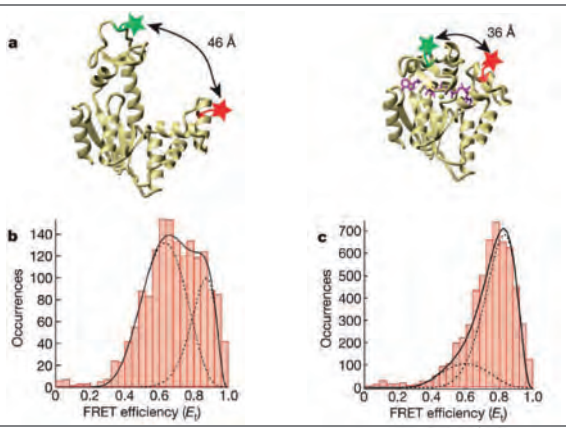
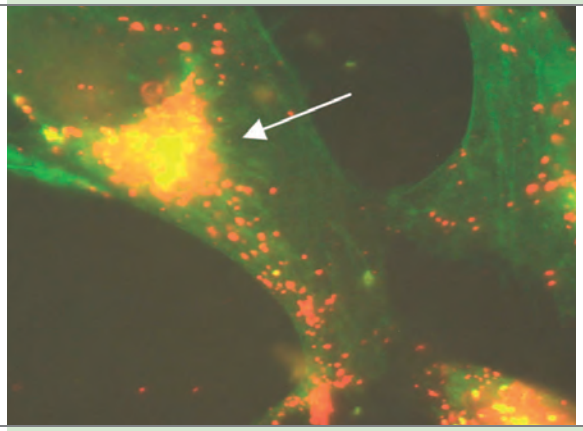
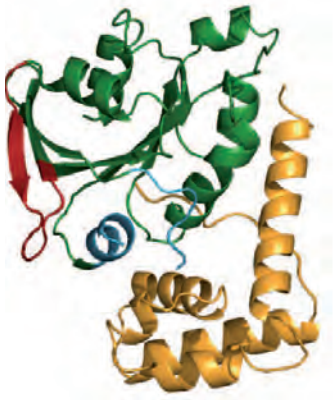
Volume 2







Center for Structural and Cell Biology in Medicine (CSCM) at the University of Lübeck

CENTER FOR STRUCTURAL AND CELL BIOLOGY IN MEDICINE

Im Focus das Leben
Universität zu Lübeck

Content:

Dear reader,

This booklet presents the Center for Structural and Cell Biology in Medicine (CSCM) at the University of Lübeck, its aims and the achievements of the member institutes since 2007.

The Center for Structural and Cell Biology in Medicine was founded in 2005 by departments and research groups of the Medical Faculty, the Faculty of Technical and Natural Sciences, and the Research Center Borstel (FZB), to form a joint research platform. The aim of the CSCM is to combine and develop local research expertise, to support joint research projects, and to present the results of the cooperative work to the public.
The CSCM is tightly linked to other local structures promoting molecular life science – the teaching program in molecular life science of the Faculty of Technical and Natural Sciences of the University Lübeck, the club "Life Science in Lübeck e.V." which supports several of the activities of the CSCM, as well as the "Lübeck Open Laboratory" (LOLA), all of which assist us in our efforts for public outreach, especially to the younger generation.

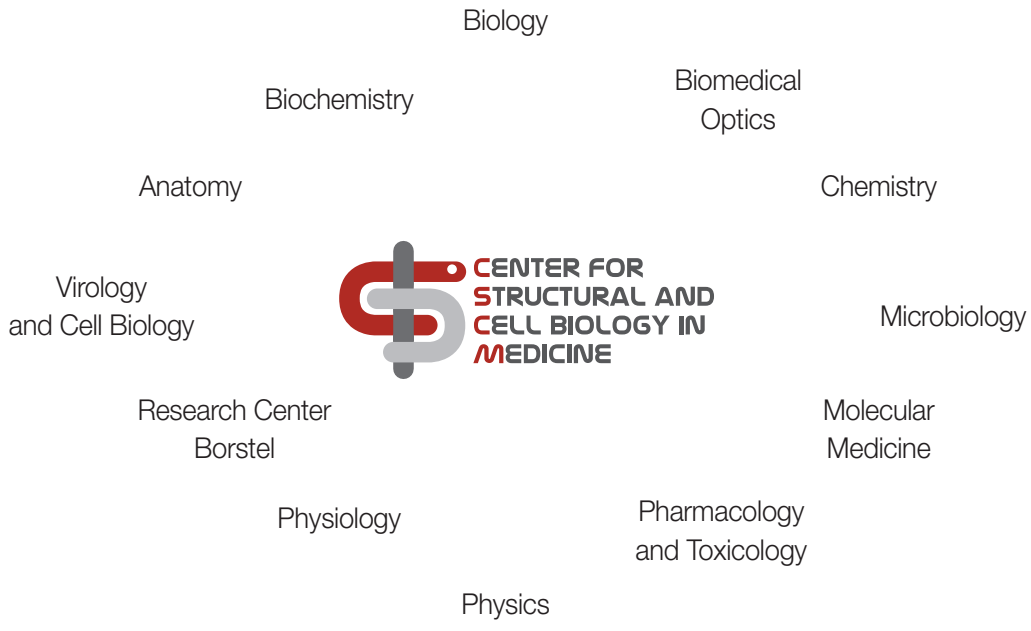
The CSCM is centrally embedded in the Life Science research community in Germany. It is a cornerstone of the "Inflammation at Interfaces" network linking biomedical research institutions at Lübeck, Borstel, and Kiel. The connections to the University and the Bernhard Nocht Institute for Tropical Medicine in Hamburg are particularly close. The CSCM is also active within European and international networks. One indication for its lively international activities is the organization of international conferences hosted by the CSCM.

The main focus of the CSCM is the analysis of structure, function and biogenesis of supramolecular and subcellular assemblies that are important for intracellular parasitism, such as viruses, mammalian phagosomes, or signalling complexes important for infection and inflammation. These studies pave the way for the discovery of drugs interfering with the pathogenic activities of such assemblies. The researchers in the CSCM combine a broad spectrum of expertise ranging from membrane biophysics, methods for structure analysis such as X-ray crystallography and NMR, drug design and chemical synthesis, biochemical assays in the fields of signal transduction and protein topogenesis, optical methods, like life cell imaging as well as in-vitro and in-vivo models of infectious disease caused by viral and intracellular bacterial pathogens. Various measures were undertaken by the members to further develop the CSCM. As a highlight, our access to synchrotron radiation was further improved by the inauguration of an outstation at the DESY (Deutsches Elektronen-Synchrotron) site in nearby Hamburg. Moreover, Master courses and a PhD program in Molecular Life Science were established, which are a prerequisite to involve motivated young researchers in our projects.

The Center for Structural and Cell Biology in Medicine is dedicated to closely link excellence in structural and cell biology to progress in medical research, in particular in the fields of infection and inflammation. It is our hope that this initiative will lead to breakthroughs in science and medicine that will help create a better tomorrow.

For more information, please visit our web page , or contact us via email. You are also invited to visit the CSCM during our "open house" days, or to contact our "Lübeck Open Lab" (LOLA)

info@cscm.uni-luebeck.de
www.cscm.uni-luebeck.de



Institute of

Prof. Dr. med. Jürgen Westermann

1976-1982 Studies of Medicine in Hannover, Germany
1982 House Physician in Internal Medicine, Walsgrave Hospital, Coventry, UK
1983-1984 Obligatory service in the army as physician
1984-1996 Research Associate in the Department of Functional and Applied Anatomy, Hannover Medical School, Hannover, Germany
1997-2001 Research Associate, Department of Functional and Applied Anatomy, Hannover Medical School
Since 2001 Full Professor and Chair, Institute of Anatomy, Medical Faculty, University of Lübeck
Since 2002 Dean for Student Affairs, Medical Faculty, University of Lübeck

Awards, memberships and others:
Anatomische Gesellschaft
Gesellschaft für Immunologie
Gesellschaft für medizinische Ausbildung

Dr. rer. nat. Kathrin Kalies

1981-1986 Studies of Biochemistry in Halle
1989-1992 PhD, Institute of Biochemistry, Swiss Federal Institute of Technology, Zurich (Switzerland)
1993-1995 Postdoctoral Fellow, Department of Cell Biology, Harvard Medical School, Boston, USA
1995-1996 Postdoctoral Fellow, Max Planck Institute of Molecular Genetics, Berlin, Germany
1997-1998 Product manager for DNA Sequence analysis in Northern Germany at the MWG Biotech Inc., Ebersberg, Germany
2000-2001 Research Associate at the Centre of Biochemistry and Molecular Cell Biology, Georg-August-University Göttingen, Germany
Since 2001 Research Associate and group leader, University of Lübeck

Director: Prof. Dr. med. Jürgen Westermann
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Main research topics in the CSCM:

Functional anatomy of the immune system.
Generation of CD4 T cell memory and autoimmunity.
Modulating immune responses by changing the cytokine milieu within lymphoid organs.
Interaction of epithelial cells and cells of the immune system on mucosal membranes.

Research group: Jürgen Westermann

T cell memory

We developed a novel concept of CD4 T cell memory. Now the implications of the new memory concept are tested in men and mice. We investigate how molecules such as prolactin, IL-6, and cortisol influence CD4 T cell memory. By unraveling the molecular mechanism involved in this process it will be possible to improve current vaccination strategies.

Identification of mechanisms required for production of pathogenic autoantibodies against type VII collagen

To diminish autoantibody production and/or to induce a switch to the production of non-pathogenic isotypes, mice with ongoing epidermolysis bullosa acquisita (EBA) will be immunized with autoantigen and different adjuvants by various administration routes. This is done in cooperation with the Clinic of Dermatology (D. Zillikens) in the framework of the Excellence Cluster "Inflammation at Interfaces".

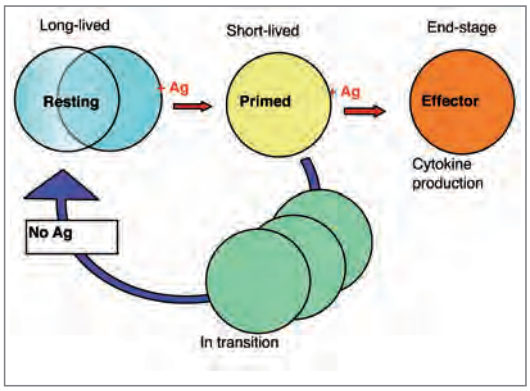

Fig. 1: Recycling CD4 T cells account for immunological memory. The CD4 T cell population may be divided into four major subsets based on surface phenotype and function. The resting subset contains long-lived CD4 T cells that recirculate in search of antigen. Antigen stimulation generates short-lived, metabolically active primed T cells whose fate hangs in the balance. A second encounter with antigen transforms primed T cells into terminally differentiated, cytokine secreting effector T cells. Failure to find specific antigen allows primed T cells to modify their cytokine capabilities to become T cells in transition with a slightly extended lifespan. In the absence of antigen they default to resting T cells that retain no memory of a previous antigen encounter.

Research group: Kathrin Kalies

Tracking immune responses locally at consecutive time points after activation

The course of adaptive immune responses is controlled by a number of checkpoints. The first checkpoint takes place in the T cell zone of lymphoid organs, where stable contacts of antigen presenting cells with naïve T cells lead to a clonal expansion of antigen specific T cells. These activated T cells migrate either to peripheral sites to exert their effector functions or to the T-B border to provide B cell help. The interaction of antigen specific T cells with antigen primed B cells represents a second checkpoint. Finally, the B cells become short-lived plasma cells or enter the germinal centres to become high affinity B cells. The contact of matured B cells with follicular antigen specific T cells within the germinal centres defines a third checkpoint.
My group aims to investigate the events at all critical checkpoints from the initiation of adaptive immune responses to the resulting effects. The main focus is the analysis within the complex tissue environment to maintain the local information. Using the technique of laser microdissection we analyse the expression of genes in the T and B cell zones in lymph nodes and spleens. CD3 as T – and CD19 as B cell maker are used to confirm accurate identification and isolation. As subcutaneously applied antigen that induces an immune response in the lymph node we infect different mice strains with the intracellular parasite Leishmania major. Sheep red blood cells are used as intravenously applied antigen that induces an immune response in the spleen. The effect of adding activated cells, apoptotic cells, isolated cell components, adjuvants or different antigen doses on the resulting immune response are studied.

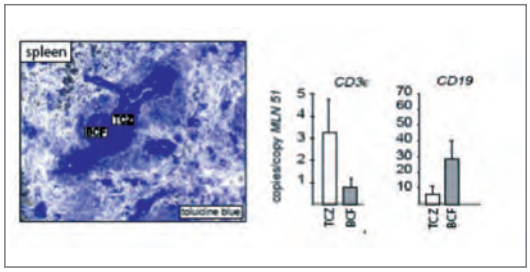

Fig. 2: Staining with toluidine blue allows the identification of functionally different lymphoid compartments of the spleen. Cryosections of a rat spleen were stained with toluidine blue. The T-cell zone (TCZ), B-cell follicle (BCF) are easily distinguishable. Bar, 100 µm. The cDNA copies for CD3 and CD19 were analyzed by real-time RT-PCR after microdissection of the TCZ and BCF and normalized to the house keeping gene metastatic lymph node gene 51 (MLN51).

Anatomy

Research group: Andreas Gebert

Our group is interested in the interaction of epithelial cells and cells of the immune system during ongoing immune reactions. We investigate mucosal membranes, with special interest in the small intestine, by advanced microscopic and molecular methods. The aim is to elucidate which cells of the mucosae interact in which manner so that the organism is effectively protected against potentially pathogenic microorganisms, antigens, and toxins. In this context, the local microenvironment and its specific composition of different cell types plays an important role. To investigate these issues, our group established a novel experimental setting for intravital 2-photon microscopy in mice. Based on tissue autofluorescence, the system provides 3D images of the gut mucosa and the associated lymphoid tissue at a sub-micron resolution. In collaboration with the physicists of the Institute of Biomedical Optics Lübeck, we develop multi-dimensional imaging methods which allow cell types, subcellular structures and dynamic processes to be observed in the living tissue. Using computer-based analysis of multi-dimensional data sets (xyz, wavelength, time), the uptake of antigens by the specialized M cells of the gut epithelium, the migration of lymphocytes in the mucosa, and the interaction of lymphocytes with epithelial and dendritic cells is examined quantitatively. This unique model is also applied to studies on the uptake of nanoparticles and the regeneration of the epithelial layer in the gut. In addition, we develop new methods for isolating tiny tissue fragments by laser micro-dissection techniques, and quantitatively analyse the contained mRNA in molecular assays. This combination of in-vivo and ex-vivo imaging with molecular techniques gives us a new understanding of the dynamic processes involved in the maintenance of tissue integrity under physiological and pathological conditions.

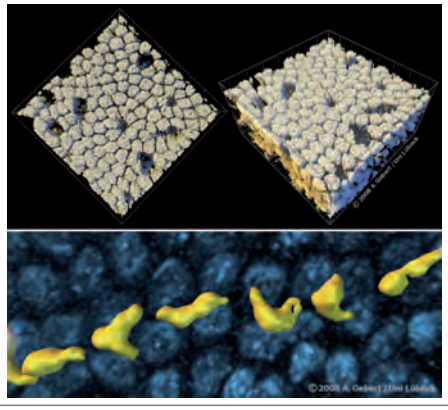

Fig. 3: Three-dimensional rendering of the living mucosa of the murine small intestine. Using intravital 2-photon microscopy based on tissue autofluorescence, an image stack was recorded and digitally analysed (upper figure). The lower figure shows an intraepithelial lymphocyte (yellow) which moves across the gut epithelium (blue). The image is a time lapse overlay of 3D snapshots recorded within only 7 minutes. Quantitation of this dynamic behaviour revealed that each intestinal epithelial cell is contacted by bypassing lymphocytes every two minutes and thus underlies an extremely dense immunological surveillance.

Research group: Peter König

Every day a human inhales more than 10,000 litres of air which contain a vast variety of pathogens and other particles such as dust and pollen. Thus, the airways need refined mechanisms to remove these particles and to prevent infection or damage. On the other hand, allergens that are harmless for the airways should not initiate an inflammatory reaction. The first line of defense in the airways is the airway epithelium. It has a variety of methods to defend itself such as motile cilia that can remove pathogens and particles and mucus which binds bacteria. Upon activation it also releases a variety of mediators to call other parts of the immune system into action.

Our group is interested in the following questions:

How does the airway epithelium manage to keep intruders from harming the epithelium and the airways?

When and how do cells of the immune system come into action as the second line of defense?

How does the epithelium control airway repair after destruction?

To gain insight into how these mechanisms work, we use advanced microscopic methods to directly observe these events. We use high speed videomicroscopy to analyze cilia-driven transport and 2-photon microscopy to follow the action of immune cells in and below the airway epithelium in several disease models and in genetically modified mice. Our lab also employs a variety of standard microscopic techniques such as immunohistochemistry, confocal laserscanning microscopy, transmission and scanning electron microscopy and other methods such as RT-PCR and Western blotting for further molecular analysis.

We have close collaborations with other labs on campus and with the Research Center Borstel as well as German and overseas colleagues. We welcome motivated students interested in lung biology!

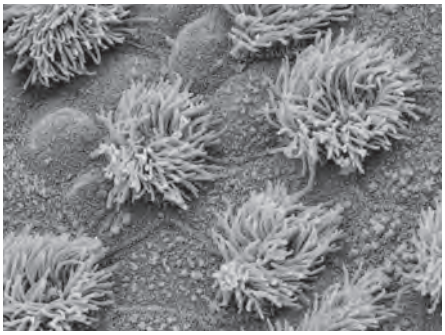

Fig. 4: Image of the airway epithelium using a scanning electron microscope.

Selected Publications:

1. Bell EB, Westermann J (2008) CD4 memory T cells on trial: Immunological memory without a memory T cell. Trends Immunol 29: 405-411.
2. Gebert A, Steinmetz I, Fassbender S, Wendlandt KH (2004) Antigen transport into Peyer's patches: increased uptake by constant numbers of M cells. Am J Pathol 164: 65–72.
3. Kalies, K., P. Konig, Y. M. Zhang, M. Deierling, J. Barthelmann, C. Stamm, and J. Westermann (2008) Nonoverlapping expression of IL10, IL12p40, and IFNgamma mRNA in the marginal zone and T cell zone of the spleen after antigenic stimulation. J Immunol 180: 5457-65.
4. M. K. Klein, R. V. Haberberger, P. Hartmann, P. Faulhammer, K. S. Lips, B. Krain, J. Wess, W. Kummer, P. König (2009). Muscarinic receptor subtypes in cilia-driven transport and airway epithelial development. Eur Respir J, 2009; 33 1113-1121

Prof. Dr. med. Andreas Gebert

1981-1988 Studies of Medicine in Hannover, Germany
1988-1989 Research Associate, Department of General Anatomy, Hannover Medical School, Germany
1989 PhD
1989-1992 Research Associate, Institute of Anatomy, University of Munich, Germany
1991-1992 Medical doctor in the German army
1992-2001 Research Associate and research group leader in the Department of Anatomy, Hannover Medical School, Germany
Since 1998 Specialist (Facharzt) for Anatomy
2000 Habilitation for Anatomy, Title: "Morphology, function and differentiation of M-cells"
Since 2001 Scientist and research group leader in the Institute of Anatomy, University of Lübeck

Awards, memberships and others:
Fellow of the Studienstiftung des deutschen Volkes
Scholarship for the doctoral thesis by the Studienstiftung des deutschen Volkes
Member of the Anatomische Gesellschaft
Teacher of the year, 2008, Medical Faculty, University of Lübeck

Dr. med. Peter König

1992-1999 Studies of Medicine, Philipps-University, Marburg, Germany and Justus-Liebig-University, Giessen, Germany
1997 Research stay at Department of Anatomy and Histology, Center for Neuroscience, Flinders University, Adelaide, Australia
1999-2002 Scholar at the DFG Research Training Group "Biological Basis of Vascular Medicine" and Intership at the Department for Anaethesiology, Intensive Care and Pain Medicine, Justus-Liebig-University, Giessen, Germany
2002-2006 Research Associate, Institut of Antomy and Cell Biology, Justus-Liebig-University, Giessen, Germany
Since 2006 Research Associate and group leader, Institute for Anatomy, University of Lübeck

Awards, memberships and others:
Anatomische Gesellschaft
European Respiratory Society
Society for Autonomic Neuroscience

Institute of

Prof. Dr. Dr. h.c. Rolf Hilgenfeld

1980 „Diplom" in Chemistry, Universität Göttingen
1986 Dr. rer. nat. in Chemistry, Freie Universität Berlin Thesis: „High-resolution crystal structure of a plant cysteine proteinase" Thesis advisor: Prof. Dr. W. Saenger

Professional experience
1986-1987 Head of Protein Crystallography, Hoechst AG, Frankfurt
1987-1988 Post-Doc, Biocenter, University of Basel, Switzerland
1988-1995 Group leader, Structural Biology and Drug Design, Hoechst AG
1995-2002 Professor (C4) of Structural Biochemistry, University of Jena
Head of Department of Structural Biology & Crystallography, Institute of Molecular Biotechnology e.V., Jena
1998-2000 Director, Institute of Molecular Biotechnology e.V., Jena
since 2003 Professor (C4) of Biochemistry, University of Lübeck
since 2003 Director, Institute of Biochemistry, University of Lübeck

Fellowships, Honours, Awards:
Tiburtius Prize of the Senate of Berlin, 1987
Visiting fellowship of the Japanese Society for the Promotion of Science, 2004
Visiting Professor, University of South Bohemia at Ceske Budejovice, Czech Republic, since 2005
Visiting Professor, Beijing Genomics Institute, Beijing, China, since 2007
Dr. honoris causa, University of South Bohemia at Ceske Budejovice, Czech Republic, 2009

Service in the scientific community:
International Organization for Biological Crystallization (Founding President, 2002-2004)
European Synchrotron Radiation Facility (ESRF), Grenoble, Scientific Advisory Committee (1998-2002)
German Chemical Society (GDCh), Biochemistry Special Interest Group, Board (1998 – 2007)

Director: Prof. Dr. Dr. h.c. Rolf Hilgenfeld
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Main research topics in the CSCM:

Structural basis of intracellular infections

When viruses, bacteria, or eukaryotic parasites infect human cells, they mis-use them to replicate to large numbers and, after exit from the host cell, start another round of infection. Researchers at the Institute of Biochemistry study the molecular basis of intracellular infections. One question they seek to answer is, which proteins are essential for entry of the pathogens into or their replication inside the host cell? Such proteins would be suitable targets for new anti-infective compounds. In the institute, such virulence proteins are identified by comparative proteomics and mutational analysis. Proteins emerging as interesting from this approach are prepared by recombinant DNA technology, purified, crystallized, and examined by X-ray crystallography. In an increasing number of cases, the study objects are large protein complexes formed upon host-pathogen interaction. X-ray diffraction data are measured using an in-house diffractometer or the Institute's beamline at the DESY synchrotron in Hamburg (where the Institute runs a fully equipped outstation – the "Laboratory for Structural Biology of Infection and Inflammation"). The resulting high-resolution crystal structures are used for the discovery of small-molecule inhibitors that interfere with the functions of the virulence proteins. To this end, either de-novo design of such compounds in the active site of the target protein, virtual screening of chemical libraries, or fragment-based design methods are applied. Initial "hits" (weak binders) are subsequently modified by the Institute's synthetic chemistry group, until "leads" (potent inhibitors) are obtained. These are tested by collaboration partners for activity in pathogen-infected cell cultures and small-animal models. The most successful anti-infectives discovered this way are offered to the pharmaceutical industry or to non-government organizations for clinical development. The pathogens investigated include RNA viruses such as Coronaviridae (example: the SARS coronavirus), Enteroviridae (poliovirus and coxsackievirus), Flaviviridae (Hepatitis C Virus, Dengue Virus), influenza virus, and the Human Immunodeficiency Virus (HIV). Among the bacteria, the Institute focuses on *Legionella pneumophila* and *Chlamydia trachomatis* as well as *Chlamydia pneumoniae*.
In summary, the Institute has all elements of the preclinical drug discovery pipeline implemented in house, from proteomics via molecular biology, structural biology, drug design to synthetic chemistry. It therefore employs scientists from many disciplines and provides training in many fields, such as proteomics, recombinant DNA technology, protein chemistry, X-ray crystallography, drug design and synthetic chemistry. The Institute's goal is to reach and maintain excellence in functional and structural studies on large complexes involved in pathogen replication and host-pathogen interactions, and to use the insights obtained for structure-based discovery of new anti-infectives.

Research group: Rolf Hilgenfeld

Structural Virology: From viral protein structure to function

The number of viral outbreaks has been increasing dramatically recently. In the past twelve years, the world has seen at least one major outbreak of either a new virus or a new variant of a known virus per year. Incidentally, almost all of these outbreaks were caused by RNA rather than DNA viruses. Unfortunately, for most known viral diseases of humans, and let alone for newly emerging ones, no drug treatment is available. We believe that in view of this scenario, it is necessary to develop lead compounds with activity against all major families of viruses, both those that infect humans and those that so far have been restricted to animals but may cross the species-barrier by zoonotic transition. Ultimately, we should aim at discovering antiviral compounds active against a broad range of viruses.

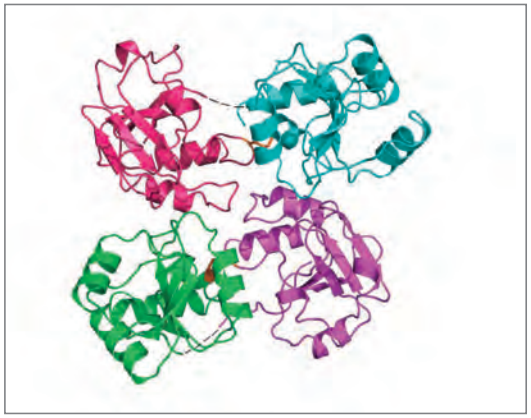

Fig. 1: Structure of the "SARS-unique domain" (SUD) dimer, a module that only occurs in the SARS virus and no other coronavirus. Is it responsible for the extraordinarily high human pathogenicity of SARS-CoV?

We believe in the merits of structure-based approaches to discover new compounds with activity against RNA viruses. We focus on components of the viral replicase complexes as targets for antiviral compounds, and in particular on the proteases that play essential roles in the viral "life cycle". But we have to realize that even seemingly relatively simple enzymes such as viral proteases tend to have more than one function. In addition to cleaving the viral polyproteins, many of them are also involved in binding to other replicase components including viral RNA. Therefore, our studies are being expanded towards larger subcomplexes of the viral replicase/transcriptase.

The largest protein produced as part of the SARS-coronavirus polyprotein is non-structural protein 3 (Nsp3). One of its seven subdomains is called the SARS-unique domain (SUD), because it is only present in SARS-CoV but no other coronaviruses. Therefore, it has been suspected to be responsible for the extraordinary high pathogenicity of the SARS virus. We have

Biochemistry

determined the crystal structure of the SUD and found it to consist of two so-called macrodomains. The observed SUD dimer (Fig. 1) binds to oligo(guanosine) stretches (G_{10} – G_{14}) in RNA. Such "G-stretches" fold into G-quadruplexes, i.e. four-stranded nucleic-acid structures formed by contiguous guanines. By binding to the G-stretches in their mRNA, SUD might block the synthesis of pro-apoptotic host proteins, to the benefit of the virus. Thus, on the basis of our biophysical studies, we were able to come up with some hypotheses which can now be tested.

Nsp9 is a single-stranded RNA-binding protein found only in coronaviruses. We determined the structure of the protein from Human Coronavirus 229E and were surprised to find a hexamer consisting of three disulfide-bonded dimers. As coronavirus replication occurs in the cytoplasm where the overall milieu is reducing, cysteine residues should be in the reduced, free form. However, disulfide-bond formation may be a way for the viral replicase components to cope with the oxidative stress caused in the host cell by the viral infection (Fig. 2).

Fig. 2: Disulfide formation in Nsp9 – a way to cope with oxidative stress? Featured on the cover of the Journal of Molecular Biology, November 28, 2008.

monitor information and react in diversified ways (i.e. to combat viruses), it is intriguing to see that some viruses still manage to affect processes in the brain, the reason of which is not sufficiently understood.

Within the Institute of Biochemistry with its strong focus on the "Molecular Basis of Intracellular Infections", the research group "Structural Neurobiology" concentrates on processes in the brain and on pathogen-brain interactions. The group is a member of the DFG clinical research group "Selfish Brain" (http://www.selfish-brain.org/). Of particular interest are viruses such as HTLV-1, which are able to affect/control the brain. In addition, the research group works on proteins that play a role in brain metabolism in general, such as glutamate carboxypeptidase II (Fig. 3) that hydrolyses the abundant neuropeptide N-acetyl-aspartylglutamic acid (NAAG). Inhibition of this enzyme is of interest for the therapy of stroke.

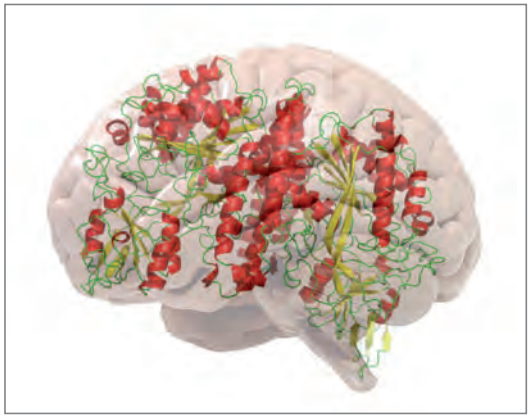

Fig. 3. Brain glutamate carboxypeptidase II (GCPII) is responsible for the release of the neurotransmitter L-glutamate.

Research group: Jeroen R. Mesters

Structural neurobiology: Viruses and the brain

So far, more than 1938 virus species (287 genera of 73 families) have been identified. Viruses can quickly and efficiently adapt to changes in the host, its immune response and drug treatment. Once a virus is inside the target host-cell, it tries to take control over the cell (and/or host?) while initiating, in a time-controlled manner, viral replication and viral mRNA-translation processes. The intrusion by viruses typically evokes an immune response by the host that many viruses strive to challenge. Interestingly, in several cases, viruses have an effect on the brain. The brain occupies a special hierarchical position in the organism. It is separated from the general circulation by the blood-brain barrier, it has a high energy-consumption and a low energy storage-capacity, it uses only specific substrates (e.g. glucose), and it can record information from the peripheral organs and control them. Despite the brain's ability to

Selected Publications:
Structural Virology Group:
1. J. Tan, C. Vonrhein, O. Smart, G. Bricogne, M. Bollati, Y. Kusov, G. Hansen, J.R. Mesters, C.L. Schmidt & R. Hilgenfeld: The SARS-unique domain (SUD) of SARS coronavirus contains two macrodomains that bind G-quadruplexes. PLoS Pathog. 5 (2009), e1000428
2. Y. Piotrowski, G. Hansen, A.L. Boomaars-van der Zanden, E.J. Snijder, A.E. Gorbalenya & R. Hilgenfeld: Crystal structures of the X-domains of a Group-1 and a Group-3 coronavirus reveal that ADP-ribose-binding may not be a conserved property. Prot. Sci. 18 (2009), 6-16
3. R. Ponnusamy, R. Moll, T. Weimar, J.R. Mesters & R. Hilgenfeld: Variable oligomerization modes in coronavirus non-structural protein 9. J. Mol. Biol. 383 (2008), 1081-1096
4. I. Robel, J. Gebhardt, J.R. Mesters, A. Gorbalenya, B. Coutard, B. Canard, R. Hilgenfeld & J. Rohayem: Functional characterization of the cleavage specificity of the Sapovirus chymotrypsin-like protease. J. Virol. 82 (2008), 8085-8093
5. Y. Piotrowski, R. Ponnusamy, S. Glaser, A. Daabach, R. Moll & R. Hilgenfeld: Production of coronavirus nonstructural proteins in soluble form for crystallization. Meth. Mol. Biol. 454 (2008), 139-159

Structural Neurobiology Group:
1. J.R. Mesters & R. Hilgenfeld: Glutamate Carboxypeptidase II. In: Handbook of Metalloproteins Online Edition (A. Messerschmidt, ed.), Wiley Interscience (2008)
2. J.R. Mesters, K. Henning & R. Hilgenfeld: Human glutamate carboxypeptidase II inhibition: structures of GCPII in complex with two potent inhibitors, quisqualate and 2-PMPA. Acta Cryst. D63 (2007), 508-513
3. J.R. Mesters, C. Barinka, W. Li, T. Tsukamoto, P. Majer, B.S. Slusher, J. Konvalinka & R. Hilgenfeld: Structure of glutamate carboxypeptidase II, a drug target in neuronal damage and prostate cancer. EMBO J. 25 (2006), 1375-1384

Dr. math. et dis. nat.
Jeroen R. Mesters

1987 Diploma degree in Biochemistry, University of Leiden
1994 Ph.D. in Biochemistry, University of Leiden
1994-1998 Post-Doc, Department of Structural Biology & Crystallography, Institute of Molecular Biotechnology e.V., Jena
1998-2003 Research Associate, Department of Structural Biology & Crystallography, Institute of Molecular Biotechnology e.V., Jena
Since 2003 Senior Research Associate and Group Leader, Institute of Biochemistry, University of Lübeck

Awards, memberships and others:
Visiting Professor of the University of South Bohemia at Ceske Budejovice (since October 2002)
Secretary of the International Organization for Biological Crystallization (since August 2006)

Institute of

Prof. Dr. rer. nat. Holger Steuber

1997-2001 Studies of Pharmacy, University of Regensburg
2002 Research Internship in Molecular Modelling, ETH Zürich
2003-2006 Ph.D. with Prof. G. Klebe, Pharmaceutical Chemistry, University of Marburg
2006-2008 Group Leader Protein Crystallography and Fragment Screening, Proteros Biostructures, Martinsried
Since 08/08 W1-Professor Structure-based Drug Design

Dr. rer. nat. Ksenia Pumpor

1994-1999 Studies of Chemistry, St. Petersburg State University, Russia
1999-2000 Research Associate, Department of Organic Chemistry, St. Petersburg State University, Russia
2000-2004 Research Associate, Institute of Organic Chemistry, University of Leipzig
2004 PhD University of Leipzig
since 2005 Group Leader Synthetic Chemistry, Institute of Biochemistry, University of Lübeck

Research topics:
Synthetic Organic chemistry
Synthesis of Heterocycles
Development of anti-viral drugs

Membership:
German Chemical Society (GDCh)

Research group: Holger Steuber

Structure-based drug design: What drives ligands into protein pockets?

Even though a profound understanding of protein-ligand recognition provides the basis for successful structure-guided ligand design and optimization, currently only limited knowledge is available about the principles driving ligands to bind to their targets. In particular, protein flexibility, the role of water for ligand affinity, as well as changes in protonation of protein residues or ligand functional groups strongly influence the driving forces, but are difficult to consider in the design process.
In the junior research group "Structure-based drug design", we address these questions by a combination of experimental and computational methods. Protein X-ray crystallography is used to elucidate binding mode and interaction pattern between the biomolecular target molecule and the ligand as well as to suggest putative optimization strategies. To increase the understanding of driving forces that cause binding of the ligand to the receptor site, the binding event is characterized thermodynamically by means of isothermal titration calorimetry (ITC). This technique enables to factorize the Free Enthalpy of binding into enthalpic and entropic contributions and thereby allows to correlate structural features such as hydrogen bonds and salt bridges to the thermodynamic driving forces. Multiple X-ray structures in complex with different ligands can provide insight into the conformational space a binding pocket is able to adopt. These experimental observations can be exploited via computational methods by docking virtual small-molecule libraries into the binding pocket conformers. Another approach for lead identification is screening of "fragments". This method builds on the higher ligand efficiency of smaller molecules compared to larger ligands and due to the reduced ligand complexity, smaller libraries can be used to cover the chemical space.
These techniques are currently used in the research group for the design of inhibitors against disease-relevant viral targets such as proteases of Hepatitis C, West Nile (Fig. 4) and Dengue virus.

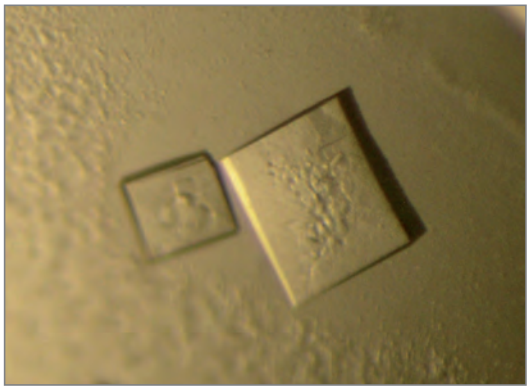

Fig. 4. Crystals of the West Nile Virus NS3 protease.

Research group: Ksenia Pumpor

Synthetic chemistry: Creating the antiviral drugs of tomorrow

Viral proteases are attractive drug targets because of their central role in processing the viral polyproteins and because their catalytic activity can be easily assayed. The synthetic chemistry group designs and synthesizes inhibitors for the cysteine proteases of coxsackieviruses and other enteroviruses including poliovirus, as well as coronaviruses. More than 300 candidate inhibitors have been synthesized over the past few months. Because all of these compounds have been designed on the basis of the three-dimensional structure of their target protein, the overall success rate of the inhibitor discovery program is good and certainly much larger than what can be expected from high-throughput screening approaches.

Within the coronavirus main protease project, the crystal structure of the SARS-CoV M^{pro} was determined after acylation of its active-site cysteine by benzotriazole esters that act as suicide inhibitors (Fig. 5). The results help now to design benzotriazole inhibitors with improved specificity.

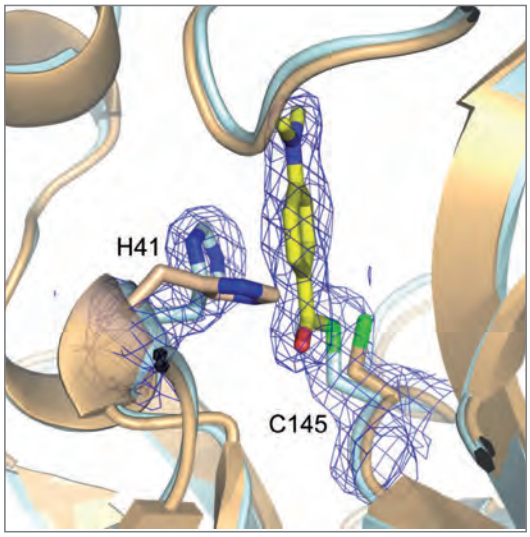

Fig. 5. Comparison of the active site of the free enzyme (light orange) with the active-site environment of the SARS-CoV M^{pro} reacted with 1-(4-dimethylaminobenzoyloxy)-benzotriazole (light blue).

Research group: Guido Hansen

Molecular Basis of Bacterial and Protozoal Infections

The research in the group is focused on virulence proteins from bacteria and protozoa. Using X-ray crystallography, atomic structures of these proteins are determined and analyzed, leading to a detailed understanding of the underlying mechanisms at a molecular level. Structural information is used as a basis for ra-

Biochemistry

tional design of new, potent anti-infectives, e.g. against malaria, tuberculosis, and legionellosis.
The causative agent of malaria, *Plasmodium falciparum*, is able to invade human erythrocytes and to feed on intracellular hemoglobin. Falcipain-2 is a protease of the parasite that is essential for hemoglobin degradation. The activity of falcipain-2 is supposed to be regulated by the plasmodial protease inhibitor ICP. The interplay of falcipain-2 and ICP presumably allows the coordinated breakdown of hemoglobin and other host-cell proteins and might be crucial for the survival of *Plasmodium* in human hosts. The group recently succeeded in structure determination of falcipain-2 in complex with ICP from *P. berghei* (Fig. 6). The molecular characterization of this important virulence mechanism will be a major step forward in the understanding of the pathogenicity of the parasite.

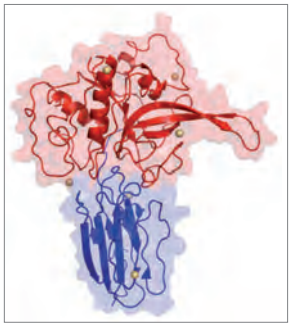

Fig. 6. Structure of falcipain-2 (red) in complex with the C-terminal domain of the cysteine protease inhibitor ICP from P. berghei (blue).

Another area of interest is focused on the clinical pattern of legionellosis leading to severe pneumonia with a case fatality rate of up to 15%. We are working on the identification of new virulence factors of *Legionella* as targets for drug therapy.
Recently, the group successfully determined the three-dimensional structure of the FeoB from *E.coli* and *L. pneumophila*, a protein that enables the uptake of iron from the environment. These structural studies are important steps towards an understanding of survival strategies of Legionella in human hosts.

Laboratory for Structural Biology of Infection and Inflammation

Synchrotron radiation at the Institute's outstation at DESY, Hamburg

Together with the University of Hamburg, the Institute runs the "Laboratory for Structural Biology of Infection and Inflammation" (LSBI&I, Fig. 7) on the DESY premises in Hamburg. The Institute's researchers use the synchrotron radiation provided by DESY for investigating their crystals of virulence proteins. In collaboration with the University of Hamburg and the European Molecular Biology Laboratory (EMBL), they also operate the synchrotron beamline X13. In the future, further investment of the Institute at DESY is planned, together with the DFG Cluster of Excellence "Inflammation at Interfaces" (Borstel – Kiel – Lübeck), in order to use the new storage ring "PETRA III", which will produce even more brillant synchrotron radiation.
The Institute's scientists are also specializing on novel methods for macromolecular crystallization and have recently constructed a pyroelectric microcalorimeter that allows the determination of all relevant thermodynamic properties of a "hanging crystallization droplet". This instrument combines calorimetry with Dynamic Light Scattering for the observation of crystal nucleation in the microdroplet.

Fig. 7. Laboratory for Structural Biology of Infection and Inflammation

Selected Publications:
Research group "Structure-based drug design"
1. H. Steuber, A. Heine & G. Klebe: Structural and thermodynamic Study on Aldose Reductase: nitro-substituted inhibitors with strong enthalpic binding contributions, J. Mol. Biol. 368 (2007), 618-638
2. H. Steuber, M. Zentgraf, C. La Motta, S. Sartini, A. Heine & G. Klebe: Evidence for a novel binding site conformer of aldose reductase in ligand-bound state. J. Mol. Biol. 369 (2007), 186-197
3. H. Steuber, P. Czodrowski, C. A. Sotriffer & G. Klebe: Tracing changes in protonation: A prerequisite to factorize thermodynamic data of inhibitor binding to aldose reductase. J. Mol. Biol. 373 (2007), 1305-1320

Synthetic Chemistry Group
1. K.H.G. Verschueren, K. Pumpor, S. Anemüller, S. Chen, J.R. Mesters & R. Hilgenfeld: A Structural View of the Inactivation of the SARS-coronavirus main proteinase by benzotriazole esters. Chem. Biol. 15 (2008), 597-606
2. M.F. Schmidt, A. Isidro-Llobet, M. Lisurek, A. El-Dahshan, J. Tan, R. Hilgenfeld & J. Rademann: Sensitized detection of inhibitory fragments and iterative development of non-peptidic protease inhibitors by Dynamic Ligation Screening. Angew. Chem. Int. Ed. Engl. 47 (2008), 3275-3278
3. R. Hilgenfeld & K. Pumpor: Sometimes intermediates do the job! Chem. Biol. 13 (2006), 235-236

Research Group "Molecular Basis of Intracellular Infection by Bacteria and Protozoa"
1. E. Liebau, J. Höppner, M. Mühlmeister, C. Burmeister, K. Lüersen, M. Perbandt, C. Schmetz, D. Büttner & N. Brattig: The secretory omega-class glutathione transferase OvGST3 from the human pathogenic parasite Onchocerca volvulus. FEBS J. 275 (2008), 3438-3453
2. T. Hogg, K. Nagarajan, S. Herzberg, L. Chen, X. Shen, H. Jiang, M. Wecke, C.J. Blohmke, R. Hilgenfeld & C.L. Schmidt: Structural and functional characterization of falcipain-2, a hemoglobinase from the malarial parasite Plasmodium falciparum. J. Biol. Chem. 281 (2006), 25425-25437
3. J. Rupp, J. Gieffers, M. Klinger, G. van Zandbergen, R. Wrase, M. Maass, W. Solbach, J. Deiwick & T. Hellwig-Burgel: Chlamydia pneumoniae directly interferes with HIF-1 stabilization in human host cells. Cell. Microbiol. 9 (2007), 2181-2191

Crystallogenesis Group
1. T. Klupsch, A. Walter, P. Mühlig & R. Hilgenfeld: Combined kinetic osmometry and pyrometric microcalorimetry: Direct measurement of the protein-precipitant (salt) interaction. Coll. Surf. A Physicochem. Eng. Aspects 318 (2008), 9-23
2. T. Klupsch, A. Walter, P. Mühlig & R. Hilgenfeld: Combined kinetic osmometry and pyrometric microcalorimetry on protein solutions: Setup and data evaluation. Coll. Surf. A Physicochem. Eng. Aspects 318 (2008), 24-44
3. J.R. Mesters & R. Hilgenfeld: Protein glycosylation, sweet to crystal growth? Cryst. Growth Des. 7 (2007), 2251–2253

Dr. rer. nat. Guido Hansen

2001 „Diplom" in biology, Institute of Genetics, University of Cologne
2005 PhD in biochemistry, Institute of Biochemistry, University of Cologne
2005-2007 Postdoc, St. Vincent's Institute, Melbourne, Australia
Since 2007 Group leader, Institute of Biochemistry, University of Lübeck

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Prof. Dr. rer. nat. Enno Hartmann

1981-1986 Studies of Technical Hydrobiology,
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1986-1991 Research Associate, Academy of Science of the GDR, Berlin
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1998-2001 Full Professor, University of Göttingen
Since 2001 Full Professor and Chair, Institute of Biology, University of Lübeck

Awards, memberships and others:
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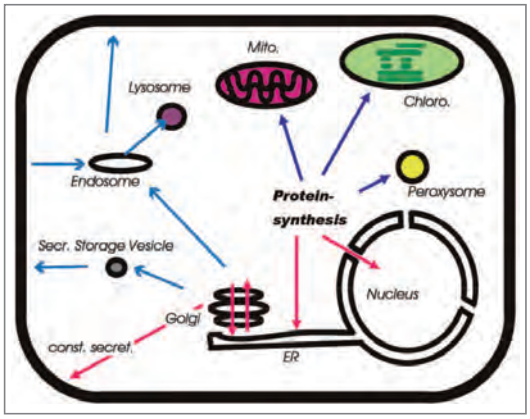
Main research topics in the CSCM:

protein transport at the endoplasmic reticulum
nucleo-cytoplasmic transport
vesicular traffic and toxin transport
Connexin assembly and interacting partners
High resolution imaging of cellular compartments and FRET in living cells

Protein Topogenesis

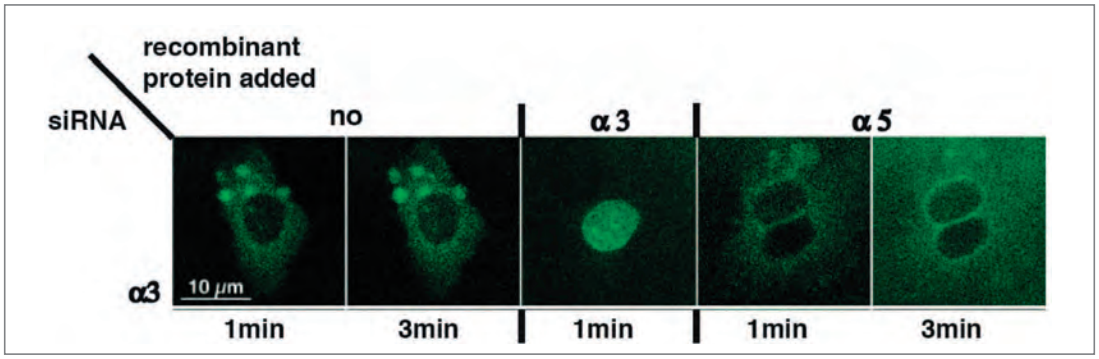
The aqueous interior of cells – the protoplasm - is separated from its surrounding by a hydrophobic biological membrane, the plasma membrane, that in the absence of appropriate transporters is impermeable for hydrophilic macromolecules like proteins. Many bacteria have additional membrane(s) outside of the plasma membrane, forming a hydrophilic area, the periplasmic space, that enwraps the plasma membrane. In eukaryotic cells, the protoplasm is partitioned by inner membranes into compartments. The exterior of the cell, the different cellular compartments and the membranes separating them are characterized by specific of sets proteins which cause their distinct biochemical properties. However, only few of these compartments – the cytoplasm, the matrix of mitochondria or the stroma of plastids - contain the machinery needed for protein synthesis. A central problem is therefore the correct distribution of proteins from their site of synthesis to their site of function - a process called protein topogenesis.
The institute of biology studies three pathways of protein topogenesis – the translocation across the endoplasmic reticulum (ER), the transport between cytoplasm and nucloplasm, and steps of the vesicular traffic along the secretory pathway



Protein translocation at the endoplasmic reticulum (ER)

Transport of newly synthesized proteins at the ER is a key step in the biogenesis of secreted proteins, plasma membrane proteins and of proteins of organelles of the secretory pathway. These proteins contain signal sequences, which direct them to translocation sites at the ER. Transport occurs either co-translationally with the ribosome tightly bound to the ER or post-translationally (1). During co-translational translocation the signal sequence triggered targeting of the nascent polypeptide is mediated by the SRP/SRPR-pathway. At the end, the nascent chain is integrated into a protein conducting channel formed by the Sec61 complex which also binds the ribosome. In post-translational transport no signal sequence dependent targeting mechanism is known. These polypeptides also traverse the membrane via the Sec61 complex. However, post translational translocation sites contain additional elements important for targeting and for directed transport.
In the last decades we and others identified all components that form the translocation site and determined the function of some of the key players. However, many features of the transport are still unknown. Presently, we are concentrating on two problems - the analysis of the detailed transport pathway of substrates with atypical primary structure and the investigation of the detailed function and mechanistic properties of components present in co-translational translocation sites.
An analysis (2) of the tertiary structure of the central component of translocation sites, the Sec61 complex, indicated that one Sec61 complex is probably sufficient to form the polypeptide conducting channel, a hypothesis which later was confirmed by biochemical approaches (3). In the light of this result it is difficult to explain why in eukaryotes translocation site seem to consist of four heterotrimeric Sec61 complexes.
Interestingly, binding studies using purified Sec61 complex as well as other biochemical methods showed, that this complex is not only able to bind ribosomes during co-translational translocation, but that it is also able to bind 26S proteasomes and other cytosolic factors which are believed to be involved in the degradation of proteins from the ER (4). This supports the hypothesis, that the protein conducting channel formed by the Sec61 complex is also involved in the back-translocation of proteins from the ER destined for degradation by the proteasome in the cytoplasm.

Biology



Coinjection of recombinant importin 3 but not of importin 5 restores nuclear RCC1 accumulation in HeLa cells with decreased expression of importin 3. RCC1 was microinjected either alone or in combination with recombinant importin 3 or 5, and its subcellular distribution was analyzed by confocal microscopy at the time points indicated. Coinjection of importin 3 caused fast RCC1 import, in less than 1 min, in 100% of importin 3 knockdown cells. Coinjection of importin 5 resulted in nuclear accumulation after 1 or 3 min in 12 or 28% of cells depleted of importin 3.

Protein transport between cytoplasm and nucleoplasm

Protein transport across the nuclear envelope is mediated by nuclear transport receptors (importins or exportins) of the importin ß-like superfamily. Some of these proteins perform the transport of their cargo with the help of adaptor molecules. In particular importin ß itself uses adaptors – importin s – to from import complexes with substrates of the "classical" import pathway (5). During the last years we were involved in the identification of most of the importins and exportins and in the search for membrane-anchor nuclear pore proteins. Currently we are especially interested in the analysis of the function of the different importin s found in mammals.
Most of these 6-7 forms are ubiquitously expressed. Although they display up to 50% divergence in their primary structure, nearly all of the residues shown to be important for interaction with the nuclear localization sequences (NLSs) of cargos or for the binding to importin ß are conserved. However, binding studies and in vitro import assays demonstrated that specific cargos, like RCC1 preferentially interact with distinct importin s (6).

First experiments trying to understand the structural basis for binding specificity showed that the major determinant providing importin specificity of a cargo like RCC1 resides in its folded part which is distinct from the NLS (7). Currently we are cooperating with the group of M. Bader, Berlin to get a deeper understanding on the in vivo relevance of the different importin (8).

Selected Publications:
1 Osborne AR, Rapoport TA, van den Berg B. Protein translocation by the Sec61/SecY channel (2005). Annu Rev Cell Dev Biol. 21, 529-550.
2 Van den Berg B, Clemons WM Jr, Collinson I, Modis Y, Hartmann E, Harrison SC, Rapoport TA. (2004) Xray structure of a proteinconducting channel. Nature(London) 427, 3644.
3 Kalies K.-U., Stokes V., Hartmann E. (2008)A single Sec61-complex functions as a protein-conducting channel Biochimica et Biophysica Acta 1783, 2375-2383.
4 Kalies KU, Allan S, Sergeyenko T, Kroger H, Romisch K. The protein translocation channel binds proteasomes to the endoplasmic reticulum membrane (2005). EMBO J. 24, 2284-2293.
5 Gorlich D, Kutay U. (1999) Transport between the cell nucleus and the cytoplasm. Annu Rev Cell Dev Biol. 15, 607-660.
6 Quensel C, Friedrich B, Sommer T, Hartmann E, Kohler M. (2004) In vivo analysis of importin alpha proteins reveals cellular proliferation inhibition and substrate specificity. Mol Cell Biol. 23, 1024655.
7 Friedrich B, Quensel C, Sommer T, Hartmann E, Köhler M (2006) Nuclear localization signal and protein context both mediate importin alpha specifity of nuclear import substrates Mo Cell Biol. 26, 8697-8709.
8 Shmidt T., Hampich F., Ridders M., Schultrich S., Hans V.H., Tenner K., Vilianovich L., Qadri F., Alenina N., Hartmann E., Köhler M., Bader M. (2007) Normal brain development in importin-alpha5 deficient-mice Nature Cell Biology 12, 1337-8.

PD Dr. rer. nat. Kai Kalies

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1990-1991 Research Associate, Academy of Science of the GDR, Berlin
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2001-2003 Research Associate and group leader, Institute of Biology, University of Lübeck
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1992 PhD, University of Heidelberg & EMBL Heidelberg
1992-1996 Postdoc, University of California, Berkeley, USA
1996-2004 Group Leader, Cambridge Institute for Medical Research, UK
2004-2008 Chair of Cell Biology & Biochemistry, Royal Holloway University of London, UK
Since 2008 Associate Professor, Institute of Biology, University of Lübeck

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1996-2004 Wellcome Trust Senior Fellow
Member of the Editorial Board of the journals "Traffic", "Histochemistry and Cell Biology" and "European Journal of Cell Biology"

Membrane traffic between the Golgi complex and the endoplasmic reticulum (ER)

Cytoplasmic coat proteins shape membranes of organelles in the secretory and endocytic pathways into vesicular or tubular transport carriers that mediate intracellular protein sorting and transport. COP I coat proteins and their regulators play essential roles in secretory protein traffic in all eukaryotic cells. They mediate a Golgi-to-ER retrieval pathway and transport within the Golgi complex, and are required for maintenance of the interphase Golgi complex. Because of this, COP I – mediated membrane trafficking and protein sorting underpins diverse and distinct physiologically important pathways such as protein secretion, ER quality control, and efficient ER – associated degradation, MHC class I antigen presentation, proper maturation of numerous cell surface receptors and ion channels, e.g. GABAB receptor, K-ATP channels, T-cell receptor or CFTR, and many others that are relevant to human disease [1-4, 7, 8].

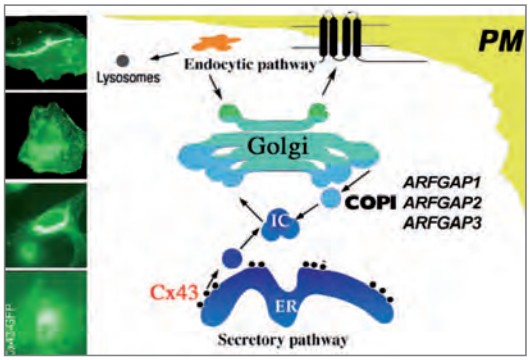

Fig. 1: Left: Connexins pass anterogradely from the ER to the Golgi complex and then to the plasma membrane to form gap junctions between contacting cells. Gap junction plaques get stabilized, e.g. by drebrin, and ultimately internalized and degraded by lysosomes and proteasomes. Right: COP I is involved in ER quality control and a retrograde pathway from the ER to the Golgi complex [8].

The minimal cytosolic machinery to form COP I vesicles consists of the small GTPase ARF in its GTP-bound form and coatomer, an ~800 kDa heptameric protein complex comprising -, -, '-, -, Δ, -, and -COP. ADP-ribosylation factors (ARFs) are ~20 kDa ras – like GTP-binding proteins that are central regulators of trafficking through and from the Golgi. ARFs are required for cargo sorting, membrane deformation, and vesicle coat release. In their inactive GDP-bound form, an N-terminal myristoyl residue is hidden and the protein is soluble in the cytosol. Nucleotide exchange to GTP triggers a conformational change, exposing the N-terminal amphipathic helix and allowing ARF's association with Golgi membranes. ARFs have a low intrinsic GTPase activity, so helper proteins, ARF GTPase-activating proteins or ARFGAPs, are essential for switching them to their inactive form. ARFGAP activity drives removal of the coat during a late step of COP I vesicle formation which is a prerequisite for membrane fusion with the target membrane and thus for cargo delivery. However, cargo sorting into COP I vesicles also requires ARFGAP activity. ARFGAP proteins selectively interact with SNARE proteins involved in specificity of vesicle fusion, p24 proteins, and with ERD2, a transmembrane receptor for escaped ER lumenal proteins that mediates ER retrieval. We hypothesize that individual ARFGAPs are involved in uptake of subsets of cargo into COP I vesicles. In yeast two distinct ARFGAPs, Gcs1p and Glo3p provide overlapping, essential functions in COP I transport. These ARFGAPs share a conserved catalytic zinc finger domain that engages ARF but are unrelated in their non-catalytic domains. The mammalian Gcs1p-ortholog ARFGAP1 harbors two lipid-packing sensor domains that sense membrane curvature. In collaboration with Jennifer Lippincott-Schwartz's lab at NIH we characterized the dynamic behavior of ARFGAP1 using live cell imaging techniques [2]. Through sequence searches we identified ARFGAP2 and ARFGAP3 as the Glo3p orthologs encoded in the human genome [1]. ARFGAP2/3 and Glo3p possess a conserved signature motif, which can be used for the unambiguous identification of Glo3p orthologs. ARFGAP2 and ARFGAP3 co-localize closely with COP I proteins and associate with COP I vesicles produced in vitro. We identified strong interactions of ARFGAP2 and ARFGAP3 with subunits of coatomer in the two-hybrid system and in vitro. Our in vivo data establish that ARFGAP2 and ARFGAP3 act in COPI - dependent Golgi-to-ER and intra-Golgi trafficking.

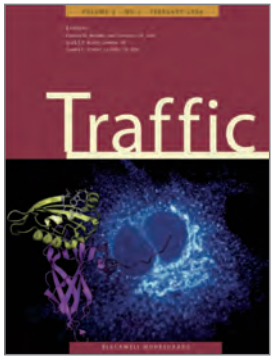

Fig.2; Immunofluorescence of the COP I coat in a Vero cells, using an antibody against the beta-COP subunit. Left: crystal structure of the gamma-COP appendage that is involved in interactions with ARFGAP2 and ARFGAP3 [4]. Featured on the cover of TRAFFIC (see Watson et al., 2004 (Feb. 2004 issue)).

We recently discovered a novel link between intracellular transport via the ARFGAP/ COPI/ARF1/cargo system and nuclear envelope stability and mitotic signalling. Dramatically, dominant mutants of ARFGAP2 or ARFGAP3 cause strong impairment of retrograde trafficking in the secretory pathway, affect nuclear envelope structure, and rapidly induce formation of giant multinucleate cells. Our data suggest a cell cycle dependence for this dramatic phenotype. Our findings set the stage for a molecular dissection of the role of COP I in cell cycle – dependent organelle assembly and for unravelling the functions of ARFGAPs in COP I – dependent traffic.

Biology

FRET, Connexins and Interacting proteins.

FRET (Fluorescence Resonance Energy Transfer) is so far the best "Spectroscopic ruler" technique to measure protein-protein interactions in Living Cells. This nano-scale precise tool can resolve proximity of Donor-Acceptor pairs at distances of 2-7 nm. Using FRET for live cell biology we can monitor functionally important protein-protein interactions [5] and host-pathogen interactions [6]. Measuring interactions that occur in millisecond to minutes using FRET gives us a detailed understanding of how proteins work in their native environment.

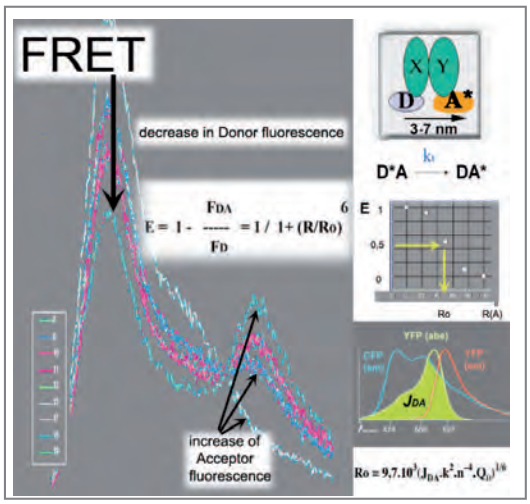

Fig.3. FRET is a "spectroscopic ruler" for live cell analyses. Principle of FRET: upon protein-protein interaction the fluorescence of the Donor decreases and the fluorescence of the Acceptor increases. This change only occurs in a distance range between Donor and Acceptor molecules of 3-7 nm. Cellular proteins are tagged with genetically encoded fluorescent proteins – GFPs to measure protein complex formation. Example of the CFP- and YFP-tagged FRET pair is shown as an integral overlap, (J DA). Experimental data are used to calculate cellular distances between proteins of interest.

Cell-cell communication in tissues would be impossible without connexins. These short-lived four-transmembrane proteins are synthesized in the ER and assemble into hexameric hemi-channels. At the plasma membranes of contacting cells these link up to form regulated cell-to-cell channels, gap junctions, that are permeable to metabolites and small signaling molecules such as Ca2+, cAMP, ATP, and cGMP. 21 connexin isoforms are encoded in the human genome. Several connexins are expressed in brain and retina, namely Cx-26, Cx-32, Cx36, Cx43, Cx45, and Cx57, where they mediate direct cell-cell coupling. Human diseases, including neurological diseases, arise from naturally occurring mutations in connexins. E.g. X-linked Charcot-Marie-Tooth disease (CMTX) - a form of hereditary neuropathy with demyelination is linked to Cx32 mutations.
The cytoplasmic loop and C-termini of connexins are regulated by phosphorylation and undergo important protein-protein interactions. Using a proteomics approach in combination with FRET, we identified a novel Connexin 43 interacting protein, Drebrin (developmentally regulated brain protein) [7]. Drebrin interacts with the C-terminus of Cx43 as a part of the submembrane cytoskeleton to stabilise cell-cell contacts [8]. Stable Cx43 gap junctions are important in cardiac protection as well as in retina, brain and other tissues. Currently we are testing the role of drebrin (and its interactors) in regulating gap junction formation/stability of neuronal connexins.

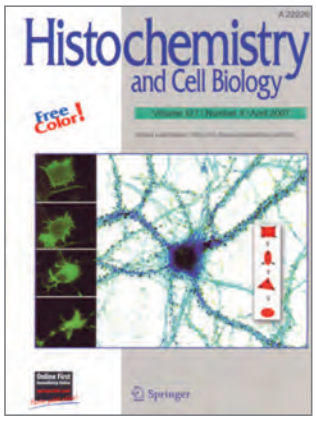

Fig. 4: Drebrin is a morphogenic protein that interacts with connexins and helps remodel dynamic cell-cell contacts. Upon overexpression drebrin can dramatically change cell shape and induce formation of neurite-like cell processes. In hippocampal neurons it is concentrated in dendritic spines. Cover of "Histochemistry and Cell Biology" (April 2007 issue) [8].

As models we use cell lines transfected with connexins and primary cells from retina, brain, and heart that naturally express Cx43, Cx32, Cx36 or Cx45. We combine high resolution live cell microscopy, FRET, RNAi, dye transfer, electrophysiology, biochemistry and yeast two-hybrid to study domain-specific interactions of connexins and their regulation in normal and pathologic cell conditions, including in inflammation.
Our research expertise includes nano-scale resolution of cellular processes with photo-activatable-GFPs, FRET-FLIM spectroscopy, and fluorescence anisotropy. We have established international (UK, USA, Japan, Israel, Italy, France, Russia) and on-campus collaborations covering a range of interdisciplinary research linking Cell Biology to Medicine. Our long-term goal is to functionally characterize novel proteins involved in cellular transport and regulation of neuronal connexins and neuronal circuit formation.

Selected Publications (Research Group Duden & Majoul):
1 Frigerio, G., Grimsey, N., Dale, M., Majoul, I., Duden, R. (2007). Two human ARFGAPs associated with COP I – coated vesicles. Traffic 8, 1644-1655
2 Liu, W., Duden, R., Phair, R.D. , Lippincott-Schwartz, J. (2005). ArfGAP1 dynamics and its role in COP I coat assembly on Golgi membranes of living cells. J. Cell Biol. 168, 1053-1063
3 Eugster, A., Frigerio, G., Dale, M., Duden, R. (2004). The - and '-COP WD40 domains mediate cargo-selective interactions with distinct dilysine signals. Mol. Biol. Cell 15, 1011- 1023
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5 Majoul, I., Straub, M., Hell, S., Duden, R., Soling, H.D. (2001). KDEL-cargo regulates interactions between proteins involved in COPI vesicle traffic. Measurements in living cells using FRET. Dev. Cell 1, 139-153
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1982 Diploma, Biological Faculty of the State University Minsk, Belarus
1983-1985 Staff Scientist, Minsk Academy of Sciences. Belarus
1986-1991 Lecturer, State Medical Institute Minsk, Belarus; Ph.D. in Biochemistry
1997 Ph.D. degree in Biophysics, Russian Academy of Sciences, Puschino, Russia
1991-1998 Research Scientist, Göttingen University; Official recognition of both
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2003 Professorship degree (d. b. s.) in Biophysics; Russian Academy of Sciences, Russia
2003-2004 Research Scientist, Cambridge Institute for Medical Research, UK
2004-2008 Honorary Lecturer Royal Holloway University of London, UK
Since 2008 Research Associate, Institute of Biology, University of Lübeck

Memberships
Royal Microscopical Society (RMS), UK, British Neuroscience Association (BNA), German Society for Cell Biology (DGZ), German Neuroscience Society (GNS)

Institute of

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1960-1963 Studies of Electrotechnical Engineering, Polytechnical School, Munich (Dipl. Ing. FH)
1965-1971 Studies of Physics, Technical University of Munich
1979 PhD
1985 Habilitation in Medical Biophysics at Ludwig-Maximilian University of Munich
1972-1980 Research Associate, GSF (Company of Radiation and Environmental Research) Munich
1980-1991 Head of the Hermann Wacker Laboratory for Medical Laserapplications of the LMU Munich
1987-1988 Visiting scientist (Ophthalmology) at Harvard University, Boston
1988-1990 Director of research and co-director of the Laser Application Program of the Wellman Laboratories of Photomedicine at Massachusetts General Hospital, Boston
since 1988 Visiting Professor (Dermatology) at Harvard University
1989-1991 Visiting Professor of the Division of Health Science & Technology at the Massachusetts Institute of Technology (MI), Cambridge
1987-1991 Professor of Medical Biophysics at Ludwig Maximilian University of Munich
Since 1992 Director of research and chairman of the MLL and Full Professor of Biophysics and Laser Medicine at the University of Lübeck
Since 2005 Director of the Institute of Biomedical Optics at the University of Lübeck

Awards, memberships and others:
1981: Award of the Society of the German Biomechanical and Optical Industry
1982: Senator Hermann Wacker Award
2009: Junius Kuhnt Medaille

Member of Deutsche Physikalische Gesellschaft, Deutsche Ophthalmologische Gesellschaft, Deutsche Gesellschaft fuer Lasermedizin, Deutsche Gesellschaft fuer Biomedizinische Technik, American Society for Laser Medicine and Surgery, Association for Research in Vision and Ophthalmology, International Society for Optical Engineering, Optical Society of America

Member of the Editorial board of Lasers in the Life Science, Lasers in Surgery and Medicine, Klinische Monatsblätter für Augenheilkunde, Ophthalmologe, IEEE Journal of Quantum Electronics (Special Issue on Lasers in Biology and Medicine, 1990), Ophthalmic Surgery, Lasers & Imaging

Director: Prof. Dr. phil. nat. Reginald Birngruber
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Main research topics in the CSCM:

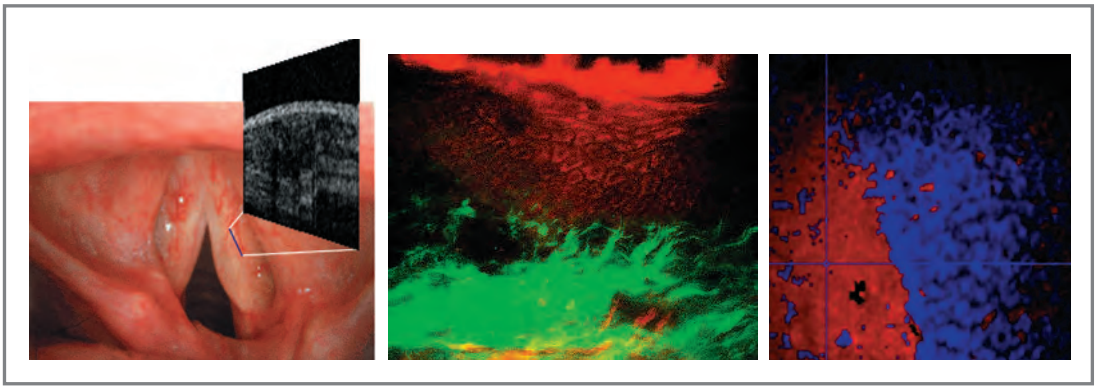
In vivo imaging of cells and tissues, linked with their manipulation
Nanoscalpels for cells using focused laser irradiation, or chromophore- and nanoparticle-assisted targeting
Laser-based procurement of biological samples for genomic analysis
Antimicrobial therapy combining photodynamic therapy and antiseptics for pathogens exhibiting resistance against antibiotics
Tumor-selective dye-enhanced photothermal therapy
Cell-specific laser therapy at the ocular fundus

Nonlinear in vivo Microscopy

Microscopy has changed biology and medicine in the last 200 years by visualizing cells and subcellular structures. However, conventional light microscopy is restricted to imaging individual cells in culture or tissue sections because in-vivo imaging of cells within the tissue is limited by strong scattering. We are working on and with two techniques capable to overcome this obstacle: Images with a resolution of 5-15 µm up to a depth of one millimeter are obtained by interferometry with a broad band light source (optical coherence tomography, OCT). In-vivo 3D imaging with subcellular resolution is achieved by means of femtosecond laser pulses that excite endogeneous fluorescence or exogeneous markers (fluorescent dyes or nanoparticles) by the collective action of two or more photons. This nonlinear microscopy provides larger imaging depth with less photobleaching and photodamage compared to laser scanning confocal microscopy (CLSM) and can visualize the action and interaction of cells in there native tissue environment.

The BMO and a spin-off company (Thorlabs Lübeck) developed the first commercially available second-generation spectral-domain OCT that, in contrast with the first generation devices, contains no moving parts. The BMO promotes OCT applications through cooperations with various preclinical and clinical research groups [1,2]. Current projects include intraoperative visualization of tissue structures of the vocal cord, middle ear (Prof. Pau, ENT, University Rostock), and eye (Ophthalmology, Univ. of Lübeck).
Multiphoton laser scanning microscopy is used for the visualization of immunological processes in intestines (Prof. Gebert, Anatonomy, Univ. of Lübeck), cornea and conjunctiva (Dr. Steven, Ophthalmology, Univ. of Lübeck) [3], and lung tissue (Dr. König, Anatomy, Univ. of Lübeck), as well as tissue remodelling in skin (Dr. Fleischer, Dermatology, Univ. of Lübeck), and diagnosis of infections (Dr. Rupp, Microbiology and Hygiene, Univ. of Lübeck).

Fig. 1: In-vivo imaging by nonlinear microscopy. Left: Non-contact cross-sectional visualization of Rainke edema at the vocal fold (OCT). Center: Discrimination of cellular structures (red) from collagen (green) at the limbus of the eye by different emission wavelengths of multiphoton excited fluorescence from cells and second harmonic generation by collagen. Right: Discrimination of tumor cells (blue) from healthy brain tissue (red) by multiphoton excited fluorescence lifetime measurements (FLIM).



Biomedical Optics

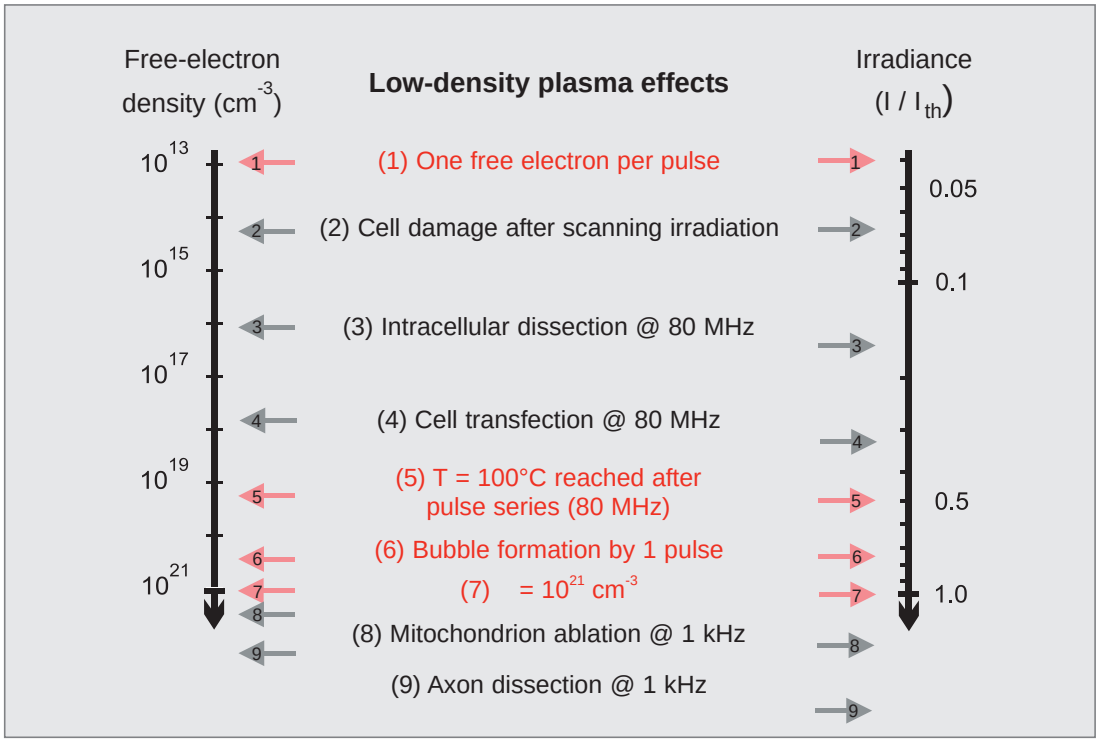


Fig. 2: Overview of fs low-density plasma effects and breakdown phenomena compared to experimental data on cell surgery

Nanoscalpels for Cells

Nonlinear absorption of tightly focused ultrashort laser pulses (plasma formation) can be used to achieve very fine and highly localized laser effects inside of biological media regardless of their local optical absorption properties, i.e. even in cells and tissues that are transparent at low irradiance. Researchers at the BMO studied for many years the physical mechanisms, tissue effects and applications of plasma-mediated intraocular microsurgery and short-pulsed laser tissue ablation [4]. In recent years the exploratory focus shifted towards laser microdisection and manipulation on a cellular and subcellular level. The advent of femtosecond laser pulses has made it possible to achieve surgical effects on a nanometer scale at arbitrary location within the cell. After proving that this kind of nanosurgery relies on plasma generation [5], the process was studied in detail both experimentally and by numerical simulations of plasma-, stress wave and bubble formation at the laser focus [5]. By comparing the theoretical threshold values to experimentally determined thresholds for various cellular and tissue effects we elucidated the working and damage mechanisms of nanosurgery at kHz and MHz laser repetition rates (Fig. 2) [6, 7]. We recently showed that not only femtosecond lasers are suitable for nano-surgery but also highly cost-effective nanosecond microchip lasers emitting at 355 nm and 532 nm wavelength [8].

Cell surgery with focused laser pulses is used for functional studies in cell physiology or developmental biology and to induce a transient permeabilisation of the cell membrane for the tranfer of genes and other substances, e.g. nanoparticles, into the cell. The BMO developed a technique for time-resolved detection of the size of the laser-produced bubbles down to a bubble radius of only 150 nm [7]. This technique has recently been advanced towards an online dosimetry for gentle cell membrane permeabilisation [9].

A disadvantage of cell surgery with focused laser irradiation is that the exact geometrical position of the target structure has to be known and to be aimed at with high precision. Furthermore, it can be applied in reasonable time only to a limited number of locations. These problems can be overcome by selective targeting making use of absorbing nanostructures, which can be brought to the target structure by coupling them to antibodies or other selectively binding molecules. Irradiation of gold nanoparticles with short laser pulses of fs to ns duration can create localized high temperatures that can be used to selectively destroy cells or even single biomolecules [10]. Depending on the laser parameters used, we can either transiently permeabilize cell membranes [11] or selectively destroy cells when gold nanoparticles are coupled to membrane proteins specific for

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1980 Degree in Physics and Sociology for teachers ('Gymnasium')
1987 PhD in Physics, University of Göttingen
1988-1992 Research Associate, laser laboratory of the University Eye Clinic Munich (LMU)
1992-2004 Research Associate and group leader, Medical Laser Center Lübeck (MLL)
1999 Habilitation in Physics, University of Lübeck
1999-2004 Vice chairman of the MLL
Since 2005 Privatdozent and group leader at the Institute of Biomedical Optics (BMO), University of Lübeck
2006 Associate Professor in Physics, Vice Director of the BMO

Membership and others
Fellow of the Optical Society of America and of SPIE, Member of Deutsche Physikalische Gesellschaft and Acoustical Society of America

Cochair of the Gordon Research Conference "Lasers in Medicine and Biology" 1996 Cochair of the Conference on Laser Ablation (COLA) 2009

Associate editor of "Optics Express," Member of the editorial board of the "Journal of Biomedical Optics"

Research topics
Mechanisms and clinical applications of pulsed laser tissue ablation, of plasma-mediated tissue dissection, and cell surgery, bio-applications of ultrashort laser pulses, nanosurgery and automated transfection of cells, laser-based procurement of biological samples for genomic analysis

Dr. rer. nat. Gereon Hüttmann

1988 Diploma in Physics, University of Göttingen
1992 Dr. rer. nat. in Physical Chemistry, University of Göttingen
1992-2004 Group leader at the Medical Laser Center Lübeck (MLL)
Since 2005 Group leader at the Institute of Biomedical Optics (BMO), University of Lübeck

Memberships and others
Member of the SPIE.

Research topics
Nanoparticle-mediated surgery of cells, optical coherence tomography, fluorescence spectroscopy and imaging, nonlinear in-vivo microscopy

Dr. rer. nat. Heyke Diddens

1975 Diploma in Biology
1979 PhD in Biology, University of Tübingen
1979-1988 Research Associate at the Children's Hospital, University of Tübingen
1981 Guest scientist at Indiana University, Indianapolis, IN, U.S.A.
1984 Guest scientist at Stanford University, Stanford, CA, U.S.A.
1988-2004 Group leader, Medical Laser Center Lübeck (MLL GmbH)
1992 Guest scientist at the Wellman Laboratories of Photomedicine, Harvard Medical School, Boston, MA, U.S.A.
1999/2000 Guest scientist at the Royal Military College of Canada, Kingston, ON, Canada
Since 2005 Group leader, Institute of Biomedical Optics (BMO), University of Lübeck

Memberships and others
Member of the European Society for Photobiology, American Society for Photobiology, International Society for Analytical Cytology.

Research topics:
Cytostatic drug resistance mechanisms in human cells, experimental, preclinical and clinical photodynamic therapy, dye-enhanced photothermal therapy, antimicrobial photodynamic therapy

certain cell types (Fig. 3). In cooperation with the Research Center Borstel (Prof. Gerdes), we elucidated the function of the proliferation-associated nuclear protein Ki-67 by means of chromophore-assisted light inactivation of pKi-67 that led to inhibition of ribosomal RNA synthesis via photochemical effects [12]. Currently, the BMO investigates the destruction mechanims of cells targeted by nanoparticles and nanorods, in cooperation with the Institute of Molecular Medicine and Experimental Immunology, University Bonn (Dr. Endl).

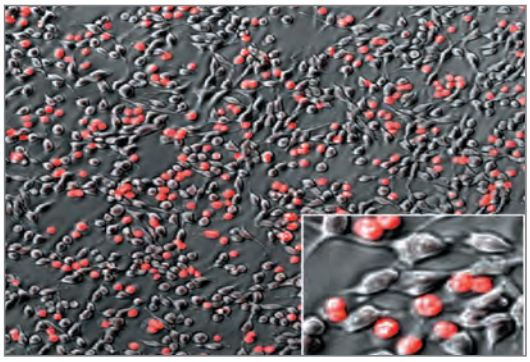

Fig 3: Selective destruction of adherent cells in a mixed culture shows the precision of NPCS. Dead cells are marked red.

Combination of Photodynamic Therapy and Antiseptics breaks Resistance against Antibiotics

Due to the extensive and widespread use of antibiotics progressive emergence and spread of microbial resistance to antibiotics is currently the most serious problem in the treatment of bacterial and fungal infections. We pursue a novel approach for selective destruction of microorganisms without harming the host tissue by combining the application of a local wound antiseptic (Octenisept) with antimicrobial photodynamic therapy (PDT). Based on a synergistic interaction, the combination of the photosensitizer toluidine blue O (TBO) with a low concentration of Octenisept results in highly efficient killing of the Gram-positive pathogenic S. aureus and the multiresistant strain MRSA, as well as of Gram-negative bacteria and Candida species. Furthermore, investigations on an organ culture model of the pig ear showed that the combination therapy caused no cytotoxic damage to human skin. Thus, TBO-PDT plus Octenisept may have potential for decontamination of extensive wounds and large areas of damaged tissue, like burns. Due to the mechanisms involved in PDT, the emergence of resistance is unlikely to develop.

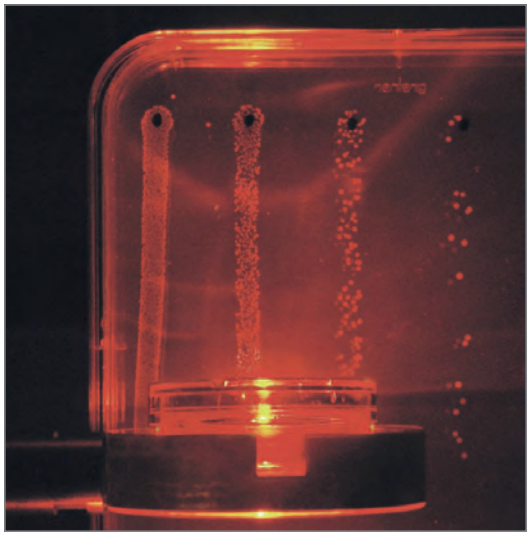

Fig 4: Photodynamic therapy combined with an antiseptic for elimination of human pathogens

Tumor-Selective Dye-Enhanced Photothermal Therapy

Chromophore-enhanced photothermal therapy is a novel approach for the treatment of tumors promising good spatial confinement. Exploiting the increased absorption caused by a tumor-localizing absorber in conjunction with infrared light irradiation for photothermal therapy, we achieved highly localized tumor necrosis that even surpassed the spatial confinement predicted by calculations of temperature distribution. This opens new perspectives for the curative and palliative treatment of nodular cutaneous and subcutaneous or submucosal neoplasia, and for treatment of embedded tumors in vital tissue, where surgical resection is not an option.

Biomedical Optics

Cell-Specific Laser Therapy at the Ocular Fundus

Gentle and highly cell-selective laser therapies are of growing interest for a variety of applications in medicine. Especially for laser therapies at the fundus of the eye, the preservation of the highly sensitive neural retina plays an important role. New treatment concepts like Selective Retina Therapy (SRT) [13], as well as well established retinal therapies like laser photocoagulation are investigated towards this goal. SRT is a new and very gentle laser method solely targeting the pigmented cells of the retinal pigmented epithelium (RPE). The RPE is a monocellular layer located between the photoreceptors and the vasculature and plays a major role in the retinal metabolism. RPE-degradation is associated with a variety of retinal diseases as age related macular degeneration (AMD), the leading cause of blindness in the industrial nations. In initial projects [14] the irradiation parameters required to achieve the desired RPE specific damage while sparing the adjacent photoreceptors and the choroid were investigated (Fig. 1). It was shown that µs-laser pulses are best suited to limit thermo-mechanical damage owing to microvaporisation at the intracellular melanin granules to the RPE cells [15]. Therefore, the nucleation and microbubble dynamics around nanometer to micrometer sized absorbing targets are theoretically and experimentally explored in more detail by optoacoustics and optical interferometry [16]. Both methods have the potential to serve as an online dosimetry control during treatment. The pathways of RPE rejuvenation and metabolic stimulations by expressed growth factors, metallomatrix-proteins (MMP's) and other cytokines at the chorio-retinal junction is investigated in cell and organ cultures. SRT itself is currently undergoing clinical trials in an international multicenter study.

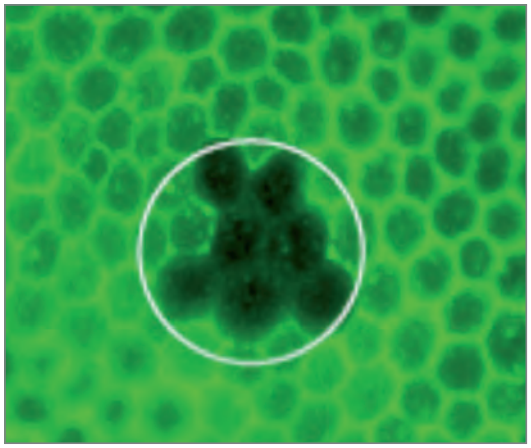

Fig 5: Pattern of hexagonal RPE cells visualized with a vitality stain (calcein AM) after SRT in vitro. Vital cells fluoresce green, dead cells appear dark.

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16 J. Neumann & R. Brinkmann: Boiling nucleation on melanosomes and microbeads transiently heated by nanosecond and microsecond laser pulses. J. Biomed. Opt. 10 (2005) 024001 1-12

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1993-2004 Research Associate and group leader, Medical Laser Center Lübeck (MLL)
2005 PhD in Physics, University of Lübeck
Since 2005 Group leader at the Institute of Biomedical Optics (BMO), University of Lübeck
Since 2005 Vice chairman of the MLL

Awards, memberships and others
2002, 2004, 2006 BMBF-Innovationspreis zur Förderung der Medizintechnik

Member of Deutsche Physikalische Gesellschaft and SPIE
2006 Cochair of the Gordon Conference of Lasers in Medicine and Biology

Research topics
Laser physics, laser tissue interactions, laser applications in ophthalmology, selective laser effects on a cellular level, smart feedback-controlled laser applications, minimally invasive laser surgery

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Prof. Dr. rer. nat. Thomas Peters

1977-1983 Studies of Chemistry at the Universities of Kiel and Hamburg
1986 PhD, University of Hamburg
1986-1987 Research Associate at the National Research Council of Canada, Institute of Biological Sciences, Ottawa
1992 Habilitation for Biophysical Chemistry, Frankfurt/Main
1992-1994 Privatdozent, Goethe-University of Frankfurt/Main
Since 1994 Full Professor and Chair, Institute of Chemistry, University of Lübeck
1996-1998 Dean of the Faculty of Natural Sciences, University of Lübeck
2004 Visiting Professor, Alberta Ingenuity Center for Carbohydrate Sciences, University of Alberta, Edmonton, Canada

Fellowship and awards:
1980-1983 Fellow of the Studienstiftung des Deutschen Volkes
1983-1985 Fellow of the Verband der Chemischen Industrie
1988-1990 Liebig-Fellow of the Verband der Chemischen Industrie
1994 Prize of the Hermann Willkomm-Stiftung
2004 Roy L. Whistler Award of the International Carbohydrate Organization

Professional Membership
Member of the Advisory Committee for the Resource for Integrated Glycotechnology, Complex Carbohydrate Research Centre, University of Athens, GA, USA.
Member of the Editorial Boards:
"Journal of Carbohydrate Chemistry"
"Carbohydrate Research"
"Glycoconjugate Journal"

Patents
T. Peters, B. Meyer, Verfahren zum Nachweis biologisch aktiver Substanzen in Substanzbibliotheken. Ger. Pat. No. DE19649359.
Swiss Pat. No. 690695.
T. Peters, B. Meyer, Method for detecting biologically active compounds from compound libraries, U.K. Pat. No. GB23211401.
U.S. patent No. US6214561.

Main research topics in the CSCM:

NMR studies of protein-carbohydrate interactions (monoclonal antibodies, lectins, selectins, siglecs, glycosyltransferases)
NMR studies of ligand binding to native viruses and viral coat proteins. Design of novel entry inhibitors
Recombinant production of isotope labeled glycosyltransferases for NMR spectroscopic experiments
Studies of thermodynamic and kinetic aspects of carbohydrate-protein recognition reactions using NMR, surface plasmon resonance and microcalorimetry
structural and biophysical characterization of skin matrix proteins involved in autoimmunity

Protein-Carbohydrate Interactions

Nature uses posttranslational modifications to orchestrate a diversity of complex biological functions such as cell-cell recognition or signal transduction. Among these modifications, glycosylations represent the perhaps most colorful and certainly the most diverse repertoire of distinct structures. The multitude of different glycostructures found in organisms from bacteria to man is far from being completely analyzed or understood. It is clear, however, that glycoproteins are key players in biological recognition events. Some examples for biological processes where protein-carbohydrate interactions play a fundamental role are:

- Viral and bacterial infections
- Cell-cell recognition and cell development
- Cancer and metastasis
- Development of Alzheimer's disease
- Diabetes type II

Our research focuses on the application of NMR spectroscopic techniques in conjunction with other biophysical methods such as surface plasmon resonance and molecular modeling for the analysis of protein-carbohydrate interactions at atomic resolution. Data from these investigations help to understand the nature of protein-carbohydrate interactions, and may enable us to modulate the biological function associated with the particular interaction. This is of importance for instance for the entry of viruses into host cells, and some of the projects target glycostructures that are associated with such viral infection processes. In that context we also study other viral proteins that are not glycosylated. A unifying theme of all of these topics is the NMR investigation of glycosyltransferases since these enzymes are the key to understand the variety of glycostructures in nature.

A very important experimental technique to analyze protein-carbohydrate complexes as well as other protein-ligand interactions is high-resolution NMR spectroscopy. In general, NMR provides a variety of tools to investigate such interactions at atomic resolution, and also has the potential to reveal dynamic aspects of the binding process. Experiments that are able to target large protein assemblies such as whole viruses, or membrane embedded proteins are attracting our special interest, since this constitutes a largely unexplored area. Here, ligand-based NMR techniques such as STD NMR and transferred NOE experiments are especially powerful. Fig. 1 shows how a blood group B trisaccharide is recognized by a viral coat protein, and depicts the experimental transfer NOE data that yield the bioactive conformation.

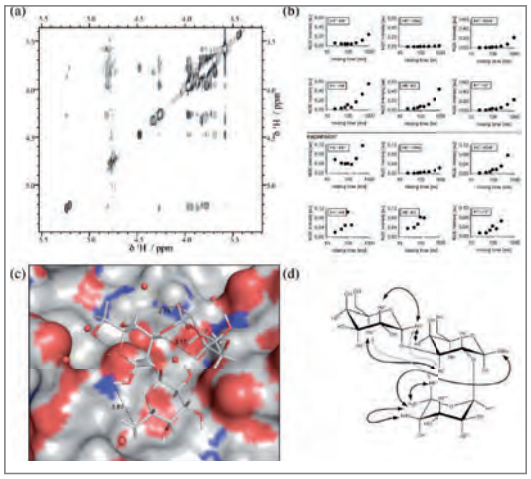

Fig. 1: Recognition of a blood group B determinant (BAG) (stick model in (c) and scheme (d)) into a shallow groove of a viral coat protein of a Norovirus. In (a) the transferred NOESY spectrum is shown that reflects the bioactive conformation of BAG. TrNOE build up curves are shown in (b) and the trNOEs are depicted schematically in (d). From C. Rademacher, Disseration, Lübeck, 2009.

Glycostructures in Virus-Host Recognition Processes

The attachment of viruses to hosts usually involves specific interactions of viral coat proteins with host cell receptors. In a couple of key experiments we showed that STD NMR is ideally suited to map the binding of low molecular weight ligands to native human rhinoviruses (HRV-2) at atomic resolution. From STD NMR we obtained the binding epitopes and relative dissociation constants for synthetic entry inhibitors that bind into the canyon binding site of the virus demonstrating that this novel technique provides a direct route to the rational design of entry inhibitors against viral infections. The approach is now extended to investigate the binding of fragments of the human LDL and VLDL receptors to HRV-2, and thus to map the viral entry process at a molecular level. First experiments have shown that it is possible to use STD NMR to resolve the binding epitope of VLDL receptor fragments at atomic resolution. This work is underway and will pave the way for a fast characterization of binding properties of different virus serotypes to the human LDL and VLDL receptors at atomic resolution.

These experiments are a proof of principle, and therefore we have applied this approach to other viruses. At the present, Norovirus infections are in the focus of our interest. To date, only very few entry inhibitors against

Chemistry

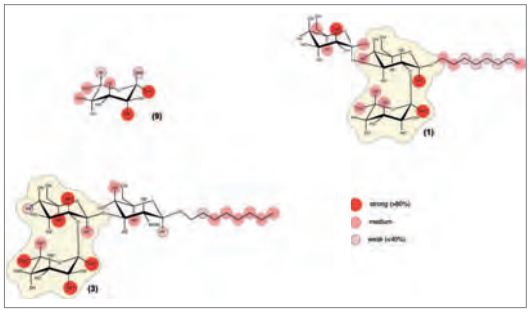

Fig. 2: Binding epitopes from STD NMR experiments for O-methyl- -L-fucopyranoside (9), blood group B trisaccharide (1) and H-type I trisaccharide (3). The binding epitopes serve as a basis for the design of potent entry-inhibitors against Norovirus infections.

viral infections have been identified. On the other hand, the availability of such compounds would offer a variety of novel therapeutic strategies to combat viral infections. As an example we have chosen Noroviruses that belong to the family of Caliciviridae. Noroviruses are non-enveloped positive stranded RNA viruses that bind to histo-blood group antigens (HBGAs) on the surface of host cells. Noroviruses are subdivided into two genogroups I and II and are extremely contagious, i.e. only 10 to 100 virions are sufficient to infect a person. Therefore, infections are usually epidemic and have been declared as B-agents by the NIH/CDC bidodefense program. There are no known cures, and vaccination strategies are not within sight. Our goal is to understand the structural and dynamic constraints of the virus-host recognition process as a basis for the rapid micro-evolution of the virus, and to finally design potent entry-inhibitors against Norovirus infections. So far, we have successfully determined the binding epitopes of several natural receptor molecules (so called human histo-blood group antigens, HBGAs) as shown in the example in Fig. 2. With so called interligand NOEs we have identified other binding fragments from so called fragment libraries. Using a novel design concept we have already synthesized an entry inhibitor with nanomolar affinity (collaboration with Prof. Bundle, Alberta Ingenuity Centre for Carbohydrate Sciences, Edmonton, Alberta, Canada).

Myelin associated glycoprotein (MAG) – modulation of neurite outgrowth

Another example where NMR spectroscopy plays a key role is provided by studies that are part of a joint project that involves research groups in Basel (Prof. B. Ernst), in Bremen (Prof. S. Kelm), in Hamburg (Prof. B. Meyer), and in Lübeck (Prof. Mailänder and Prof. Peters). This project focuses on the investigation of the interaction of gangliosides with a glycoprotein called myelin associated glycoprotein (MAG) that is responsible for the inhibition of neurite outgrowth upon injuries of the central nervous system (CNS). The overall goal in this project is the design of inhibitors of MAG that would eventually allow to promote neurite outgrowth and to finally heal pathological conditions such as quadriplegia. One major issue within this project is to analyze the bioactive conformation of natural ligands of MAG that may serve as templates for the design of much more potent inhibitors. To this end we have been investigating synthetic fragments of the most potent natural inhibitor of MAG, a ganglioside called GQ1b . Our work allowed to define the bioactive conformation of the key tri- and tetrasaccharides that are now further modified to generate more drug like compounds with a high potency to inhibit MAG. An example is shown in Fig. 3 that depicts the binding of the key tetrasaccharide to MAG. In collaboration with the plastic surgeons (Prof. Mailänder) in Lübeck we have succeeded to establish neurite outgrowth assays to test the activity of potential inhibitors of MAG before they are tested in animal models. It is hoped that these efforts finally will lead to a drug that is capable of curing quadriplegic.

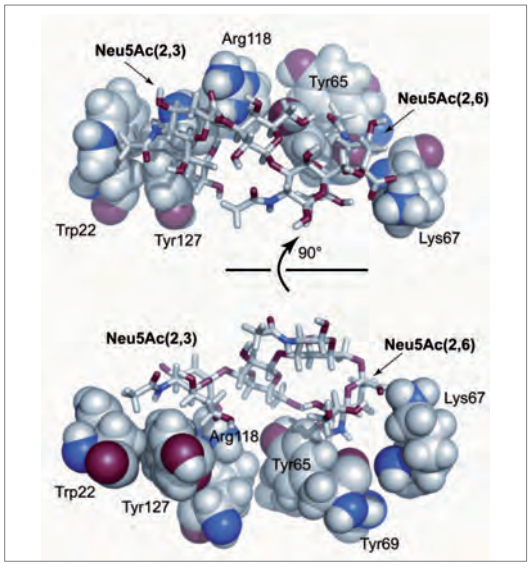

Fig. 3: NOE-based docking model of tetrasaccharide 2 in the binding site of MAG. Only selected amino acids of the binding pocket are shown. The (2→3)-glycosidic linkage prefers a "syn" orientation. It is seen that the carboxy groups of the (2→6)-linked and the (2→3)-linked Neu5Ac residues are close to the side chains of the amino acids Lys67 and Arg118, respectively, allowing for corresponding salt bridges. The image was produced with the program PyMOL (http://www.delanoscientific.com). From reference 2.

Glycosyltransferases as key players in posttranslational modifications

More than 75% of all proteins are glycoproteins, and it is unlikely that nature undergoes such a biosynthetic effort without a defined need. Yet, the biological function of carbohydrate structures and their biosynthesis is not well understood. A main obstacle had been the lack of suitable analytic techniques to perform structural and functional analyses. With the advent of high sensitivity NMR techniques in the past ten years this scenario has dramatically changed. The analysis of glycosylation patterns of glycoproteins with NMR is therefore an

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1990-1993 Study of Mechanical Engineering at the Universität of Aachen.
1993-1998 Study of Chemistry at the University of Cologne
2003 PhD in Biophysical Chemistry at the Universität of Lübeck.
Since 2003 Scientist at the Institute of Chemistry, University of Lübeck.
2006-2008 Training at the "Freie Journalistenschule" in Berlin

Memberships and others:
Gesellschaft Deutscher Chemiker, Deutscher Fachjournalisten Verband

Research topics: NMR spectroscopy, Metabonomics

PD Dr. phil. nat. Thomas Weimar

1983-1989 Studies of Chemistry, University of Frankfurt/Main
1993 PhD, University of Frankfurt/Main
1993-1995 Research Associate, Department of Chemistry at the Simon Fraser University, Canada
Since 1996 Institute of Chemistry, University of Lübeck
2002 Habilitation in Organic Chemistry
Since 2002 Privatdozent, Institute of Chemistry University of Lübeck

Research topics: NMR spectroscopy, surface plasmon resonance, protein-ligand interactions

Membership:
Gesellschaft Deutscher Chemiker, GBM, Deutsche Gesellschaft für Biophysik

Institute of

Dr. phil. nat. Hannelore Peters

1975-1980 Studies of Food Chemistry at the University of Frankfurt/Main.
1980-1981 Practical training at the Food Chemistry Department in Kassel and second examination to "State Approved Food Chemist".
1982-1983 Studies of Chemistry at the University of Hannover.
1983-1989 Dissertation in Biophysical Chemistry Department at the University of Frankfurt/Main.
1989-1997 Scientist in Research & Development Diagnostica and group leader for waste management at the Merck KG, Darmstadt (1995 Research and production transfer to Merck, France).
1997-1999 Scientist at the Institute of Chemistry, University of Hamburg.
Since 2000 Scientist at the Institute of Chemistry, University of Lübeck.
2004 Visiting researcher at the Ingenuity Center for Carbohydrate Sciences, University of Alberta, Canada.

Research topics: Recombinant expression of isotope labeled proteins, protein purification and characterisation

emerging field. We have concentrated on the analysis of human glycosyltransferases since these are the key enzymes that are responsible for specific glycosylation patterns. An understanding of their function will significantly enhance our options to interfere with pathological processes in which glycostructures are involved.
At the present we focus on the human blood group B galactosyltransferase (GTB). In collaboration with Prof. Palcic from the Carlsberg Laboratory in Copenhagen we aim at deciphering especially the enzyme dynamics that take place during catalysis. Nothing is known so far about this important issue. Understanding the dynamics of such enzymes at atomic level will greatly enhance the repertoire of approaches to modulate the function of these proteins for biotechnological or pharmaceutical purposes. NMR yields data on the complete time scale of molecular motions from the picosecond into the second time scale. We have also started a collaboration with Prof. Hübner from the Institute of Physics where we correlate NMR data with measurements from single molecule fluorescence spectroscopy. As a first success we have identified the bioactive conformation of donor substrates binding to GTB and we have come to a hypothesis that explains the exquisite specificity with which this enzyme processes its donor substrate. In Fig. 4 we show the dramatic change that donor substrates undergo when binding to GTB, and in Fig. 5 we show how an aspartate and a glutamate act as molecular tweezers to correctly position the donor substrate.

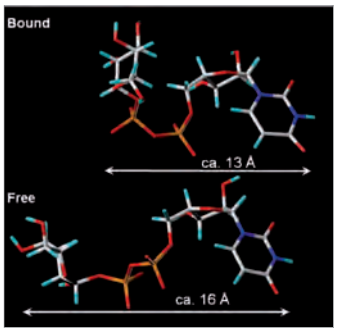

Fig. 4: Free and bound conformations of the donor substrate UDP-Gal are dramatically different. The bound conformation is not found in solution and has to be "stabilized" by the enzyme (see reference 6 for details).

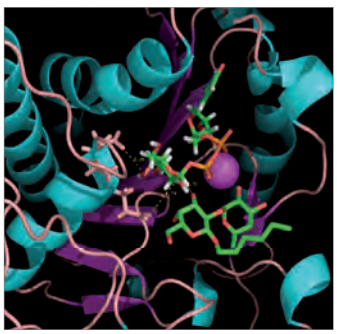

Fig. 5: NMR based model to explain the specificity with which GTB processes UDP-Gal but not UDP-Glc. UDP-Gal is shown as stick model with hydrogen atoms attached (see reference 6 for details).

The research projects require access to several techniques:

Protein expression and purification (Dr. Hanne Peters):
Recombinant expression of proteins is routinely performed in E.coli. For NMR experiments with proteins it is required to isotope label the protein with NMR active stable isotopes, i.e. with ^{13}C, ^{15}N and ^{2}H. We have established these techniques in our laboratory and constantly improve the conditions in order to improve the yields of isotope labeled proteins. This is mandatory since isotope labeling can be extremely costly, especially if ^{2}H and ^{13}C isotopes are involved, or if amino acid selective labeling is performed. We have succeeded in substantially improving known protocols such that e.g. GTB is produced at concentrations of 100 mg per L of culture. Our current focus is on glycosyltransferases, viral proteases and viral coat proteins.

NMR spectroscopy (Dr. Thorsten Biet)
High resolution NMR spectroscopy allows to analyze (bio)molecules in solution. NMR complements crystallography in that it adds dynamic information to the static picture. Another asset is the ease with which molecular recognition processes can be analyzed by NMR. Therefore, NMR nowadays plays a major role in the analysis of receptor-ligand interactions and in the drug discovery process. We have focused in our projects on carbohydrate-protein recognition processes.
Our NMR instruments include:
- 250 MHz Bruker DRX
- 500 MHz Bruker DRX with TCI cryogenic probe
- 700 MHz Bruker DRX with TXI cryogenic probe (located at our outstation at the University of Hamburg)

Biacore and Microcalorimetry (PD Dr. Thomas Weimar)
For a deeper understanding of molecular recognition processes it is important to have access to the thermodynamic signature of such reactions. In our institute surface plasmon resonance experiments (Biacore) are well established and deliver kinetic and thermodynamic information at the same time. As a gold standard we employ microcalorimetry to unravel the thermodynamics of binding events. The combination of Biacore and microcalorimetry has proven to be very powerful.
The Institute of Chemistry houses a Biacore 3000 and a Biacore J instrument and has access to an isothermal and differential scanning calorimetry equipment in the Institute of Biochemistry.

Chemistry

Structural changes of proteins causing autoimmunity

Inflammatory diseases are a research field of growing interest, because very often a direct linkage between inflammations and other diseases exists. An inflammation is an immune response of the body to exogenous substances like pathogens or, in the case of autoimmune diseases, a misled response to endogenous proteins.
Why an endogenous protein present in the human body for a long time is suddenly recognized as exogenous is not yet understood.
Therefore, detailed structural information at a molecular level are needed to understand why an endogenous protein is recognized by antibodies. Knowing the three dimensional structure of the protein, structural changes upon antibody binding can be investigated by NMR and will help to understand the mechanisms of antibody recognition.

Epidermolysis bullosa acquisita (EBA) is an autoimmune disease of the skin which is characterized by autoantibodies directed against type VII collagen. Type VII collagen links different skin layers together and is therefore an important skin protein (figure 1). Autoantibody binding to type VII collagen results in skin blistering and skin lesions. In contrast to other autoimmune diseases, EBA offers the advantage that both the autoantibodies and the target of the antibodies are known. Thus, EBA presents an adequate model to study structural changes involved in autoimmunity. The knowledge about the nature of these changes will finally help to understand the mechanisms that lead to autoimmune diseases.

Methods which will be employed are heterologous protein expression and purification (including labeling with stable isotopes), NMR spectroscopy and crystallography.

Metabolomic investigations

In a disease state different proteins are newly synthesized while the expression of other proteins is repressed. However, not only the protein composition and levels are changed during a disease, there are also changes in metabolite concentrations (figure 2). Comparing the metabolome from the healthy state and the disease state will identify metabolic pathways related to the disease and will provide clues to diagnosis as well as to a deeper understanding of the disease itself.

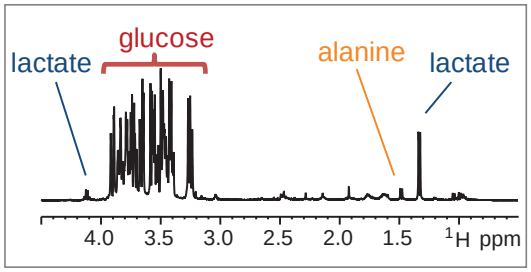

Fig. 2: Typical NMR spectrum of mice serum. Highly abandoned metabolites as glucose and lactate give intense NMR signals, where as metabolites present in lower concentrations show only small NMR signals.

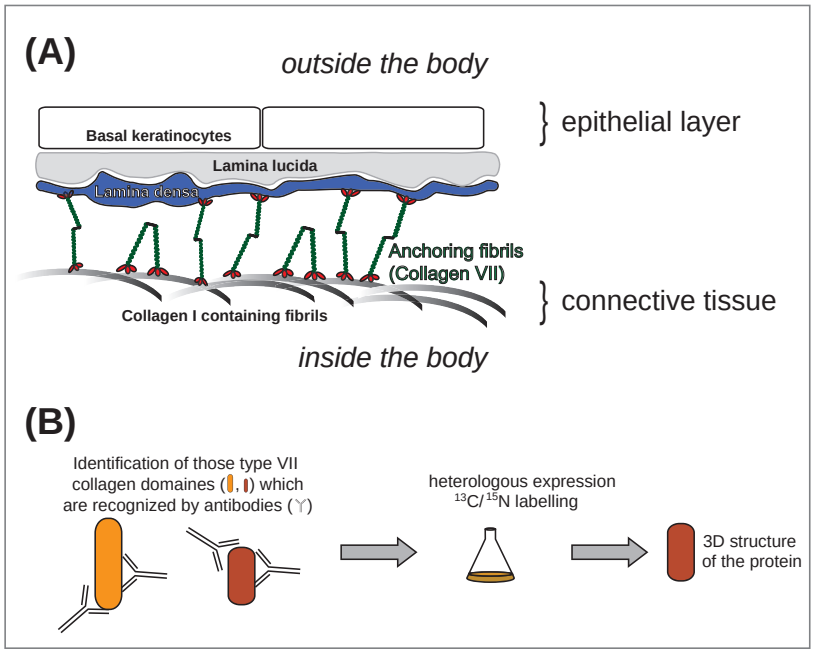

Fig. 1: A) Schematic representation of the skin. Type VII collagen links different skin layers together.
B) Typical approach for structure determination of a protein. The protein of interest is expressed in bacterial cells allowing the incorporation of stable isotopes (^{13}C, ^{15}N) and high yields of the protein. Labelled protein is needed for the structure determination.

Karsten Seeger

1998-2003 Studies of Biochemistry at the Universität Leipzig
2003-2006 PhD studies, Universität Leipzig including a three month research stay at the University of Alberta (Canada)
2006-2008 Postdoctoral fellow, CEA Saclay, France
Since 2008 Assistant professor in „Structural biology of Autoimmunity" at the Universität Lübeck

Selected Publications:
1 Rademacher, C., Krishna, N.R., Palcic, M., Parra, F., and Peters, T., NMR experiments reveal the molecular basis of receptor recognition by a calicivirus. J Am Chem Soc 130, 3669-3675. Highlighted by: Frank, A.O., and Kessler, H. 2008. Biophysics: the sweetest candy for the virus. Nature 452, 822-823.
2 Bhunia, A., Schwardt, O., Gathje, H., Gao, G.P., Kelm, S., Benie, A.J., Hricovini, M., Peters, T., and Ernst, B.. Consistent bioactive conformation of the Neu5A-calpha(2-->3)Gal epitope upon lectin binding. Chembiochem, 2008, 9, 2941-2945.
3 Rademacher, C., Shoemaker, G.K., Kim, H.S., Zheng, R.B., Taha, H., Liu, C., Nacario, R.C., Schriemer, D.C., Klassen, J.S., Peters, T., et al. . Ligand specificity of CS-35, a monoclonal antibody that recognizes mycobacterial lipoarabinomannan: a model system for oligofuranoside-protein recognition. J Am Chem Soc 2007,129, 10489-10502.
4 Munch, J., Standker, L., Adermann, K., Schulz, A., Schindler, M., Chinnadurai, R., Pohlmann, S., Chaipan, C., Biet, T., Peters, T., et al. . Discovery and Optimization of a Natural HIV-1 Entry Inhibitor Targeting the gp41 Fusion Peptide. Cell 2007,129, 263-275.
5 Angulo, J., Rademacher, C., Biet, T., Benie, A.J., Blume, A., Peters, H., Palcic, M., Parra, F., and Peters, T.. NMR analysis of carbohydrate-protein interactions. Methods Enzymol 2006, 416, 12-30.
6 Angulo, J., Langpap, B., Blume, A., Biet, T., Meyer, B., Krishna, N.R., Peters, H., Palcic, M.M., and Peters, T.. Blood Group B Galactosyltransferase: Insights into Substrate Binding from NMR Experiments. J Am Chem Soc 2006, 128, 13529-13538.

Institute of

Prof. Dr. rer. nat. Heinrich Terlau

1980-1990 Studies of Biology, University of Münster and Göttingen
1990 PhD, University of Tübingen
1990-1992 Post doctoral fellow at the Max-Planck-Institute for Biophysical Chemistry in Göttingen
1992-1999 Group leader at the Max-Planck-Institute for Experimental Medicine in Göttingen
1998 Habilitation (venia legendi) in Physiology, University of Göttingen
1999-2005 Leader of the research group "Molecular and Cellular Neuropharmacology", Max-Planck-Institute for Experimental Medicine, Göttingen
2005 Privatdozent, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck
2006 Habilitation in Physiology and Pharmacology
Since 2006 Associate Professor, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck

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Main research topics in the CSCM:

Molecular pharmacology of ion channels
Molecular endocrinology and signal transduction of orexins
Function and regulation of hypoxia-inducible factors (HIFs)
Interaction between the renin-angiotensin-system and stress
Mechanisms of cardiac anti-ischemic preconditioning

Research group Heinrich Terlau:

Molecular pharmacology of ion channels

Ion channels are proteins which are embedded in the outer membrane of nearly all cells of an organism. These proteins mediate the fast, selective transport of ions through the membrane and thereby many diverse physiological functions like electrical excitability of cells or absorption and secretions within the epithelia. Ion channels can be activated by different signals and malfunction of these proteins can lead to diseases. Several hereditary diseases like cystic fibrosis or special forms of epilepsy, deafness or heart arrhythmia are known to be correlated with mutations of certain ion channels.

Our research focuses on the structure-function relations of voltage gated ion channels and the identification and characterization of pharmacological active substances interacting with these proteins (1, 3). Due to their special role in different areas of cellular function, ion channels are „popular" targets of biological active substances from different venomous organisms. Accordingly during evolution a great variety of specific ligands interacting with ion channels have been evolved. The identification and characterization of these ligands can be very useful for studying the features of a given protein.

The main focus of our research is the investigation of the mechanism of action of toxins from cone snails interacting with voltage gated ion channels (1, 3). Several new families of conotoxins interacting with Na^+- or K^+-channels have been identified already. A biophysical description and the investigation of the potential physiological implications of the interaction pharmacological active substance – ion channel is performed by using mainly electrophysiological techniques and expression systems. The aim is to establish new tools for studying the physiological role of a given target and to create the basis for a potential pharmacological or even clinical use of these substances.

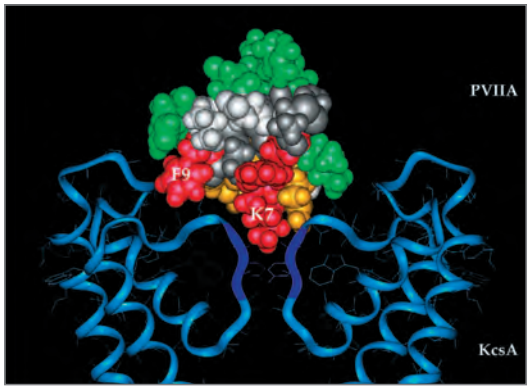

Fig.1: Hypothetical docking of conotoxin -PVIIA in the outer vestibule of the pore of a K^+ channel

For the interaction of conotoxins that bind to voltage activated K^+ channels we could demonstrate that this interaction depends on several parameters: Voltage-gated ion channels are activated by a depolarized membrane potential resulting in a conformational change, allowing ions to permeate. From this open state voltage-gated ion channels can either be inactivated by an additional conformational change which leads to a non-conducting state of the channel or they may be deactivated by a repolarized membrane potential (Fig. 2). We are studying in how far this gating of ion channels has an impact on the interaction of pharmacological active substances with these proteins.

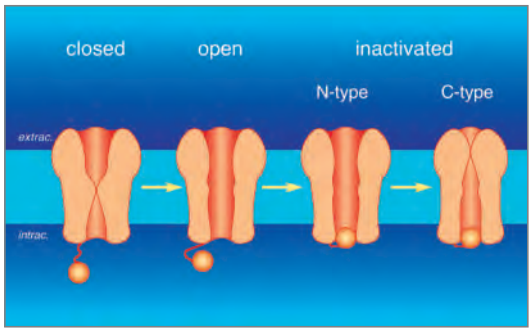

Fig. 2: Gating of K^+ channels

In general, the conformational changes of voltage-gated ion channels during activity can lead to changes in the interaction of drugs or toxins with the channel protein due to changes in the binding pocket during gating. This so called activity or state dependent binding is known for local anesthetics and other channel targeted substances that are used in the clinic, where besides a tonic blocking action, a phasic or use-dependent component for the block of Na^+ currents is observed. State-dependent interaction has the advantage, for certain therapeutic applications, that only the channels in a certain conformation are affected, whereas the same channel in a different conformation is less affected. We have demonstrated that also the binding of different K^+ channel-targeting conotoxins depends

Experimental and Clinical Pharmacology and Toxicology

on the conformational state of the channel. This indicates that state dependence might be a more general phenomenon for the interaction of substances binding to ion channels.

Due to their specific mode of action several conotoxins do have a therapeutic potential and six different peptides have reached human clinical trials. For conotoxin -PVIIA, a K^+ channel targeting peptide where the molecular interaction with the K^+ channel pore has already been studied (Fig. 1) it recently has been shown that it is effective in decreasing cardiac damage in animal models of myocardial infarction. The underlying mechanisms of this protective effect will be a main subject of our future research.

Research group Olaf Jöhren:

Orexins

The final sequencing of the entire human genome has revealed new molecular drug targets for the pharmaceutical industry. Many of these new targets are analogous to already-known targets like neurotransmitter or hormone receptors. A pharmaceutically important class of drug targets are G-protein coupled receptors (GPCRs). By a strategy called reverse pharmacology a variety of physiological ligands for orphan GPCRs (receptors without known ligands) were identified during the past decade, most of them being peptides. Moreover, 9 novel neuropeptide families were discovered. Orexins represent such a novel family. These peptides are highly expressed in the hypothalamus and regulate feeding behavior, neuroendocrine and autonomic functions, as well as sleep-wakefulness. Furthermore, orexins are significantly involved in the pathophysiology of narcolepsy and may play a central role in metabolic disorders. Two orexin GPCR subtypes, OX1 and OX2 receptors, were described thus far.

One major research focus of our group is the exploration of biological and pathophysiological functions of orexins and orexin receptors. We have described the existence of a peripheral orexin system and found a high expression of orexin receptors in adrenal glands. In ongoing experiments we investigate the cellular effects of orexins and the downstream signal pathways that finally lead to an altered production of steroid hormones such as cortisol (Fig. 3). Using qPCR and reporter-gene-assays we are analyzing the orexin-effects on the gene expression of steroid synthesizing enzymes like 21-hydroxylase (CYP21) or type II 3 -hydroxysteroid dehydrogenase (HSD3B2). The use of specific inhibitors and siRNA revealed the involvement of intracellular signal molecules such as protein kinase C (PKC) and MAPK/ERK kinase (MEK). In addition, we have cloned OX2 receptor splicing variants from NCI-H295 cells and investigate possible functional differences of these clones in various cell systems.

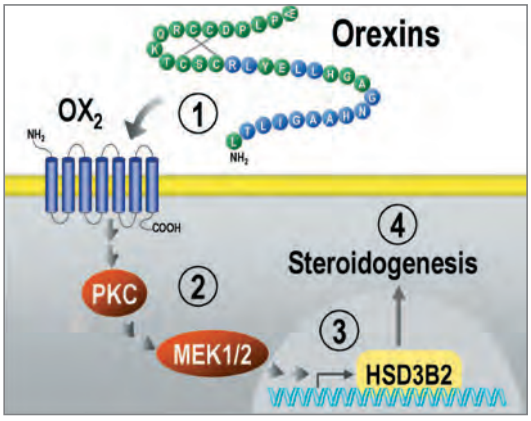

Fig. 3: Cellular actions of orexins in human adrenocortical cells. We are analyzing the specificity of various peptidic and non-peptidic agonists and antagonist of orexin receptors (1), downstream signal pathways (2), effects on the activity of genes coding for steroid-synthesizing enzymes such as HSD3B2 (3), and the final cellular and physiological alterations (4).

Selected Publications:
Research group Terlau

1 Terlau H, Shon KJ, Grilley M, Stocker M, Stühmer W, Olivera BM: Strategy for rapid immobilization of prey by a fish-hunting marine snail, Nature 381, 1996, 148-151
2 Finol-Urdaneta RK, Strüver N, Terlau H: Differential redox sensitivity in Kv1.7 channel isoforms
J General Physiology 128, 2006, 133–145
3 Terlau H, Olivera BM: Conus venoms: A rich source of novel ion channel-targeted peptides, Physiological Reviews 84, 2004, 41-68

Research group Jöhren

1 Jöhren O, Neidert SJ, Kummer M, Dendorfer A, Dominiak P (2001) Prepro-orexin and orexin receptor mRNAs are differentially expressed in peripheral tissues of male and female rats. Endocrinology 142: 3324-3331.
2 Jöhren O, Brüggemann N, Dendorfer A, Dominiak P (2003) Gonadal steroids differentially regulate the messenger ribonucleic acid expression of pituitary orexin type 1 receptors and adrenal orexin type 2 receptors. Endocrinology 144: 1219-1225.
3 Heidbreder M, Fröhlich F, Jöhren O, Dendorfer A, Qadri F, Dominiak P (2003) Hypoxia rapidly activates HIF-3 mRNA expression. FASEB J 17: 1541-1543.
4 Raasch W, Wittmershaus C, Dendorfer A, Voges I, Pahlke F, Dodt C, Dominiak P, Jöhren O (2006) Angiotensin II inhibition reduces stress sensitivity of hypothalamo-pituitary-adrenal axis in spontaneously hypertensive rats. Endocrinology 147: 3539-3546.

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2004 Habilitation in Pharmacology and Toxicology
Since 2004 Privatdozent, Institute for Experimental and Clinical Pharmacology and Toxicology, University of Lübeck

Institute of Medical

Prof. Dr. med. Werner Solbach

1972-1979 Studies of Medicine University of Mainz
1981 Guest Researcher Imperial Cancer Research Fund London
1987 Medical Specialist for Medical Microbiology, Virology and Hygiene
1989-1997 Deputy Director Institute for Medical Microbiology and Hygiene, University Erlangen-Nürnberg
Since 1997 Chair Institute for Medical Microbiology and Hygiene, University Lübeck

Awards, memberships and others:
Founding member and project leader DFG-SFB 654 (Plasticity and Sleep)
Founding member and Executive Board of DFG-Graduate School "Computing in Medicine and Life Science"
Founding member and Executive Board of DFG-Excellence Cluster "Inflammation at interfaces"
Member of the Academy of Science at Hamburg
Dean of the Medical Faculty

Director: Prof. Dr. med. Werner Solbach
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Main research topics in the CSCM:

Innate immune responses to intracellular pathogens
The healing power of granulocytes
Circadianicity and adaptive immune responses
Virulence of pathogenic staphylococci
Pathogenomics of Chlamydia
Inflammation and hypoxia

Research group Werner Solbach/ Thomas Bollinger:

T cells and clock genes

In mammals, the 24-h rhythm of physiological and behavioural activity is controlled by a hierarchical network of cellular circadian clocks that are active in all tissues. The master circadian pacemaker is found in the hypothalamic suprachiasmatic nucleus (SCN). The SCN is synchronized to external time by e.g. daily changes in the light/dark cycle. Peripheral oscillators, described in numerous tissues, are reset via the SCN, but are to a certain degree self-sustaining and autonomous.

On the molecular level, these clocks are based on a set of inter-locked transcriptional/translational autoregulatory feedback loops. A core loop is formed by the transcription factors CLOCK and BMAL1 (positive limb) that drive the expression of Per (Per1and Per2) and Cry (Cry1 and Cry2) genes, which negatively regulate their own expression (Fig. 1). This core loop is stabilized by two ancillary loops involving the nuclear orphan receptors Rev-erb and Ror and the transcription factors Dbp and E4bp4, respectively. The molecular oscillator is responsible for the downstream regulation of the expression of numerous clock controlled genes that translate time information into physiologically relevant signals.

We could previously show that the function of T cells and regulatory T cells follows a 24 h rhythm which is in part dependent on sleep (Fig. 2). However, the molecular mechanism for this functional rhythm is unknown. A likely mechanism is the molecular clock of T cells. The molecular clock has been described in several peripheral cells but never in T cells.

Subsequently, we are investigating the molecular clock in freshly isolated CD4+ T cells and how this clock is sustained in vitro.

Circadian clock gene expression has been shown to be sustained for 3-7 days in several peripheral cells ex vivo and can even be induced in vitro. The in vitro analysis of circadian clock gene expression enables to investigate subsequent circadian changes of cellular functions under defined conditions. Therefore, we investigate the influence of cytokines on the expression of clock genes and the resulting circadian immune activity of T lymphocytes. Conversely, the relationship of adaptive immune functions to clock gene expression is explored, especially under T cell activation conditions.

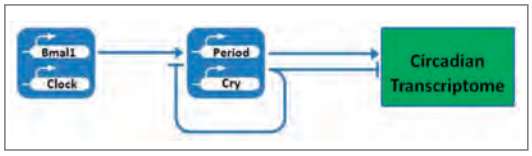


Fig. 1: Core Loop of the molecular clock. (Modified from Hastings et al., 2007).

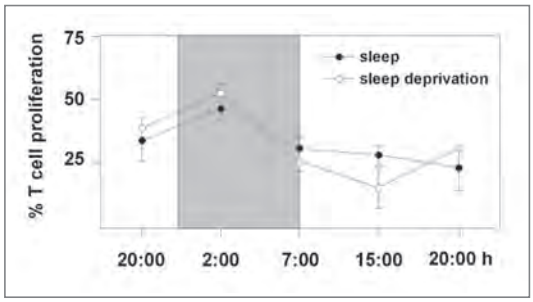


Fig. 2: CD4+CD25- T cells were isolated from peripheral blood of healthy young men with sleep (closed circles) or sleep deprivation (open circles), labelled with CFDA and polyclonally stimulated. The percentage of proliferated T cell was measured through the reduction in CFDA fluorescence (Bollinger et al., 2009)

Microbiology and Hygiene

Prof. Dr. med.
Johannes Karl-Mark Knobloch

1989-1996 Studies of Medicine, University of Erlangen-Nürnberg
1996-2006 Research Associate, Institute for Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf
1999 PhD in Medicine
2004 Medical Specialist for Medical Microbiology, Virology and Hygiene
2005 Habilitation
Since 2006 Full Professor and Deputy Director of the Institute of Medical Microbiology and Hygiene, University of Lübeck

Awards, memberships and others:
2004 Research award „clinical microbiology“of the German society for Hygiene and Microbiology (DGHM)
2004 Young investigator award of the Federation of Infectious Societies, UK
Member of the Excellence Cluster Inflammation@Interfaces
Founding member of the Academic Cluster Program “Visualisation of pathogenic processes”

Research group Johannes Knobloch:

Virulence determinants of pathogenic Staphylococci

Foreign body-associated infection, which are triggered by the use of artificial medical devices (for example prostheses) are most frequently caused by Staphylococcus epidermidis and Staphylococcus aureus. They are increasing in number, leading to substantial morbidity and mortality. Staphylococci are also increasingly resistant to antimicrobial therapy. The ability to form a multilayered biofilm is of outmost importance for their pathogenic potential.

The intercellular polysaccharide adhesin (PIA) is one of the major determinants for the formation of biofilms. PIA is synthesized by the gene products of the icaADBC operon. PIA synthesis is influenced by a variety of environmental factors and is part of a complex regulatory network. The DNA-binding protein IcaR, which is encoded by a gene upstream of the icaADBC operon, is a negative regulator of PIA synthesis and biofilm formation.

In S. epidermidis, at least four other regulatory elements are involved in the control of biofilm formation. In our group we identified the alternative sigma factor (SigB) as an important regulator of PIA synthesis. It could be demonstrated, that SigB acts via the repression of icaR transcription. The SigB -dependent regulation of icaR transcription could be bypassed by environmental signals like ethanol in the growth medium. Besides biofilm formation the alternative sigma factorB regulates a variety of other virulence factors of S. epidermidis. Mechanisms by which SigB affects the expression of virulence factors are currently investigated in our research group.

In addition to the SigB operon a second regulatory operon, the barAB operon, has been identified in S. epidermidis by our research group. This operon is partially transcribed from a SigB -dependent promoter. Inactivation of these genes results in a complete repression of PIA synthesis and biofilm formation, indicating that at least BarB is essential for biofilm formation by S. epidermidis. Therefore, BarB is a potential new target for anti-biofilm strategies for prevention and therapy of foreign body-associated infections. The characterization of the BarAB-dependent regulation of biofilm formation forms a further project of the research group.

Cells embedded in a biofilm cannot be efficiently controlled by the immune system during infection, which often results in protracted chronic inflammation. The identified potential target structures for the inhibition of biofilm formation in S. epidermidis are global regulators involved in the adaption of bacterial cells to changes in the environmental conditions. A wide variety of other virulence factors of S. epidermidis are co-regulated by these regulators. To investigate the consequence of differential expression of other virulence factors in the case of suppression of biofilm formation targeting global regulators we investigate the interaction of defined mutants of the respective regulators with human neutrophil granulocytes, the first line of defense in the human immune system.

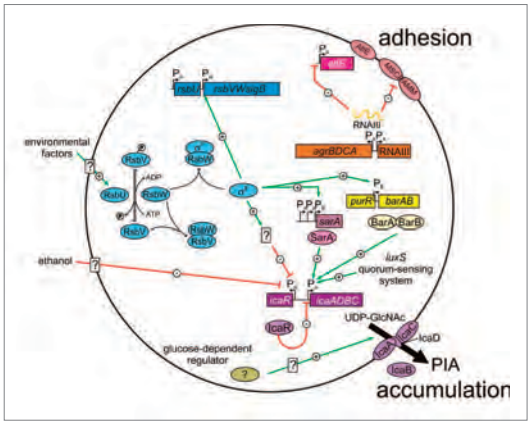
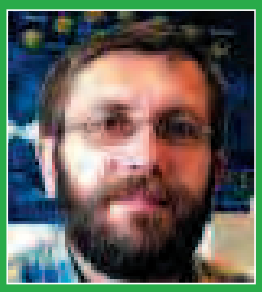


Fig. 1: Model of the regulation of primary adhesion and PIA synthesis in S. epidermidis

Institute of Medical



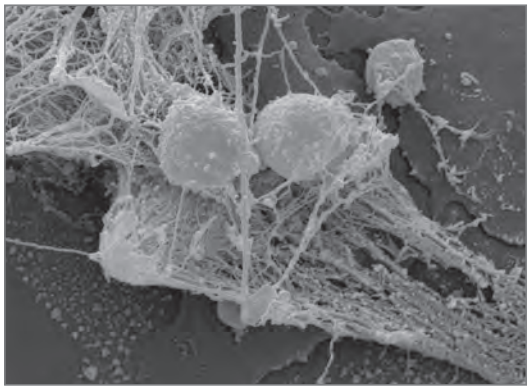
Prof. Dr. univ. (Universität Budapest) Tamás Laskay

1975-1980 Studies of Biology, Eötvös-University in Budapest
1980-1984 Research Associate, National Institute for Haematology and Blood Transfusion, Budapest
1985-1986 PhD student at the Institute of Immunology, Karolinska Institute, Stockholm, Sweden
1987 PhD in Immunology at the Eötvös-University, Budapest
1987 Visiting scientist at the Department of Tropical Public Health, Harvard School of Public Health, Boston, USA
1988-1990 Research Associate, Armauer Hansen Research Institute (AHRI), Addis Abeba, Ethiopia
1990 Awarded with the title Honorary Assistant Professor by the Department of Microbiology, Addis Abeba University, Ethiopia
1991-1997 Research Associate at the Institute of Clinical Microbiology and Immunology at the University Erlangen
Since 1997 Research Associate, head of the „Innate Immunity" research unit at the Institute of Medical Microbiology and Hygiene at the University of Lübeck
2000 Habilitation, Immunology and Infection Biology
Since 2005 Full Professor, Institute of Medical Microbiology and Hygiene, University of Lübeck

Research group Tamás Laskay:

Innate immune response to intracellular pathogens

The innate immune system controls infections within the first hours/days after microbial challenge. In our research group we investigate the innate immune response to intracellular pathogens. The focus of our research is the interaction of the pathogens with neutrophil granulocytes, since neutrophils are the first cells of the innate immune system that are recruited from the blood circulation to the site of an infection. Microbial pathogens are phagocytosed and in most cases rapidly killed by neutrophil granulocytes. However, a group of intracellular pathogens such as the parasite *Leishmania major* and the bacteria *Anaplasma phagocytophilum* and *Chlamydia pneumoniae* escape the intracellular killing and survive in granulocytes. The research work of the group aims to clarify the mechanisms how these pathogens can survive in neutrophil granulocytes. The formation of neutrophil extracellular traps (NETs) is a recently described defence mechanism mediated by neutrophil granulocytes. NETs, that are generated by neutrophils undergoing a novel form of cell death called NETosis, can efficiently capture and kill microbial pathogens. In our ongoing projects we investigate the induction of NETs by various groups of microbial pathogens with the aim to obtain insights into the molecular mechanisms of NET-formation.



Neutrophil extracellular traps (NETs) released by activated neutrophil granulocytes. Scanning electron microscopy by Dr. Martina Behnen-Härer (Institute of Medical Microbiology and Hygiene) and PD Dr. Matthias Klinger (Institute for Anatomy).

As major projects of the group we investigate:

- the escape mechanisms of intracellular pathogens
- the role of apoptosis of both pathogens and host cells for the outcome of an infection
- the immunoregulatory role of neutrophil granulocytes
- the phagocatosis of apoptotic cells by leukocytes
- the induction of NETs by microbial pathogens

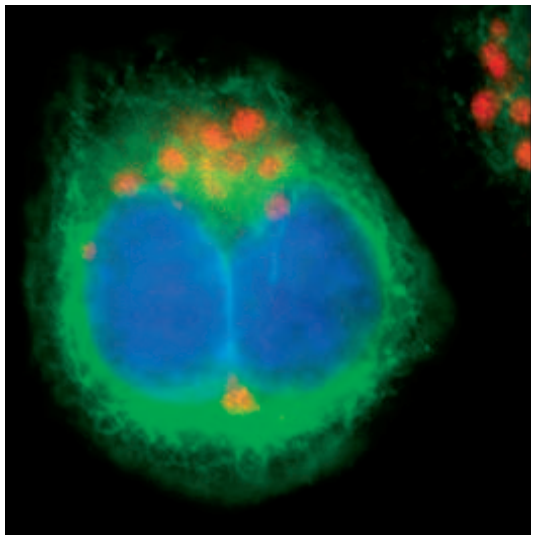
Microbiology and Hygiene

Research group Jan Rupp:

Host-pathogen interactions and inflammation in hypoxia

Sites of inflammation are characterized by an influx of inflammatory cells that drive a high metabolic turnover in an environment of fading oxygen availability. Oxygen concentrations in non-diseased tissues vary between 4 – 15% under physiological conditions and easily become hypoxic (O2< 5%) during chronic but also acute inflammatory process. Many bacterial pathogens survive well and even grow under anaerobic conditions, thus host-defense mechanisms must be adapted to function effectively when oxygen tension is low. To compensate for environmental changes in O2 supply, host cells get under the control of "hypoxia-inducible factors (HIF)", of which HIF-1 regulates most of the hypoxia-dependent genes involved in cell metabolism, apoptosis and innate immunity. The hypoxia-inducible factor-1 is a ubiquitously and constitutively produced transcription factor composed of an oxygen-labile -subunit and an oxygen-resistent -subunit, with the latter being identical with the aryl hydrocarbon nuclear translocator. HIF-1 possesses two central oxygen dependent degradation domains (ODDs) and two transactivation domains. In the presence of O2, human HIF-1 is hydroxylated at the proline residues Pro-402 and Pro- 564 within the ODDs, immediately captured by the von Hippel–Lindau protein, and degraded via the E3 ubiquitin ligase pathway.

Interestingly, obligate intracellular chlamydiae *(C. pneumoniae, C. trachomatis*) evolved strategies to directly interact with the oxygen-sensing mechanisms of infected cells to meet their needs on energy and nutrients, and to actively manipulate cell survival and proliferation. To investigate the metabolome and interactome of chlamydia-infected human host cells with respect to the surrounding O2 availability is in the focus of our current studies.



Expression of the chlamydial protease CPAF (red) in a C. pneumoniae infected epithelial host cell

Selected Publications:
Research group Knobloch

1 Knobloch, J. K.-M., K. Bartscht, A. Sabottke, H. Rohde, H. H. Feucht, and D. Mack. 2001. Biofilm formation of Staphylococcus epidermidis depends on RsbU, a functional activator of the sigB operon: differential activation mechanisms due to ethanol and salt stress. J. Bacteriol. 183:2624-2633.
2 Knobloch, J. K.-M., S. Jäger, H. Rohde, M. A. Horstkotte, S. Dobinsky, and D. Mack. 2004. RsbU dependent regulation of Staphylococcus epidermidis biofilm formation is mediated via the alternative sigma factor B by repression of the negative regulator gene icaR. Inf. Immun. 72:3838-3848.
3 Knobloch, J. K.-M., S. Jäger, J. Huck, M. A. Horstkotte, H. Rohde, and D. Mack. 2005. mecA is not involved in the B dependent switch of the expression phenotype of methicillin resistance in Staphylococcus epidermidis. Antimicrob. Agents Chemother. 49:1216-1219.
4 Knobloch, J. K.M., H. von Osten, M. A. Horstkotte, H. Rohde, and D. Mack. 2008. Biofilm formation is not necessary for development of quinolone resistant persister cells in an attached Staphylococcus epidermidis population. Int. J. Artif. Organs 31:752-760

Research group Laskay

1 Laskay T, van Zandbergen G, Solbach W. 2008. Neutrophil granulocytes as host cells and transport vehicles for intracellular pathogens: Apoptosis as infection-promoting factor. Immunobiology 213:183-91.
2 van Zandbergen G, Solbach W, Laskay T. 2007. Apoptosis driven infection. Autoimmunity 40:349-52.
3 van Zandbergen G., Bollinger A., Wenzel A., Kamhawi S., Voll R., Klinger M., Müller A., Hölscher C., Herrmann M., Sacks D., Solbach W., Laskay T. 2006. Leishmania disease development depends on the presence of apoptotic promastigotes in the virulent inoculum. Proc Natl Acad Sci USA 103:13837-42.
4 van Zandbergen G, Klinger M.,, Dannenberg S., Müller A, Solbach W and T. Laskay. 2004. Cutting edge: Neutrophil granulocytes serves as a vector for Leishmania entry into macrophages. J Immunol. 173:6521-6525.

Research group Rupp

1 Rupp J, Solbach W, Gieffers J. Variation in the mutation frequency determining quinolone resistance in Chlamydia trachomatis serovars L2 and D. J Antimicrob Chemother 2008 Jan;61(1):91-4.
2 Rupp J, Gieffers J, Klinger M, van Zandbergen G, Wrase R, Maass M, Solbach W, Deiwick J, Hellwig-Burgel T. Chlamydia pneumoniae directly interferes with HIF-1alpha stabilization in human host cells. Cell Microbiol. 2007 Sep;9(9):2181-91.
3 Rupp J, Solbach W, Gieffers J. Single-nucleotide-polymorphism-specific PCR for quantification and discrimination of Chlamydia pneumoniae genotypes by use of a "locked" nucleic acid. Appl Environ Microbiol. 2006 May;72(5):3785-7.
4 Rupp J, Hellwig-Burgel T, Wobbe V, Seitzer U, Brandt E, Maass M. Chlamydia pneumoniae infection promotes a proliferative phenotype in the vasculature through Egr-1 activation in vitro and in vivo. Proc Natl Acad Sci U S A. 2005 Mar 1;102(9):3447-52.
5 Rupp J, Berger M, Reiling N, Gieffers J, Lindschau C, Haller H, Dalhoff K, Maass M. Cox-2 inhibition abrogates Chlamydia pneumoniae-induced PGE2 and MMP-1 expression. Biochem Biophys Res Commun. 2004 Jul 30;320(3):738-44.

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1999-2001 Research fellow, University of Lübeck, Department of Internal Medicine
2001 PhD in Medicine (MD), University of Lübeck
2001-2006 Post-doctoral fellow, University of Lübeck, Institute of Medical Microbiology and Hygiene
2005 Specialist licensure for Medical Microbiology and Infectious Diseases
Since 2005 Independent group leader at the Institute of Medical Microbiology and Hygiene, University of Lübeck
2007: Habilitation for Medical Microbiology, Certificate as "Clinical Infectiologist"
Since 2009: Professor (W2) for Molecular Pathogenesis of Infections

Memberships:
Junior Research Group Leader in the Excellence Cluster "Inflammation@ Interfaces"
Founding member of the intramural research cluster: "Inflammation of epithelia"

Awards, memberships and others:
2002 ICAAV Program Committee Award
2006 Young investigator award of the German Society of Hygiene and Microbiology (DGHM)
2006 Heinrich Dräger research award

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Prof. Dr. rer. nat. Georg Sczakiel

1976-1978 Undergraduate Chemistry, University of Kaiserslautern
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1983-1986 PhD in Molecular Biology, Department of Biophysics, Max-Planck-Institute for Medical Research, University of Heidelberg
1986 Postdoctaral fellow, Max-Planck-Institute for Medical Research, University of Heidelberg
1987-2000 Postdoctaral fellow and head of group, Applied Tumorvirology, „Deutsches Krebsforschungszentrum" (DKFZ), Heidelberg
1994-1995 Sabbatical, Tsukuba University, Japan
Since 2000 Full Professor and Chair, Institute of Molecular Medicine, University of Lübeck

Awards, memberships and others:
Member of Gesellschaft Deutscher Chemiker (GDCh)
Member of Gesellschaft für biologische Chemie (GBM)
Member of Deutsche Arbeitsgemeinschaft für Gentherapie e.V.
Co-founder and Vice President Oligonucleotide Therapeutics Society, Boston, USA
Member of the Editorial Board, ´Oligonucleotides´
Guest Executive Editor, ´Current Pharmaceutical Design´
Executive Editor, ´Nucleic Acids Research´

Prof. Dr. rer. nat. Tobias Restle

1988-1991 PhD University Heidelberg / MPI for Medical Research
1992-1993 Research Associate, MPI for Medical Research Heidelberg
1993-1996 Research Associate, Yale University Department of MB&B
1996-2003 Group leader MPI of Molecular Physiology Dortmund
2001 Habilitation in Biochemistry
Since 2003 Associate Professor, Institute of Molecular Medicine, University of Lübeck

Director: Prof. Dr. rer. nat. Georg Sczakiel
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Main research topics in the CSCM:

RNA-RNA recognition, mechanisms and delivery of oligomeric nucleic acid drugs (aptamers, antisense, siRNA, miRNA), non-viral vectors, Argonaute proteins and mechanism of RNA interference.

Central projects at the Institute of Molecular Medicine include:

- Structure-function relationship and therapeutic application of siRNA, miRNA, aptamers and antisense nucleic acids
- RNA biochemistry, kinetics and structure
- Very short oligonucleotides as novel target-specific tools – HIV-1 reverse transcriptase
- New concepts and non-viral vectors for the cellular delivery of therapeutic nucleic acids
- The biology of extracellular RNA *in vivo*
- RNA-based non-invasive tumor diagnostics
- Human Argonaute proteins
- Polymerases as drug targets – e.g. HIV and HCV
- Structure-function relationship of DNA polymerases

Research

Controlled expression of genetic information is crucial to normal development of cells and organisms. In the course of regulated gene expression from genes via gene products to phenotypes, nucleic acids represent two major classes of natural biologically important macromolecules, deoxyribonucleic acids (DNA) and ribonucleic acids (RNA), respectively. Dysregulated or aberrant gene expression, however, may lead to altered phenotypes including uncontrolled cell proliferation or functional defects that may be causatively related to diseases.

As the human genome and its transcribed sequences become increasingly revealed more light is shed onto the molecular level of the pathomechanisms of human diseases which may include fatal changes of DNA and, hence, RNA transcribed thereof. At this level, the analysis of genomic DNA and of transcribed sequences including mRNA (transcriptome) enables one in principle to obtain diagnostic information on the risk or state of human diseases. As uncontrolled and also viral gene expression may cause the development of serious diseases including cancer and lethal infections such as AIDS, specific DNA segments and mRNAs represent new and promising drug targets for novel therapeutic concepts on the molecular level. Interestingly, short nucleic acids (oligonucleotides) may be used to interfere with the biological function of DNA and RNA and several classes of oligonucleotides are currently under investigation as new drugs. Oligonucleotides are synthesized by chemical means. They are not expressed endogenously and, thus, have to be delivered to target cells and target tissues *in vivo* which requires new methodologies for the cellular uptake. The studies are performed at the level of biochemistry, molecular biology, and cell biology. In collaboration with clinical partners, we also conduct pre-clinical studies and studies *in vivo*.

Nucleic acid delivery:

The cellular delivery of siRNA is one of the major technical hurdles of its application in living cells.

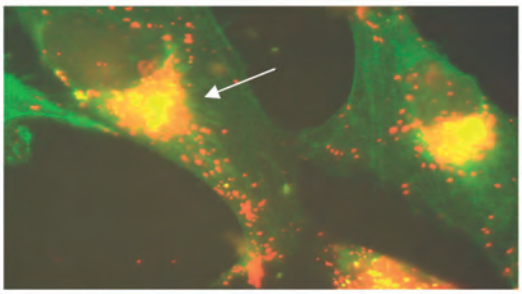

Fig. 1: The picture shows cells harbouring biologically active siRNA in vesicles (orange, indicated by a white arrow), delivered according to a novel principle (Overhoff & Sczakiel, 2005). The green fluorescence shows the actin filaments.

Array-based search for target-specific hexanucleotides:

As a spin-off activity of the ´Kompetenzzentrum für Drug Design and Target Monitoring´ we developed a novel chip onto which the complete sequence space of hexanucleotides is printed. This approach enables a systematic array-based search for hexanucleotides which specifically and tightly bind to any target of interest thereby leading to lead compounds for subsequent drug development. The enormous target selectivity of this method is reflected by species that distinguish between the highly conserved reverse transcriptases (RT) of HIV-1 and HIV-2 (see figures 2 and 3).

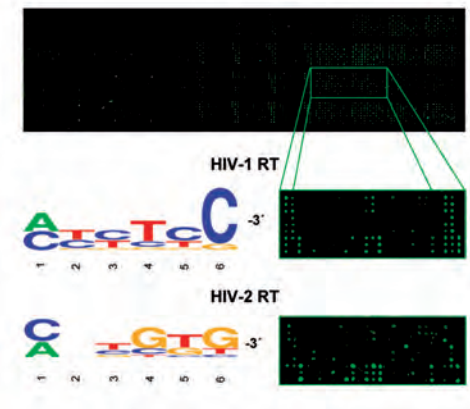

Fig. 2: Hexanucleotide array. The signal pattern produced in the use of closely related target proteins is different and shows the high specificity of the array-based system. This is further supported by the analysis of the tightest binders which reveals different consensus sequences for HIV-1 RT and HIV-2 RT.

Molecular Medicine

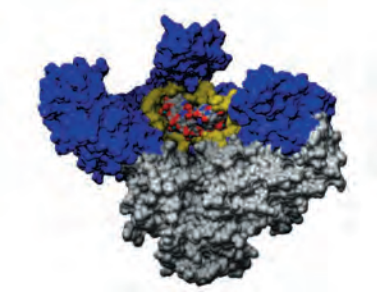

Fig. 3: Molecular modelling. Hypothetical three-dimensional model of the HIV-1 reverse transcriptase binding site of an inhibitory hexanucleoticle. Colour code: blue, large subunit p66; grey, small subunit p51, surface residues < 5 Å away from the hexamer are shown in yellow.

Molecular diagnostics:

The existence of RNA outside cells (exRNA) is known for a long time. Presence and amount of individual extracellular RNA species are correlated to the extent of necrosis and apoptosis. *In vivo*, some of these exRNAs bind to blood cells thereby "travelling" through the human organism. Surprisingly, extracts containing extracellular nucleic acids derived from tumour cells have been shown to induce tumour growth *in vivo*. Therefore, exRNAs provide a diagnostic as well as therapeutic target.

We apply methods from molecular biology, cell biology as well as biochemistry to investigate mode of action of these exRNA molecules. A beneficial application of these investigations is the possibility to use the presence of exRNAs for the early diagnosis of cancer. In collaboration with clinical partners, we work on the validation of novel tumour markers for the non-invasive diagnosis of bladder and breast cancer.

Human Argonaute proteins and their role in the RNA interference process:

Gene expression in eukaryotes is regulated at the post-transcriptional level by a process termed RNA interference (RNAi). Key players are short double stranded RNAs which upon interaction with the RNA induced silencing complex (RISC) lead to degradation or functional blockage of perfectly or partially complementary mRNAs. Within the RISC, the so called Argonaute (Ago) proteins play a crucial role. However, their exact function is currently not well characterized. In this context diverse experiments are performed at the levels of biochemistry, molecular biology, and cell biology trying to unravel the molecular details of these complex protein/RNA interactions.

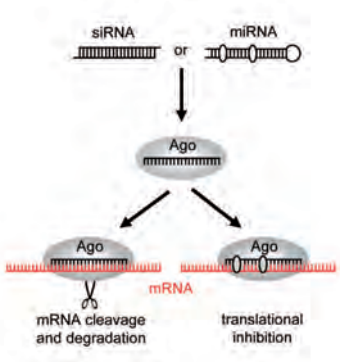

Fig. 4: Simplistic depiction of the RNAi process. The gene silencing is guided by short double stranded RNA and involves the Ago proteins.

Polymerases:

The nature of DNA replication fidelity is of immense biological importance due to the fundamental requirement for accurate DNA synthesis, in both replicative and repair processes. We are interested in mechanistic and structural properties enabling polymerases to catalyze nucleotide incorporation with selectivity far greater than dictated by the thermodynamic differences between base pairs in free solution. Furthermore, polymerases represent an important drug target in the fight against viral diseases.

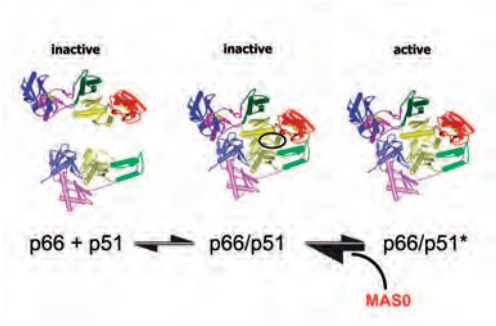

Fig. 5: Proposed mode of action of recently identified small molecule HIV-1 RT dimerization inhibitors (Grohmann et al., 2008). The inhibitors are supposed to interfere with the dimer equilibrium by shifting the equilibrium from an active to an inactive dimer by trapping the enzyme in the inactive conformation.

Selected Publications:

1 Overhoff, M. and Sczakiel, G., Phosphorothioate-stimulated uptake of short interfering RNA by human cells, EMBO reports 6, 1176-1181 (2005).
2 Wünsche, W. and Sczakiel, G., The biological activity of siRNA is related to the kinetics of siRNA-target RNA recognition in vitro: mechanistic implications, JMB 345, 203-209 (2005).
3 Overhoff, M., Alken, M., Kretschmer-Kazemi Far, R., Lemaitre, M., Lebleu, B., Sczakiel, G. and Robbins, I., Local RNA target structure influences siRNA efficacy: a systematic global analysis, JMB 348, 871-881 (2005).
4 Cramer, J., Jaeger, J. and Restle, T., Biochemical and Kinetic Characterization of the Hepatits C Virus RNA Polymerase (NS5BD21, HC-J4). Biochemistry 45 (11), 3610-3619 (2006).
5 Mescalchin, A., Wünsche, W., Laufer, S.D., Grohmann, D., Restle, T. and Sczakiel, G., Specific binding of a hexanucleotide to HIV-1 reverse transcriptase: a novel class of very small bioactive oligonucleotides, NAR 34 (19), 5631–5637 (2006).
6 Veldhoen, S., Laufer, S.D., Trampe, A. and Restle, T., Cellular delivery of small interfering RNA by a non-covalently attached cell-penetrating peptide: quantitative analysis of uptake and biological effect. NAR 34 (22), 6561-6573 (2006).
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8 Cramer, J., Rangam, G., Marx, A. and Restle, T., Varied active site constraints in the Klenow Fragment of E. coli DNA polymerase I and the lesion-bypass Dbh DNA polymerase. ChemBioChem 9, 1243-1250 (2008).
9 Grohmann, D., Godet, J., Mély, Y., Darlix, J-L. and Restle, T., HIV-1 nucleocapsid traps reverse transcriptase on nucleic acid substrates. Biochemistry 47, 12230-12240 (2008).
10 Laufer, S.D. and Restle, T., Peptide-mediated cellular delivery of oligonucleotide-based therapeutics in vitro: quantitative evaluation of overall efficacy employing easy to handle reporter systems. Curr. Pharm. Design 14(34), 3637-3655 (2008).
11 Detzer, A., Overhoff, M., Rompf, M., Mescalchin, A. and Sczakiel, G., Phosphorothioate-stimulated cellular uptake of siRNA: a cell culture model for mechanistic studies, Curr. Pharm. Design 14, 3666-3673 (2008).
12 Detzer, A., Overhoff, M., Wünsche, W., Rompf, M., Turner, M., Ivanova, G., Gait, M. and Sczakiel, G., Increased RNA interference is related to intracellular release of siRNA from the perinuclear space via a covalently attached signal peptide. RNA 15(4), 627-636 (2009).
13 Hanke, M., Höfig, K., Merz, H., Feller, A.C., Kausch, I., Jocham, D., Warnecke, J.M and Sczakiel, G. A robust methodology to study urine miRNA as tumor marker: miR-126 and miR-182 are related to urinary bladder cancer. Urol. Oncol., (2009) in press.

Dr. rer. nat.
Rosel Kretschmer-Kazemi Far

1983-1989 Graduate Biology, FU Berlin
1993 PhD, Institute of Biochemistry, FU Berlin
1993-1995 Research Associate, Institute of Biochemistry, FU Berlin
1995-1997 Research Associate, Department of Medical Oncology and Applied Molecular Biology, MDC Berlin
Since 1997 Research Associate, Institute of Molecular Medicine, University of Lübeck

Dr. rer. nat. Sandra Laufer

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2004-2008 PhD, Institute of Molecular Medicine, University of Lübeck
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1994-2001 Studies of Biology, University of Padua
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2004-2007 PhD, Institute of Molecular Medicine, University of Lübeck
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Institute of

Prof. Dr. rer. nat. Christian Hübner

1989-1994 Studies of Physics, University of Halle
1997 Research stay at the UC Berkeley
1998 PhD, University of Halle
1998-2003 Research Associate, ETH Zürich and University of Mainz,
2003-2006 Junior-Professor University of Halle,
Since 2006 Full Professor and Chair of the Institute of Physics at the university of Lübeck

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Main research topics in the CSCM:

- Protein folding
- Function and dynamics of enzymes
- Virus assembly
- Single molecule sequencing
- Mössbauer spectroscopy in energy and time domain
- Density functional calculations and molecular modelling

Protein folding

Proteins (derived from the Greek word *protos* = first) are the most important fundamental units of all creatures. No other class of cell components has so diverse functions: catalysis of chemical reactions, transport of ions and of metabolic products and educts through the cell membrane, mechanical stabilisation, active movement, signal transduction. This enormous diversity is possible due to particular 3-dimensional conformations of the macromolecular proteins. This 3-dimensional conformation is determined by the sequence of amino acids, the basic components of proteins. Yet it is not clear how the macromolecule 'finds' in a process called folding the exact conformation out of the incredible large set of possible conformations. Conformations, which exist only for a short time during the folding, cannot be observed if a large number of molecules (ensemble) are studied. For this reason the method of single molecule detection is used to investigate protein folding. This method allows to 'watch' a single protein during the folding process. Furthermore, the folded and unfolded fraction in a folding reaction at equilibrium can be distinguished allowing for a population-specific conformational analysis.

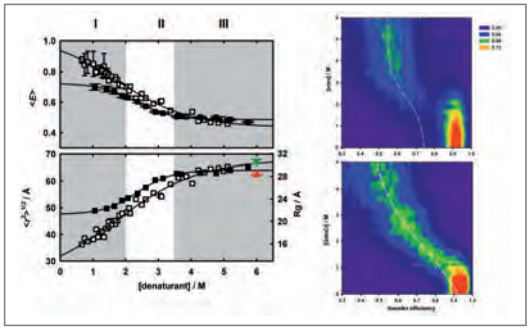

Fig. 1: Left panel: Average transfer efficiency and hydrodynamic radius of barstar upon unfolding in urea (filled squares) and GdnHcl (open squares). Right panel: Transfer efficiency distributions upon unfolding.

Function and dynamics of enzymes

For biophysicists, enzymes are the most interesting proteins due to their function in the regulated catalysis of metabolic processes in cells. It is not the pure catalytic power but rather the subtle regulation of the rate of catalysis, which is in the center of our interest. A reaction catalyzed by enzymes usually has several steps: first, the educts are bound to the enzyme, afterwards the reaction takes place, and, finally, the products dissociate from the enzyme. The velocity of the reaction is determined by the slowest of these steps. Our working hypothesis is that for many enzymes this step is associated with a conformational transition, i.e., a change of the structure of the enzyme. We already found that this holds true for the enzyme Adenylate kinase from Aquifex aeolicus. We now further aim to elaborate all steps in the catalytic cycle by direct observation of their sequence, which is impossible by ensemble methods.

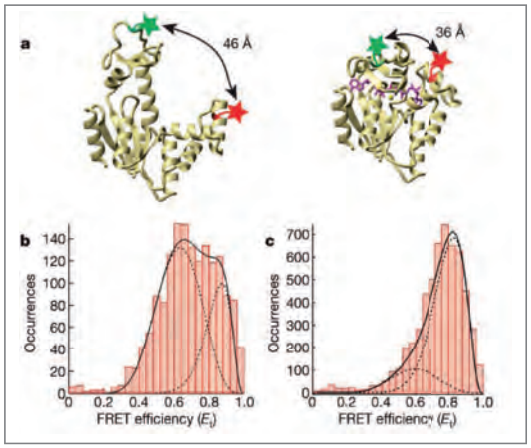

Fig. 2: a: Structure of Adenylate kinase from aquifex aeolicus in the open and closed conformation. b: Transfer efficiency distribution of the apo enzyme. c: Transfer efficiency distribution of the inhibitor-bound enzyme.

Virus assembly

The capsid of a virus is a fascinating example of self-organisation in nature. We aim to understand how the capsid is assembled. Is a dimer of building blocks the nucleus where monomers are successively attached? Or is the assembly a hierarchical process? Single particle fluorescence the perfect tool to answer these questions. We can directly follow the assembly process on a single particle level, thus unraveling transiently populated states. Also, processes like "breathing", the exchange of building blocks between the capsids, can be studied.

Physics

Single molecule DNA sequencing

The development of most characteristics of living organisms is coded in a universal way in the DNA. Many pathogens can now be identified on the basis of their genetic code. To do so, the available DNA is multiplied by the polymerase chain reaction (PCR) to obtain sufficient amounts for sequencing. The disadvantage of this method is the necessary time that is lost for life saving actions and the error rate of the PCR. Direct sequencing of single DNA molecules would circumvent these problems. We are working on the realization of such single molecule sequencing. To this end, DNA labeled specifically at every base is degraded by the action of an exonuclease, and the labeled nucleotides are subsequently identified.

Mössbauer spectroscopy in energy and time domain

Mössbauer spectroscopy is based on the recoilless nuclear -resonance discovered by Rudolf Mössbauer in 1958. A -quantum emitted by a Mössbauer nucleus can be absorbed in resonance by another nucleus of the same kind if the nuclear recoil energy is small compared to the linewidth of the transition. If the emitting and the absorbing nuclei do not have the same chemical environment, resonance can be destroyed by small shifts of the nuclear energy levels due to hyperfine interactions with surrounding nuclei and electrons and external fields. Such shifts can be detected by tuning the energy of the -quanta, and in this way valuable information can be obtained about the geometric and electronic structure of the molecule or crystal that hosts the Mössbauer nucleus (1). In the last several decades this method has developed into a powerful tool in solid-state physics and chemistry, including bioinorganic chemistry.

With the advent of third-generation synchrotron radiation sources the technique of nuclear resonant forward scattering (NFS) of synchrotron radiation has been developed which can be regarded as Mössbauer spectroscopy in the time domain. The high brilliance and the extremely collimated synchrotron beam lead to a large flux of photons through a very small size of the sample (0.1 – 1 mm^2) that make it possible to measure extremely small samples of bioinorganic compounds or metalloproteins. Another related technique, nuclear inelastic scattering (NIS) of synchrotron radiation, can be regarded as an extension of the conventional, energy-resolved Mössbauer spectroscopy (in the range 10^{-9}–10^{-7} eV) to energies of the order of molecular vibrations (in the range 10^{-3}–10^{-1} eV). This method is highly site-selective: the intensity of individual peaks in the spectrum is roughly proportional to the mean-square displacement of the Mössbauer nucleus arising from the corresponding molecular vibration (see also Fig. 3 as an example).

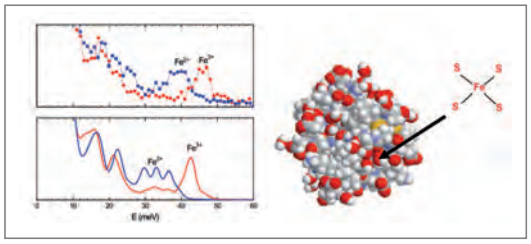

Fig. 3: Measured (left upper panel) and simulated (left lower panel) NIS spectrum (in arbitrary units) of Rubredoxin (right).

Density functional calculations and molecular modelling

Density functional theory (DFT) is currently the method of choice for calculating the electronic structure of inorganic or biological molecules containing transition metals. Next to the electronic structure also the geometric structure of molecules in its ground state, in metastable states or in transition states can be calculated by DFT methods (3). Moreover these methods can be used for the calculation of the molecular dynamics as studied for instance by vibrational spectroscopies like IR, Raman or NIS. In order to assess large biological molecules DFT methods can be combined with molecular mechanics methods (see for example Fig. 3).

Selected Publications:

1 H. Hofmann, R. P. Golbik, M. Ott, C. G. Hübner and R. Ulbrich-Hofmann: Coulomb Forces Control the Density of the Collapsed Unfolded State of Barstar. Journal of Molecular Biology 376 (2008) 597-605.

2 K. A. Henzler-Wildman, V. Thai, M. Lei, M. Ott, M. Wolf-Watz, T. Fenn, E. Pozharski, M. A. Wilson, G. A. Petsko, M. Karplus, C. G. Hübner and D. Kern: Intrinsic motions along an enzymatic reaction trajectory. Nature 450 (2007) 838-U13.

3 T. Teschner, L. Yatsunyk, V. Schünemann, H. Paulsen, H. Winkler, C. Hu, W.R. Scheidt, F.A. Walker, A.X. Trautwein: Models of the Membrane-Bound Cytochromes: Mössbauer Spectra of Crystalline Low-Spin Ferriheme Complexes Having Axial Ligand Plane Dihedral Angles Ranging from 0° to 90°. J. Am. Chem. Soc. 128 (2006) 1379-89.

Prof. Dr. rer. nat. Alfred X. Trautwein

1961-1967 Studies of Physics, TU München
1969 PhD, TU München
1972 Research Associate, University of Utah, Salt Lake City
1973-1980 Associate Professor at the "Universität des Saarlandes"
1972-1976 Several research stays in Kyoto and Salt Lake City,
1980-2006 Full Professor and Chair of the Institute of Physics at the University of Lübeck
Since 2006 Professor emeritus

Awards, memberships and others:
Max-Planck Research Prize 1993 of the German Alexander von Humboldt-Foundation (together with Professor Raymond Weiss, Strasbourg, France)
Gay-Lussac Research Prize 1999 of the French Ministery of Education Research and Technology

Vice-President, President and Past-President of the Society of Biological Inorganic Chemistry (1998-2004)
Secretary of the German Biophysical Society (1995-2002)
Member of the German Physical Society (since 1970), German Chemical Society (since 1994), Steering Committee of the Network „Transition Metals in Biology" of the European Science Foundation (1989-1997), Editorial Board of several Journals, International Board on the Applications of the Mössbauer Effect (since 1995), several Networks of the European Community
Coordinator of the Priority Programme of the German Research Foundation (DFG) on „Bioinorganic Chemistry: Transition Metals in Biology and their Coordination Chemistry" (1990-1996)

Institute of

Prof. Dr. med. Wolfgang Jelkmann

1967-1973 Studies of Medicine, Medical School of Hannover;
1974 M.D.,
1973 Fellow: Dept. Clin. Biochem., Med. School of Hannover, Germany
1975 Dept. Physiology, University of Regensburg
1978 Dept. Pharmacol., Tulane University New Orleans, USA
1980 Privatdozent: Dept. Physiol., University of Regensburg
1986 Associate Professor: Dept. Physiol., University of Lübeck
1990 Full Professor: Dept. Physiol., University of Bonn
Since 1995 Full Professor and Chair of the Institute of Physiology, University of Lübeck

Awards, memberships and others
Member of the Editorial board of the journals: Journal of Interferon and Cytokine Research, BloodLine, Annals of Hematology, Current Medicinal Chemistry
Member: German Physiol. Soc., Am. Physiol. Soc., Am. Soc. Hematol., German Soc. Hematol. Oncol., Int. Cytokine Soc.
Advisory Boards:
Natl. Inst. Med. Pharmaceut. Examin. (IMPP, Mainz)
PRCA Global Scientific Advisory Board
Edited books:
Erythropoietin (W. Jelkmann, A.J. Gross, eds.) Springer, 1989
Pathophysiology and Pharmacology of Erythropoietin (H. Pagel, C. Weiss, W. Jelkmann, eds.) Springer, 1992
Erythropoietin: Molecular Biology and Clinical Use (W. Jelkmann, ed.) F.P. Graham, 2003
Rekombinante Arzneimittel (I. Krämer, W. Jelkmann, eds.) Springer, 2008

Director: Prof. Dr. med. Wolfgang Jelkmann
www.physio.uni-luebeck.de

Main research topics in the CSCM:

Production and action of inflammatory cytokines and hemopoietic growth factors with emphasis on erythropoietin,
Oxygen supply to tissues,
Cardiovascular physiology,
Renal physiology

Cellular O_2 sensing and transcriptional control of hypoxia-inducible genes

Cells are permanently threatened by hypoxia, i.e. limited O_2 supply. Hypoxia causes cellular dysfunction and, eventually, cell death. Here, the activation of genes encoding adaptive proteins is of crucial importance. Some of these proteins act systemically, such as erythropoietin (EPO), which increases the O_2 capacity of the blood, and others locally, such as vascular endothelial growth factor (VEGF), which stimulates angiogenesis. At the single cell level, the expression of glucose transporters and of glycolytic enzymes is enhanced on hypoxic stress. The expression of all of these genes is under the control of hypoxia-inducible transcription factors (HIF-1 and HIF-2).
HIF are heterodimeric transcriptional complexes (HIF- /ß). The HIF- subunits possess 2 central oxygen-dependent degradation domains (ODD) and 2 trans-activation domains (TAD). In a pO2-dependent way, human HIF- is hydroxylated at distinct proline residues in the ODDs. This reaction is catalyzed by specific HIF- prolyl hydroxylase domain containing, 2-oxoglutarate- and Fe2+-dependent, dioxygenases (PHD-1, -2 and -3). Prolyl-hydroxylated HIF- is captured by von-Hippel tumor suppressor protein and degraded by the 26S proteasome. Furthermore, HIF- can be hydroxylated at asparagine residues in the C-terminal TAD, which reduces its ability to bind the transcriptional coactivator CBP/p300. Our research has shown that 2-oxoglutarate competitors and iron chelators inhibit the degradation of HIF-1 (Fig. 1)

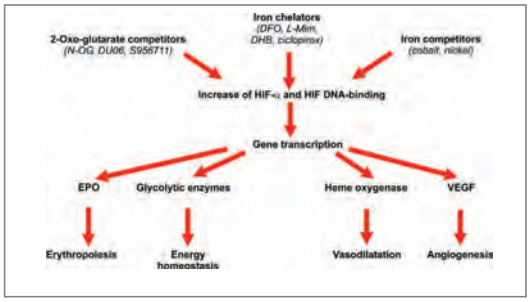

Fig. 1: Effects of drugs stabilizing HIF-1

EPO: The fountain-head in studies of hypoxia-inducible proteins

The glycoprotein hormone EPO is essential for red blood cell production. EPO mRNA is expressed in several organs, with the kidneys being the main production site in adults. The EPO enhancer is activated by HIF-1 and HIF-2 (Fig. 2). EPO inhibits apoptosis and promotes the proliferation of erythrocytic progenitors. EPO signalling involves tyrosine phosphorylation of EPO-R and JAK-2, the activation of anti-apoptotic proteins and of STAT factors. Lack of EPO results in normochromic normocytic anemia as in patients with end-stage renal failure. Our studies have revealed that endogenous EPO levels are also low in patients with chronic inflammatory and malignant diseases, because inflammatory cytokines (IL-1, TNF-) suppress EPO gene expression.

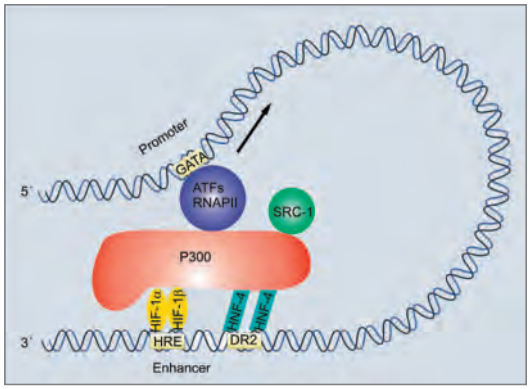

Fig. 2: Transcriptional control of the EPO gene

Ligand-induced activation of HIF-1

We have identified peptidic mediators that can induce HIF-1 accumulation and HIF-1 DNA-binding in normoxic cells, including IL-1, TNF- , and insulin. Our seminal finding that IL-1 and TNF- stimulate HIF-1 dependent gene expression in normoxia and hypoxia has opened a new field in immunological research, with HIF-1 acting as a central player in inflammatory processes.

Both the hypoxic and the ligand-induced activation of HIF-1 involve the phosphatidylinositol-3-kinase (PI-3-K) and the mitogen activated protein kinase kinase-1 (MAPKK-1) pathways.
Activation of PI-3-K/Akt results in increased HIF-1 protein levels. The MAPKK-1 pathway increases the trans-activation ability of HIF-1. Thus, there is a fine control of HIF-1 activity, involving (i) feedback-control of HIF- by PHD-2 and -3, (ii) ligand-induced HIF-1 activation and (iii) modulation by kinases and transcriptional co-factors.

Physiology

Therapeutic implications

On stabilization of HIF, the production of EPO increases which is of benefit in anemia. Stimulation of HIF-dependent gene expression accelerates angiogenesis for application in ischemic diseases. Furthermore, HIF activation augments cell proliferation, wound healing, and distinct inflammatory reactions. HIF suppression, on the other hand, is potentially beneficial to prevent tumor angiogenesis and tumor cell adaptation to hypoxia (Fig. 3). Our present research aims at studying the role of the HIF system in the resistance of tumor cells towards radiation and chemotherapeutics.

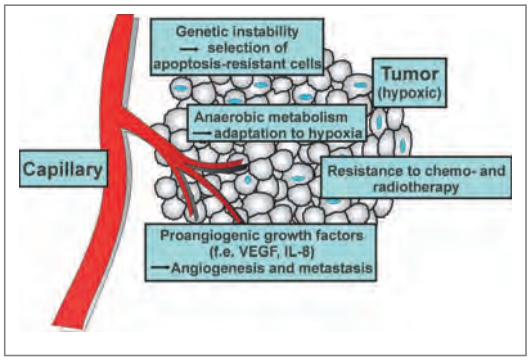

Fig. 3. Promotion of tumor growth by hypoxia

Vascular control and microcirculation

Oxygen requirements of the organs depend on work load and blood perfusion is continuously matched to the changing needs. This adaptation is achieved by changes of vascular diameters and most pronounced in the skeletal muscle in which perfusion can increase up to 20-fold with heavy work load. The behaviour of the smallest arteries (arterioles) is of special importance herein, because the highest resistance resides in these vessels. The inner cell layer (endothelium) is crucial for dilation, because it releases mediators that relax the adjacent smooth muscle and ultimately dilate the vessel. These mediators include nitric oxide (NO), prostaglandins, and an additional, still elusive mediator termed 'endothelium-derived hyperpolarizing factor' (EDHF). EDHF activates potassium channels and thus induces hyperpolarization and relaxation.

EDHF does not only induce dilation in the local vicinity of its release, but it also exerts remote effects and thus relaxes the vessel at distant, up- and downstream sites. This is termed an ascending or conducted dilation and the hyperpolarization is of special importance, because it can be transferred through intercellular contacts along the vessel wall. These contact sites consist at the molecular level of intercellular channels composed of connexin proteins that connect neighboring cells directly. Clusters of these channels are termed gap junctions, which allow communication along the endothelial and/or the smooth muscle cell layer and thus along the vascular tree. By this means a coordination of vascular behaviour over long distances is enabled, which leads to a steady uniform dilation or constriction over a comparably long range of the vessel. Such a coordination of diameter changes along the vessel is an indispensable requirement for large changes of blood flow.

We are studying the role of different connexins as well as other mechanism that contribute to the conduction of vasomotor responses (dilation or constriction) along the arteriolar tree and their functional role in blood flow regulation. We have identified connexin40, which is expressed mostly in the endothelium (Fig. 4), as an important molecule to support communication in the vessel wall. Interestingly, mice deficient for this connexin are hypertensive implicating a role for cell communication in the regulation of blood pressure.

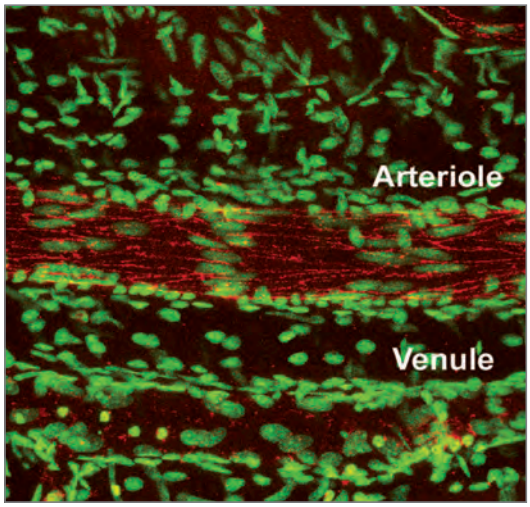

Fig. 4. Gap junctions (connexin40, red) within the vessel wall delinate the plasma membrane of endothelial cells in arterioles, but not in venules (nuclei in green)

Selected Publications:
Research group Jelkmann:
1 Bruegge K, Jelkmann W, Metzen E: Hydroxylation of hypoxia-inducible transcription factors and chemical compounds targeting the HIF- hydroxylases. Curr Med Chem 14: 1853-1862, 2007
2 Krajewski J, Batmunkh C, Jelkmann W, Hellwig-Bürgel, T: Interleukin-1 inhibits the hypoxic inducibility of the erythropoietin enhancer by suppressing hepatocyte nuclear factor-4 . Cell Mol Life Sci, 64: 989–998, 2007
3 Laugsch M, Metzen E, Svensson T, Depping R, Jelkmann W: Lack of functional erythropoietin receptors of cancer cell lines. Int J Cancer 122: 1005-1011, 2008
4 Wagner AE, Huck G, Stiehl DP, Jelkmann W, Hellwig-Bürgel T: Dexamethasone impairs hypoxia-inducible factor-1 function. Biochem Biophys Res Comm 372: 336-40, 2008
Research group de Wit:
1 Siegl D, Koeppen M, Wölfle SE, Pohl U, de Wit C: Myoendothelial coupling is not prominent in arterioles within the mouse cremaster microcirculation in vivo, Circ Res 97, 781-788, 2005
2 Wölfle SE, Schmidt VJ, Hoepfl B, Gebert A, Alcoléa S, Gros D, de Wit C: Connexin45 (Cx45) cannot replace the function of Cx40 in conducting endothelium-dependent dilations along arterioles, Circ Res 101, 1292-1299, 2007
3 de Wit C, Boettcher M, Schmidt VJ: Signaling across myoendothelial gap junctions - Fact or fiction? Cell Commun Adhes 15: 231-245, 2008
Research group Pagel:
1 von Wussow U, Klaus J, Pagel H: Is the renal production of erythropoietin controlled by the brain system? Am J Physiol Endocrinol Metabol 289: E82–E86, 2005
2 Groesdonk HV, Bauer A, Kreft B, Heringlake M, Paarmann H, Pagel H: Urodilatin and pentoxifylline prevent the early onset of Escherichia coli-inducted acute renal failure in a model of isolated perfused rat kidney. Kidney Blood Press Res 32: 81–90, 2009

Prof. Dr. med. Cor de Wit

1982-1989 Studies of Medicine, FAU Erlangen, University of Lübeck
1990 M.D.
1990-1998 Research Associate in Physiology, University of Lübeck and University of Mainz
1996 Accreditation as medical specialist for Physiology
1998-2003 Group leader, Physiology, LMU München
2001 Habilitation for Physiology at the faculty of Medicine, LMU München
2003-2006 Associate Professor, Institute of Physiology, Univ. of Lübeck
Since 2007 Full Professor, Univ. of Lübeck

Awards, membership and others:
1999 Servier Award
2002 Christian Crone Award
2003 President of the German Soc. for Vascular Biology and Microcirculation
2009 Vice-Chairman of the Working Group for Coronary Pathophysiology and Microcirculation of the European Society for Cardiology

Member of the Editorial Board of the Journals: Microcirculation, Journal of Vascular Research
The Open Nitric Oxide Journal

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1981 Final Examination in Biology; University of Hannover
1985 PhD, Department of Nephrology, Medical School of Hannover
1986 Research Associate, Institute of Physiology, University of Lübeck
1995 Habilitation
1996 Fellow, Institute of Molecular Medicine, University of Oxford / England
Since 2001 Associate Professor, Institute of Physiology, University of Lübeck

Member of the Editorial Board of the Open Physiology Journal
Official Anti-Doping Representative of the Cycling Association Schleswig-Holstein/Germany
Member of the Scientific Advisory Board of enformax International Inc./ Germany

Institute of

Prof. Dr. rer. nat. Norbert Tautz

1984-1990 Studies of Biology, University of Regensburg
1990-1994 PhD student, Federal Research Centre for Viral Diseases of Animals, Tübingen
1994 PhD in Biology, Eberhard Karls University Tübingen
1994-1994 Postdoctoral fellow, Federal Research Centre for Viral Diseases of Animals, Tübingen
1995-2000 Scientist, Justus-Liebig-University in Gießen
2000-2007 Assistant professor, Justus-Liebig-University in Gießen
2003 "Habilitation" in Virology
Since 2007 Full Professor and Chair, Institute of Virology and Cell Biology, University of Lübeck

Member of the editorial board of the Journal of General Virology

Dr. rer. nat. Olaf Isken

1992-1997 Studies of Biochemistry, University of Leipzig
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2002 PhD in Biology, Justus Liebig University Giessen
2002-2005 Research Associate at the Institute for Cancer Research, Fox ChaseCancer Center, Philadelphia, USA, Research Associate at the Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, USA
Since June 2008 Research Associate at the Institute of Virology and Cell Biology, University of Lübeck, Germany

Research topics: Virus-host interactions, viral RNA replication and cellular gene expression

Director: Prof. Dr. rer. nat. Norbert Tautz
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Main research topics in the CSCM:

replication control of positive strand RNA viruses
virus host cell interactions
connective tissue research
cartilage tissue engineering

Research group Norbert Tautz:

Replication of members of the Flaviviridae family

This family contains the genera Flavivirus (yellow fever virus, dengue virus, tick borne encephalitis virus), *Hepacivirus* (hepatitis C virus, HCV) and *Pestivirus* (classical swine fever virus, bovine viral diarrhea virus, BVDV).
While HCV and many flaviviruses represent a major threat to human health pestiviruses are highly important pathogens of live stock. Moreover, HCV and BVDV have the capacity to establish lifelong persistent infections. About 130 million people suffer from chronic HCV infection and are at risk to develop liver cirrhosis and hepatocellular carcinoma. BVDV persists in about 1-2% of cattle world wide causing severe reproduction problems in stock farming. Both represent also highly interesting models to study the molecular basis of viral persistence and virus host interaction involved in this process.
A common feature of the viruses of the family *Flaviviridae* is the translation of one large polyprotein directly after entering the host cell. This viral polyprotein is further processed by host cell derived and virus-encoded proteases into the mature viral proteins. Proteolytic processing is a highly complex and ordered process with regulatory importance for viral replication.

Our main interests are:
– to understand the details of these processing events
– the regulation of the enzymes involved
– host cell proteins with regulatory activity

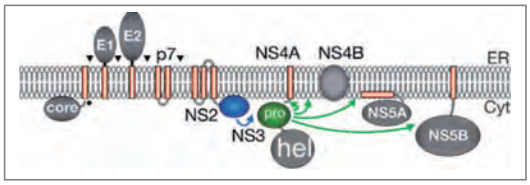

Fig. 1
Processing of the HCV polyprotein
Black arrow heads: processing by cellular ER resident proteases. Blue arrow: NS2-3 Protease cleavage. Green arrows: Processing events catalyzed by NS3/4A protease.
Mod. after Lorenz et al., Nature 442, 831-835.

A detailed understanding of these subjects will enable us to develop novel strategies to interfere with viral replication. Innovative concepts for the treatment of infections with HCV and several flaviviruses are urgently needed since these viruses are a constant risk for human health.

HCV:

The non-structural protein 2 (NS2) encodes a cysteine protease which generates its own C terminus and thereby releases NS3 which is an essential component of the viral replicase. Interestingly, NS2 requires for its proteolytic activity the N-terminal domain of NS3. Accordingly, the enzyme is referred to as NS2-3 protease. Since its activity is crucial for viral replication it represents an interesting drug target. We recently observed that activation by NS3 may also occur *in trans* but the function of NS3 in protease activation remains obscure.

Therefore we aim at establishing:
- the role of the NS3 part in NS2-3 protease activity
- an *in vitro* system to test activation by NS3 *in trans*
- setting up an in vitro assay for inhibitor screening

A serine protease in NS3 catalyzes processing at the remainder of the cleavage sites. NS4A serves as a cofactor. We are interested in the interplay between these molecules in protease activation.

BVDV:

Persistence of BVDV in cattle depends on the non-cytopathogenic phenotype of the virus. We observed that this phenotype relies on a strict downregulation of viral RNA replication. Mutant viruses without this restriction lead to cell lysis in cell culture and a lethal disease in persistently infected animals. The critical balance in RNA replication efficiency is maintained by limiting amounts of a cellular chaperone serving as an essential cofactor a cysteine protease in NS2.

Main goals are:
– to visualize protease activation at the atomic level
– understand regulation of cofactor expression in the host and correlation with viral tissue tropism
– sites of and mechanisms involved in particle formation

Flaviviruses:

We observed that overexpression of the cellular chaperone which acts as cofactor of the pestiviral NS2 protease and thereby stimulates pestiviral RNA replication acts as negative regulator of flaviviral RNA replication. We aim at the identification of the viral interaction partners and the molecular basis for the down- regulation of flaviviral RNA replication. The underlying mechanism may lead the way to novel antiviral therapies.

Virology and Cell Biology

Prof. Dr. rer. nat. Holger Notbohm

1966-73 Studies of Physics, University of Wien and Kiel
1973 Research Associate, University of Kiel
1978 PhD in Biophysics, University of Kiel
1987 Habilitation in Molecular Biophysics
Since 1998 Associate Professor, Institute of Medical Molecular Biology, University of Lübeck

Regenerative Medicine

Laboratory group Prof. Dr. H. Notbohm: Matrix biology

Current concepts of tissue regeneration are based on the utilisation of donor cells, their in vitro expansion and matrix integration prior to the replacement of injured or damaged tissues such as bone, cartilage or skin. To progress in this area, the following aims are in the focus of our research and development efforts:

First hypothesis:

Molecular assembly of proteins of the extracellular matrix is governed by the relative proportions of individual constituents forming the functional tissue (-alloy's).

We study assembly mechanisms of various collagen types (fiber forming collagens, plasma membrane inserted collagens) and analyse, additionally, the functional contributions of posttranslational modifications on the assembly process.

Most recently, in collaboration with CRM, Kiel, we characterized molecular features and studied assembly mechanisms of a novel marine collagen, which may prove to be suited for cartilage repair.

Second hypothesis:

The extracellular matrix, in particular in anatomical niches of the body, provides the biological containment for stem cell differentiation and functional maintenance of differentiated mesenchymal cells. We study in close collaboration with the group "'Stem cell biology" and the Fraunhofer group the potential of various extracellular matrices on the onset and direction of stem cells differentiation.

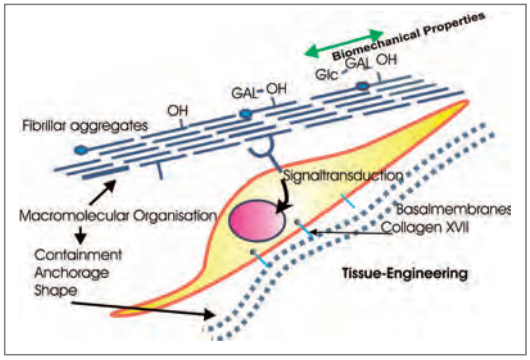

Fig 1: Structure and function of the extracellular matrix

Selected publications:
Research group Notbohm

1 Areida SK, Reinhardt DP, Müller PK, Fietzek PP, Köwitz J, Marinkovich MP, Notbohm H: Properties of the collagen type XVII ectodomain. Evidence for n- to c-terminal triple helix folding, J Biol Chem. 2, 276(2), 2001, 1594-601
2 Kassner A, Tiedemann K, Notbohm H, Ludwig T, Morgelin M, Reinhardt DP, Chu ML, Bruckner P, Grässel S: Molecular structure and interaction of recombinant human type XVI collagen, J Mol Biol. 339(4), 2004, 835-53
3 Brinckmann J, Notbohm H, Müller PK (eds): Collagen: Primer in Structure, Processing and Assemby, 2005, Springer Verlag Berlin, Heidelberg, New York

Publikationen Tautz:

1 Lackner T., A. Müller, A. Pankraz, P. Becher, H.-J. Thiel, A.E. Gorbalenya, and N. Tautz. 2004. Temporal modulation of an autoprotease is crucial for replication and pathogenicity of an RNA virus. J. Virol. 78:10765-10775.
2 Lackner T., A. Müller, M. König, H.-J. Thiel, and N. Tautz. 2005. Persistence of bovine viral diarrhea virus is determined by a cellular cofactor of a viral autoprotease. J. Virol. 79: 9746-9755.
3 Lackner T., H.-J. Thiel, and N. Tautz. 2006. Dissection of a viral autoprotease elucidates a function of a cellular chaperone in proteolysis. Proc. Natl. Acad. Sci. U S A. 103 (5): 1510-5.
4 Schregel V., Jacobi S., Penin F., and N. Tautz. 2009. Hepatitis C virus NS2 is a protease stimulated by cofactor domains in NS3. PNAS Proc. Natl. Acad. Sci. U S A. 2009 Mar 31; 106 (13): 5342-7

Institute of

Prof. Dr. med. Jürgen Brinckmann

1979-1986 Study of human medicine, Mainz and Lübeck
1987 Graduated MD
1987-1989 Research assistant, Department of Dermatology, University of Lübeck
1989-1991 Research assistant, Department of Medical Molecular Biology, University of Lübeck
Since Juli 1991 Research assistant, Department of Dermatology / Department of Virology and Cell Biology, Universitäty of Lübeck
1998 Habilitation in Dermatology
Since 2006 Associate professor
2008 Visiting professor McGill University, Montreal, Canada

Research group Jürgen Brinckmann (Department of Dermatology)

Regeneration and fibrosis

The center of interest is the analysis of collagen and microfibrillar proteins in regeneration and fibrotic / sclerotic skin diseases (systemic sclerosis, lipodermatosclerosis, keloids). Wound healing is characterized by an overlapping sequence of events comprising inflammation, proliferation, migration, differentiation and tissue remodelling. These tightly regulated processes resemble in some aspects those found in embryonic development and also cause sclerosis when out of control. Especially, posttranslational modifications of collagen and the pattern of collagen cross links stabilizing the extracellular matrix are in the center of our studies. We could show that sclerotic skin is characterized by a shift from cross-links primarily observed in adult skin to cross-links predominantly found in fetal skin. This shift seems to be involved in a reduction of proteolytic breakdown and tissue hardening and is induced by profibrotic cytokines (i.e. TGF-ß, IL-4). TGF-ß is synthesized as an inactive precursor which is bound to fibrillin-1 and fibrillin-2 and thus stored in the extracellular matrix. In cooperation with Prof. Reinhardt (McGill-University, Montreal) we show that an enhanced expression of fibrillin-2, which is typically expressed in the embryo-fetal period but only scarcly found in adult skin, is a general feature of activated fibroblasts found in wound healing and sclerotic tissues. The higher expression of fibrillin-2 in wound healing and sclerotic tissue is another example for the “embryonic reprogramming” of fibroblasts in these conditions.

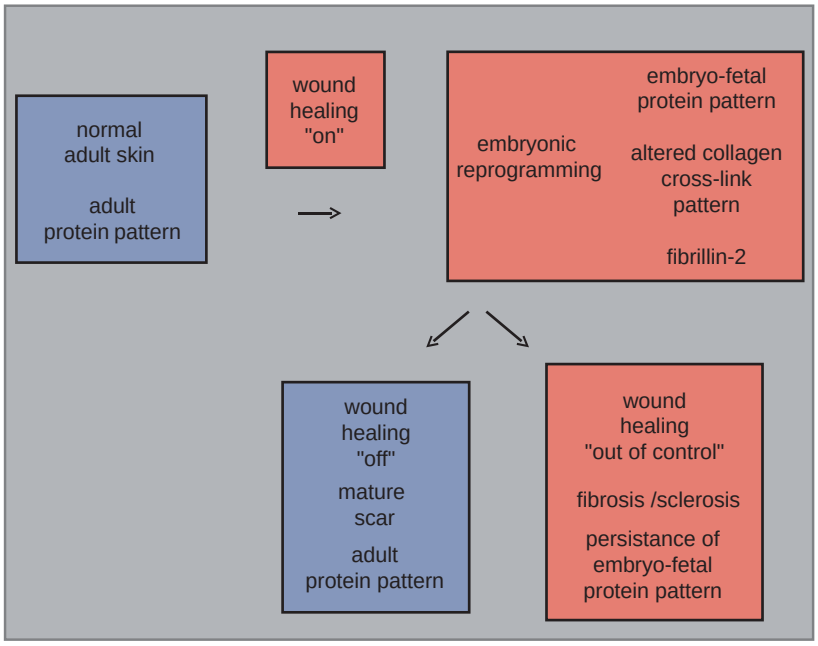

Fig. 1: Embryonic reprogrammin in wound healing and fibrosis

Virology and Cell Biology

Research group Jürgen Rohwedel and Jan Kramer

Stem Cell Biology

The focus of our research is the differentiation of embryonic stem (ES) cells *in vitro* as well as the isolation and characterization of somatic stem cells. In particular, we are interested in (i) the program of differentiation of ES cells into different cell types and (ii) the use of ES cell differentiation as a model system to study cellular development (see Fig. 2). With regards to future clinical aspects we (iii) identify and isolate stem/progenitor cells from different organs and (iv) analyze the role of somatic stem cells during regeneration after tissue injury.

During ES cell differentiation in vitro cellular processes of embryonic development are recapitulated. We have characterized in detail differentiation of mouse ES cells *via* mesenchymal precursors into chondrogenic and osteogenic cells *in vitro*. Recently, we demonstrated that ES cells differentiate into different renal cell types *in vitro*. Moreover, cellular interaction and the *in vitro* formation of cartilage nodules and renal multi-cellular structures can be observed, respectively.

Stem cells mediate regeneration of damaged tissue. We isolated mesenchymal stem/progenitor cells from different tissues and characterized these cells *in vitro*. In human we demonstrated that mesenchymal stem cells can be guided to a cartilage defect using extracellular matrix components. One goal of our efforts is to understand which factors play a role during the local recruitment of reparative cells. Recently, we found evidence that mesenchymal tissue stem/progenitor cells and fibroblasts are closely related. Currently, we analyse the role of these cells during regeneration after kidney injury.

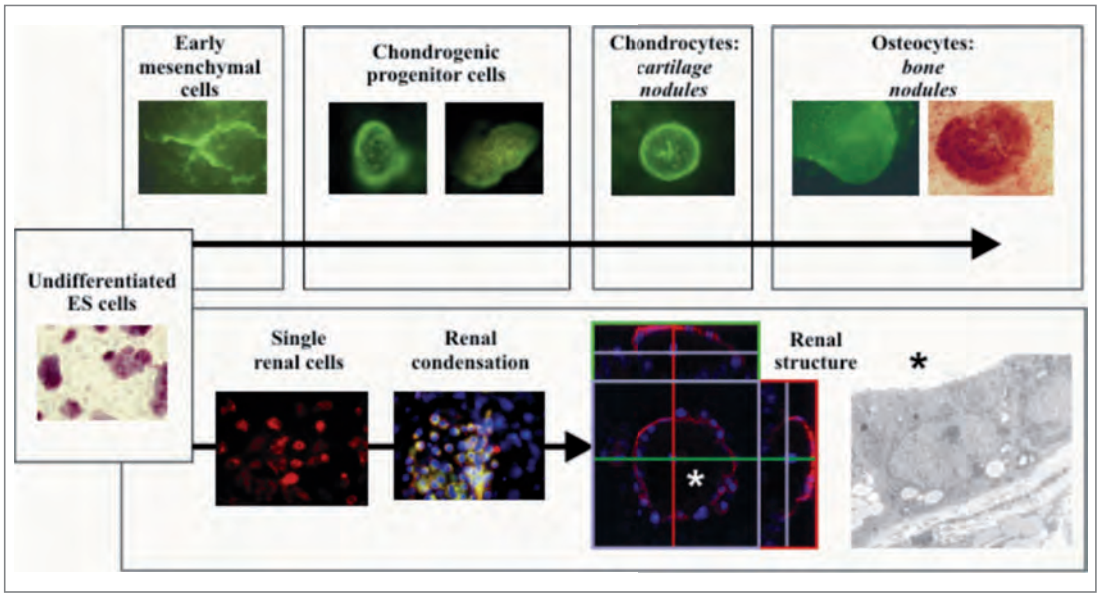

Fig. 2: The ES cell model system recapitulates cellular processes of embryonic development.

Selected publications:
Publikationen Brinckmann:

1 El-Hallous E, Sasaki T, Hubmacher D, Getie M, Tiedemann K, Brinckmann J, Bätge B, Davis EC, Reinhardt DP. Fibrillin-1 interactions with fibulins depend on the first hybrid domain and provide an adaptor function to tropoelastin. J Biol Chem. 2007 Mar 23;282(12):8935-46.

2 Wu J, Reinhardt DP, Batmunkh C, Lindenmaier W, Far RK, Notbohm H, Hunzelmann N, Brinckmann J. Functional diversity of lysyl hydroxylase 2 in collagen synthesis of human dermal fibroblasts. Exp Cell Res. 2006 Nov 1;312(18):3485-94.

3 Brinckmann J, Hunzelmann N, El-Hallous E, Krieg T, Sakai LY, Krengel S, Reinhardt DP.
Absence of autoantibodies against correctly folded recombinant fibrillin-1 protein in systemic sclerosis patients. Arthritis Res Ther. 2005;7(6):R1221-6.

4 Brinckmann J, Kim S, Wu J, Reinhardt DP, Batmunkh C, Metzen E, Notbohm H, Bank RA, Krieg T, Hunzelmann N. Interleukin 4 and prolonged hypoxia induce a higher gene expression of lysyl hydroxylase 2 and an altered cross-link pattern: important pathogenetic steps in early and late stage of systemic scleroderma? Matrix Biol. 2005 Oct;24(7):459-68.

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Silvia Bulfone-Paus

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1996 Deputy Director Institute of Immunology, Benjamin Franklin University Hospital, Free University of Berlin
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1992
John Enders Award, Yale University
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1999
Young Investigators Award, International Cytokine Society
Heisenberg-Stipend (DFG)
2001
Minerva Price, Rome

Member of the Scientific Advisory Board (Health Politics) of the Ministry for Education and Research
Member of the Scientific Advisory Board of the Norddeutsche Life Science Agentur GmbH, Norgenta
Member of the Curatorship of the Forschungsverbund Berlin
Chairwoman of the Scientific Advisory Board, Bernhard-Nocht-Institut, Hamburg
Member of Scientific Advisory Board, Leibniz-Institut für Arteriosklerosеforschung, Münster

Director: Prof. Dr. med. Dr. rer. nat. Silvia Bulfone-Paus
www.fz-borstel.de

Main research topics

The cellular and molecular basis of infection, allergy and inflammation.
Promoting the development of novel forms of therapy for the diseases target

The Department takes an unusually comprehensive and synergistic approach by addressing both innate and adaptive immunity, and to combine whenever possible studies in the murine and human system. Both systems comprise soluble and cellular components, which are regulated by receptor-dependent mechanisms, and exhibit multiple levels of cross talk and cross-regulation.

In this ever-increasing complexity of innate and adaptive immunity interactions, two teams in the Department focus on the mechanisms by which the immune system recognizes selected microbial pathogens (e.g. via LPS and other endotoxins) and on the subsequent signaling events, using a complementary array of particularly instructive *in vitro*- and *in vivo*-assays. Several other scientists in the Department, instead, focus on the role of chemokines and cytokines in inter- and intracellular signaling in both arms of the immune system. To this end, the expression, regulation, signal transduction and function of chemokines released primarly by thrombocytes, as well as key cytokines and their receptors at the cross-roads of innate and adaptive immunity, such as IL-15, are analysed. In particular, these investigators pursue the challenge to clarify how selected cytokines and chemokines regulate intercellular communication within the innate component of immunity, or study, alternatively, how these cytokines and chemokines regulate the differentiation and effector functions (e.g. T cell priming) of antigen-presenting cells in adaptive immune responses.

In addition, we are investigating the regulation and biology of membrane trafficking in an immunological context e.g. the function of the trafficking/sorting machinery in signal transduction and the molecular mechanisms that regulate endocytic pathways employing the death receptor Fas (CD95) as our model system. We hope to better understand signaling pathways during normal immune responses and how perturbations in these pathways can lead to immune deficiency or autoimmunity.

Epigenetics is an emerging discipline that concerns the mechanisms that bring about the stable cell-to-cell inheritance of gene expression patterns. One is the so-called epigenetic “histone code”, which is founded upon the observation that states of gene activitv are associated with particular post-translational modifications of the histones. HP1 is known to recognise and bind one of the best characterised determinants of the histone code, namely tri-methylated lysine 9 of histone 3 (Me(3)K9H3), which is an evolutionary conserved marker of silent heterochromatin domains within the nucleus. Our work is focused on elucidating how HP1 proteins regulate genome organisation and expression in cells of the innate and acquired immune system.

Forschungszentrum Borstel

A third, traditional focus of the Department, which extends upon this latter theme and carries it into tropical veterinary medicine, is constituted by studies that elucidate the genetics, cellular and molecular biology of an intracellular protozoan parasite (*Theileria*). These studies, for which the Department is internationally recognized center, aim at clarifying host-pathogen interactions and at developing effective vaccines.

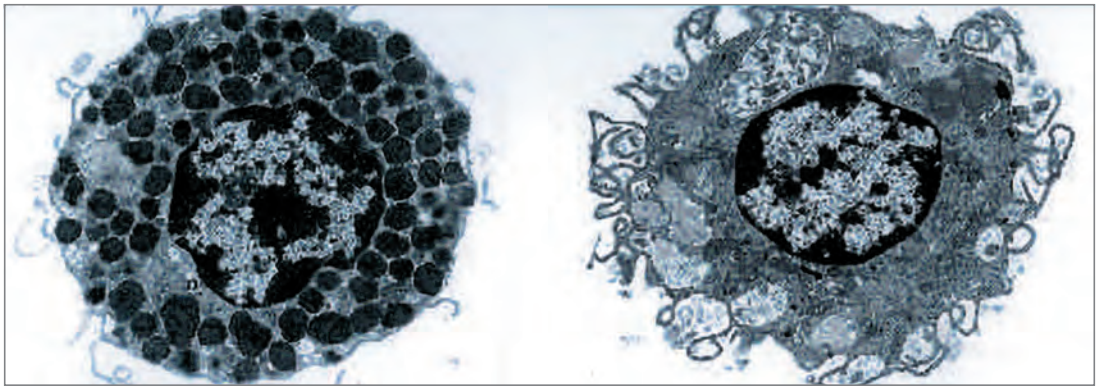

Fig 1: Resting mast cells (left). Crosslinking of IgE antibodies on the surface leads to activation and release of granule contents (right). (Garland Publishing / Elsevier Science 2000)

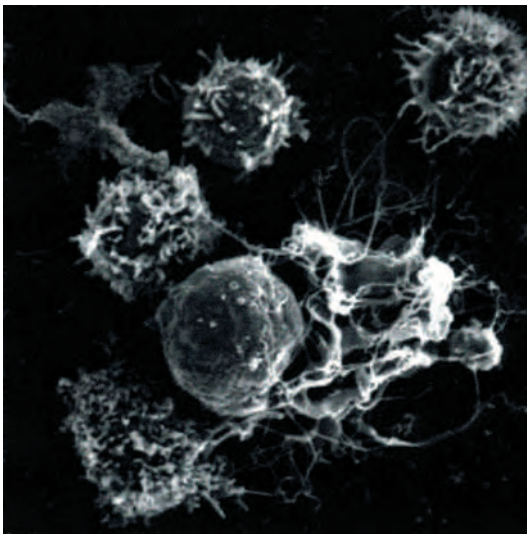

Fig 2: High resolution electron microscopy of Dendritic-cell-T-cell cluster (MD Anderson Cancer Center, Texas, USA)

Department of Molecular Infection Biology of the (Leibniz-Center for Medicine and Biosciences)

Prof. Dr. rer. nat. Ulrich E. Schaible

1991 Doctor of Philosophy MPI for Immunobiology, Freiburg
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2006-2008 Professor, Chair in Immunology, London School of Hygiene & Tropical Medicine, UK
Since 2008 Honorary Professor, London School of Hygiene & Tropical Medicine, UK
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since 2004 Editorial Board "Microbes and Infection"
2005 GlaxoSmithKline Foundation, Award for Clinical Research
since 2005 Editorial Board "Faculty of 1000 Biology"
2006-2011 Royal Society Wolfsohn Research Merit Award
03-09/2006 Wellcome Trust Value in People Award

Referee German Research Foundation, WHO, MRC, Wellcome Fund, Swiss Cancer Fund
Advisory Board "Microbiology & Immunology", German Research Foundation

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The Department of Molecular Infection Biology (MIB) combines basic research on the many facets of bacterial infections. The research agenda reaches from molecular analysis of bacterial structures and their genetic basis to studies on interactions between host and pathogen and immune responses against infection and further to translational approaches together with the clinical department. Thereby, an infection can be studied with a holistic view. The prime focuses of MIB are on pulmonary pathogens, in particular those, which cause tuberculosis and cystic fibrosis, but also on environmental bacteria and their influence on lung immunity including allergic reactions.

The main topics in the CSSM are

- Host genetics, host-pathogen interaction and immunity in tuberculosis,
- Structural, genetic and functional characterization of bacterial virulence factors,
- Membrane biophysics of receptor-ligand interactions in bacterial infection,
- Immune modulation by bacterial compounds.

The broad range of state of the art techniques as well as the expertise and interdisciplinary approach of MIB's scientific staff allows for a detailed analysis of a pathogen and the disease it causes. From the characterization of individual bacterial molecules and their interactions with host cell structures to their influence on the host and its defense responses, we can globally study the infectious process. Nuclear magnet resonance and mass spectroscopy are used for structural analysis of bacterial compounds. Atomic force microscopy and micro calorimetry allow assessment of molecular interactions between pathogen and host molecules. Using advanced life cell imaging and other microscopy techniques combined with tissue culture and *in vitro* organ models, the host cell-pathogen interplay can be dissected. A large collection of gene knock-out and transgenic mouse strains paired with safety level 3 labs for work on tubercle bacilli *(Mycobacterium tuberculosis)* provides the great opportunity to study the host response to tuberculosis, one of the most important human infections world-wide.

Several groups within MIB analyze various bacterial molecules including lipoproteins, lipopolysaccharides (of Gram-negative bacteria), lipoteichoic acids (of Gram-positive bacteria), cell wall glycolipids (of mycobacteria) and proteins (U. Zähringer, O. Holst, B. Lindner) as well as their genetic basis (U. Mamat). Interactions of these structures with host receptors and membranes are studied within the groups of biophysics and immune biophysics (T. Gutsmann, A. Schromm). Immune modulating molecules of environmental and commensal bacteria are explored with respect to their influence on allergy (O. Holst, B. Lindner) and infection (U. Schaible). Genetically engineered antibodies are employed to specifically detect these structures and use them as targets for diagnosis and therapy (H. Brade, S. Müller-Loennies). The cellular microbiology of infection, in particularv of tuberculosis, is the research topic of several groups using both *in vitro* and *in vivo* models (N. Reiling, U. Schaible). The micro ecology of bacterial lung infections is explored with respect to the influence of commensal bacteria and micronutrient availability (U. Schaible). Genetically engineered murine models are generated and employed to study immunity in tuberculosis with a particular interest in immune modulating cytokines such as interleukins 15 and 17 (C. Hölscher). Induction and control of inflammatory reactions are studied using the murine tuberculosis model (S. Ehlers). Host genetic factors for susceptibility or resistance to tuberculosis as derived from human cohort studies are tested in genetically defined murine models (S. Ehlers). Finally, in collaboration with other groups within the Clinical and Immunology/Allergy departments of the Research Center Borstel, we will direct our research towards translational approaches. The allocation of novel findings and inventions to benefit medical progress is facilitated by their translation into patents and clinical applicability, by which we hope to contribute to the development of novel diagnostics and therapeutics including anti-tuberculosis and anti-allergy drugs.

Forschungszentrum Borstel

Fig 1: TB-infection studies.

Together with the Heinrich-Pette-Institute (Virology) and the Bernhard-Nocht-Institute (Tropical Medicine and Parasitology) in Hamburg, MIB comprises the Leibniz Center for Infection Research, thereby covering the whole of infection biology and medicine. Research of MIB is embedded in numerous regional cooperative projects with the universities in Kiel, Lübeck and Hamburg, including DFG-funded collaborative Research Centers (SFB 470, SFB 617, SFB 415) as well as DFG- and BMBF-funded national (Transregio 22, NGFN-2) and EU-funded international consortia (Gabriel, GA-2LEN, PAN-TB-NET). MIB is an integral part of the Cluster of Excellence on Inflammation Research (Kiel-Borstel-Lübeck) promoting collaborative studies on inflammation, allergy and infection. There are numerous international collaborations with other scientific institutions including a close link to the London School of Hygiene and Tropical Medicine, one of the world-leading universities in public health issues.

The Department of Molecular Infection Biology within the Research Center Borstel is dedicated to innovative basic, applied and translational research in infection biology for the improvement of human health in the world.

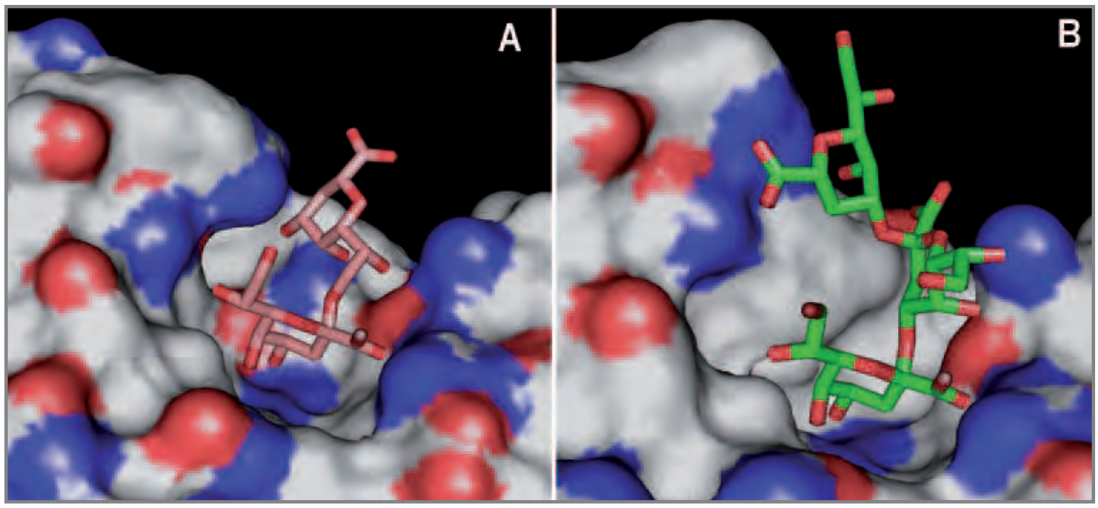

Fig 2: Binding pockets of two monoclonal antibodies (Fab fragments) recognizing as a minimal structure a disaccharide (A) or a trisaccharide (B) of 3-deoxy-D-manno-oct-2-ulosonic acid.

Central Facilities support for the CSCM:
Isotopes Laboratory of the TNF

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1968-1977 Studies of Chemistry at the Universities of Stuttgart and Tübingen
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1981-1984 Postdoctoral fellow at the Dept of Chemistry, UC Berkeley USA
1984-1992 Research Associate, Dept of Biology, University of Tübingen
1989 Visiting scientist at the Dipartimenta di Biologia Cellulare e dello Svilluppo, University of Palermo, Italy
1990 Habilitation
1993-1995 Group leader at the University of Tübingen, and scientist at the "Hans Knoell Institut für Naturstoffforschung", Jena
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Habilitation fellowship (DFG)
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Organizer and Chairman of the conference Biometals 2004, 09/3-5/2004; Garmisch-Partenkirchen, Germany

GdCh (Gesellschaft deutscher Chemiker)
ACS (American Chemical Society)
IBS (International BioIron Society)
SBIC (Society of Biological Inorganic Chemistry)
VAAM (Vereinigung für Allgemeine und Angewandte Mikrobiologie)
Deutsche Gesellschaft für Biophysik
Fachverband für Strahlenschutz e.V.

Prof. Dr. rer. nat. Berthold F. Matzanke-Markstein

Main research topics in the CSCM:

Iron transport in eubacteria, archaea and fungi imperfecti
Iron homeostasis and oxidative stress response
Biomineralization

Iron Transport

For all animals and plants, and for virtually all microbes, life without iron is impossible. In response to this, microorganisms secrete high affinity iron-binding compounds, called siderophores. More than 600 naturally occurring siderophores have been isolated and characterized, and the discovery of new siderophores is continuing at a good rate(1-3). Siderophores serve as drugs for the treatment of tranfusional iron overload (desferal). They found therapeutic application for various pathological conditions due to aluminum overload. For improved enhancement inMRI, different paramagnetic ions like Mn2+, Fe3+ and Gd3+ have been utilized. In this context, chelators are required that prevent release of the free cation in vivo. Siderophores and their synthetic anlogs serve as principal models for such chelators. Semisynthetic siderophore-antibiotic adducts may emerge as a completely new class of antibiotics('Trojan-horse' concept) which are urgently required given the growing multiple resistance of bacteria like *Mycobacterium tuberculosis* or certain *Pseudomonas* strains. Studies on iron transport, on the specificity of siderophores and on the thermodynamic and kinetic features of siderophores are important prerequisites for these investigations. Our group is working in this field for 25 years. During that time period we developed time resolved in situ Mössbauer and EPR spectroscopy as analytical tools to follow the intracellular fate of iron (1) .

Iron homeostasis and oxidative stress response

In order to prevent OH*-radical formation and Fenton chemistry, intracellular iron must be tightly regulated and secured (2). An array of iron-storage and –detoxification compounds has been detected including Ftn A, Ftn B, Bfr, Dps1, and Dps 2. Up to four of these multi-subunit proteins are expressed in a variety of Eubacteria and Archaea. Up to now, the diversity of functions and regulation patterns have been merely partially uncovered. Whereas some Dps proteins display increased expression under conditions of oxidative stress and in stationary growth phase, Ftn-protein levels are enhanced in many Eubacteria at high intracellular iron concentration in the logarithmic growth phase. The role of Bfr is completely enigmatic and some Dps proteins behave more like Ftn- than like Dps-proteins. We could demonstrate this in the case of Dps A from the *Euryarchaeon Halobacterium* salinarum. Currently, an extensive study is carried out on *Erwinia chrysanthemii* with ΔBfr, ΔBfrΔFtn, and ΔFtn,ΔDps constructs. Low-molecular mass iron, which is required for the biosynthesis of iron-containing enzymes must also be Fenton-inactive. In addition, the binding should not be too tight in order to allow facile ligand exchange. Initial experiments of our laboratory have shown that a ferrous oligomeric sugar phosphate, which we call ferrochelatin, is the major low-molecular mass iron-pool in *E.coli* and in many other Gram-negative bacteria (3). A detailled structural analysis of ferrochelatin started in late 2008 including spectroscopic analyses (UV-Vis, Mössbauer, EXAFS) as well as determination of thermodynamic stability and of mineral formation kinetics.
We believe that a compound with similar functions should be also abundant in all eukaryotes.

Biomineralization

Iron storage kinetics and mineral structures of classical ferritins are well characterized. Much less understood is the mechanism of iron release from ferritin. Moreover virtually nothing is known about the biomineralization of iron in Dps-proteins. In 2007 we started a program analyzing Dps biomineralization in vitro and in vivo.
A functionally and structurally distinct process of biomineralization is found in magnetotactic bacteria. Magnetite serves in these mainly microaerophilic bacteria as a device to find the appropriate oxygen partial pressure in sediments. We are currently analyzing (together with the group of Prof. Schüler, Maximilians University Munich) the process of magnetosome formation and intracellular iron trafficking (4). Biosynthesized magnetite particles (20nm in size) are of potential importance in various biotechnologic and technologic applications.

Selected Publications:
1 B.F. Matzanke: Iron Transport: Siderophores. In: Encyclopedia of Inorganic Chemistry, R. B. King, ed; Wiley publishers, 2nd edition September 2005, Vol IV,2619-2646.
2 A. Boughamoura, B.F. Matzanke, L. Böttger, S. Reverchon, E. Lesuisse, D. Expert, T. Franza: Differential role of ferritins in iron metabolism and virulence of the plant pathogenic bacterium Erwinia chrysanthemii 3937. Journal of Bacteriol 2008, 190, 1518-1530
3 R. Böhnke, B.F. Matzanke : The mobile ferrous iron pool in E. coli is bound to a phosphorylated sugar derivative. BioMetals 8, 1995, 223-230
4 D. Faivre, L. Böttger, B.F. Matzanke, D. Schüler: Intracellular magnetite biomineralization in bacteria proceeds via a distinct pathway involving membrane-bound ferritin and ferrous iron species . Angewandte Chemie, Int. Edition 2007, 46, 8495-8499

Figures on page 1:

Structure of Adenylate kinase from aquifex aeolicus in the open and closed conformation. b: Transfer efficiency distribution of the apo enzyme. c: Transfer efficiency distribution of the inhibitor-bound enzyme.
Institute of Physics

The picture shows cells harbouring biologically active siRNA in vesicles (orange, indicated by a white arrow), delivered according to a novel principle (Overhoff & Sczakiel, 2005). The green fluorescence shows the actin filaments.
Institute of Molecular Medicine

Three-dimensional structure of FeoB from L. pneumophila.
Institute of Biochemistry

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