

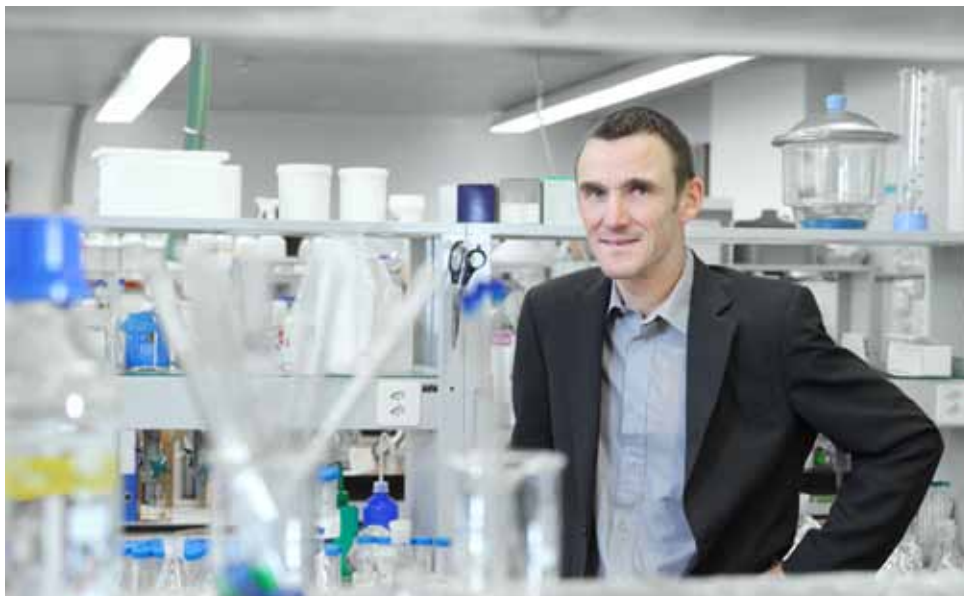
TransCure

TRANSLATIONS

SWISS NATIONAL CENTER OF COMPETENCE IN RESEARCH

NEWSLETTER NO.2, JULY 2013

A MESSAGE FROM THE DEPUTY DIRECTOR



Jean- Louis Reymond

Dear Readers,

Transporter-related proteins include the classical transporters (also called solute carriers, SLCs), ion channels, aquaporins, ABC transporters and ATP-driven pumps. They reside in the membranes of cells and organelles and control the flow of a myriad of minerals and nutrients throughout the cells of our body. Our genome encodes almost one thousand transporter-related proteins. This includes about 500 SLCs, many of which are essential to the basic cellular metabolism. However, as of today, many of the transporters and related proteins are still poorly understood. TransCure aims to advance the basic understanding of SLCs and their role in diseases, as well as to develop innovative therapies in critical areas such as cancer. To achieve this goal we have created a scientific network at the interface between medicine, structural biology and chemistry.

Each research group is engaged in interdisciplinary collaborations that can bring the SLC field forward in a "trias" logic: 1) to clone and express a transporter to enable the search for small molecule inhibitors and activators with a screening assay in a therapeutic context; 2) to select and synthesize the molecules to test and optimize for transporter activity for possible therapeutic benefit; 3) to investigate the three-dimensional structure of a transporter to understand its mechanism of action and disease relevance as well as the molecular interactions with activators or inhibitors. As an example, my own group utilizes both in-silico and synthetic chemistry in many of the screening assays around the network to find inhibitors and activators.

This overall approach requires the understanding of each other's methods and the formulation of common working hypotheses and goals.

Three years into the project, we are discovering that this interdisciplinary approach brings results much faster than expected. The summary on the SNF view on our network described by our Director, Matthias Hediger later in this newsletter, testifies to the progress during Phase I of the NCCR. The article from Jürg Gertsch highlights one of the many exciting TransCure projects regarding the understanding of the endocannabinoid transport system. Collaborating with chemists, he has identified novel and quite potent transport inhibitors which do not act on endocannabinoid metabolizing enzymes, and produce the expected analgesic effects in vivo. Similar efforts are underway in several other transporter projects, which also include advances towards protein structure determination. This Newsletter also updates you on our educational and communication activities, which include training courses for TransCure fellows, public outreach and the mentoring for women (M4W) program launched last November. This session serves to inspire women to embrace science careers which is strategically important to Swiss universities. M4W supports female scientists early in their careers, and organizes courses and a workshop. A key TransCure event in 2013 will be the Bioparadigms Conference in St-Moritz this summer, a prominent event in transporter research worldwide.

Jean-Louis Reymond, Deputy Director

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BioMedical Transporters
Conference 2013



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UNIVERSITÄT
BERN



NCCR TransCure PROGRESSES, THE DIRECTOR'S PERSPECTIVE

“To apply excellence in membrane transporter research to the treatment of human diseases”

Each year, the NCCR TransCure presents its progress to a review Panel sponsored by the Swiss National Science Foundation. At the annual Site Visit held in October 2012, the review Panel provided feedback indicating that the scientific progress of TransCure has been strong and that it clearly reflects an increasing interaction and collaboration among the disciplines of the Trias. They noted that, for many of the transporter targets addressed, two to three of the Trias disciplines are involved. Much of this success has been the result of intensive communication and collaboration across the network. To further enhance communication, TransCure will establish an intranet to facilitate the interactions and keep the teams across the network updated on the most recent results. The Panel Members also emphasized how much they enjoyed the informative dialog with the young TransCure fellows during the three poster sessions. They were very pleased by the motivation and the quality of the young scientists.

Some of the TransCure research projects have excelled in developing promising hit compounds. Important next steps will include testing of their specificity, seeking improved potency and assessing their performance in both in vitro and in vivo disease related models. The latter task will be forwarded and strengthened by the recently appointed new TransCure tenure track Assistant Professor, Roch-Philippe Charles.

The Panel charged the researchers with continuing studies to validate the targets. In general, structural biology projects are on the right track, having made significant progress. The findings will contribute greatly to a mechanistic and functional understanding of the transporters addressed. The quality

of work from the chemical groups is also high, and this area will be strengthened to allow greater capacity. It will be important to find molecules with high potency, excellent pharmaceutical properties, and to round out the intellectual property.

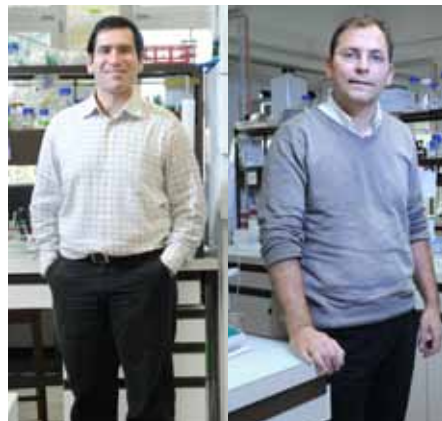
In the coming years, the major responsibilities and challenges for the Management Committee and the International Advisory Board will be to prioritize targets and to agree on milestones with each project leader. Over time, the major funding and scientific focus is expected to gradually shift from a broader to a more limited number of the most promising targets. Combining the passion of the researchers, diversity of technologies available, and hard work by all the TransCure staff will all be critically important for the overall success and sustainability of TransCure.

I would like to bring to your attention that a novel transporter internet database has been established (www.bioparadigms.org) with information on genomic, functional and therapeutic aspects of human transporters derived from the recently published solute carrier (SLC) mini-reviews. This series, for which I was the guest editor, is entitled “The ABC’s of Solute Carriers: Physiological, pathological and therapeutic implications of human membrane transport proteins”. It has just been published in the Journal Molecular Aspects of Medicine (JMAM) earlier this year.

I am also very pleased to announce that two TransCure colleagues, Dimitrios Fotiadis and Jürg Gertsch were both promoted from tenure track Assistant Professor to Full Professor (Prof. Extraordinarius, with tenure) at the Medical Faculty meeting in Bern in July, 2013. Dimitrios Fotiadis started as an Assistant Professor (tenure track) in “Structural Biology of

Membrane Proteins” at the Institute of Biochemistry and Molecular Medicine (IBMM) on October 1, 2008. His research interests are related to structural work on transporters of therapeutic importance including amino acid, peptide, lactate, and vitamin transporters. His goals are to the development of new medications based on the protein structures for the treatment of a number of clinically relevant cancers. Jürg Gertsch began as Assistant Professor (tenure track) in “Membrane Biology” on May 1, 2009 as part of the IBMM in Bern. His research interests focus on the function of the endocannabinoid system as well as the CNS and the immune system. Ultimately he plans to develop strategies for the treatment of depression, anxiety and immune diseases. The TransCure team and I want to congratulate them and warmly wish them a distinguished career with continued successes at the IBMM and as important investigators in NCCR TransCure.

Matthias Hediger, Director



Dimitrios Fotiadis and Jürg Gertsch promoted to full professorship. Congratulations!

NEW TransCure ASSISTANT PROFESSOR



Roch-Philippe Charles

Dr. Roch-Philippe CHARLES started at the University of Bern as TransCure assistant professor with tenure track for “In Vivo Cancer Biology” at the beginning of 2013.

Roch-Philippe studied in France and started his PhD in the field of signal transduction in the group of Dr. Schmitz at the University of Bern. He continued his PhD in the field of skin biology under the direction of Dr Hummler at the University of Lausanne in the department of Pharmacology and Toxicology where he also stayed for post-doctoral training. He then moved to UCSF in Prof. McMahon’s lab working on the oncogenic potential of the mutant protein BRAFV600E. He made use of endogenous

mouse models as a pre-clinical platform for pathway-targeted drug therapies (lung cancer, pancreatic cancer, melanoma, thyroid cancer). Roch-Philippe has an expertise with xenograft models and genetically manipulating mice for in vivo modeling of tumors in different organs. He brings in vivo transporter target validation studies that are required to progress NCCR TransCure programs, while setting up his own independent research group at our Institute.

We warmly welcome Roch-Philippe Charles in NCCR TransCure and are looking forward to collaborating with him on many interesting projects.

EDUCATION AND TRAINING

The Advanced Training Course entitled “*Basic statistics and analysis of small and large data sets made easy: A practical paradigm for solving common problems in the wet lab*” took place in Bern on April 22-23, 2013.

The course was organized by Pascale Anderle (University of Bern and Swiss Institute of Bioinformatics), Benjamin Cléménçon (University of Bern), Nicolas Montalbetti (University of Bern), Fred Schütz (Swiss Institute of Bioinformatics) and Matthias Hediger (University of Bern). Invited speaker Jean-Louis Reymond (Department of Chemistry, University of Bern) spoke about data mining and visualization in chemical space.

Tamas Hegedus (Department of Biophysics, Semmelweis University Budapest, Hungary) presented his methods on large scale literature data screening.

During this very interesting course, students learned how to select basic statistic tests and how to make use of in silico data analysis in their research projects.

A Hands-On Workshop on Image Processing of Electron Crystallography Data with 2dx was also offered to the students. It was organized by Henning Stahlberg during the summer break 2012.



Advanced Training Course

NEW IFP FELLOWS

We welcome our new post-doctoral fellows financed with the International Fellowship Program (IFP) TransCure:

- Arjun Jain (Albrecht)
- Salomé Gachet (Gertsch)
- Jennifer Hemmings (Lochner)
- Guy Giuffredi and Emilyne Blattes (Reymond)
- Meritxell Costa (Fotiadis)
- Michael Fine and Gergely Gyimesi (Hediger)

Some fellows already part of TransCure are now also supported by the IFP TransCure, including:

- Benjamin Cléménçon (Hediger)
- Yassine Amarouch (Abriel)

We wish them all great success in their research project.



TransCure fellows

NEW PRINCIPAL INVESTIGATOR

Bruno Vogt was recently appointed as the new Chairman and Head of Nephrology and Hypertension, Inselspital and University of Bern in August 2012. He has established a distinguished career with training in Geneva, Paris, Lausanne and Berne. Since 1988 Bruno has been working on the clinically relevant problem of renal sodium retention and edema formation in nephrotic syndrome and cirrhosis of the liver. He developed a new mouse model for this purpose. In his clinical research, he focuses on the

problem of renal oxygenation and perfusion using magnetic resonance imaging techniques as well as classic renal hemodynamic studies in humans.

Bruno is participating with the NCCR TransCure network on a pilot project in which a new and important target, ZIP8 a zinc transporter, will be investigated. Bruno's expertise in renal biology will be broadly utilized across the network.



Bruno Vogt

TransCure PUBLIC OUTREACH LABORATORY

We recently established, in collaboration with the chemistry department of the University of Bern, a “School Lab” to promote science to young students. We wish to encourage them to choose science options during their studies and eventually pursue with a scientific career.

The students can participate, with their teachers, in laboratory activities with active researchers in the disciplines of chemistry and biology.

Our hands-on laboratory is open to groups of

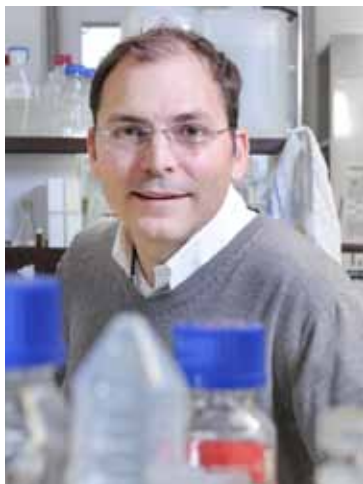
10-25 students aged between 14 and 17 years old. The scientific level is that of secondary schools. The program is available outside the academic semesters.

For more information, please visit our website www.transcure.org.



Students at the laboratory

FROM SCREENING TO MOLECULAR PHARMACOLOGY AND DRUG DISCOVERY



Jürg Gertsch

Education & Appointments

- 2013 Professorship, University of Bern
- 2010 PI NCCR TransCure
- 2009 Appointment Tenure-Track IBMM, University of Bern
- 2008 Visiting professorship University of Cagliari, Italy
- 2005 group leader IPW, D-CHAB, ETH Zürich
- 2002 Postdoc in group of Prof Karl-Heinz Altmann ETH Zürich
- 2002 PhD Natural Products Chemistry, ETH Zürich
- 1999 Ethnopharmacological fieldwork Venezuela
- 1997 MSc Biochemistry, University of Sussex, England
- 1994 Studies in Neurosciences and Biochemistry, University of Sussex, England

Field of Research

- Molecular pharmacology

Selected memberships

- ASBMB
- ACS
- GA
- ICRS

Molecular screening and profiling is a good starting point to do basic research as it allows the discovery and development of novel tool compounds. Using chemical tools to study biological questions is often termed chemical biology and the application of molecules to manipulate physiological or pathophysiological processes is called molecular pharmacology. When molecular pharmacology is applied to generate optimal molecular probes to interfere with disease processes it may lead to drug discovery.

A major focus of our group is the endocannabinoid system (ECS), which plays a pivotal regulatory role in the central nervous system and in peripheral tissues. During cellular stress, the ECS is activated and exerts major modulatory effects on neurotransmission, the immune system, bone remodeling and lipid metabolism in the liver. The ECS comprises arachidonic acid-derived endocannabinoids, which are directly synthesized from phospholipid precursors at the inner plasma membrane leaflet of certain cells. There are different known cytoplasmic binding proteins that direct the fate of endocannabinoids. Once outside, they can interact with the cannabinoid receptors CB1 and CB2 which are G-protein coupled receptors (GPCRs) that modulate the release of major neurotransmitters and modulate important immune factors.

Besides projects dedicated to cannabinoid receptors (CB2 receptor collaboration with Roche), within NCCR TransCure, we also study the mechanisms of endocannabinoid trafficking across cellular membranes. In a recent study from our lab we provide evidence in favor of a passive cell membrane transporter which facilitates cellular release and uptake of these lipids at physiological concentrations¹. The best tool to study a particular protein (known or unknown) is probably a potent and selective ligand. We are therefore developing novel highly potent and selective inhibitors of endocannabinoid membrane transport which may also serve as probes to identify the putative endocannabinoid membrane transporter.

Currently, Dr. Andrea Chicca, Simon Nicolussi (PhD student) and Mark Rau (MSc student) are working on different subprojects including novel methodologies, screening and profiling. Endocannabinoid membrane transport has become a competitive research field since no membrane protein has been cloned to date which regulates the cellular transport of endocannabinoids. Using our technologies within NCCR TransCure and with the help of industry collaborations we hope to be able to identify the target of our potent inhibitors within the next several years. This would lead to the elucidation of the mechanism of endocannabinoid membrane transport, finally clarifying the controversy about endocannabinoid trafficking across cellular membranes.

Our group is closely collaborating with the chemists Jean-Louis Reymond (Uni Bern) and Karl-Heinz Altmann (ETH Zurich) on two exciting projects. In one industry collaboration we have already generated highly potent and apparently selective inhibitors for endocannabinoid uptake.

Thanks to a successful novel polypharmacophoric similarity search algorithm (collaboration Jean-Louis Reymond) we were able to screen several hundreds of drug-like pre-selected molecules, which led to the identification of the first lead structures for TransCure. We now want to develop these molecules further for preclinical studies (see Fig. 1).

In our screening efforts we also found a natural product that is highly active on endocannabinoid transport and shows potent CNS effects in mice. Chemical synthesis of this molecule and the generation of analogs with improved properties are currently ongoing in the lab of Karl-Heinz Altmann. Molecules targeting the ECS system trigger specific behavioral effects which can also be studied in mice lacking CB1 receptors or the enzymes involved in endocannabinoid catabolism and metabolism. The combination of chemical probes and genetic animal models is thus a powerful tool in drug discovery and development. As it becomes increasingly clear that biochemical pathways are networks rather than isolated single targets we have started to also put efforts into the development of analytical methods to study more complex systems. The acquisition of a Hybrid Triple Quadrupole/Linear Ion trap mass spectrometer (LC-MS/MS) enables us to carry out metabolic profiling within the NCCR TransCure. The ultrafast LC-MS is ideal for drug discovery and development, metabolic profiling, and metabolite identification.

Currently, Dr. Salome Gachet, Dr. Janine Marazzi and Jelena Simovic (analytical technician) are developing analytical methods for the endocannabinoid projects, but also for other projects within the NCCR TransCure. Transporters are often non-selective and metabolites may take different routes in order to get to the same place. We believe that understanding molecular networks through metabolic profiling will add value to transporter research as nature works with synergies and whole metabolite families. Using metabolic profiling we are able to study the biochemical effects of an inhibitor of a membrane transporter to both obtain evidence for its mechanism of action *in vivo* and further explore its range of activity.

¹ Chicca A, Marazzi J, Nicolussi S, Gertsch J. Evidence for bidirectional endocannabinoid transport across cell membranes. *J Biol Chem.* 2012 Oct 5;287(41):34660-82

High Content Screening

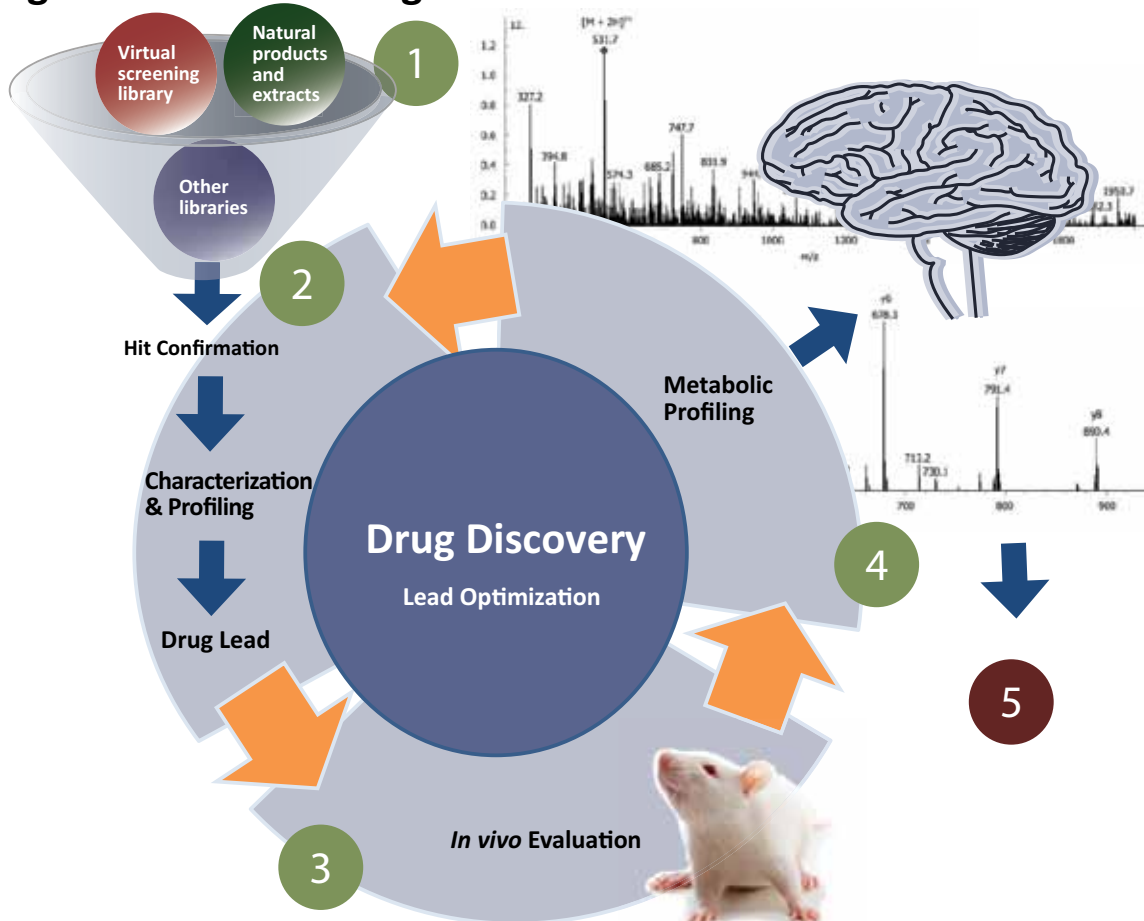


Fig. 1 Generation of potent and selective inhibitors of endocannabinoid membrane transport.

1. Different libraries of small organic molecules (purchased compounds from similarity searches derived from polypharmacology algorithm; natural products and extracts; other libraries) are screened in a semi-automated (Biomek 3000 robotics) high content screening measuring 3HAEA uptake into U937 cells. 2. Positive molecules (as good as or better than the currently available non-specific endocannabinoid uptake inhibitors) are extensively characterized on endocannabinoid transport (competitive vs. non-competitive) and on all known targets of the endocannabinoid system (receptors, enzymes, binding proteins) in a series of biochemical assays. 3. Once a drug lead is defined it is subjected to a behavioral test battery in BALB/c mice (5 mg/Kg i.p.) and specific readouts are generated. 4. Drug leads that show the desired CNS effect pattern will be studied in preliminary pharmacokinetics assays (amount of drug found in CNS) and in GC/MS and LC-MS metabolic profiling (changes of relative endocannabinoid tissue levels, lipidomic analyses, etc.). The insights from this iterative process are fed into a medicinal chemistry program in which desirable parameters are increased by changing the molecular properties of the lead compounds. In the preclinical phase of the drug discovery project everything is related to lead optimization. 5. Leads are also molecular tools that can be used for target identification as they should be potent and apparently selective binders to the putative endocannabinoid membrane transporter.



ADVANCEMENT OF WOMEN

Mentoring Program: support to women aiming at a successful academic career and work-life balance



Mentorship is a personal developmental relationship in which a more experienced or more knowledgeable person helps to guide a less experienced or less knowledgeable person. It offers a platform centered on the professional and personal development of the mentee. Our program is one of the actions NCCR TransCure has established within its strategy for the advancement of women in collaboration with the medical faculty of the University of Bern. Women's promotion is supported by the Rectors' Conference of the Swiss Universities (crus.ch) which states that "If Swiss universities wish to remain competitive and attract the best qualified researchers, they need to focus on gender equality as a quality factor." Several studies confirm that a lack of mentoring is among

the main reasons for a deficiency in career success in academics, especially for women. Mentoring offers to women a one-to-one relationship with a mentor from a related discipline. The mentoring relationship lasts two years and applications can be submitted at any time during the year, allowing a relationship to start when appropriate for the mentee. The mentors will focus on transferring their experience to mentees and help them to plan and pursue an academic career and build a professional network.

To support the mentoring program and the advancement of women, a course entitled "communication skills and self-confidence" was organized on December 7, 2012. The theoretical aspect related to the prepara-

tion, methods and organization of an oral presentation, including dos and don'ts specific to women. Practical exercise for each participant allowed practice of the theory and interaction with the group and trainer. Individual feedback on self-confidence was also provided by the trainer. Overall, the course was of high quality, and well appreciated by the participants.

To participate in the program, please visit our webpage and find out more about our conditions and application process.

The mentoring program is funded by the Bundesprogramm Chancengleichheit, the NCCR TransCure and the Medical Faculty of the University of Bern.

Mini-Symposium "Excellence in Women's Science"

On June 11, 2013, the NCCR TransCure held its second mini-symposium featuring outstanding female speakers who shared their professional and personal experience with our young researchers. The scientific part of the day allowed the audience to discover new research topics: Eilika Weber-Ban from the Institute of Molecular Biology and Biophysics, ETH Zurich, spoke about "the Pup-Proteasome-System, a Ubiquitin-like Degradation Pathway in Bacteria" and Nathalie Rouach from the Center for Interdisciplinary Research in Biology at Collège de France, Paris provided highlights on "Unraveling the Role of

Astroglial Connexions in Neurotransmission. Rajini Rao, Department of Physiology, The Johns Hopkins University, Baltimore spoke about "An Unconventional Interaction between a Calcium Pump and Channel in Lactation and Breast Cancer" and Liz Carpenter from the Structural Genomics Consortium (SGC), Oxford University, Oxford gave a presentation on the "Structural Biology of Human Membrane Proteins from ABCB10 to ZMPSTE24".

The panel addressed the academic career and family life. The four speakers provided a great deal of advices to the audience, sharing

enthusiastically their personal experiences and being very positive about successfully combining work and family. The feedback provided by both genders in the audience clearly established that the younger generation of researchers considers caring for children as a shared responsibility between both partners, and no longer as "female-only" burden. Most likely, this shift in attitude towards childcare from the younger generation will change the academic environment in the future. Finally networking breaks and lunch allowed everyone to share experiences, discuss science, career development and life balance.



Emilyne Blattes

Main advices from the panel:

Academic Career

- Be driven by the joy of defining and answering fundamental scientific questions.
- Plan your research with a fundamental question in mind.
- Don't be afraid to tackle new things and don't follow the beaten track.
- Work smart and know what you need to do to stay on track.
- Be confident and determined in what you do.
- Publications and grants are the most critical aspects of your work.
- Travel to conferences and get as much scientific exposure as possible.
- Network actively: go out there and talk to people.

Life balance

- Do not let others tell you that you have to choose between family and career.
- Use professional daycare solutions.
- Remember that nothing is ever perfect.
- Above all enjoy our life and be happy.

NCCR TransCure EXHIBITS AT BEA 2013 IN BERN

For the first time tunBern.ch took place at this year's BEA 2013 in May. BEA 2013 is the annual Bernese spring fair which is opened to the public for 10 days. 72 classes and a total of 3200 children and young people visited the adventure show and took the opportunity to experiment and try out sciences. The University of Bern participated with several institutes and departments, and our

NCCR. TransCure made an appealing and well-attended exhibition at BEA during two days thanks to the great efforts of Christine Deisl, Manuel Anderegg and Michèle Frey. Our students led activities featuring pipetting, taking apart and rebuilding a human body torso model, folding a DNA origami or watching a video on TransCure's automated Xenopus oocytes screening technologies.

The exhibition team was very busy both days explaining to groups of children how to absorb and transfer fluids with a pipette, how to set up a microscope to look at tiny crystals and how to perform surgery on "Doktor Bibber". It was an enriching experience for both children, teenagers and students. We thank our scientists for making this event a success.



At the exhibition booth

3rd ANNUAL TransCure RETREAT

The beautiful city of Murten hosted us for two days on May 30 and 31, 2013. The first day of our retreat included poster sessions and a guided tour of the city of Murten under a fabulous sunny sky. Plenty of time was available to the whole team to discuss projects and share ideas. Discussions were especially

important this year since the pre proposals for Phase 2 must be submitted to the SNSF this coming August. The apéro was held in the bar of the hotel Murten, with great wine and beers. During dinner, Bob Burrier spoke about "Metabolomics and Biomarkers". The second day was rainy and allowed the

team to focus on the future of the NCCR portfolio of projects and targets. Selected projects' proposals were presented and discussed by all. The retreat finished early in the afternoon on May 31 and everyone went home, satisfied with the many interesting exchanges.



BioMedical Transporters 2013

8th International
Research Conference

August 11 – 15, 2013

St. Moritz, Switzerland

Concert Hall,
Hotel Laudinella

The conference occurs biennially and has a long history of bringing together scientists (from thought leaders to trainees) in a very interactive and engaging program. This year, the event will be held in the world-famous Swiss mountain village of St. Moritz, from August 11-15, 2013, and will focus on several important topics related to membrane transporter research and therapeutic perspectives. Dedicated sessions will address most recent breakthroughs in basic science as well as newest achievements derived from applied studies. The conference will be the ideal forum to connect with the world renowned leaders in this field from both academia and industry and it presents great opportunities for discussing novel industrial applications.

Session Topics:

- Membrane Transporters and Channels in Drug Discovery.
- Transporters from a Basic Science Perspective.
- How to Discover New Targets / Ligands: Bioinformatics on Membrane Transporters.
- Transporters and Drug Safety.
- Use of Transporters for Drug Delivery.
- International Young Investigators Session: SFB35 (Austria), NCCR TransCure (Switzerland), SFB807 (Germany).
- Structure, Function and Imaging of Transporters.
- Transporters of the Intestine, Liver, Kidney and Brain.
- Systems Biology and Transporters.
- Poster Session and Vendor Fair.
- Downstream of Transporters: Second Messengers / Signalling Pathways.
- Transporters and Cancer.



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