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The Life & Medical Sciences Institute
at the University of Bonn

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PhD student Eva Jentgens
in the zebrafish facility
photo by Nick van Veenendaal

Foreword

The Life & Medical Sciences Institute (LIMES) is an internationally oriented center for biomedical research and higher education at the University of Bonn. The main scientific focus of the institute is to explore the regulation of lipid metabolism and the immune system in health and disease, and decipher the signaling processes that take place both within and on biomembranes.

The LIMES Institute is involved in several national and international research networks and offers talented young students and researchers an ideal environment for interdisciplinary training, innovative research and development to independence.

This brochure is supposed to give you an overview on the LIMES research groups and their scientific activities, on the LIMES study and training programs for students and young investigators, and on our scientific interactions with local and international partners.

Enjoy reading!

Prof. Michael Hoch
Managing Director

View on the city of Bonn with the University building in the foreground and the river Rhine and the "Siebengebirge" in the background, photo by Matthias Zepper

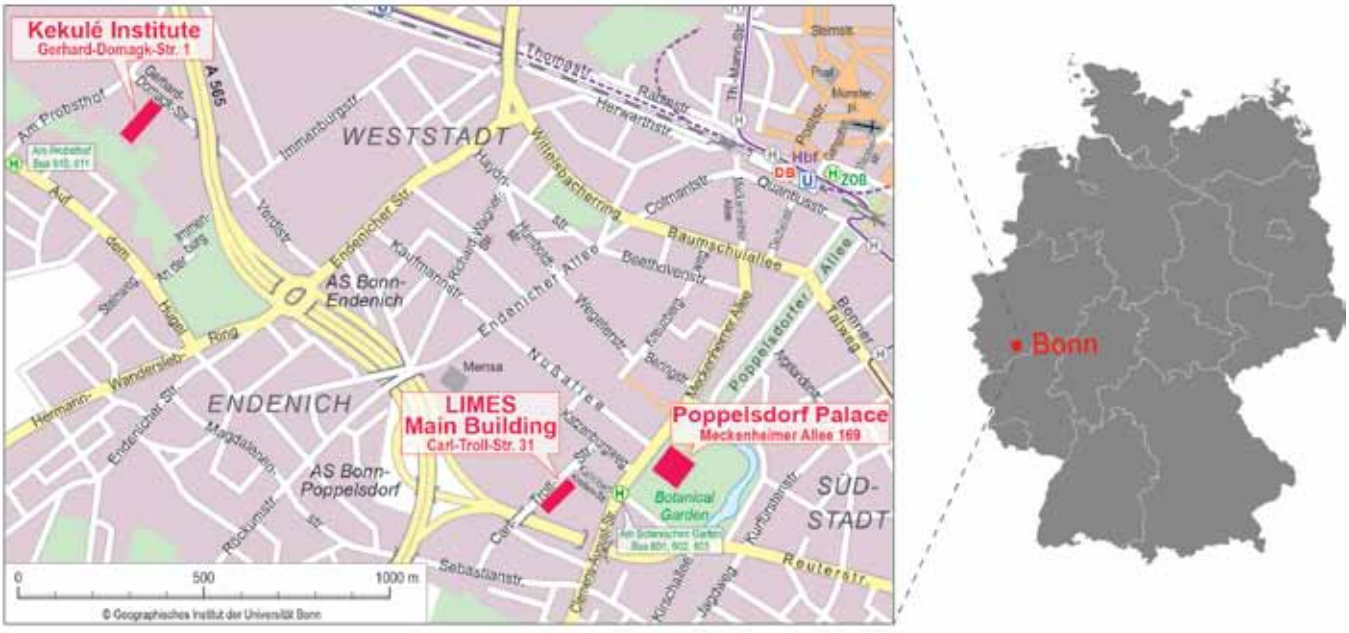
About us

Bonn - City of United Nations

Located in the heart of the city Bonn, the LIMES institute is perfectly positioned within a hop and a skip to the beautiful botanical gardens, Poppelsdorf Palace and a great selection of restaurants, bakeries, cafés and bars.
Eighteen United Nations organizations have made Bonn their home and it's easy to see why. Whether it be the international silent film festival, world class exhibitions found in galleries and museums along the "Museum Mile", Carnival celebrations, the nostalgic Christmas Markets, or festivals including Bonn's personal ode to joy for their son Ludwig van Beethoven, this small city always has something on offer.

The LIMES Institute

The Life & Medical Sciences (LIMES) Institute was founded in 2006 as a new scientific institution of the Mathematics and Natural Sciences Faculty of the University of Bonn (Molecular Biomedicine Division).
Currently, the staff comprises over 240 scientists, postdoctoral fellows, PhD students and support staff from 19 different countries. This number is further increased by up to 150 Bachelor's and Master's students at any one time.
Eleven Departments/Professors, two senior professors and the LIMES institute's central infrastructure are spatially distributed across two sites in Bonn: The LIMES Main Building in Bonn-Poppelsdorf (main site of the Institute since 2010), and the Kekulé Institute of the Department of Chemistry at Bonn-Endenich.



MISSION

Core objectives of our institute are to:

- *Practice scientific excellence in basic biomedical research in a working atmosphere that stimulates inter-departmental collaboration and mutual success in a resource-rich and harmonious environment.*
- *Provide goal-oriented interdisciplinary training for students and early career researchers, and provide a forum for promotion of talented scientists at all career levels.*
- *Systematically develop national and international research associations and networks, and promote the international reach of the institute through the establishment of international study programs.*
- *Actively promote public outreach programs to enrich communication with our public.*

Our Research Focus

The scientific focus of the LIMES working groups are: exploring the interaction of the immune system with metabolism in health and disease, and the study of cellular membrane processes using methods of lipid biochemistry, chemical biology and biophysics. The working groups of the Institute are organized in four Research Units according to their scientific expertise.
Collaboration is integral to the LIMES Institutes innovative research. Our research activities are further integrated into national and international cooperation networks.

At the LIMES Institute: Study, work, inspire

The Life & Medical Sciences Institute is committed to the development of young researchers. The interdisciplinary structure of the Institute provides a great environment for training. We currently offer internationally recognized Bachelor's study program "Molekulare Biomedicine" (German language); Master's "Life & Medical Sciences" (English language); and Doctoral study programs.
In addition, the institute hosts and trains students through internships, and is regularly involved in public outreach programs; working with schools and the local community to stage "science rally's", lab visits and student/parent information days.

History and Development

The LIMES Institute was founded in 2006 as part of the Mathematics and Natural Science Faculty of the University of Bonn. The initial concept for the LIMES Institute was established in 2000, by two newly recruited professors, Michael Hoch (Biology) and Michael Famulok (Chemistry). Their vision was to create a framework for interdisciplinary and internationally competitive research at the interface of biology, chemistry, and medicine at the University of Bonn. They presented their concept to the University of Bonn Rectorate (Rector, Prof. Klaus Borchard; Chancellor, Dr. Reinhardt Lutz), who gave their support.

THE LIMES CONCEPT

The **core objectives** of the "LIMES-concept" were:

- To establish interdisciplinary DFG (German Research Funding) collaborative **research initiatives** at the interface of biology, chemistry and medicine.
- To establish new **biomedical education and training** programs for the promotion of talented young scientists.
- To establish a **new LIMES Institute building** to house internationally competitive biomedical research and teaching programs.

In recent years, the three objectives have been systematically implemented through the concerted efforts of the LIMES steering group (Hoch, Famulok and Prof. Waldemar Kolanus, who was recruited in 2002) and colleagues from the Faculty of Mathematics & Natural Sciences and the Faculty of Medicine.
External funding networks have been successfully obtained, study programs have been implemented, and in 2010 the first LIMES building was unveiled.

Ground-breaking for the new LIMES building in December 2008 (from left): Barbara Ludwig-Leylabi, Bau- und Liegenschaftsbetrieb NRW, Prof. Dr. Matthias Winiger, Rector of the University of Bonn, Dr. Uwe Günther, Bau- und Liegenschaftsbetriebes NRW, Andreas Pinkwart, NRW- Minister for Innovation, Helmut Joisten, Mayer of the city of Bonn, Prof. Michael Hoch, Scientific Head of the LIMES-project

The LIMES Institute Main Building

The new Life & Medical Sciences (LIMES) Institute building was unveiled in January, 2010. Perfectly located in the center of Bonn (Bonn-Poppelsdorf), it will neighbor a new university campus that will be developed over the next decade. With ~3,700 square meters, it accommodates the 10 current working groups with laboratory and office space, as well as central social areas and a common infrastructure for research and teaching. Researchers and engineers worked in partnership to design a building for the future. Its unique atmosphere is a magnet, especially for young talent emerging in the field of life sciences.

An Innovative Concept for Lab, Office and Meeting Space

A flexible laboratory and office concept was developed to accommodate the changing needs of the working groups. Meeting rooms and public areas encourage scientific exchange and social interaction. Lounge areas and coffee rooms attract staff and students – acting as a central meeting space where new ideas are born.

Laboratories are found on the northwest side of the building, while offices line the southeast side. An atmosphere has been created where experienced scientists and young people alike find inspiration.

Gender Equality

We are aware of the challenges facing women in academia and are dedicated to take internal measures to support, promote and recruit excellent women scientists at all career levels.

New initiatives support women scientists

A challenge in research world-wide, is to counter the trend that the number of women drastically decreases as you go up in seniority.

As part of our commitment to address the gender imbalance at senior levels, the LIMES institute has recently taken several measures: we recruited Prof. Irmgard Förster - an expert known for her work on functional characterization of macrophages and dendritic cells - to head a new Immunology and Environment group at the LIMES institute. Furthermore, we recently appointed a Gender Equality committee for **LIMES-WiS (Women in Science)**, also lead by Prof. Förster.

LIMES-WiS aims to provide support for staff and students to make the transition from undergraduate training, to more independent positions and senior research roles.

The LIMES-WiS committee initiated a bi-monthly seminar series featuring emanent local and international scientists, as well as various soft-skill workshops. As part of the LIMES-WiS seminar series, some lecturers share their experiences and lend career development advice. Discussions continue with regular get-togethers of female scientists after the seminars. "We hope that such a forum will help to identify and enforce suitable measures for childcare and a stable work-life balance, as well as fostering scientific exchange and mentoring to improve career development among our institute's women scientists." says Prof. Förster.

LIMES-WiS initiatives are proudly sponsored by SFB's 645, 704 and TRR83. Members of these cooperative research centres can utilize additional services currently being piloted by LIMES-WiS including: the flying nanny service, which offers staff emergency child care, as well as the possiblilty to apply for lab support during maternity leave.

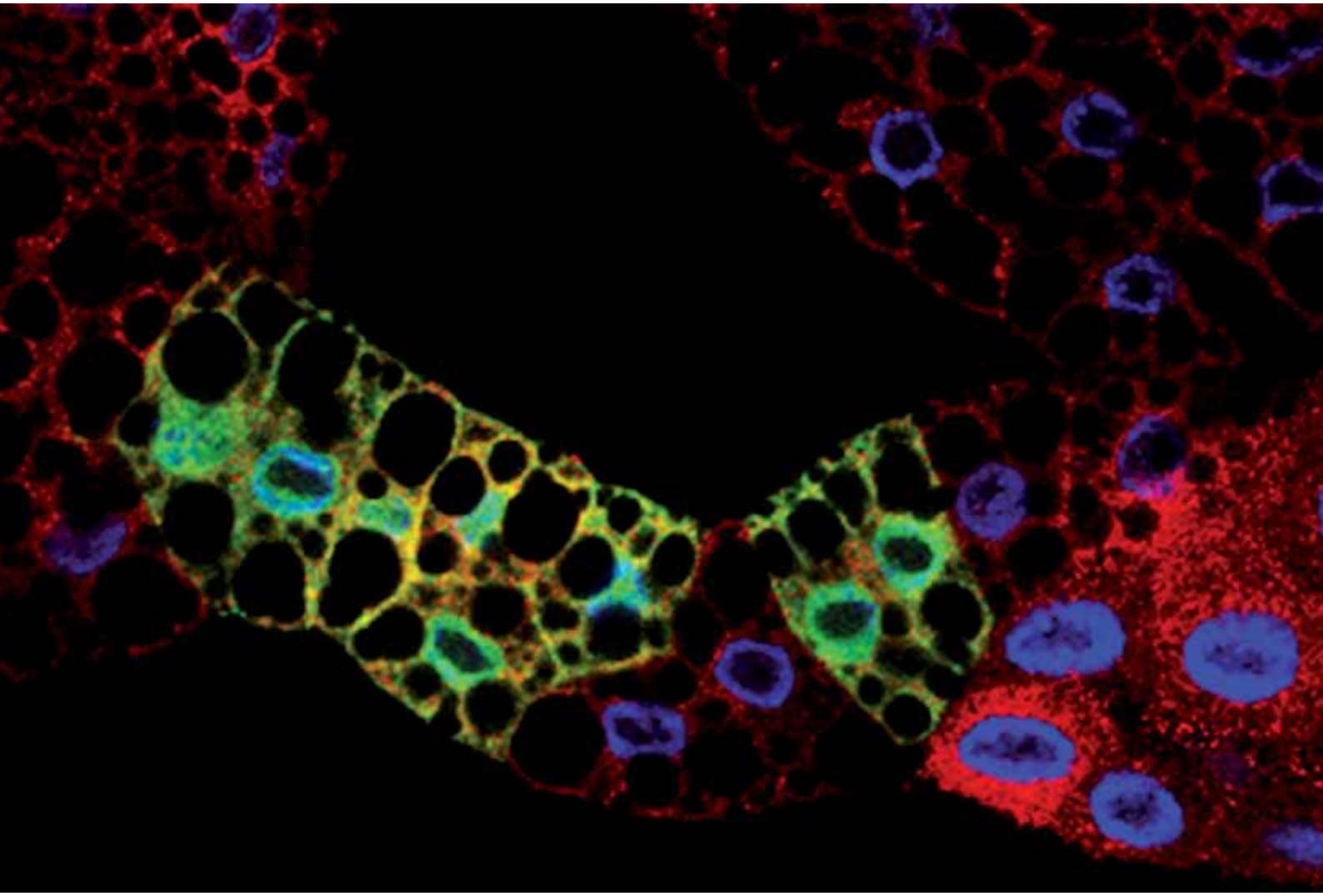
Prof. Irmgard Förster (far left) and members of the LIMES-WiS committee, planning the next WiS seminar.

Japanese exchange students from Waseda University, Tokyo

International LIMES

At the Life & Medical Sciences Institute you will find students and experienced scientists at the forefront of biomedical research at the University of Bonn. We currently accommodate academic staff from 19 different nations at the level of PhD student or postdoc. Our international Master's Course in Life and Medical Sciences, and lively exchange with our international partner institutions further add to this dynamic environment.

Master students 2013
Some of the LIMES international staff members 2014



Heat-shock-inducible, GFP-labeled cell clones in the Drosophila fatbody

Research

The research groups of Unit 1 utilize a combination of genetics and molecular cell biology approaches to identify key regulatory genes and genetic networks which control the cross regulation of metabolism, innate immunity and brain functions such as feeding in the model systems fruit fly, mouse and zebrafish. They also study the impact of nutrition and the gut microbiome on organ physiology in health and disease.

The Program Unit „Molecular Immune- and Cell Biology" comprises four groups with a dedicated focus on interdisciplinary immunological research. Featured methods shared among these labs include modern molecular genetics, cellular immunology, mouse models and human cells for the study of immune related diseases, macromolecular interactions, visualization of cell dynamics and motility, as well as functional genomics and bioinformatics.

The unifying object of Unit 3 is the biological membrane. Membranes are formed by lipids and are the basis for membrane protein insertion, structure, and function. In Unit 3, lipid synthesis and degradation, lipid signaling, and the structure and dynamics of membrane protein assemblies are intensely studied in systems ranging from reconstituted liposomes to intact animals.

The groups of Unit 4 explore the potential of aptamers and small molecules as tools for addressing a large variety of biological questions. These biological questions are mostly, but not exclusively, related to the general topics of interest in Units 1-3. Examples are the chemical biology of receptor tyrosin kinase signalling, (photo)-switchable aptamers, riboswitches and regulatory RNAs, and chemoinformatics.

Molecular Developmental Biology

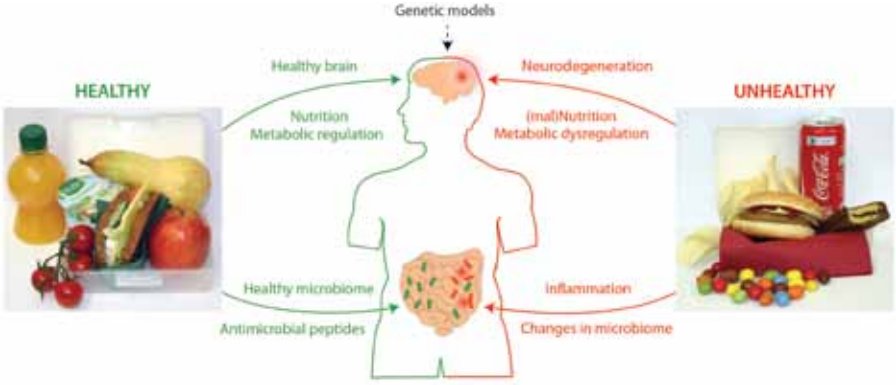
How do the organs of our body develop and how are their physiological functions controlled? Are energy homeostasis, innate immunity and ageing linked? Do fasting or overeating affect neural functions? What roles do diet and gut microbiome play? To address these and other questions, we utilize the genetic model systems fruit fly, mouse and zebrafish in combination with biochemistry and molecular cell biology techniques.

Our overall goal is to understand how nutrition and metabolism influence the immune and the nervous systems in health and disease.

Prof. Michael Hoch
Director

Prof. Michael Hoch (middle) with Dr. Anna Aschenbrenner (left) and Dr. Elvira Mass (right)

What's in your lunch box?



Identifying new key regulators and genetic networks

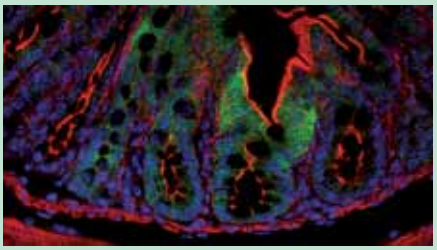
We aim to identify new key regulators and genetic networks that control metabolism and cell and organ physiology. In particular, we investigate the metabolism – innate immunity – gut microbiome axis, to elucidate how nutrition influences brain function, and how the onset and progression of neurodegenerative diseases can be modulated in response to diet. We are interested in peroxisome and lysosome functions and dysfunctions, and aim to identify new regulators of cell signaling and stress responses (unfolded protein response, osmoregulation).

Technology

- Genetic models: Fruit fly, Mouse, Zebrafish
- Genetic screening, Genetic Engineering (TALEN, CRISPR/Cas9, Cell Biology and Biochemistry, Nutrient and Lipid Analysis, Microscopy

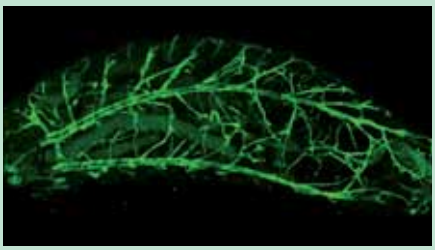
HIGHLIGHT

Malformation of cardiac valves accounts for a large proportion of congenital heart diseases. The initiation of heart valve formation requires calcineurin/nuclear factor of activated T-cells c1 (NFATc1) signaling in the cardiomyocytes and endocardial cell layer. We recently identified the murine Cysteine-Rich with EGF-Like Domains 1 (mCreld1) gene as a new and essential regulator of heart valve formation. We could show that mCreld1 directly interacts with the regulatory subunit B of the phosphatase calcineurin at the endoplasmic reticulum, thereby controlling the nuclear translocation of NFATc1. Since human CRELD1 was found to be associated with the pathogenesis of atrioventricular septal defects that constitute about 5% of all recognized congenital heart diseases, our results suggest a conserved function of Creld1 genes in regulating heart valve morphogenesis. Mass E. et al., Dev Cell, 2014.



Cross-section through an embryonic mouse heart

In a recent study, we found that antimicrobial peptide (AMP) can be activated by the transcription factor FOXO, a key regulator of stress resistance, metabolism and ageing, independently of the immunoregulatory TOLL and IMD pathways. Our results indicate a new mechanism of cross-regulation of metabolism and innate immunity by which AMP genes can be activated under normal physiological conditions in response to the oscillating energy status of cells and tissues. The sparse production of AMPs in epithelial tissues in response to FOXO may help modulate the defence reaction without harming the host tissues, in particular when animals are suffering from energy shortage or stress. Becker T. et al., Nature, 2010.



Antimicrobial peptide expression in the airway system of a Drosophila larva

Top 5 Publications

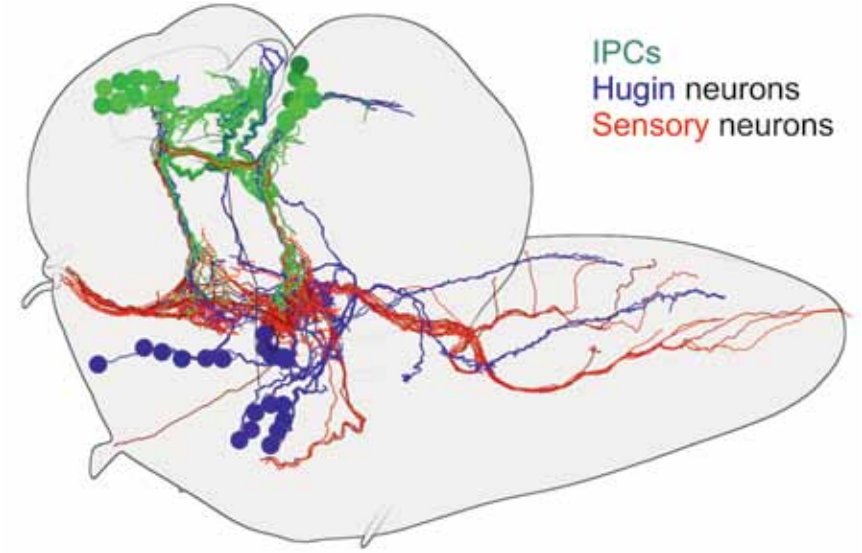
1. Mass E, Wachten D, Aschenbrenner AC, Voelzmann A and Hoch M. Murine Creld1 Controls Cardiac Development through Activation of Calcineurin/NFATc1 Signaling. Dev Cell 2014; 28(6): 711-726.
2. Becker T, Loch G, Beyer M, Zinke I, Aschenbrenner AC, Carrera P, Inhester T, Schultze JL, Hoch M. FOXO-dependent regulation of innate immune homeostasis. Nature 2010; 463: 369-373.
3. Bauer R, Voelzmann A, Breiden B, Schepers U, Farwanah H, Hahn I, Eckardt F, Sandhoff K, Hoch M. Schlank, a member of the ceramide synthase family controls growth and body fat in Drosophila. EMBO J 2009; 28: 3706-3716.
4. Behr M, Wingen C, Wolf C, Schuh R, Hoch M. Wurst is essential for airway clearance and respiratory-tube size control. Nat Cell Biol 2007; 9: 847-853.
5. Fuss B, Becker T, Zinke I, Hoch M. The cytohesin Steppke is essential for insulin signalling in Drosophila. Nature 2006; 444: 945-948.

Molecular Brain Physiology and Behavior

Our lab is studying how the brain controls behavior and metabolism, using Drosophila as a model organism. We focus on the neural circuits that mediate feeding behavior and internal metabolic and external sensory factors that modulate feeding motor programs. Utilizing a combination of genetic, behavioral, imaging and electrophysiological tools, we aim to identify the feeding neural circuitry and to elucidate the central mechanisms by which specific motor programs are selected to achieve meaningful behavior.

Prof. Michael Pankratz
Director

Prof. Michael Pankratz (right) and Dr. Andreas Schoofs, discussing how neural circuits function.



EM-Reconstruction of a Micro Circuit in the Drosophila Brain: *Based on serial-section TEM of an entire larval CNS, we found that neuropeptide Hugin-producing neurons connect the sensory system to insulin-producing cells (IPCs).*

Mapping the feeding connectome

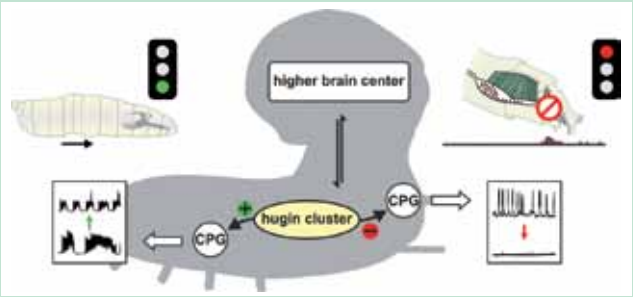
Our major goal is to elucidate the complete synaptic connectivity pattern underlying the larval feeding system. In collaboration with the Cardona lab at HHMI Janelia Farm Research Campus in the USA, we are mapping the sensory inputs and the motor and endocrine outputs of neurons involved in feeding, based on serial electron microscope reconstructions of the larval brain. These include gustatory sensory neurons, pharyngeal motor neurons, insulin-producing cells (IPCs) and higher order Hugin neurons.

Technology

- Two-photon and confocal microscopy
- GCaMP imaging of neuronal ensembles
- Optogenetics and electrophysiology
- High-resolution quantitative behavioral analysis

HIGHLIGHT

Many factors regulate the decision to eat or not, including hunger state, quality of odor and taste, prior experience and memory, and the immune response such as, when fighting an infection. The various conflicting, competing or complementary signals are processed by higher brain centers, which then execute an appropriate behavioral response. In the animal kingdom two of the most essential behaviors are feeding and locomotion. Our recent work provides a model for the functional role of central neurons in relaying inputs from higher brain centers onto motor centers for feeding and locomotion in Drosophila. Activation of a small cluster of neurons in the brain of Drosophila larvae caused the animals to simultaneously stop eating and start moving. These neurons express the neuropeptide hugin, which is homologous to the mammalian neuromedins. The cluster of hugin neurons is functionally divided into distinct subgroups that accelerates the motor program for locomotion and decelerates the motor program for feeding. We propose that hugin neurons represent a relay center between information-processing higher brain circuits and those executing motor programs. Schoofs *et al.* (2014). *PLoS Biol,* 12:e1001893.



Model for the selection of motor programs: The 20-cell hugin cluster relays information processed by the higher brain centers to simultaneously suppress feeding and initiate locomotion motor programs.

Top 5 Publications

1. Schoofs A, Hückesfeld S, Schlegel P, Miroschnikow A, Peters M, Zeymer M, Spiess R, Chiang AS and Pankratz MJ. (2014). Selection of motor programs for suppressing food intake and inducing locomotion in the Drosophila brain. *PLoS Biol,* 12:e1001893.
2. Bader R, Sarraf-Zadeh Peter M, Moderau N, Stocker H, Köhler K, Pankratz* MJ and Hafen* E. (2013). The IGFBP7 homolog Imp-L2 promotes insulin signaling in distinct neurons of the Drosophila brain. *J Cell Sci,* 126, 2571-2576. *corresponding authors
3. Buch S, Melcher C, Bauer M, Katzenberger J and Pankratz MJ. (2008). Opposing effects of dietary protein and sugar regulate a transcriptional target of Drosophila insulin-like peptide signaling. *Cell Metabolism* 7, 321-332.
4. Melcher C, Bader R, Walther S, Simakov O and Pankratz MJ. (2006). Neuromedin U and its putative Drosophila homolog hugin. *PLoS Biol,* 4:e68.
5. Melcher C and Pankratz MJ. (2005). Candidate gustatory interneurons modulating feeding behavior in the Drosophila brain. *PLoS Biol,* 3:e305.

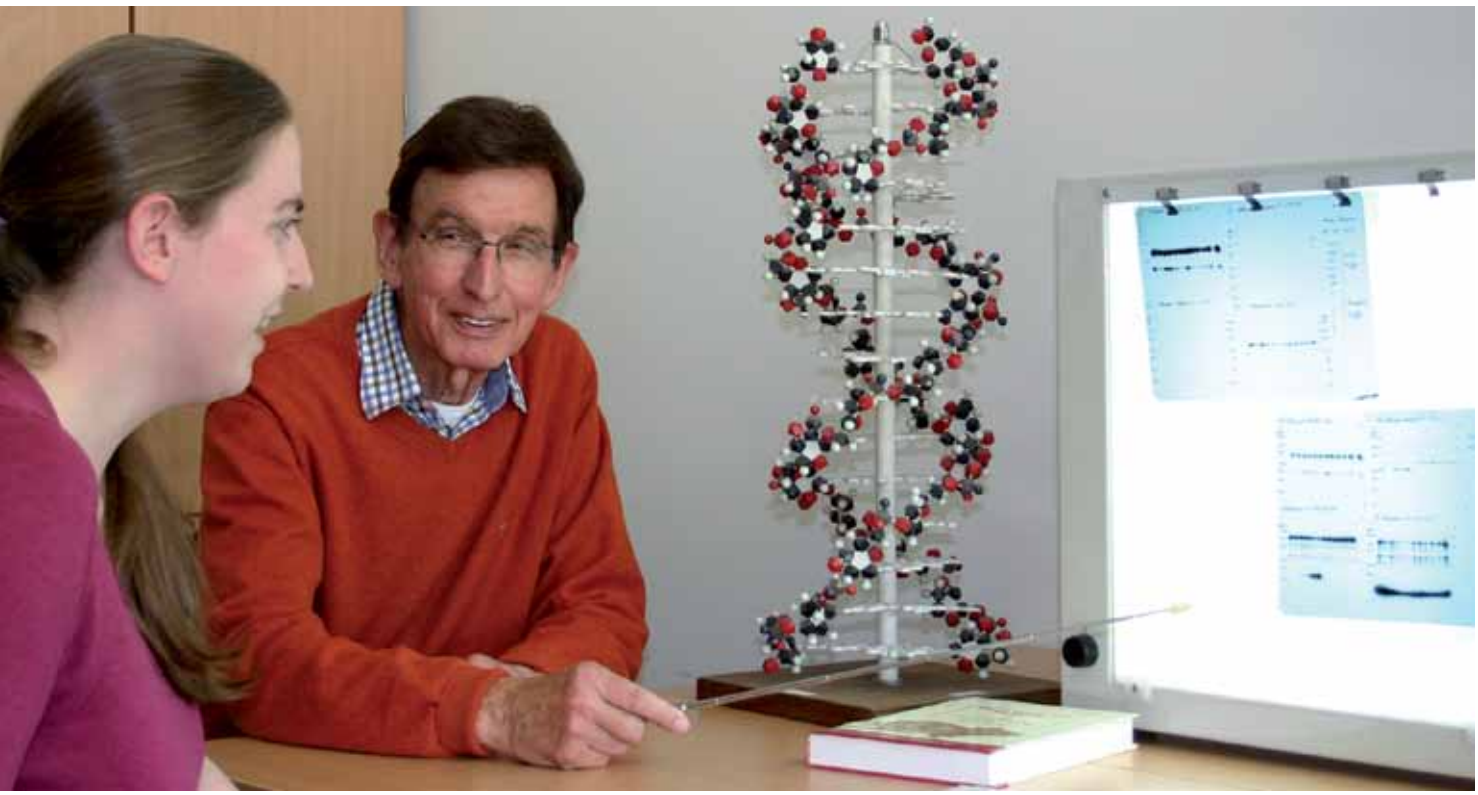
Molecular Genetics and Cell Biology

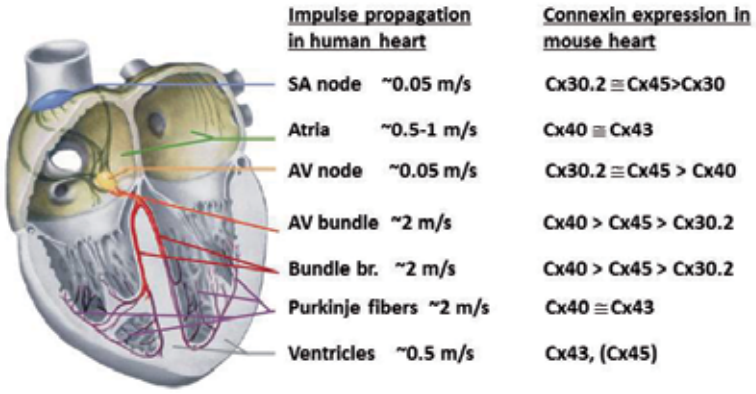
We investigate the biological functions of connexin proteins and certain sphingolipids in targeted transgenic mouse mutants generated in our group.

In the mouse genome, 20 different genes code for connexin proteins that form conduits (channels) for intercellular communication of ions and metabolites in distinct cell types. Six enzymes catalyze the synthesis of ceramides (and sphingolipid derivatives) with distinct fatty acyl chains as membrane anchors. Our current projects focus on connexin functions in the heart, skin and embryonic stem cells, as well as the biological functions of ceramides in skin, lung and neural tissue.

Prof. Klaus Willecke
Laboratory Head

Prof. Klaus Willecke and PhD student, Christiane Kremser discussing immunoblots of protein expression levels.





Cross-section of a heart indicating expression of different connexin (Cx) proteins in distinct cardiac areas. The various connexin channels show different rates of impulse propagation during a heartbeat. Ablation of distinct connexins in the heart leads to abnormalities in the electrocardiogram.

Biological functions of connexin proteins and sphingolipids

During recent years we have characterized several transgenic mouse mutants that harbor mutations in connexin genes taken from patients who suffer from genetic diseases. Since mouse and human connexins are largely orthologous, we can utilize mouse models to study the mechanisms and physiological consequences of human genetic disease. Recent examples include connexin mutations that cause Sudden Infant Death or the Clouston Syndrome in skin. In addition, we have generated and analyzed mouse mutants with defects in ceramide synthase 1, 2, 4 or 6, which express distinct phenotypic abnormalities in liver, brain and skin. Our long-term goal is to unravel the molecular mechanisms of these functional abnormalities.

Technology

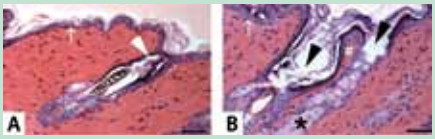
- Generation of transgenic mouse mutants: deletions or point mutations with ubiquitously expressed or conditional gene defects
- Knock-in mice with reporter genes coding for ß-galactosidase or fluorescent proteins. Tamoxifen- or Doxycylin- inducible transgenic mice
- Sphingolipid analyses, histochemical analyses of various mouse tissues

Research

HIGHLIGHT

Ceramide Synthase 4 deficiency in mice causes lipid alterations in sebum and results in alopecia. Ebel et al. *Biochem Journal, 2014, PMID: 24738593*

In order to further unravel the effect of the distinct membrane anchor of various ceramides, we studied the biological function of Ceramide Synthase 4 in newly generated CerS4-deficient mice (CerS4-/-). In addition, we raised specific antibodies to analyze the expression of CerS4 in suprabasal epidermal layers of footpads and in sebaceous glands of dorsal skin. Loss of CerS4 protein leads to altered lipid composition of the sebum, which is more solidified and therefore might cause progressive hair loss due to physical blocking of the hair canal. Wax diesters in the sebum of CerS4-/- mice are strongly diminished. Mass spectrometric analyses revealed a large decrease in C20 containing sphingolipids. From these and results with other ceramide synthase deficient mice recently published by our laboratory we conclude that the biological roles of ceramide synthases are manifold. Ceramides with C20 fatty acid residues provided by the enzymatic activity of CerS4 are essential for the proper viscosity of sebum and its effect on enduring hair growth.



Enlarged sebaceous gland and sebum-filled piliary canal in wild type (A) compared to CerS4-deficient skin (B).

Recent Publications

From a total of 353 publications listed in PubMed under Willecke K

1. Ebel P, Imgrund S, Vom Dorp K, Hofmann K, Maier H, Drake H, Degen J, Dörmann P, Eckhardt M, Franz T, Willecke K: Ceramide Synthase 4 deficiency in mice causes lipid alterations in sebum and results in alopecia, Biochem J 2014, 1; 461(1): 147-58
2. May D, Tress O, Seifert G, Willecke K: Connexin47 protein phosphorylation and stability in oligodendrocytes depend on expression of Connexin43 protein in astrocytes, J Neurosci 2013, 33:7985-7996
3. Lübkemeier I, Andrie R, Lickfett L, Bosen F, Stockigt F, Dobrowolski R, Draffehn AM, Fregeac J, Schultze JL, Bukauskas FF, Schrickel JW, Willecke K: The Connexin40A96S mutation from a patient with atrial fibrillation causes decreased atrial conduction velocities and sustained episodes of induced atrial fibrillation in mice, J Mol Cell Cardiol 2013, 65: 19-32
4. Ebel P, Vom Dorp K, Petrasch-Parwez E, Zlomuzica A, Kinugawa K, Mariani J, Minich D, Ginkel C, Welcker J, Degen J, Eckhardt M, Dere E, Dörmann P, Willecke K: Inactivation of ceramide synthase 6 in mice results in an altered sphingolipid metabolism and behavioral abnormalities, J Biol Chem 2013, 288:21433-21447
5. Ginkel C, Hartmann D, vom Dorp K, Zlomuzica A, Farwanah H, Eckhardt M, Sandhoff R, Degen J, Rabionet M, Dere E, Dörmann P, Sandhoff K, Willecke K: Ablation of neuronal ceramide synthase 1 in mice decreases ganglioside levels and expression of myelin-associated glycoprotein in oligodendrocytes, J Biol Chem 2012, 287:41888-41902

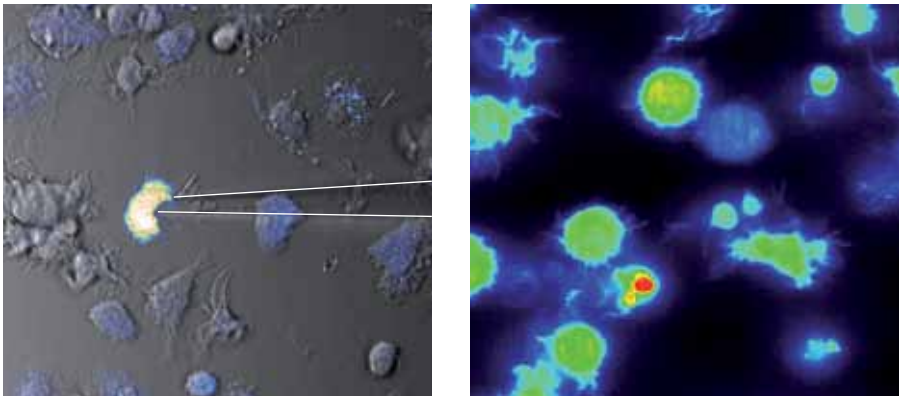
Molecular Immunology and Cell Biology

Our group investigates mechanisms of intracellular signal transduction in fundamental biological processes, including cellular activation, differentiation and migration. Because of a strong interdependence of cellular communication pathways, our main interest is to elucidate circuits that link immune pathways to the control of renewal/maintenance and metabolic stress regulation. We specialize in basic research; the pathways we study provide fundamental information to better understand important disorders including autoimmunity and cancer.

Current research topics include: (i) The identification of intracellular regulators and second messengers in immune cell adhesion and migration, (ii)ARF-GTPase signaling in the control of general and immune cell metabolism, (iii) Trim-NHL proteins and their roles in embryonic/adult stem cell biology and cancer.

Prof. Waldemar Kolanus
Director

Prof. Waldemar Kolanus (middle) with PhD students, Angrit Namislo (left) and Felix Tolksdorf (right).



$Ca2^+$ flashes in dendritic cells triggered by a chemokine. Left panel: single cell response triggered by a micropipette. Right panel: Bulk response stimulated by a chemokine in solution

Visualization and quantitative analysis of cell motility at the population level and in single cells

Cell motility is a fundamental prerequisite for immune system function since immune cells are never stationary. We use state-of-the-art microscopic techniques with high-resolution and high-speed image acquisition for the analysis of cell migration. Current knowledge suggests that immune cells can switch between slow adhesion-dependent and fast adhesion-independent "modes", which allow them to adapt to different environments (cell surfaces and 3D interstitial matrices). To a certain extent this is analogous to the function of the gearbox and clutch in motorized vehicles. We employ the visualization of e.g. actin and $Ca2^+$ dynamics to understand the molecular pathways that are essential for this important cell function.

Technology

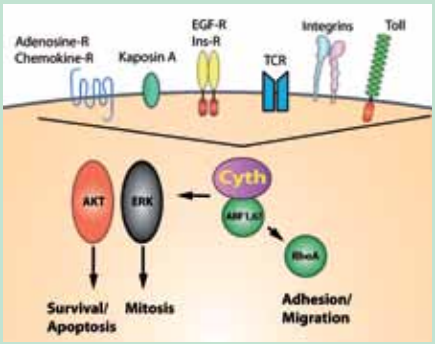
In addition to those mentioned above, other key methodologies used by our group include:

- Transgenic systems (e.g. conditional knockout and the latest technologies of genome editing in mouse and zebrafish)
- *In vitro* techniques such as siRNA-mediated "knockdown", as well as the utilization of proprietary chemical inhibitors

Research

HIGHLIGHT

We have a long-standing interest in the regulation of immune cell adhesion, which contributes to the mechanics of cellular locomotion and the dynamic re-distribution of immune cells in the lymphatic system, or in peripheral organs. The binding of integrin adhesion receptors to their ligands is regulated by cytoplasmic signals. We have discovered a family of cytoplasmic signaling molecules, the cytohesins, which can control integrin activity in several systems. These cytoplasmic proteins are so-called guanine nucleotide exchange factors (GEFs) for small cellular GTPases and regulate a number of other important signal transduction routes, including metabolic pathways (insulin signaling) as well as cell growth, differentiation and activation (e.g. EGF- and T cell receptor signaling). We have recently generated mouse knockout models to study the essential biological functions of these proteins *in vivo*.



Signaling pathways controlled by cytohesin (cyth) GEFs

Top 5 Publications

1. Bald T, Quast T, ... Forster I, Kastenmuller W, Kolanus W, Holzel M, Gaffal E and Tuting T. (2014). Ultraviolet-radiation-induced inflammation promotes angiotropism and metastasis in melanoma. *Nature,* 507(7490), 109-113.
2. Ulbricht A, Eppler FJ, Tapia VE, van der Ven PF, Hampe N, Hersch N, Vakeel P, Stadel D, Haas A, Saftig P, Behrends C, Furst DO, Volkmer R, Hoffmann B, Kolanus W and Hohfeld J. (2013). Cellular mechanotransduction relies on tension-induced and chaperone-assisted autophagy. *Curr Biol,* 23(5), 430-435.
3. Goller T, Seibold UK, Kremmer E, Voos W and Kolanus W. (2013). Atad3 function is essential for early post-implantation development in the mouse. *PloS one,* 8(1), e54799.
4. Quast T, Eppler F, Semmling V, Schild C, Homsi Y, Levy S, Lang T, Kurts C and Kolanus W. (2011). CD81 is essential for the formation of membrane protrusions and regulates Rac1-activation in adhesion-dependent immune cell migration. *Blood,* 118(7), 1818-1827.
5. Quast T, Tappertzhofen B, Schild C, Grell J, Czeloth N, Forster R, Alon R, Fraemohs L, Dreck K, Weber C, Lammermann T, Sixt M and Kolanus W. (2009). Cytohesin-1 controls the activation of RhoA and modulates integrin-dependent adhesion and migration of dendritic cells. *Blood,* 113(23), 5801-5810.

Genomics and Immunoregulation

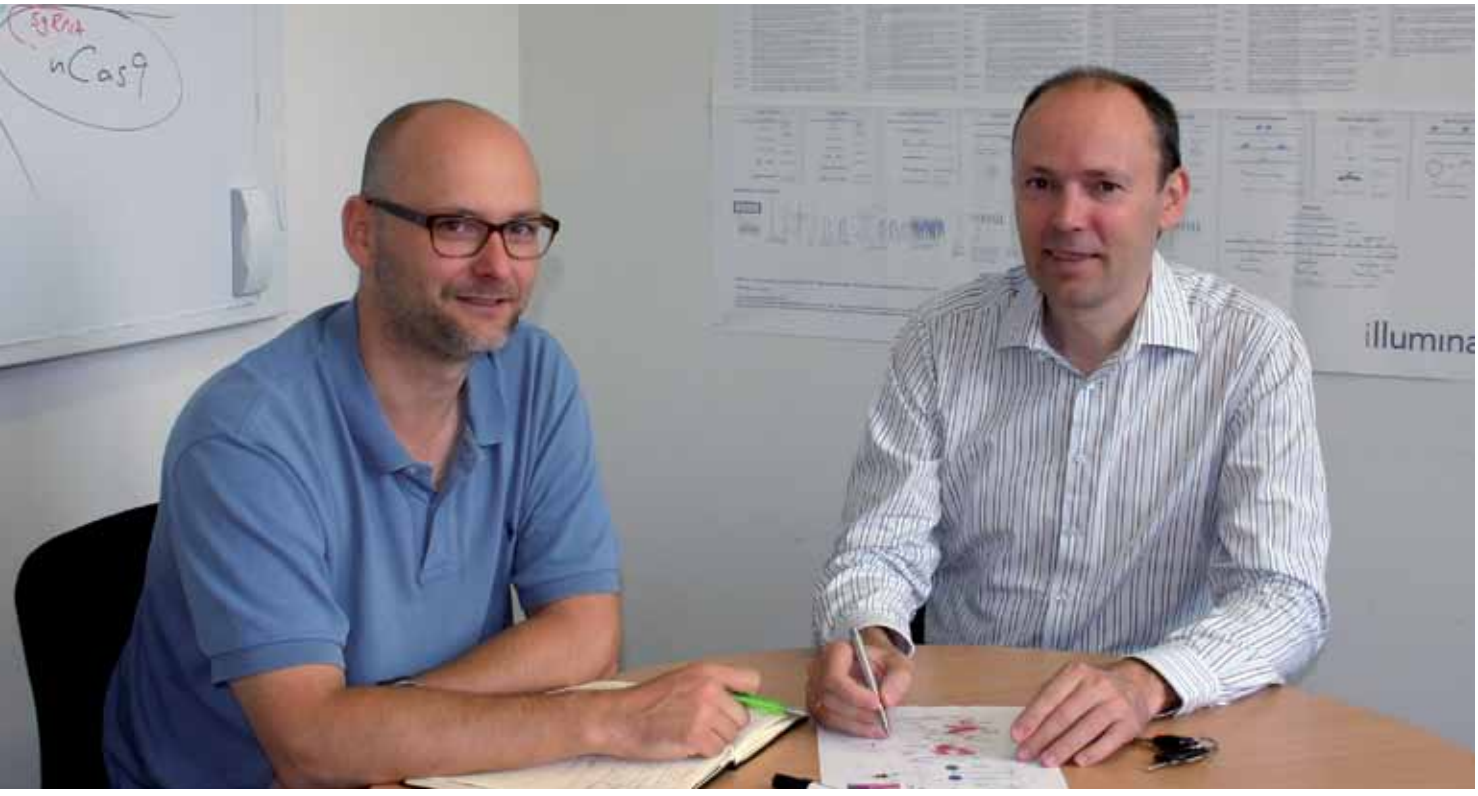
How is a phagocyte programmed during an infection or in adipose tissue during obesity? Can we reprogramm T lymphocytes by central switches? What happens during chronic inflammation? Which programs are switched on in immune cells and how is this regulated molecularly? Which transcription factors are involved in such processes?

To answer these questions, we employ systems biology approaches using genomic technologies such as microarrays or next generation sequencing combined with classical genetic, molecular, biochemical, immunological and cell biological methods.

Prof. Joachim Schultze, MD
Director

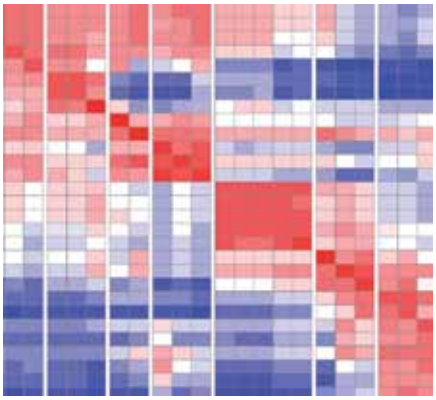
Dr. Marc Beyer
Group Leader

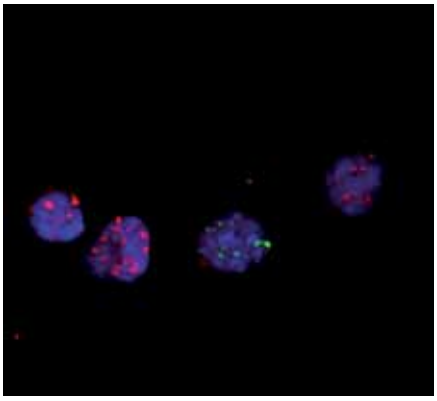
Prof. Joachim Schultze (right) and Dr. Marc Beyer, discussing transcriptomics data on regulatory T cells.



Right: An example of gene expression data from macrophages showing highly expressed genes in red and genes expressed at low levels in blue.

Far right: Expression of Satb1 (red) and Foxp3 (green) in regulatory T cells (arrow) and conventional CD4+ T cells.





Beyer Research Group

We are interested in deciphering the molecular function of regulatory and effector T cells. Using modern genomic methods in human cells and model systems, we study the transcriptional events necessary for 1) the suppressive activity of regulatory T cells and 2) the differentiation of T cells and acquisition of effector T-cell properties. For example, we want to investigate the molecular events that induce SATB1 expression in CD4+ T cells and regulate its expression. Integration of emerging technologies, such as TALENs or the CRISPR/Cas9 system allow us to elucidate the genetic and molecular mechanisms downstream of SATB1 that regulate differentiation and function of CD4+ T cells.

Technology

We utilize cutting-edge technologies to identify genes and genetic networks that regulate basic biological processes in immune cells. These include:

- Microarray-technology
- Next generation sequencing (RNA-seq, ChIP-seq)
- Multi-color flow cytometry assisted cell analysis
- Flow cytometry assisted cell sorting
- Genetic engineering (TALEN, CRISPR)/Cas9

HIGHLIGHT

Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Xue et al, Immunity 40, 274–288 (2014).
In the body, macrophages go on patrol as scavenger cells and act to eliminate intruders. According to the commonly held belief in immunology, they are divided into two groups: "classical macrophages", which spur on inflammatory processes, and "alternative macrophages", which shut down inflammation. Recently, we challenged this dogma by showing that macrophages react towards many different stimuli and do not just differentiate into cells that fuel inflammation or anti-inflammatory processes. In fact, we found that these immune cells differentiate into at least nine forms, which each use their weapons to tackle intruders in their own way. To obtain these data, blood samples from humans were used to generate as many different macrophages as possible using various growth factors. While these mature, certain genes are activated. With genome-wide transcriptome analysis and complex bioinformatic approaches, we obtained a type of fingerprint for each macrophage, which showed us which genes in the cell were directly active. Using this genetic fingerprint, we were able to deduce - in patient samples from lung tissues - which combination of stimuli influenced the macrophage to develop in a particular direction. As macrophages play a role in many widespread diseases including, atherosclerosis, obesity, diabetes, asthma, Alzheimer's disease and cancer, this will be the dawn of new therapy options directly targeting the differentiation of macrophages.

Top 5 Publications

1. Xue J. et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* 40, 274-288, (2014).
2. Sommer D. et al. Efficient genome engineering by targeted homologous recombination in mouse embryos using transcription activator-like effector nucleases. *Nat Commun* 5, 3045, (2014).
3. De Nardo D. et al. High-density lipoprotein mediates anti-inflammatory reprogramming of macrophages via the transcriptional regulator ATF3. *Nat Immunol* 15, 152-160, (2014).
4. Nino-Castro A. et al. The IDO1-induced kynurenines play a major role in the antimicrobial effect of human myeloid cells against Listeria monocytogenes. *Innate Immun*, (2013).
5. Beyer M. et al. Repression of the genome organizer SATB1 in regulatory T cells is required for suppressive function and inhibition of effector differentiation. *Nat Immunol* 12, 898-907, (2011).

Immunology and Environment

The immune system, which functions to protect our body from microbial pathogens, is also continuously exposed to non-infectious environmental challenges including food constituents, allergens, environmental pollutants, or physical stress (UV light). Such environmental stimuli are known to influence physiological immune homeostasis but may also trigger inappropriate immune responses, such as allergies or autoimmunity.

Our research focuses on the function of innate immune cells – in particular dendritic cells and macrophages – at environmental interfaces in skin and the intestine. We investigate cellular receptors and soluble mediators involved in environmental sensing, using gene targeting technology and models of allergic and inflammatory diseases.

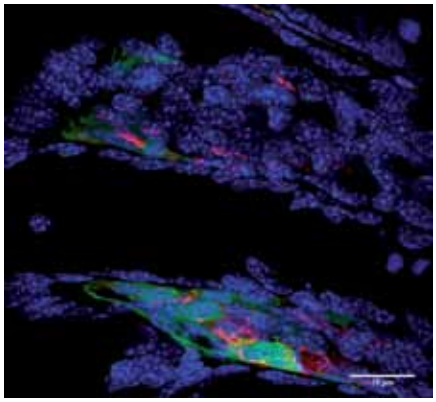
Prof. Irmgard Förster
Director

Dr. Heike Weighardt
Group Leader

Prof. Irmgard Förster (right) and Dr. Heike Weighardt preparing for a scientific presentation in the conference room of the LIMES Institute

Confocal image of the colonic mucosa showing AhRR (green) and MHC class II (red) expression. Nuclear DNA is stained in blue. (right).

Irradiation of skin-derived keratinocytes with UVB light (far right).



Weighardt Research Group

Our group investigates the interplay between environmental influences and innate immunity. The innate immune system does not only react to pathogens, but also to sterile danger signals of the environment such as UV radiation. MyD88 is a central adapter of the innate immune system involved in signaling of pattern recognition receptors and cytokines such as IL-1 or IL-18. We are analyzing the contribution of MyD88-induced signal transduction in allergic and inflammatory reactions of the skin. UVB irradiation also leads to immunosuppression and skin aging. Thus, we want to clarify if MyD88-induced signaling also influences environmentally induced skin aging, and if skin aging changes the functionality of the innate immune system.

Technology

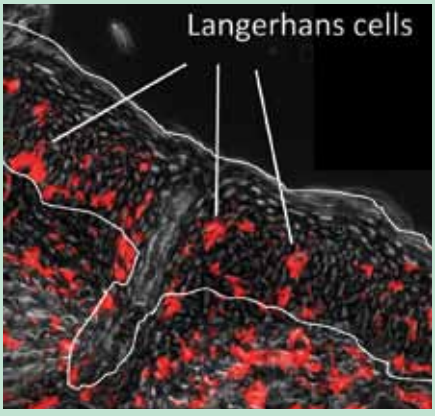
- Multi-color flow cytometry
- Cell migration assays
- Immunohistology
- Gene targeting technology
- Experimental models of atopic dermatitis, food allergy, inflammatory bowel disease and infection

HIGHLIGHT

Immunoregulation by CCL17-expressing dendritic cells in barrier organs.

Chemokines represent a subgroup of cytokines with chemotactic activity: they direct both constitutive and inducible migration of cells within the organism. We have shown that upon activation by microbial pathogens as well as allergens (such as contact sensitizers), the chemokine CCL17 is mainly produced by dendritic cells of the skin and intestine. CCL17 acts as a pro-inflammatory chemokine on antigen-specific T cells, enabling cross-presentation of soluble antigens and more efficient T cell-dendritic cell interactions. In addition, CCL17 enhances the migration of dendritic cells from the skin to the skin-draining lymph nodes in an autocrine or paracrine manner. Using pre-clinical disease models we observed that CCL17 promotes development of various inflammatory diseases, including atopic dermatitis and inflammatory bowel disease. Furthermore, CCL17-expressing cells often show activation of the aryl hydrocarbon receptor (AhR), a sensor of small environmental chemicals. We could demonstrate that dendritic cells and macrophages located at environmental interfaces strongly upregulate expression of the AhR repressor, a feedback regulator of the AhR. Our current research addresses the function of AhR/AhRR activation in immune cells of barrier organs in the context of local responses to environmental stimuli.

Skin section stained for MHC class II (red) after induction of dermatitis. In the absence of CCL17, Langerhans cells fail to migrate out of the inflamed epidermis.



Top 5 Publications

1. Globisch T, Steiner N, Fülle L, Lukacs-Kornek V, Degrandi D, Dresing P, Alferink J, Lang, Pfeffer K, Beyer M, Weighardt H, Kurts C, Ulas T, Schultze JL and Förster I. 2014. Cytokine-dependent regulation of denditic cell differentiation in the splenic microenvironment. *Eur J Immunol,* 44, 500-510.
2. Tigges J*, Weighardt H*, Wolff S, Götz C, Förster I ... and Fritsche E. 2013. Aryl hydrocarbon Receptor Repressor (AhRR) Function Revisited: Repression of CYP1 activity in human skin fibroblasts is not related to AhRR expression, *J Invest Dermatol,* 133, 87-96. *Equal contribution
3. Stutte S, Quast T, Gerbitzki N, Savinko T, Novak N, Reifenberger J, Homey B, Kolanus W, Alenius H and Förster I. 2010. Requirement of CCL17 for CCR7- and CXCR4-dependent migration of cutaneous dendritic cells. *Proc Natl Acad Sci* USA 107: 8736-41.
4. Alferink J, Lieberam I, Reindl W, Behrens A, Weiß S, Hüser N, Gerauer K, Ross R, Reske-Kunz A, Ahmad-Nejad P, Wagner H and Förster I. 2003. Compartmentalized production of CCL17 in vivo: strong inducibility in peripheral dendritic cells contrasts selective absencefrom the spleen. *J Exp Med*, 197, 585-599
5. Takeda, K*, Clausen BE*, Kaisho T, Tsujimura T, Terada N**,** Förster I* and Akira S*. 1999. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity* 10, 39-49. *Equal contribution

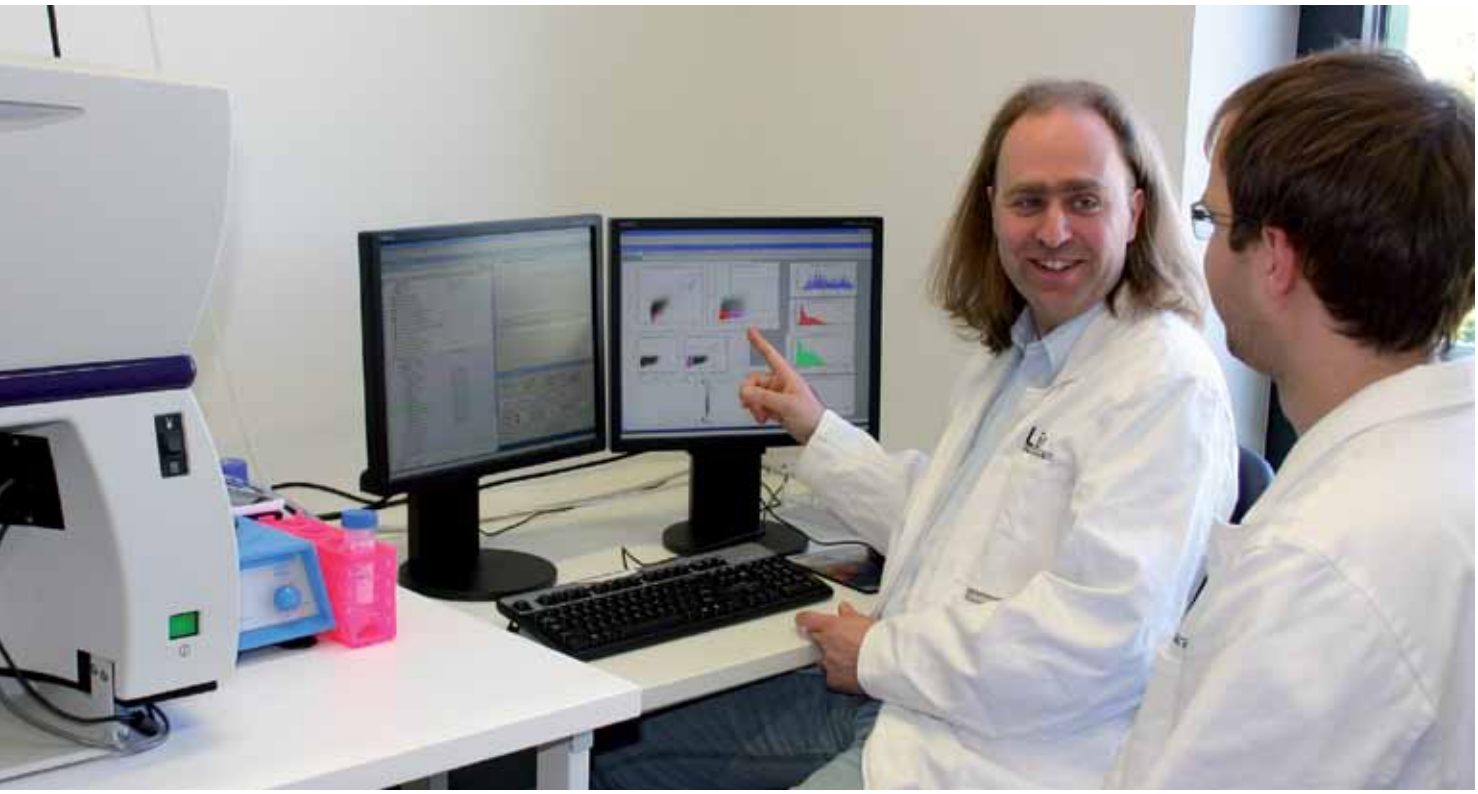
Cellular Immunology

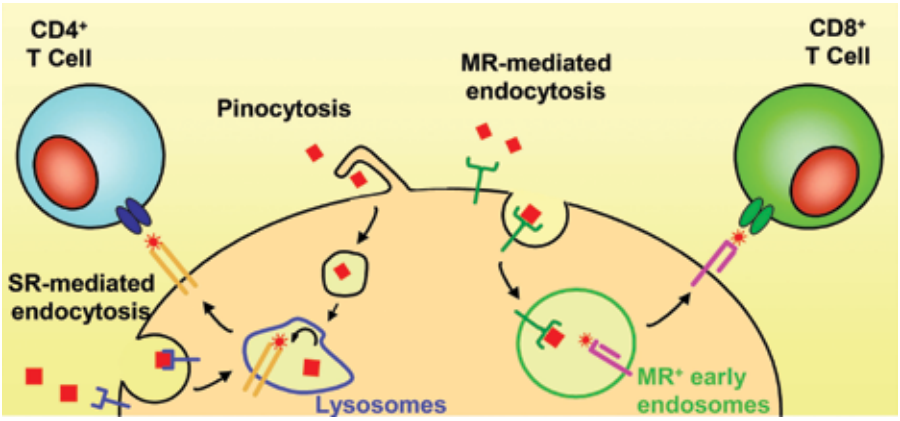
The Burgdorf group focusses on the molecular mechanisms underlying antigen cross-presentation and on the induction of T cell tolerance. Of special interest to the group is the role of endocytosis receptors in these processes.

Current research topics include, 1) the molecular mechanisms of antigen translocation into the cytosol - an important prerequisite of cross-presentation thought to be mediated by members of the ER-associated degradation (ERAD) machinery, 2) the recruitment of these ERAD proteins towards antigen-containing endosomes and their regulation by pro-inflammatory stimuli and 3) the molecular mechanisms of T cell tolerance induced by direct interaction of T cell surface proteins with endocytosis receptors on antigen-presenting cells.

Prof. Sven Burgdorf
Laboratory Head

Prof. Sven Burgdorf and PhD student, Matthias Zehner discussing the flow-cytometric analysis of individual antigen-containing endosomes.





The role of endocytosis receptors in antigen presentation: Antigens internalized by pinocytosis or Scavenger Receptor (SR)-mediated endocytosis are rapidly targeted towards lysosomes for presentation on MHC II molecules. Antigens internalized by the Mannose Receptor (MR) are targeted towards distinct endosomes for cross-presentation.

Molecular mechanisms of cross-presentation

Antigens internalized by the Mannose Receptor need to be translocated into the cytosol for proteasomal degradation. Subsequently, antigen-derived peptides are transported back into the endosomes for loading onto MHC I molecules.

Current Projects

- Export of antigens out of the endosomes into the cytoplasm
- Proteasomal degradation of antigens for cross-presentation
- Transport of ER components towards endosomes
- Influence of endotoxins on the molecular mechanisms of cross-presentation
- Induction of T cell tolerance and the regulatory role of endocytic receptors
- Influence of endocytic receptors on the activation status of dendritic cells

Technology

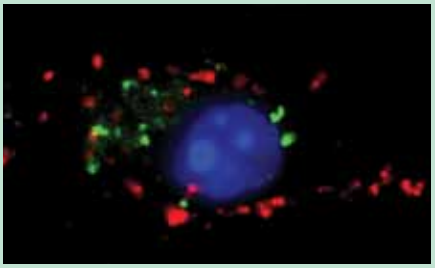
- Flow cytometry
- Analysis of antigen cross-presentation by proliferation assays and ELISA
- Cytotoxicity assays
- Isolation and flow-cytometric analysis of antigen-containing endosomes

Research

HIGHLIGHT

The mechanisms regulating whether internalized antigens are presented on MHC I or MHC II molecules, and therefore, what kind of immune response is induced by a specific antigen, were unknown for a long time. In a recent study, we could demonstrate that this decision is determined by the mechanism of antigen uptake. Whilst pinocytosis and scavenger receptor (SR)-mediated endocytosis introduce the model antigen ovalbumin (OVA) into lysosomes for presentation exclusively on MHC II molecules, the mannose receptor (MR) routed the antigen into stable early endosomes. In these endosomes, the antigen was rescued from rapid lysosomal degradation and was processed only for presentation on MHC I, demonstrating that antigens intended for presentation on MHC I or MHC II molecules are internalized by distinct endocytosis mechanisms and are targeted into different cellular organelles.

Processing for cross-presentation requires antigen transport from the endosomes into the cytosol for proteasomal degradation. In a recent study, we could demonstrate that this transport is mediated by members of the ER-associated degradation (ERAD) machinery. We showed that the recruitment of these ERAD proteins towards antigen-containing endosomes is initiated after ligand binding to the endocytosis receptor and is highly regulated by the inflammatory state of the dendritic cells.



SR- (green) and MR-internalized (red) antigens in different cellular compartments.

Top 5 Publications

1. Zehner, M. et al. (2012). Intraendosomal flow cytometry: a novel approach to analyze the protein composition of antigen-loaded endosomes. *Eur J Immunol* 42, 2187-2190
2. Zehner, M. et al. (2011). Mannose receptor polyubiquitination regulates endosomal recruitment of p97 and cytosolic antigen translocation for cross-presentation. *Proc Natl Acad Sci USA* 108, 9933-9938
3. Burgdorf, S. et al. (2010). Steady-state cross-presentation of OVA is mannose receptor-dependent but inhibitable by collagen fragments. *Proc Natl Acad Sci USA* 107, E48-49
4. Burgdorf, S. et al. (2008). Spatial and mechanistic separation of cross-presentation and endogenous antigen presentation. *Nat Immunol* 9, 558-566
5. Burgdorf, S. et al. (2007). Distinct pathways of antigen uptake and intracellular routing in CD4 and CD8 T cell activation. *Science* 316, 612-616

Biochemistry & Cell Biology of Lipids

Lipids are central for life, both at the level of a single cell and of complex organisms. While in the past research in Cell Biology has focussed on proteins and their functions, the importance of lipids as active players has attracted much interest in recent years. A key starting observation is the fact that the cellular lipid pool consists of several hundred individual lipid species that are different in hydrophobic side chains and hydrophilic head groups. The challenge is to understand this amazing diversity with respect to organization and function in the context of a living cell.

Our group is focused on the regulation of lipid metabolism, particularly on the specialized neutral lipid storage organelle, the lipid droplet.

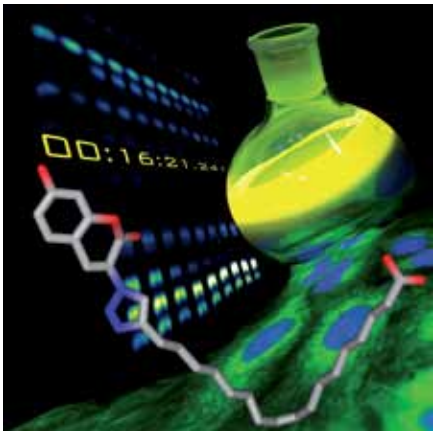
Prof. Christoph Thiele
Director

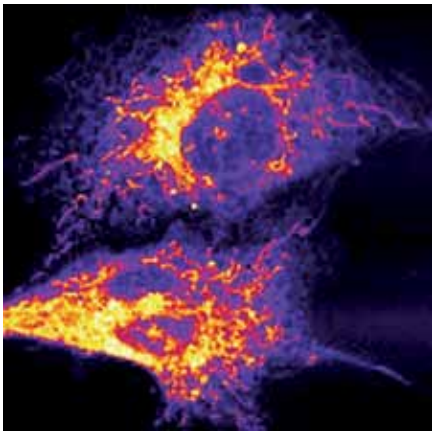
Dr. Lars Kuerschner
Group Leader

Prof. Christoph Thiele (right) and Dr. Lars Kuerschner (left) combine Chemistry, Cell Biology and Molecular Medicine in their research

An artistic illustration of how lipid metabolism can be followed by fluorescence microscopy or chromatographic lipid analysis employing a alkyne lipids and the CLiCK reaction with a fluorescent reporter molecule. (Right).

Endothelial cells incubated with a polyene ether lipid show a prominent staining of mitochondria as observed by two-photon fluorescence microscopy. (Far right)





Technology

To answer open questions in the lipid field we have developed innovative technologies that we apply to study lipid functions in living cells:

- Click-chemistry for lipid detection
- Alkyne lipid tracers
- Fluorescent lipid dye LD540
- Fluorescent Polyene-lipids
- Photoactivatable lipids
- Photoactivatable amino acids
- High-throughput screening
- *In vivo* and *in vitro* enzyme assays

These technologies have been made available to colleagues in the field and have been used worldwide.

Kuerschner Junior Group

In Cell Biology, microscopy has proven an invaluable technique. Fluorescence microscopy of living cells yields valuable information on dynamic processes, while electron microscopy provides the highest resolution data. We are working on the development of these methods for lipid tracing. In combination with biochemical, spectroscopic, cell and molecular biological techniques, we apply lipid microscopy to study lipid metabolism in various cells and organs, especially the brain.

Of special interest to our laboratory are the cell or tissue specific characteristics in lipid metabolism and how these effect other cells or organs in the body.

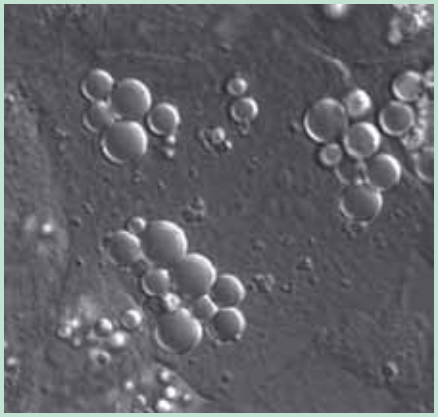
HIGHLIGHTS

What makes fat cells different?

Fat cells (adipocytes) are the major storage site for fat in mammals and many other organisms. These cells are specialized to convert sugar into fat and store it together with the fat from other sources in intracellular lipid droplets.

What is the molecular basis for these special acitivities? When we compared fatty acid metabolism of adipocytes and other cell types by metabolic tracing, it appeared that differences were rather subtle and more quantitative than qualitative: fat cells took up more fatty acid and made relatively more fat from it than other cells (see Publications 3 and 4).

Recently, after introducing a novel labeling technology with access to a much broader selection of molecular tracers, we saw a very different picture: fat cells convert fatty acids of any lenght into fat, other cells only use a small set of long chain fatty acids (2).



An adipocyte with large lipid droplets (shadow cast discs) observed by DIC-microscopy.

Yet, we do not know the molecular basis of this difference. But with our new tools we are well equipped to search for it. With the tools of biochemistry, we study uptake of fatty acids, intracellular metabolism to form fat, and its deposition in lipid droplets. We use affinity labeling techniques (5) and click-imaging (1) to learn about lipid-interacting proteins and about intracellular dynamics of lipids.

And finally, we study lipid storage in the context of tissues and organs, e.g. in liver, and its interaction with the immune system.

Top 5 Publications

1. Kuerschner L & Thiele C. (2014). Multiple bonds for the lipid interest. *Biochim Biophys Acta.*
2. Thiele C, …& Kuerschner L. (2012). Tracing fatty acid metabolism by click chemistry. *ACS Chem Biol, 7*(12), 2004-2011.
3. Kuerschner L, Moessinger C & Thiele C. (2008). Imaging of lipid biosynthesis: how a neutral lipid enters lipid droplets. *Traffic, 9*(3), 338-352.
4. Kuerschner L, …& Thiele C. (2005). Polyene-lipids: a new tool to image lipids. *Nat Methods, 2*(1), 39-45.
5. Thiele C, …& Huttner WB. (2000). Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. *Nat Cell Biol, 2*(1), 42-49.

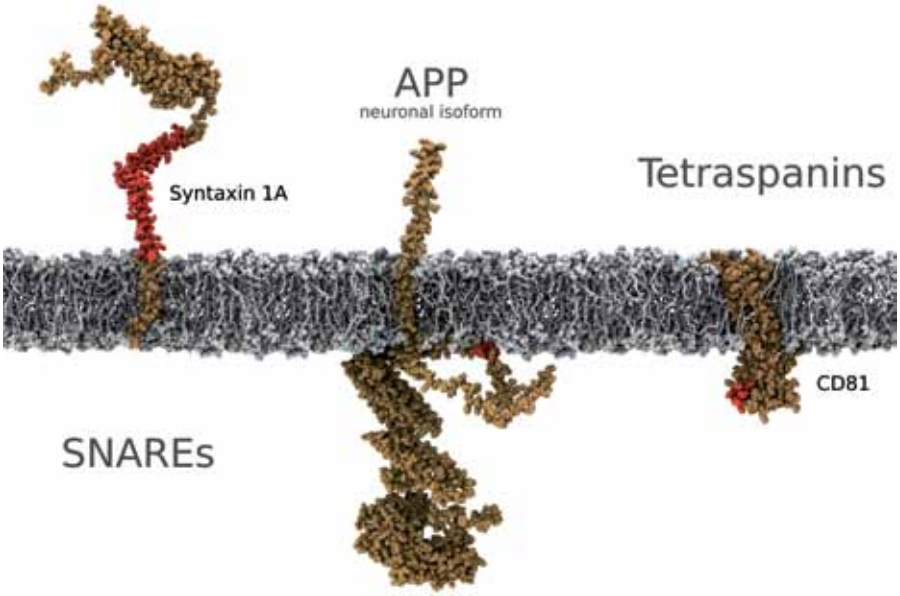
Membrane Biochemistry

Cells are the smallest units of life: their border is defined by a thin cell membrane constituting less than 1% of the cell volume. Though limited in space, manifold biological processes, for example transport, signaling and membrane trafficking events of vesicles, proteins and even lipids, occur at this plasma membrane. Since the 70's it has been assumed that biological membrane constituents mix randomly. However, in recent years many findings demonstrated that plasma membrane proteins organize in submicrometer-sized domains or clusters, of which we are just beginning to understand the anatomy, composition, dynamics and functions.

We employ a combination of biochemical and biophysical approaches to further study the properties and functions of plasma membrane proteins.

Prof. Thorsten Lang
Laboratory Head

Prof. Thorsten Lang (middle) with PhD student, Elisa Merklinger (left) and Dr. Thomas Schmidt (right).



Membrane proteins of interest, from left to right: syntaxin 1A (SNARE protein mediating membrane fusion during neurotransmission), Amyloid Precursor Protein (plays a role in Alzheimer´s disease) and CD81 (member of the tetraspanin familiy involved in the entry of several pathogens). Red indicates protein regions with a key function in membrane domain formation.

Micropatterning of membrane proteins

We investigate micropatterning mechanisms of membrane proteins and their functional consequences. Our hypothesis is that several mechanisms, including protein-protein interactions, lipid phase separation and interactions with cytosolic components and the extracellular matrix, work independently or in conjunction to form membrane protein clusters. Another aim is to understand the biological roles of protein clusters: we assume that membrane protein clustering influences the biochemical pathway of membrane protein complex formation and the stability of certain membrane protein complexes, and that the function of clustering is to separate biological processes that work in parallel in the plasma membrane.

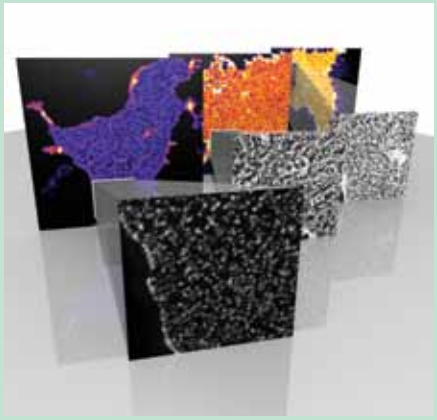
Technology

- Plasma membrane sheets
- Total internal reflection fluorescence (TIRF) microscopy
- Fluorescence recovery after photobleaching (FRAP)
- Superresolution microscopy

HIGHLIGHT

Using the protein CD81, we identified a key step in CD81 tetraspanin microdomain formation for which a small alpha-helical domain, located within the large extracellular loop of the protein, is essential (Homsi Y, et al. 2014. *Biophys J* 107(1), 100-113).

The observations made in this study have two implications: first, as the step is independent of strong binary interactions and overexpression leads to more and not larger domains, the findings support a concept explaining microdomain formation on physicochemical principles that generate cluster phases. Second, as CD81 plays a role in the entry of several pathogens, blocking pathogen entry by interfering with such small extracellular sequences is an attractive concept.



Colored pictures are low resolution confocal scans taken prior to superresolution imaging of the organization of CD81. From this experiment it was concluded that overexpression of CD81 generates more and not larger CD81 microdomains (Right).

Top 5 Publications

1. Homsi Y, Schloetel JG, Scheffer KD, Schmidt TH, Destainville N, Florin L and Lang T. (2014). The Extracellular δ-Domain is Essential for the Formation of CD81 Tetraspanin Webs. *Biophys J* 107(1), 100-113.
2. Schreiber A, Fischer S and Lang T. (2012). The amyloid precursor protein forms plasmalemmal clusters via its pathogenic amyloid-beta domain. *Biophys J, 102*(6), 1411-1417.
3. Zilly FE, Halemani ND, Walrafen D, Spitta L, Schreiber A, Jahn R and Lang T. (2011). Ca2+ induces clustering of membrane proteins in the plasma membrane via electrostatic interactions. *EMBO J, 30*(7), 1209-1220.
4. Sieber JJ, Willig KI, Kutzner C, Gerding-Reimers C, Harke B, Donnert G, Rammner B, Eggeling C, Hell SW, Grubmuller H and Lang T. (2007). Anatomy and dynamics of a supramolecular membrane protein cluster. *Science, 317*(5841), 1072-1076.
5. Sieber JJ, Willig KI, Heintzmann R, Hell SW and Lang T. (2006). The SNARE motif is essential for the formation of syntaxin clusters in the plasma membrane. *Biophys J, 90*(8), 2843-2851.

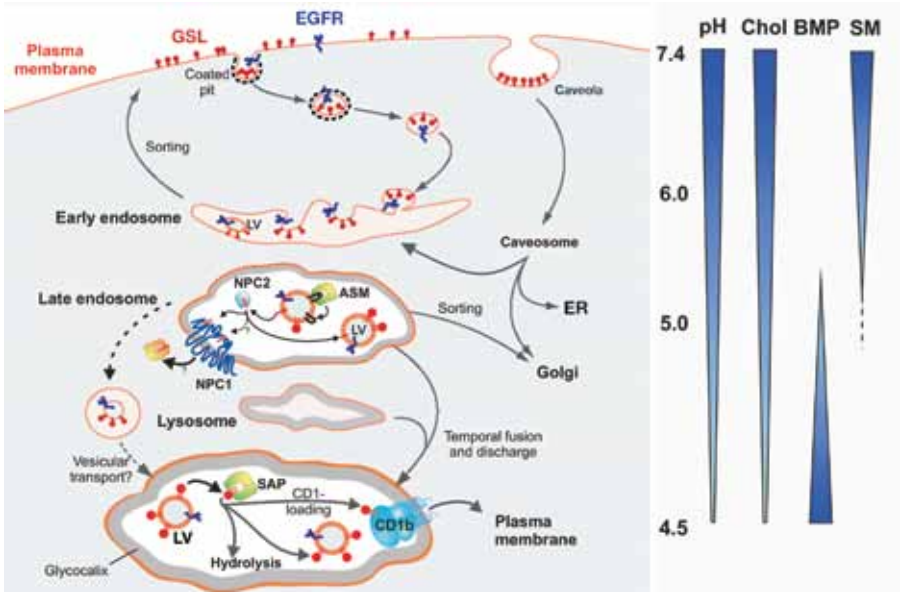
Lipid Biochemistry

Our group is interested in the biochemistry of sphingolipids and the molecular pathology of sphingolipidoses. (Glyco)sphingolipids (GLS) are stabilizing components of neuronal plasma membranes. For turnover they reach intraendolysosomal vesicles. Since lipids are not easily accessible to the water-soluble lysosomal hydrolases they need lipid transfer proteins, SAPs (Sap A, B, C, D and the GM2 activator protein (GM2AP)), to present them to the catabolic enzymes.

In the past, we clarified the molecular basis of several inherited sphingolipid storage diseases such as Tay-Sachs and Sandhoff disease, AB and B1 variant of GM2-gangliosidosis, Farber, Niemann-Pick disease and prosaposin deficiency. Current studies focus on (1) delineating the role of SAPs and lysosomal lipids in the development of these diseases, (2) investigating the role of the multifunctional glycoproteins and developing assays for uncompromised monitoring of their capabilities to mediate intervesicular lipid transfer and membrane fusion.

Prof. Konrad Sandhoff
Laboratory Head

Prof. Konrad Sandhoff and Dr. Susi Anheuser discussing results.



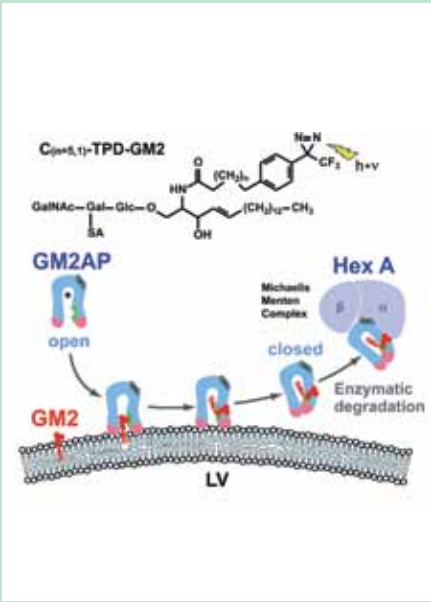
Topology of lysosomal sphingolipid catabolism and membrane digestion: *We identified luminal vesicles (LVs) as platforms for membrane and lipid degradation. At late endosomes their sphingomyelin is degraded by acid sphingomyelinase (ASM), facilitating the removal of cholesterol (an inhibitor for lysosomal GSL catabolism) by NPC2 and NPC1. In lysosomes, the cholesterol poor LVs and their lipids are digested by hydrolases and SAPs. Inherited defects of any of these proteins cause fatal diseases with lipid and membrane storage or loss of the water permeability barrier in the skin. (GSL: Glycosphingolipids, NPC: Niemann-Pick protein type C, SAP: sphingolipid activator protein).*

Technology

- Chemical synthesis of complex lipids
- Expression and purification of proteins
- Metabolic studies in cultured cells and Drosophila larvae
- Lipid analysis (thin layer chromatography and mass spectrometry)
- Assays to study lipid transfer and vesicle fusion

HIGHLIGHT

During analysis of the above mentioned sphingolipid storage diseases, we identified proteins, enzymes and SAPs required for catabolism of storage material. In the case of Tay-Sachs disease, these were the lipid binding and transfer protein, GM2AP, and the heterodimeric hydrolase hexosaminidase A, mediating the degradation of vesicle bound GM2. Mutations in the three genes coding for any of these essential proteins can cause fatal neurodegenerative diseases. However, *in vitro* reconstitution experiments using neutral liposomes yielded only negligible catabolic rates. Physiologically relevant rates, however, were obtained only after introducing anionic phospholipids (BMP, PG, PA etc.) into the substrate carrying membranes, stimulating catabolic rates up to 100 fold. On the other hand, the membrane stabilizing lipid of the plasma membrane, cholesterol (and to a small extent sphingomyelin), is a strong inhibitor of GM2 degradation (Sandhoff, K. (2013) *Biochem Soc Trans*, 41, 1562-1568; Anheuser et al. manuscript in prep.).



Right: Mechanism of GM2AP-liftase mediating ganglioside GM2 degradation by hexosaminidase A (Hex A) at intraendosomal vesicles (LV). The synthesis of the photosensitive GM2 analog served to clarify the reaction.
** hydrophobic, lipid binding cavity; pink hydrophobic feet for membrane binding*

Top 5 Publications

1. Schwarzmann G, Arenz C, Sandhoff K (2014). Labeled chemical biology tools for investigating sphingolipid metabolism, trafficking and interaction with lipids and proteins. *Biochim Biophys Acta*, 1841, 1161-73.
2. Breiden B, Sandhoff K (2014). The role of sphingolipid metabolism in cutaneous permeability barrier formation. *Biochim Biophys Acta*, 1841, 441-52.
3. Abdul-Hammed M, Breiden B, Adebayo MA, Babalola JO, Schwarzmann G, Sandhoff K (2010). Roles of endosomal membrane lipids and NPC2 in cholesterol transfer and membrane fusion. *J Lipid Res*, 51, 1747-60.
4. Kolter T, Sandhoff K (2005). Principles of lysosomal membrane digestion-stimulation of sphingolipid degradation by sphingolipid activator proteins and anionic lysosomal lipids. *Annu Rev Cell Dev Biol*, 21, 81-103.
5. Kolter T, Sandhoff K (1999). Sphingolipids -Their Metabolic Pathways and the Pathobiochemistry of Neurodegenerative Diseases. *Angew Chem Int Ed*, 38, 1532-68.

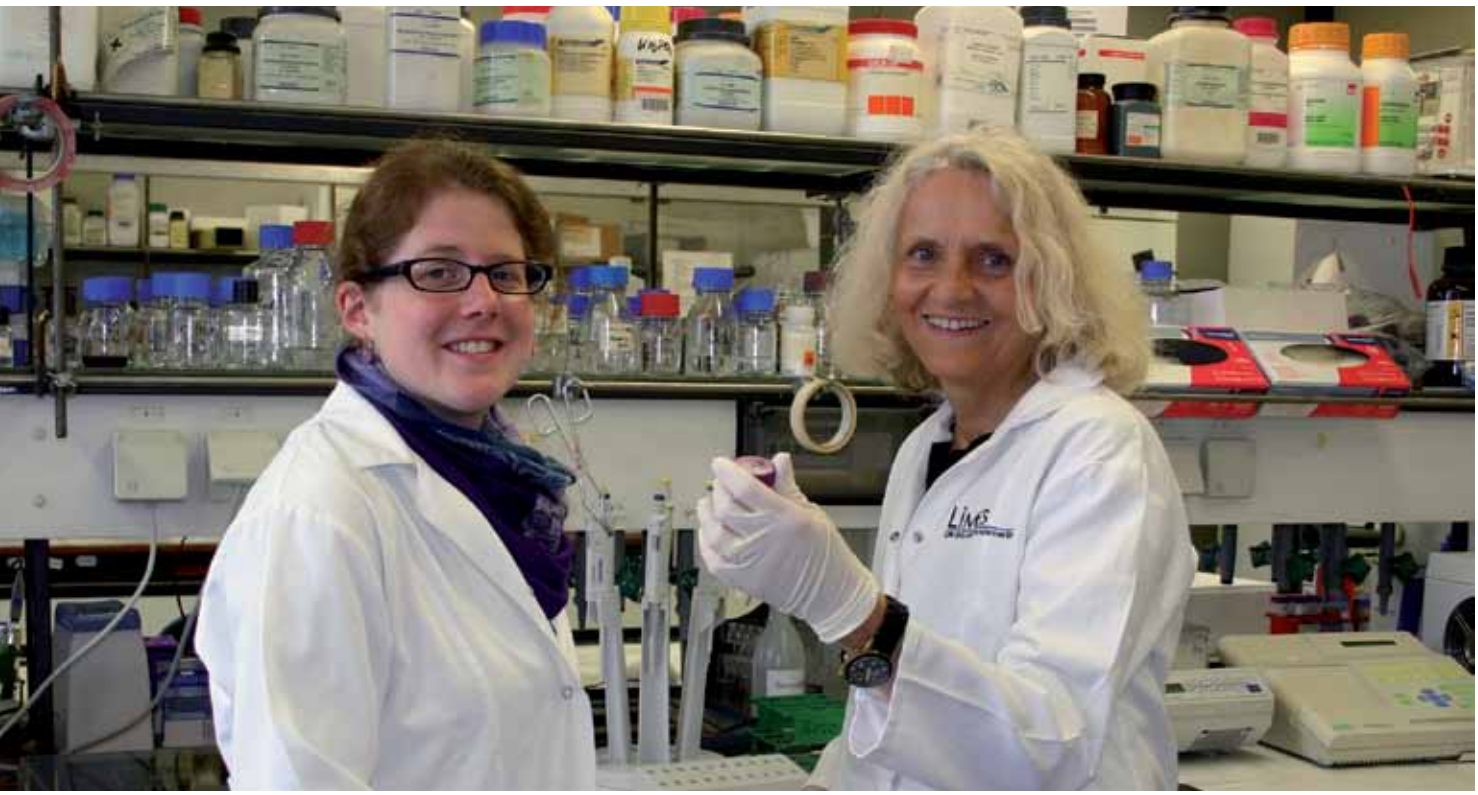
Lipid Biochemistry

In mammals, the brain exhibits the highest lipid content in the body next to adipose tissue. Complex sphingolipids are characteristic compounds of neuronal membranes. Vital neural functions including information flux and transduction occur along these membranes. It is therefore not surprising that neuronal function and survival is dependent on the metabolism of these lipids.

Our studies indicate that sphingosine-1-phosphate (S1P), a bioactive catabolic intermediate of all sphingolipids, represents a link between ganglioside metabolism and neurodegeneration in Alzheimer's disease. Verifying our hypothesis will open a new perspective in both prevention and therapy of Alzheimer's disease, by employing available tools to adequately interfere with sphingolipid metabolism.

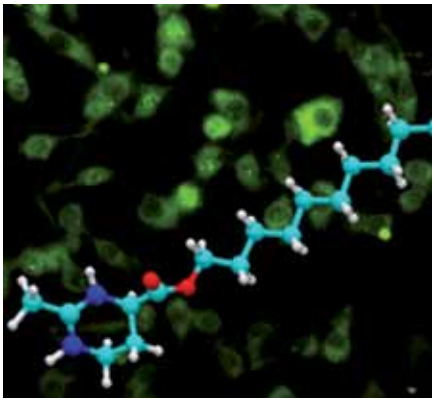
Dr. Gerhild van Echten-Deckert
Laboratory Head

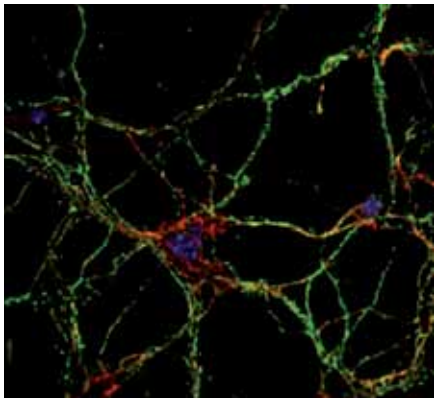
Dr. van Echten-Deckert and PhD student, Sabrina Reis are interested in investigating ectoines



(Right) Lauryl-ectoine: the lipid anchor improves the inflammation protective effect of ectoine in cultured macrophages.

(Far right) Confocal imaging of primary cultured cerebellar neurons. Immunostaining of presynaptic protein synaptophysin (green), nuclei (blue) and F-actin (red).





Research Focus

Although S1P promotes cell survival in peripheral tissues, increased S1P levels appear to be toxic in terminally differentiated primary neurons. Our results indicate a functional relation of S1P and the ß-amyloid precursor protein (APP). The accumulation of S1P in S1P-lyase deficient cells induces increased expression of APP and affects its proteolytic processing. We generated a conditional knockout mouse model with a specific deletion of S1P-lyase in neural tissue to study the role of this lipid in the brain.

We are also interested in the cell protective effect of ectoine, a natural tetrahydropyrimidine produced by aerobic bacteria to protect them against environmental stress. Others and we have shown that it also protects mammalian cells and tissues against different stressors. This protective effect was accompanied by a decrease in cellular ceramide levels. We recently reported that a lipid anchor substantially improves the inflammation protective effect of ectoine. We design and synthesize derivatives and test their inflammation protective effect in cultured macrophages.

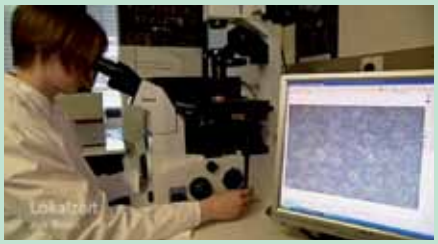
Technology

- Cell culture
- Immunoblotting
- Immunocytochemistry
- Chemical synthesis (ectoine derivatives)
- Metabolic studies in neurons
- Lipid analysis

Research

HIGHLIGHT

In the 1990s, we discovered the neurotoxic effect of a synthetic, metabolically stable analogue of S1P. We demonstrated that the short-lived natural counterpart affects the same cellular pathways, yet in a more transient and hence less effective manner. The generation of an S1P-lyase-deficient mouse model enabled us on the one hand to show that S1P is indeed neurotoxic and on the other hand to establish conditions which are essential for this neurotoxic effect. Intriguingly, S1P-induced neurotoxicity resembled that of Aβ in Alzheimer's disease. Both, S1P and Aβ induce an aberrant reactivation of cell cycle events and activation of cyclin-dependent kinase5 (CDK5). In addition, S1P- and Aβ-induced neurotoxicity involves calpain and procaspase-12 activation by disruption of ER calcium homeostasis but not by membrane- or mitochondria-targeted signals. We also assessed a correlation between S1P accumulation and hyperphosphorylation of tau in primary cultured neurons. Neuroanatomical studies revealed that neurons with abundant S1P-lyase expression are those, which degenerate first in S1P-lyase-deficient mice. Recently we showed that regardless of a rather minor impact of S1P-lyase deficiency on sphingolipid and cholesterol levels, the amount of cholesterol esters increased considerably in brains of S1P-lyase-deficient mice. The direct correlation between cholesterol ester levels and the production of Aβ reported about a decade ago highlights the potential pathophysiological relevance of S1P in Alzheimer's disease.



Dr. Nadine Hagen-Euteneuer studying the morphology of primary cultured neurons.

Top 5 Publications

1. Wedeking A, Hagen-Euteneuer N, Gurgui M, Broere R, Lentzen Tolba RH, Galinski E, van Echten-Deckert G. (2014). A Lipid Anchor Improves the protective Effect of Ectoine in Inflammation. *Curr Med Chem* 21(22):2565-72
2. van Echten-Deckert G, Walter J. (2012). Sphingolipids: Critical players in Alzheimer's disease. Prog Lipid Res 51: 378-393
3. Hagen N, Hans M, Hartmann D, Swandulla D, van Echten-Deckert G. (2011). Sphingosine-1-phosphate links sphingolipid metabolism to neurodegeneration via a calpain-mediated mechanism. *Cell Death Differ*. 18: 1356-1365
4. Hagen N, Van Veldhoven PP, Proia RL, Park H, Merill HA.Jr, van Echten-Deckert, G. (2009). Subcellular origin of sphingosine-1-phosphate is essential for its toxic effect in lyase-deficient neurons. *J Biol Chem* 284: 11346-53
5. Dragusin M, Wehner S, Kelly S, Wang E, Merrill AH.Jr, Kalff JC, van Echten-Deckert G. (2006). Effects of sphingosine-1-phosphate and ceramide-1-phosphate on rat intestinal smooth muscle cells: implications in postoperative ileus. *The FASEB J* 20, 1930-1932

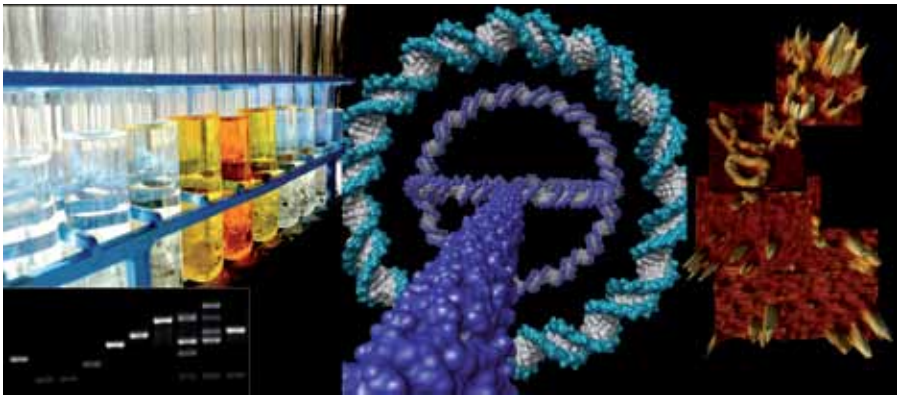
Chemical Biology

We are interested in the functions of guanine nucleotide exchange factors (GEFs), a group of proteins known as activators of small G proteins. Our primary research focuses on several GEFs for ARF, Rac and Rab GTPases for which no inhibitors are currently available. Our goal is to identify inhibitors that allow us to characterize the cellular functions of the corresponding GEFs.

Areas of interest include: the search for active drug-like compounds by aptamer displacement assays, robot supported high-throughput screening, ribozyme- and aptamer research, in vitro selection and evolution of combinatorial nucleic libraries (SELEX-Technology), and DNA nanotechnology.

Prof. Michael Famulok
Director

Prof. Michael Famulok (right) and PhD student, Finn Lohmann, discussing data obtained by mass spectrometry. Photo by Dr. Sven Freundenthal



Artistic illustration of methods employed in DNA nanotechnology: atomic force microscopy (AFM), spectroscopic functional assays, agarose gel electrophoresis and modelling of DNA sequences.

DNA Nanoarchitectures

An exciting new and rapidly emerging interdisciplinary field of research that merges the life- and engineering sciences is the discipline of Synthetic Biology. To this end, we have recently started to combine our expertise in aptamer research with DNA nanotechnology. We constructed a rotaxane molecule made from DNA. Because of DNA's programmability and structural robustness, DNA rotaxanes with interlocked, yet free-to-move parts are an exciting new approach that promise to be useful components in molecular machines. They open a new field that conjoins DNA nanotechnology and interlocked molecular architectures, providing tools that will greatly impact the fields of synthetic biology and nanorobotics.

Technology

- Chemical biology approaches
- Aptamer research
- High-throughput compatible screening assays
- Chemical Synthesis
- DNA nanostructures
- Genetic models in Drosophila and mouse

HIGHLIGHT

Chemical Genetics: Aptamer-displacement assays for screening of small molecules inhibitors of target proteins

We have established high-throughput compatible screening assays that allow conversion of the inhibitory profile of an aptamer into drug-like inhibitors. We have used these approaches to identify small organic molecules from compound collections (currently > 12,500) that displace an aptamer-protein interaction specifically, and adopt the aptamer's modulatory properties. Similarly, we can use allosteric, aptamer-regulated ribozymes for the same purpose.

These approaches provide access to all-purpose, target-independent assay systems for the identification of small molecules. We apply these compounds in various cellular systems and in model organisms (Drosophila, mouse) for the functional elucidation of target proteins. For example, to elucidate the effect of a compound in certain signaling pathways, we collaborate in analyzing compound activities by genome-wide transcriptional profiling using DNA array technology. We continuously expand our screening platform (consisting of pipetting robots, diverse fluorescence-readers, and drug-like compound collections), with the aim to increase applications of these chemical genetics approaches in our future research endeavors. Study published in Bill A, et al., Cell 2010, 143, 201-211.

Prof. Michael Famulok and Finn Lohmann setting up a HPLC run. Photo by Dr. Sven Freudenthal

Top 5 Publications

1. Vinkenborg JL, Mayer G, and Famulok M. (2012). Aptamer-based affinity labeling of proteins. *Angew Chem Int Ed*, 51, 9176-9180.
2. Bill A, Schmitz A, Albertoni B, Song J-N, Heukamp LC, Walrafen D, Thorwirth F, Verveer JP, Zimmer S, Meffert L, Schreiber A, Chatterjee S, Thomas RK, Ullrich RT, Lang T, and Famulok M. (2010). Cytohesins are cytoplasmic ErbB receptor activators. *Cell*, 143, 201-211.
3. Ackermann D, Schmidt TL, Hannam JS, Purohit CS, Heckel A, and Famulok M. (2010). A double-stranded DNA rotaxane. *Nat Nanotechnol*, 5, 436-442.
4. Hafner M, Schmitz A, Grüne I, Srivatsan SG, Paul B, Kolanus W, Quast T, Kremmer E, Bauer I, and Famulok M. (2006). Inhibition of cytohesins by SecinH3 leads to hepatic insulin resistance. *Nature*, 444, 941-944.
5. Hartig JS, Najafi H, Grüne I, Yan A, Ellington AD, and Famulok M. (2002). Protein-dependent ribozymes report molecular interactions in real-time. *Nat Biotechnol*, 20, 717-722.

Chemical Biology & Chemical Genetics

How can aptamer technology be used to understand biological phenomena? How can nucleic acids be exploited as drugs or drug targets to develop novel diagnostic and therapeutic applications? What are the underlying principles of nucleic acid evolution in the test tube and how can these be employed to generate novel compounds? To address these and other questions, we utilize in vitro selection procedures, in combination with chemical, biochemical, immunological, neurological and molecular cell biology techniques.

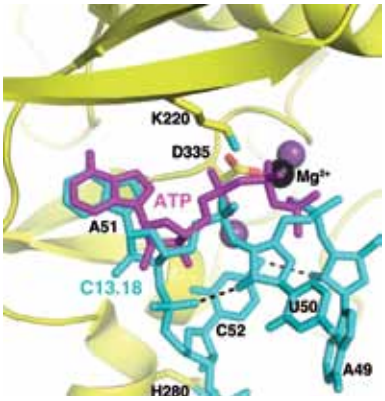
Our overall goal is to develop molecular tools that allow us to investigate biological systems with high precision. These tools also represent the foundation for implementing novel diagnostic and therapeutic strategies.

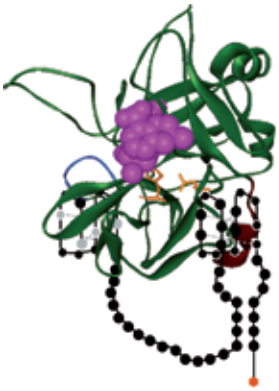
Prof. Günter Mayer
Director

Prof. Günter Mayer (standing) discussing results with PhD students, Silvana Hassel (left) and Fabian Tolle (right). Photo by Shannon Smith

Co-crystal structure of an aptamer (cyan) mimicking the binding of ATP (magenta) in the active site of the G-protein coupled receptor kinase 2 (GRK2, yellow). Tesmer VM et al. Structure, 2012 (Right)

Capturing thrombin (green)-argatroban (magenta) complexes by a bivalent aptamer (black) for measuring in patient concentrations of active thrombin. Müller et al. Angew Chem Int Ed, 2011 (Far right)





Identifying novel functional tools to address biomedical needs

Our aim is to identify sophisticated molecules that excert a specific function and utilize them to study biological systems. In this way we develop and investigate aptamers, riboswitches, small molecules and combinations thereof. Based on these compounds we seek to develop novel diagnostic and therapeutic strategies in the fields of tumour biology, haematology, bacterial infections and neurological disorders. We also develop novel methods to generate chemically modified aptamers, allowing precise control and modulation of their activites, e.g. targeting tumour tissues or other target structures depending on the presence of distinct molecular entities that are naturally not present in nucleic acids.

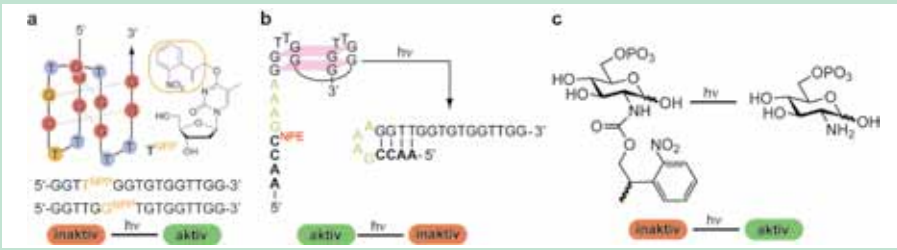
Technology

Our laboratory is an interdisciplinary research facility, equipped for performing research in the fields of molecular biology, microbiology, organic synthesis, and cell biology. The laboratory also has instrumentation to synthesize and characterize nucleic acids (e.g. DNA/RNA synthesizer, phosphorimager, HPLC) and to analyze the interaction of nucleic acids with target molecules (e.g. Biacore 3000, ITC, Microscale thermophoresis). Also, we have a cell culture laboratory for culturing eukaryotic cells, equipped with standard instruments (e.g. LS-microscope, flow cytometry) and access to an isotope laboratory to work with 32P-labelled nucleic acids.

HIGHLIGHT

Regulating nucleic acid function by light. We have long-standing experience in aptamer generation and characterization as well as their applications *in vitro* and in cellular studies. Recently, we have started to develop and investigate caged aptamers: these are equipped with photo-labile entities, such as o-nitrophenylethyl (NPE) and o-nitrophenylpropyl (NPP). We were the first group to introduce caged aptamers, whose conformation and function can be triggered by UV light (λ = 365 nm). According to our design principles, we could show that either

Approaches towards the light-regulation of nucleic acid activity.



activation **(a)** or inactivation **(b)** of aptamer function is possible by light-irradiation, thus making caged aptamers valuable tools to control biological processes via external light stimuli. Recently, we have extended this approach towards caged ligands of RNA molecules that control gene expression in bacteria so-called riboswitches **(c)**. Consequently, these caged ligands enable light-control of protein synthesis.

Top 5 Publications

1. Muller J, Becher T, Braunstein J, Berdel P, Gravius S, Rohrbach F, Oldenburg J, Mayer G and Potzsch B. Profiling of active thrombin in human blood by supramolecular complexes. *Angew Chem Int Ed*, 2011, 50, 6075-8.
2. Lünse CE, Schmidt M, Wittmann V, and Mayer G. Carba-sugars activate the glmS-riboswitch from Staphylococcus aureus. *ACS Chem Biol*, 2011, 6, 675-678.
3. Raddatz MSL, Dolf A, Endl E, Knolle P, Famulok M and Mayer G. Enrichment of cell-targeting and population-specific aptamers by fluorescent-activated cell sorting. *Angew Chem Int Ed*, 2008, 47, 5190-5193.
4. Müller J, Wulffen B, Pötzsch B, and Mayer G. Multi-domain targeting generates a high affinity thrombin-inhibiting bivalent aptamer. *ChemBioChem*, 2007, 8, 2223-2226
5. Heckel A and Mayer G. Light-regulated aptamer: An anti-thrombin aptamer with caged thymidine nucleobases. *J Am Chem Soc*, 2005, 127, 822-23.

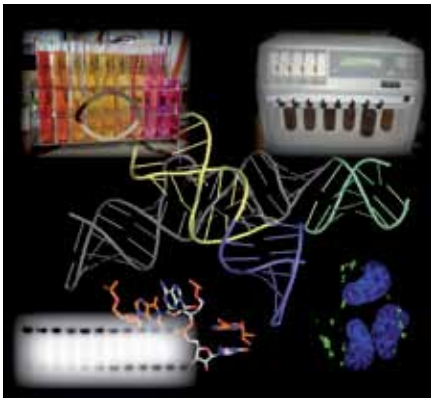
Functional RNA

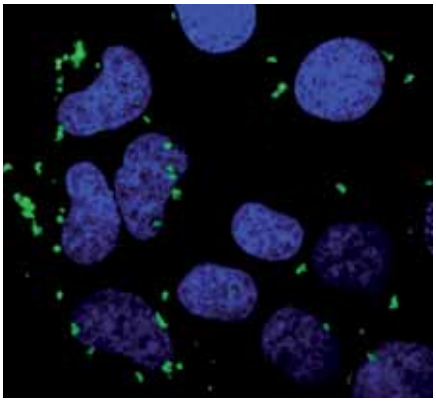
Only a small percentage of the mammalian genome encodes proteins but most of it is transcribed into RNA. Non-coding RNA molecules play major roles in transcriptional and translational regulation, mRNA processing, transport and localization, posttranslational modification and protein degradation and translocation. In all cases, their ability to fold into complex three-dimensional structures determines their function. Our research focuses on the investigation of functional RNA – the role of ribozymes, RNA-RNA interactions and the visualization of functional RNA molecules in cells.

We aim to expand the scope of RNA catalysis and detection of specific RNA molecules using a semi-synthetic approach to develop new RNA based catalysts and tools.

Dr. Stephanie Kath-Schorr
Laboratory Head

Dr. Stephanie Kath-Schorr and PhD student, Christof Domnick discussing novel tetrazine derivatives synthesized for their application in oligonucleotide labeling.





Far left: Investigation of ribozyme catalysis requires the combination of synthetic and biochemical methods. We perform solid phase RNA synthesis to incorporate modified nucleosides into RNA.

Left: iEDDA click reaction on synthetic RNA in mammalian cells using norbornene modified RNA and tetrazine-fluorophore conjugates visualized by confocal fluorescence microscopy. The RNA is localized to discrete foci in the cytoplasm (green spots).

RNA labeling

Effective attachment of reporter groups to RNA *in vitro* and as well as in cells is a prerequisite for our research on functional RNA molecules. Inverse electron demand Diels-Alder (iEDDA) cycloadditions are extremely useful tools for orthogonal labeling of biomolecules such as oligonucleotides or proteins in a cellular context. We develop novel tetrazine based fluorophor conjugates and alkene modified RNA nucleosides for copper-free iEDDA click chemistry on RNA (see Pyka AM, 2014 *Bioconjugate Chem* for further details).

Technology

- Organic synthesis
- Oligonucleotide synthesis
- RNA biochemistry
- Ribozyme catalysis

HIGHLIGHT

We are a junior research group located in the Chemical Biology & Medicinal Chemistry Unit at the LIMES Institute existing since January 2013 and currently funded by a Liebig-Fellowship of the Fonds der Chemischen Industrie. Being an interdisciplinary research group, we are combining both organic synthesis with biochemical methods to develop chemical tools for the detection and investigation of RNA functions *in vitro* and in cells.

One part of our research focuses on the investigation of catalytically active RNA molecules, termed ribozymes. We are interested in the function and role of naturally existing ribozymes, especially in human ribozymes. Here, questions regarding catalytic mechanisms as well as localization and abundance in cells are addressed.

Group members: (left to right) Christof Domnick, Katharina Kulikov, Dr. Stephanie Kath-Schorr and Frank Eggert.

We further extend our research towards the development of artificial ribozymes with novel functions. Only a few examples of evolved artificial ribozymes catalyzing reactions other than cleavage or ligation of phosphodiester bonds exist, thus, it is impossible to predict the limits of catalysis by RNA (and DNA). Here, we seek to employ a chemical approach to select for novel ribozyme functions.

Detection and localization of such functional RNA structures in cells provides a challenging task. However, progress could give new valuable insights into RNA localization, transport, degradation and RNA/RNA interaction. A further aim of our research group is to develop novel strategies to label sequence specifically folded and hence functional RNA molecules in mammalian cells using fluorescent RNA probes for detection.

Top 5 Publications

1. Pyka AM, Domnick C, Braun F, and Kath-Schorr S. (2014). Diels-Alder Cycloadditions on Synthetic RNA in Mammalian Cells. *Bioconjugate Chem.*, DOI: 10.1021/bc500302y, July 28 Epub ahead of print.
2. Kath-Schorr S, Wilson TJ, Li NS, Lu J, Piccirilli JA and Lilley DM. (2012). General acid-base catalysis mediated by nucleobases in the hairpin ribozyme. *J Amer Chem Soc, 134*(40), 16717-16724.
3. Ouellet J, Schorr S, Iqbal A, Wilson TJ and Lilley DM. (2011). Orientation of cyanine fluorophores terminally attached to DNA via long, flexible tethers. *Biophys J, 101*(5), 1148-1154.
4. Schorr S, Schneider S, Lammens K, Hopfner KP and Carell T. (2010). Mechanism of replication blocking and bypass of Y-family polymerase {eta} by bulky acetylaminofluorene DNA adducts. *Proc Natl Acad Sci USA, 107*(48), 20720-20725.
5. Schorr S and Carell T. (2010). Mechanism of acetylaminofluorene-dG induced frameshifting by polymerase eta. *ChemBioChem, 11*(18), 2534-2537.

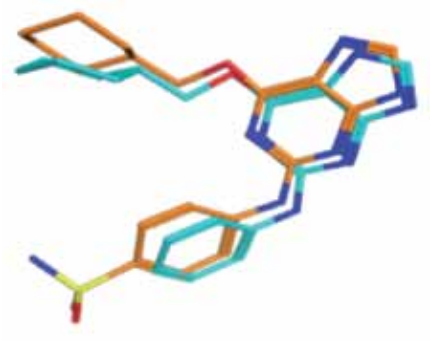
Chemoinformatics, Computational Medicinal Chemistry & Chemical Biology

How can small molecules be identified for therapeutic intervention or as probes to interrogate biological functions? How can biological targets be characterized? How can one systematically explore structure-activity relationships (SARs) and optimize chemical leads?

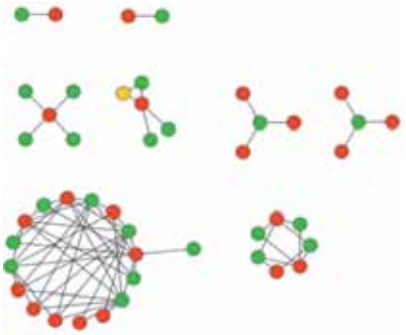
To address these and related questions, computational approaches play an increasingly important role. We develop computational methods for pharmaceutical research and chemical biology and apply these methodologies in collaborative projects with experimental groups including the pharmaceutical industry.

Prof. Jürgen Bajorath
Director

Prof. Jürgen Bajorath (right) with PhD student, Antonio de la Vega de Léon (left) and Masters student, Magdalena Zwierzyna (middle)



Bioactive conformations of two structurally analogous enzyme inhibitors are aligned. They are distinguished by only one functional group but have over 100-fold difference in potency. Such compound pairs are referred to as "activity cliffs". Activity cliffs reveal SAR determinants and are thus of high interest for medicinal chemistry.



Coordinated activity cliffs isolated from a data set of specifically active compounds and visualized in a network: compounds are represented by colored nodes (green: highly potent, red: weakly potent) and edges indicate the formation of activity cliffs; coordinated activity cliffs emerge as clusters.

Research

Concepts from computer science/informatics are adapted for the generation of computational methods to analyze and predict SARs and design novel active compounds. Predictive models of specific biological activities are developed using machine learning approaches. In addition, the entire universe of publicly available bioactive molecules and their targets is analyzed through large-scale compound data mining to elucidate molecular promiscuity patterns (the basis of polypharmacology) and establish ligand-based target relationships. Furthermore, activity landscapes of compound data sets and activity cliffs are systematically explored. For the analysis of activity landscapes and complex SARs, new visualization methods are developed.

Technology

- Compound data mining
- SAR visualization
- Machine learning
- Virtual screening
- Drug design

HIGHLIGHT

Composition and topology of activity cliff clusters formed by bioactive compounds. Stumpfe et al., *J Chem Inf Model,* 54, 451-461, 2014.

Activity cliffs were systematically extracted from all publicly available bioactive compounds on the basis of high-confidence activity data. A total of 20,080 activity cliffs were identified for 293 different target proteins. Only 769 of these activity cliffs were formed in isolation (i.e., by individual pairs of compounds); all others were formed in a coordinated manner. A global activity cliff network was generated that revealed 1303 differently sized clusters of coordinated activity cliffs. Surprisingly, 861 of these clusters could be assigned to only three basic topologies and extensions of these topologies. Activity cliff clusters are rich in SAR information and thus of prime interest for compound optimization efforts. Thus, the 1303 clusters of coordinated activity cliffs obtained from the global network provide an unprecedented knowledge base for medicinal chemistry. The study presents an instructive example for the potential impact of chemoinformatics on pharmaceutical research.

5 Recent Key Publications

1. Maggiora G, Vogt M, Stumpfe D, Bajorath J. Molecular similarity in medicinal chemistry. J Med Chem 57, 3186-3204, 2014.
2. Stumpfe D, Hu Y, Dimova D, Bajorath J. Recent progress in understanding activity cliffs and their utility in medicinal chemistry. J Med Chem 57, 18-28, 2014.
3. Gupta-Ostermann D, Shanmugasundaram V & Bajorath J. Neighborhood-based prediction of novel active compounds from SAR matrices. J Chem Inf Model 54, 801-809, 2014.
4. Hu Y & Bajorath J. How promiscuous are pharmaceutically relevant compounds? A data-driven assessment. AAPS J 15, 104-111, 2013.
5. Dimova D, Heikamp K, Stumpfe D & Bajorath J. Do medicinal chemists learn from activity cliffs? A systematic evaluation of cliff progression in evolving compound data sets. J Med Chem 56, 3339-3345, 2013.

Strategic Partners and Funding Networks

National and international partnerships and collaborations are key to our research success. We engage with government, universities, research institutions, and other organisations to facilitate linkages to world renowned experts and access to key technology platforms to maximise our research output. Local and regional networks are funded by the Germany Research Foundation (DFG), Germany's largest independent research funding organisation.

Cooperative Research Centers (SFBs)

Researchers of the LIMES Institute are linked through numerous local and regional research structures. These initiatives provide funding for collaborative research projects, seminars, career development and gender equality. The insititute's prinicipal SFBs are **SFB 645**, **SFB 704** and **TRR 83**. The institute is also a main partner of the Bonn's Excellence Cluster "**ImmunoSensation**".

SFB 645 - Speaker Prof. Hoch

An interdisciplinary research initiative of the University of Bonn, SFB 645 "Regulation and manipulation of information flow within dynamic protein and lipid environments" has been funded by the DFG since 2005. The initiative encompasses 20 research projects and 11 institutions with key objectives to: study the role of lipid metabolizing enzymes in controlling membrane composition and cellular metabolism; analyse the dynamics and organization of membrane proteins; and elucidate the molecular mechanisms that control membrane trafficking.

SFB 704 - Speaker Prof. Kolanus

Funded by the DFG since 2006, SFB 704 "Molecular Mechanisms and Chemical Modulation of Local Immune Regulation" is an initiative of the University of Bonn. Key objectives: decipher important pathways of local or organ-dependent immune regulation, with particular emphasis on the mechanisms of migration, signaling, cellular activation and the generation of functional repertoires.

TRR SFB 83 - Speaker Prof. Söllner

Transregional (TRR) SFB 83 "Molecular Architecture and Cellular Functions of Lipid/Protein Assemblies", connects experts in fields of synthetic organic chemistry, biochemistry, cell biology, virology, and immunology, from Bonn, Dresden and Heidelberg. The LIMES Institute's, Prof. Christoph Thiele is the site coordinator in Bonn.

Excellence Cluster "ImmunoSensation" - Speaker Prof. Hartmann

The LIMES institute is a major partner in Bonn's Excellence Cluster, ImmunoSensation, which in 2012, was awarded 28 Million Euros by the DFG for an initial period of 5 years. The cluster connects leading experts from the University of Bonn, the center of advanced european studies and research (**caesar**) and German Center for Neurodegenerative Diseases (**DZNE**) of the Helmholtz Society, to form a strong multidisciplinary research cluster in immunology and neighboring fields such as biophysics, sensory systems, and neurobiology.

DFG

ImmunoSensation
the immune sensory system Bonn cluster of excellence

SFB 704

SFB 645

SFB TRR 83

caesar (Center of Advanced European Studies and Research)

The caesar was established in 1995 under the umbrella of the Max Planck Society. Its main research focusses on cellular signal processing and the neural foundations of animal behavior.

DZNE (German Center for Neurodegenerative Diseases)

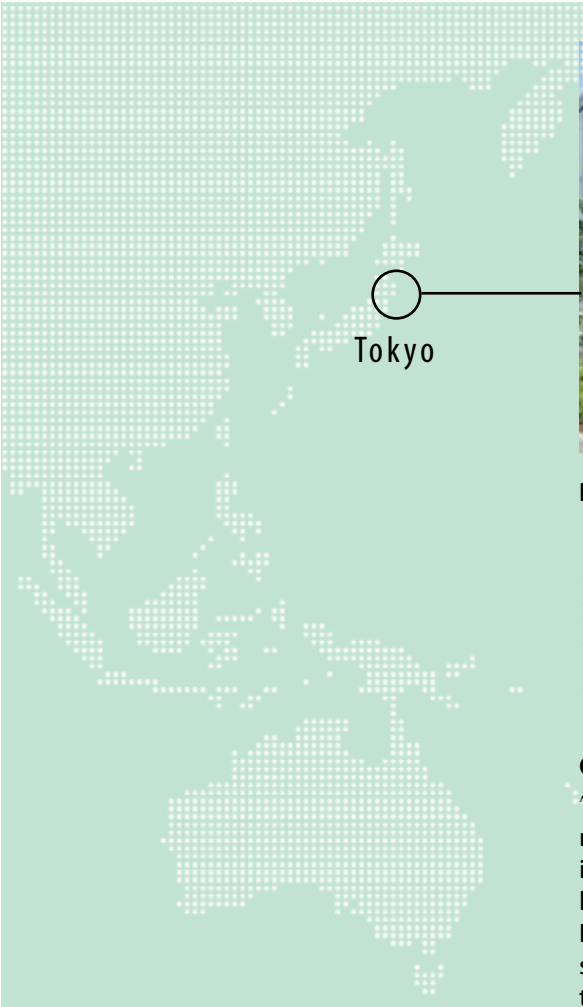
Neurodegeneration and neuroinflammation are core interests of the Helmholtz center DZNE, which was established in 2008. The DZNE has a strong translational focus, with the aim of using knowledge acquired about the similarities and differences between various brain diseases to develop new preventive and therapeutic approaches.

Medical Faculty, University of Bonn

The LIMES Institute has launched many joint programs in research and teaching together with members of the Medical Faculty of the University of Bonn. This has made the Medical Faculty a major local interaction partner.

TWIns institute in Tokyo Japan, where the LIMES institute has access to laboratory space (left). Waseda Uni delegation visit Bonn for the 13th joint symopsium in 2013 (right).

WASEDA UNIVERSITY, JAPAN



The LIMES institute's strong links with ASMeW, TWIns and TUAT, promote academic exchange, collaborations, education and training, as well as joint symposia and workshops. We can look back on many meetings hosted in Bonn and Tokyo, in which researchers came together to discuss new ideas in research and teaching in the context of global challenges in the Life Sciences.

ASMeW: Consolidated Research Institute for Advanced Science and Medical Care
TWIns: Tokyo Women's Medical University - Waseda University Joint Institution for Advanced Biomedical Sciences
TUAT: Tokyo University of Agriculture and Technology

Partnership Profile at a Glance

- **1960** Uni Bonn and Waseda Uni
- **2006** ASMeW and LIMES
- **2010** Mutual lab space: LIMES in TWIns and TWIns in LIMES
- **2011** Uni Bonn and TUAT

Our Japanese Partners

"It is important to build international networks with outstanding scientific institutions to promote research excellence", says Prof. Hoch. To this end, the LIMES institute made a strategic decision to put a focus on Asia, and in particular, Japan.

Molecular Biomedicine Students (from left): Balthasar Schlotmann, Sophia Mädler and Maximillian Bille

Education and Training

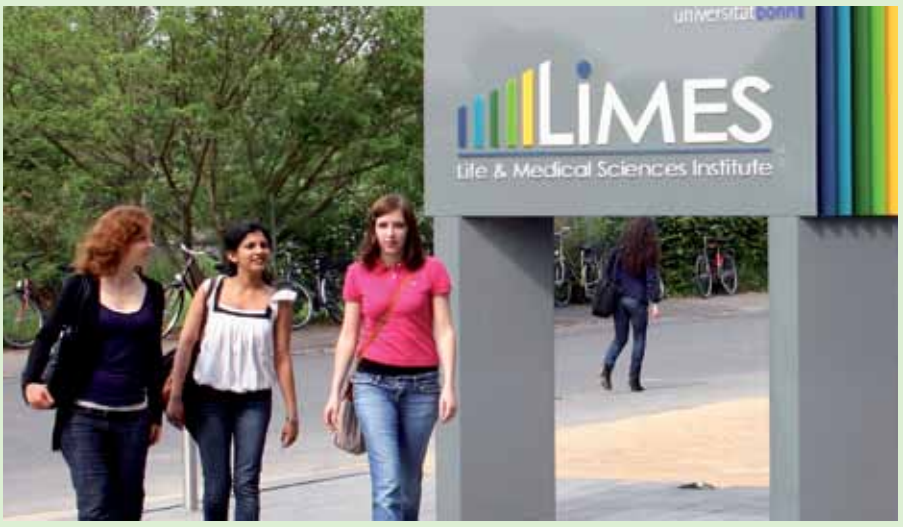
The Life & Medical Sciences Institute is committed to the development of young researchers. We currently offer internationally recognized undergraduate (Bachelor's) and graduate (Master's, PhD) study programs.

Working with schools

We offer internships for pupils and we regularly take part in interactive workshops and information sessions, such as the "Pupil-Teaser Days" or the annual "Science Rally". Our aim is to get young talents interested in life science research as early as possible. Applications for internship should be directed to the individual lab heads.

Bachelor of Science (B.Sc.) in Molecular Biomedicine

This highly competitive bachelors course "Molekulare Biomedizin" is the most successful of its kind in Germany. It was developed in 2003 in collaboration with the Medical Faculty at the University of Bonn and attracts 700-800 applications per year, with only 30 students admitted into the course (only offered in German). Since its conception, participants have featured as co-authors of over 200 peer-reviewed publications.



Graduates of the study course Molecular Biomedicine throwing their caps at the University festival

Master of Science (M.Sc.) in Life and Medical Sciences

The international Master's program "Life & Medical Sciences" is comprised of lectures, methods courses, tutorials, and 4 lab rotations over 1.5 years. Students gain multidisciplinary exposure to basic research carried out in academic or industry environments and have the freedom to explore rotations in labs of their choice. Graduates leave with enhanced technical and analytical skills, the ability to independently drive a research project, and a strong foundation for embarking on a successful PhD.

PhD (Dr. rer. nat)

PhD candidates have the opportunity to gain their biomedical research training in a multidisciplinary, highly collaborative, resource-rich environment. Students are awarded their doctoral degree from the Faculty of Mathematics & Natural Sciences at the University of Bonn. Candidates graduate with a "Dr. rer. nat." (Doctorate in Natural Sciences) degree - the German equivalent of a PhD.

New PhD program for 2016 intake

The LIMES international graduate school (LIMES-IGS) is currently developing a new five-year PhD program with a core structure. Candidates would enter with a Bachelor's degree. The program will be composed of course work, technical and soft skills workshops, laboratory rotations and an independently driven research project.

The course will be directed by Prof. Christoph Thiele.

Dr. André Völzmann reveiving a self made doctoral cap from his lab mates after his PhD defense. Photo by Dr. Ines Hahn

Public Relations

The LIMES culture

The core of our Public Relations begins with keeping our staff up-to-date while promoting strong relations with and among employees. We place special emphasis on a working atmosphere where experienced scientists and young people alike find inspiration. Modern lounge areas and coffee rooms attract staff and students, acting as central meeting spaces where new ideas are born.

The LIMES culture ensures ample opportunity for social interaction to compliment life in the lab. Be it at meetings and retreats with working groups or annual Christmas and Carnival celebrations, there is always something happening!

Coming together in the coffee room and Carnival at the LIMES-Institute

TRANSPARENCY

We offer our employees internal communication tools where information can be readily transferred in everyday life:

- an internal **newsletter "LIMES-Klaaf"** that keeps employees informed about news and events
- the **LIMES intranet** for personnel information and forms
- **information screens** found on each floor, which are regularly updated with relevant news and alerts
- **Institute seminar series** to foster the scientific exchange
- **Career development workshops** and excursions

Japanese students at the 13th Joint Symposium of WASEDA University Tokyo and Univesity of Bonn in September 2013

Reaching out

There are many ways to learn more about the Life & Medical Sciences Institute:

- **Press**
  In close cooperation with the University of Bonn press office - we report on the most interesting breakthroughs, activities and achievements of our institute.

- **Podcasts/Videos**
  We feature professional podcasts from uni-bonn.tv that highlight research and teaching within the LIMES Institute and produce short films that show our research in action.

- **Events & Seminars**
  An important activity at the LIMES institute is to organize and host both academic and community focused events. These include regular seminars by leading national and international experts, and joint conferences with our international partners.

- **Information sessions for pupils and students**
  We offer internships and regularly take part in interactive workshops and information sessions, such as the "Student-Teaser Days" or the annual "Science Rally".

- **Publications**
  We provide brochures about our study programs and scientific publications.

HOW YOU CAN SUPPORT LIMES

Basic research in the field of molecular biomedicine is vital to our understanding of the many complex mechanisms and processes of our body. Disruption to our body's normal function can lead to disease. Findings from basic research form the basis for the development of better therapeutics to help treat and prevent the progression of chronic disease states. Your donation will help our scientists to continue to make new discoveries for a future of happier, healthier people.

You can make a difference by supporting the important work of the Life and Medical Sciences (LIMES) Institute in the following ways:

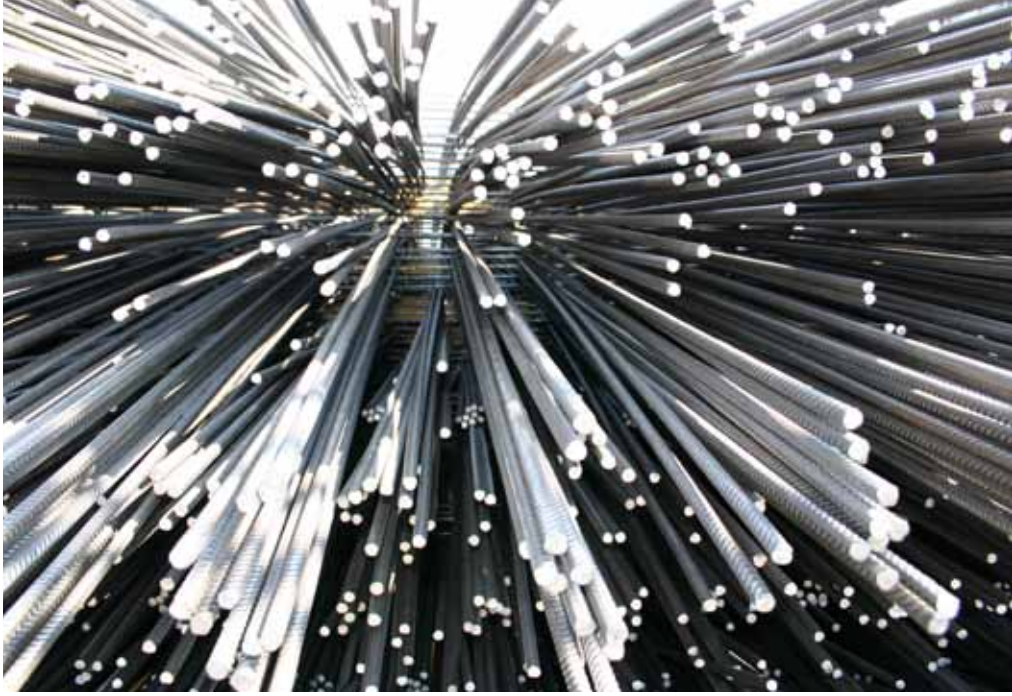
- **One-off donation**
- **Regular donations**:
  Your continued support means a great deal to the LIMES Institute. You can set up regular donations for the amount and frequency of your choice. This can be directly deducted from your credit card or bank account.
- **Special occasion**:
  You can give the greatest gift of all by requesting donations to the LIMES Institute instead of gifts at your next celebration.
- **Donate in memory**:
  Remember your loved ones by making a donation in their memory.
- **Leave a gift in your will**:
  By remembering the LIMES Institute in your will, you can leave a legacy to science and help future generations to live a happier, healthier life.
- **Trust and Foundation**:
  Your support is vital for the future of biomedical research at the LIMES Institute. If you are a trust or foundation who is interested in working with the LIMES institute, please contact us directly.

How to donate to the Institute

Account details:
Universitätskasse Bonn, LIMES-Institut
Bank: Sparkasse Köln/Bonn
IBAN: DE08 3705 0198 0000 0576 95
BIC: COLSDE33
Please specify the following transaction description:
Kapitel 6 11 16, Titel 2 82 11, Projekt-Nr. 310 600 79

For a donation receipt (for gifts of 100 Euro and over), please contact us at info@limes-bonn.de or
call +49 (0) 228 / 73 - 6 27 03.

Thank you for your support!



"Material"
construction phase of the LIMES building,
2007

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