hydrogen Exchanger NHA2 Exacerbates Obesity- and Aging-Induced Glucose Intolerance in Mice. PLoS One. 2016 Sep 29;11(9):e0163568. doi: 10.1371/journal.pone.0163568.



A recent study by the group of Daniel Fuster investigated the impact of the loss of the sodium/hydrogen exchanger NHA2 during the physiological aging process and in the setting of diet-induced obesity in mice. The study indicates that this loss exacerbates obesity- and aging-induced glucose intolerance in mice, and reveals a close link between NHA2 expression and insulin secretion capacity in pancreatic islets.

Upcoming TransCure Events

TransCure Lecture in Biology
Patrick Mehlen
(University of Lyon, FR)
19 January 2017 - Bern

UN International Day of Girls and Women in Science
11 February 2017

TransCure Lecture in Physiology
Lucia Prieto Godino
(University of Lausanne, CH)
23 February 2017 - Bern

Pre-Seed Workshop
20-21/28 March 2017 – Geneva

TransCure Lecture in Drug Design
Vittorio Limongelli
(Università della Svizzera Italiana, CH)
30 March 2017 - Bern

7th Annual NCCR TransCure Retreat
11-12 May 2017 – Baden

TransCure Lecture in Biology
Thomas Stockner
(Medical University of Vienna, AT)
22 May 2017 - Bern

10th Biomedical Transporters Conference
6-10 August 2017 – Lausanne

Please visit www.nccr-transcure.ch for more details.

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