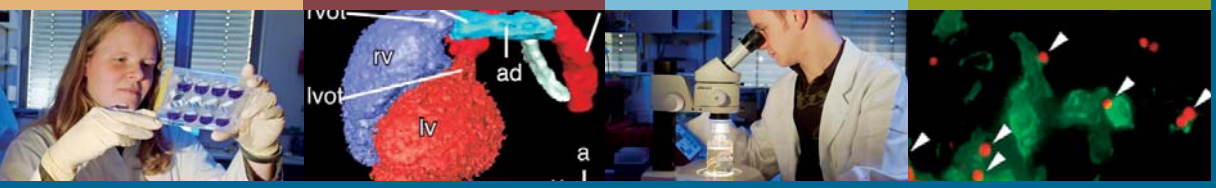
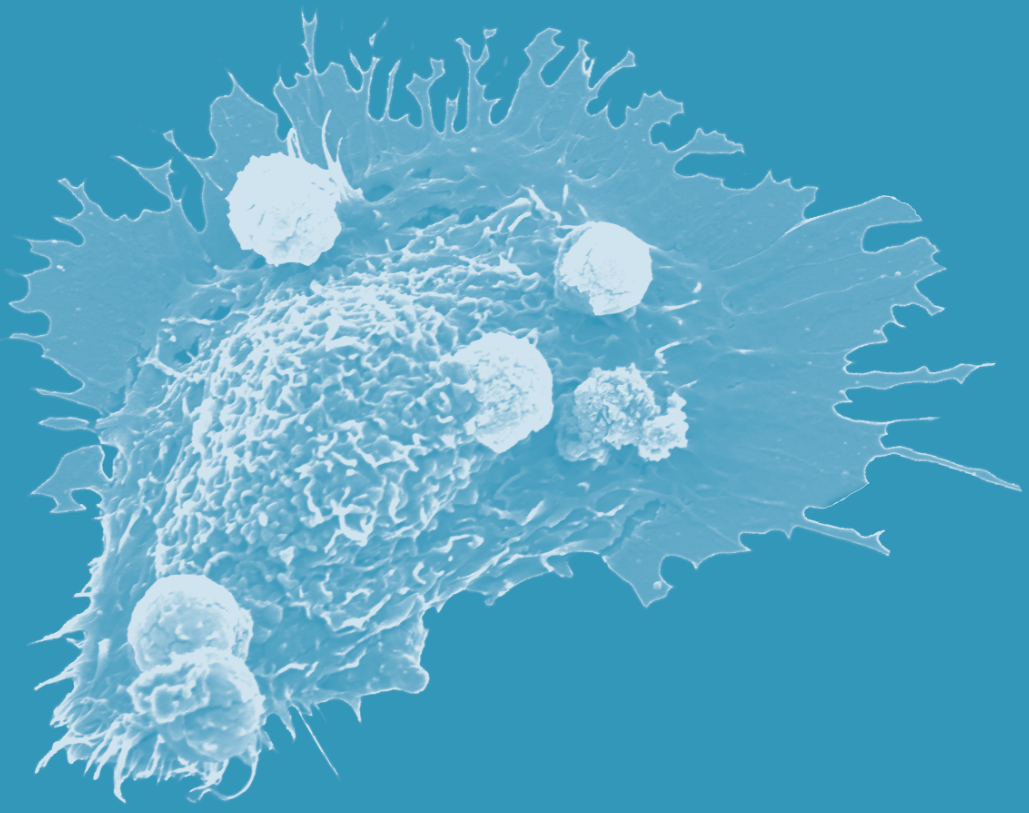


ANNUAL REPORT 2004/2005



GBF

Gesellschaft für
Biotechnologische Forschung
German Research Centre
for Biotechnology

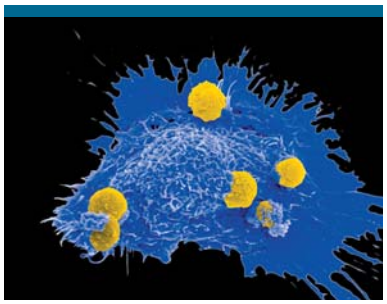


Photo title: Dr. Rohde

Phagocytosis of apoptotic cells by macrophages.
The scanning electron photomicrograph shows a macrophage (blue) that engulfs several apoptotic thymocytes (yellow). Different phases of the phagocytosis process are visible. In this particular experiment *phosphatidylserine receptor (Ptdsr)* – deficient mouse macrophages have been used, thus demonstrating that these macrophages are still capable to engulf apoptotic cells.

Photo back cover: Radde

View of the GBF Forum and the Rainwater Biotope.
The FORUM, providing venues for symposia, seminars, lectures, and exhibitions is located in the southern part of the GBF campus.

- *Cover thumbnail pictures from left to right: Stefanie Schiebe controlling the coloration of cell preparations. | Identification of cardiac malformations in Ptdsr knockout mice using magnetic resonance imaging (MRI). Shown is a 3D reconstruction of MRI data of a wild-type mouse. | Phillip Hahn analysing a skeleton of a mouse mutant under the stereoscope. | Phagocytosis of apoptotic cells by fetal liver derived macrophages from Ptdsr-deficient mice. Apoptotic thymocytes (red) were stained with TAMRA and feed to F4/80 stained fetal liver derived macrophages (green). Quantification of phagocytosis by wild-type or Ptdsr -/- macrophages revealed no differences in the efficacy of apoptotic cell engulfment.*

ANNUAL REPORT 2004/2005

GERMAN RESEARCH CENTRE FOR BIOTECHNOLOGY

Gesellschaft für
Biotechnologische Forschung
German Research Centre
for Biotechnology

SCIENTIFIC REPORTS

FACTS AND FIGURES

THE GERMAN RESEARCH CENTRE FOR BIOTECHNOLOGY (GBF)

- **The GBF is a centre for infection research** The focus of our work is the study and investigation of pathogens which are medically relevant or can be used as models for researching infection mechanisms. Ninety percent of GBF funding is provided by the Federal German Government and the other ten percent by the State of Lower Saxony. The GBF has approximately 600 employees on its staff and an annual budget of about € 50 million. The centre is a member of the Helmholtz Association of German Research Centres, Germany's largest non-university research organization.

  Infections are responsible for a third of all deaths worldwide. Global mobility, international tourism and migration accelerate the spread of infectious pathogens. In addition, growing antibiotic resistance, the increased susceptibility for infections in an aging population, the reappearance of nearly forgotten infectious diseases, and the emergence of new infectious agents generate an urgent need for the development of new therapies and medicines to combat infectious diseases. Furthermore, recent research indicates that infections are also responsible for triggering certain diseases, which were previously thought not to be connected to pathogens, such as cancer, diabetes and allergies.

  Among the scientific questions we explore are: What turns bacteria into a pathogen? Why are some people highly sensitive and others resistant to infections? How can we intervene in the infection process? Understanding these mechanisms will contribute to combating infectious diseases with new drugs and vaccines.

  The human immune system can respond with amazing speed and agility to new pathogens and yet collapse occasionally under the onslaught of bacteria or viruses. How do our natural pathogen defenses work and how these strategies can be used, or even improved, is the focus of GBF research on the immune system. For this, we also study the immune response in mice, which show high similarity in this respect to their human counterparts.

  The GBF works closely with universities and other research institutes both at the national and international level, and is also a member of the National Genome Research Network. As part of a European Union programme to support talented young researchers, the GBF, together with the Hannover Medical School, trains young scientists to become experts in infection research.

  The GBF holds certifications for the production of pharmaceutical agents based on the good manufacturing practice (GMP) guidelines. As a consequence, the GBF is able to manufacture substances for clinical testing and so bridge the gap between basic research and medical applications.

The German Research Centre for Biotechnology – GBF
Mascheroder Weg 1
38124 Braunschweig, Germany
Tel: (+49) 5 31-61 81-0
Fax: (+49) 5 31-61 81-515
info@gbf.de | www.gbf.de

GBF

Gesellschaft für
Biotechnologische Forschung
German Research Centre
for Biotechnology

FOREWORD

Prof. Dr. Rudi Balling | Scientific Director

Whether in science or industry, international acceptance is the result of hard work. The GBF continues to gain this recognition as an infection research centre. Four years ago the GBF made the decision to pursue this goal. Today, GBF scientists publish their research findings on infection biology in prestigious international journals. We have set up new cooperative efforts with highly regarded partners, such as the Pasteur Institute in France, the Riken Genomic Sciences Center in Japan and Rockefeller University in the United States. At the same time, we have also intensified our regional networking in Germany. These advances were made possible by the extraordinary commitment of our GBF staff members.

For me, the past four years have gone by so quickly. My first tournament as scientific director is now coming to a close and I would like to express my gratitude to the GBF's Scientific Advisory Committee and the Supervisory Board for extending my contract another five years.

To ensure that the GBF maintains its international profile will require great efforts. For that, our scientists need a creative environment. The future scientific strategy will be based on three pillars: experimentation and clinical and theoretical infection research. Securely establishing these three aspects will enable us, through the results of our GBF basic research, to contribute to the resolution of the clinical problems physicians must deal with on a daily basis.

- Experimental infection research is well established at the GBF – and needs to be expanded further in the coming years;
- An excellent starting point for clinical infection research is offered by our partnership with the Hannover Medical School and the joint founding of the Center for Experimental and Clinical Infection Research;
- By setting up R&D in the field of theoretical biology with special emphasis on bioinformatics and system biology, the GBF can play a pioneering role in infection research.

We want to introduce these aspects to health research at the Helmholtz Association. Only when we proceed jointly and strategically with other health research centres – especially the DKFZ, MDC, and GSF – will we have the chance to be internationally competitive. At the same time, however, it is equally important not to ignore our immediate neighbours. Of key importance is the Braunschweig Technical University. To achieve substantial results in theoretical biology it is necessary to cultivate an intensive exchange between biology and the engineering sciences. The Technical University is strong in both these fields – and we need strong partners. Only by working closely with them, can the strategy and creativity of GBF researchers lead to future scientific success.

Rudi Balling

Photos: left: Dr. Jo Schilling (right) coordinating the interview with Prof. Dr. Rudi Balling, GBF (middle) and Prof. Dr. Dieter Bitter-Suermann, MHH (left) | centre: Priv.-Doz. Dr. Dietmar Pieper (left) coordinator of the BIOTOOL international research project discussing further experiments with Dr. Howard Junca of his work group | right: Prof. Dr. Singh Chhatwal analysing agar plates with oral streptococci.

Photos: Hans, GBF (le), Sierigk (ce), Ammerpohl (ri)

Out of the Lab and into the Clinic – GBF and MHH Launch Joint Translation Centre

INTERVIEW WITH | **Prof. Dr. Dieter Bitter-Suermann (President, MHH) and Prof. Dr. Rudi Balling (Scientific Director, GBF)**

- The course has been set: the GBF and Hannover Medical School (MHH) are going to launch a joint research centre. The aim of the new "Center for Experimental and Clinical Infection Research" is to add a new quality in research by interconnecting the work of different sectors. "Translation" is the catchword here and stands for the rapid movement of research results into the clinics – a prospect that promises to make the development of new medicines and therapies more efficient. Housed in the building that is close to the MHH Campus and will be vacated by the Max-Planck-Institute for Experimental Endocrinology, the new centre will be designed to be a melting pot for research to develop new concepts to treat infections.

Infection research is a focus of the Braunschweig-Hannover region. GBF and MHH work closely on infection and epidemiology issues. What was the *modus operandi* for setting up the "Center for Experimental and Clinical Infection Research"?

- **Dieter Bitter-Suermann:** We needed to do this to bring together two research cultures – two directions. Pure scientific research on infectious diseases needs to have a stronger connection to the field of medicine in general and to applications for patients.

- *Pure scientific research on infectious diseases needs to have a stronger connection to the field of medicine in general and to applications for patients.*

Photo: Hans, GBF

- **Rudi Balling:** This process is called 'Translation' – and it goes in both directions. Medical expertise and the handling of patients generate questions for doctors that basic research can answer. However, basic research people need to know the questions. What problems does medicine have that researchers should address? When the dialogue functions, basic research can have a positive influence on medical research and, in turn, medical applications.

- **Dieter Bitter-Suermann:** And there is another aspect. At the MHH, we conduct clinical research and basic research, but what's missing at the MHH is what the Helmholtz centres, besides their scientific know-how, can provide; namely, platform technologies.

- *Medical expertise and the handling of patients generate questions for doctors that basic research can answer.*

Photo: Hans, GBF

Medical research is successful…

- **Dieter Bitter-Suermann:** … but it could still function better. With the translation centre we can achieve synergy effects that we can use for infection research. Individually, each of our facilities is good, but together we will be even better.

- *With the translation centre we can achieve synergy effects that we can use for infection research.*

Photo: Hans, GBF

When will the Translation project be launched?

- **Dieter Bitter-Suermann:** A provisional centre is already operating. We founded the Center for Infection Biology in October, 2003. This center doesn't have its own space yet, but the doctorate degree programme integrated into it already exists. As soon as the Max-Planck-Institute for Endocrinology moves out of its current building, our centre will move in and have its own facilities. At that point we won't be the Center for Infection Biology anymore, but rather the Center for Experimental and Clinical Infection Research.

When the translation centre becomes a reality, isn't there a danger that it will be just another of three facilities in the region conducting infection research?

- **Rudi Balling:** No, we are bringing special expertise from different areas under one roof. The building currently housing the Max-Planck-Institute for Endocrinology is directly adjacent to the MHH campus and easily and quickly reached from Braunschweig. The region is growing and coming together. What we're doing in infection biology is just a reflection of this and we're creating a brand name, as it were: "Infection research made in Lower Saxony." In conjunction with the centre we already have four "DFG-Sonderforschungsbreiche" (special research areas funded by the German Research Foundation), several joint research groups and a European graduate degree programme that enjoys financial support from the European Union.

There are already numerous scientists working at the GBF and MHH. In other words, we're joining the established sectors of infection biology basic research at both the GBF and MHH with the expertise at MHH in clinical infection research. The basic researchers and clinical interns will be able to have a much broader exchange of ideas and experience just by being together to talk, whether it's in the cafeteria or the laboratory. Both sides will feel responsible for the projects they jointly work on, and both sides, although now still living in different worlds, will soon learn to better understand the other. Together, they can succeed in projects where previously one discipline alone would not.

- *Both sides will feel responsible for the projects they jointly work on, and both sides … can succeed in projects where previously one discipline alone would not.*

Photo: Hans, GBF

There are two very clearly defined goals in infection research: the development of new vaccines and the development of anti-infection agents. But these goals can only be achieved when we understand the basics; that is, how the mechanisms of infection work and how anti-infection therapies or vaccinations function in relation to those mechanisms. And the best way to do that is together.

What does the focus of the translation centre actually consist of?

- **Rudi Balling:** The focus, of course, is determined by the needs of the medical profession. At the GBF, for example, we have paid close attention to the infection problems faced by transplant specialists. These issues, which arise from the daily clinical routine, are extracted, as it were, from the MHH research environment and then discussed with us. Together we then select the topics we think are the best for us to jointly tackle and solve.

- *Together we then select the topics we think are the best for us to jointly tackle and solve.*

Photo: Hans, GBF

- **Dieter Bitter-Suermann:** Naturally, we address the research issues that the GBF and MHH are involved with and then we look for the gaps. We ask ourselves such things as how we can improve or add to areas we already know well. What are we lacking on know-how, for example? And now, for the first time, we have proposed topics that are not handled at all in Germany, or only peripherally, in order to solidify the special role of this centre.

Could you name a few examples?

- **Dieter Bitter-Suermann:** In the past we have dealt with persistent and chronic infections, but only in conjunction with Listeria. However, we have not conducted research on the basic mechanisms of persistence. That would be a field for the translation centre; also, antibiotic resistance and multi-drug resistance. Another issue is biofilm formation; in other words, the ability of pathogenic microorganisms to essentially form their own habitats on endogenic and exogenic surfaces and isolate themselves from defense mechanisms. This is a highly interesting aspect, both from a medical and basic research standpoint. To date, no solution has been found to overcome this. To be able to understand how the signal transmissions function in these bacterial communities would be very exciting and something we definitely should take a closer look at.

For questions like these to be jointly tackled by the GBF and MHH, what would you need?

- **Dieter Bitter-Suermann:** The key point here is to bring clinicians in contact with the methods used in basic research to give medical research a push forward.

- *The key point here is to bring clinicians in contact with the methods used in basic research to give medical research a push forward.*

Photo: Hans, GBF

- **Rudi Balling:** And to do that there needs to be a joint effort with the people in basic research. When they have to solve a medical problem, the clinicians in the new centre have immediate access to the researchers with the necessary scientific expertise. And they can solve the problem together.

You've mentioned five areas that the new centre could focus on: genetic susceptibility, molecular mycology, biofilms, infections and cancer, persistence and chronic infections. Which of these are the most urgent?

- **Dieter Bitter-Suermann:** That depends on the personnel. The way I see it, when we begin looking for people to fill the key positions, then we have to simply go through the research landscape and decide who we would like to have at the centre. There will be two permanent departments and everything else will be third-party funded projects. We'll have young researchers coming in for a few years and then leaving. Besides two department heads, no one else will be permanent. Instead, there will be a regular fluctuation. We do need two permanent departments, though, because when you have so many doctoral candidates, post-grads and young researchers working, you need to have some constant to take on the organizational tasks and the academic aspects. At the moment, we're looking at one department for basic infection research and one for clinical infection research. These would be the two pillars supporting the centre.

How will the centre be financed?

- **Dieter Bitter-Suermann:**
Funding and the purchase of the building will come from the GBF and the Helmholtz Association. The long-term financing of our operational costs, and the research and academic appointments, will be divided.

How does the new centre fit in with the general cost-saving efforts at the MHH?

- **Dieter Bitter-Suermann:** If we only try to save money, we'll end up saving ourselves to death. We've set up a structural package that tapers off toward the end and allows us more funding at the top.

- **Rudi Balling:** … And infection research gets some of the best grades at MHH ...

When will the "Center for Infection Biology and Clinical Infection Research" be ready to operate?

- **Rudi Balling:**
The preparations are under way. The research programme is currently being developed and the building is being purchased. By mid-2006 it will be ready for occupancy. By the end of 2005, we'll launch the personnel selection process and at the end of 2006 life will begin in the new building

- *The research programme is currently being developed and the building is being purchased.*

Photo: MPG

Dr. Jo Schilling is a freelance science journalist, writing for national and international newspapers, magazins and radio stations. She is specialized in biotechnological, medical and chemical topics.

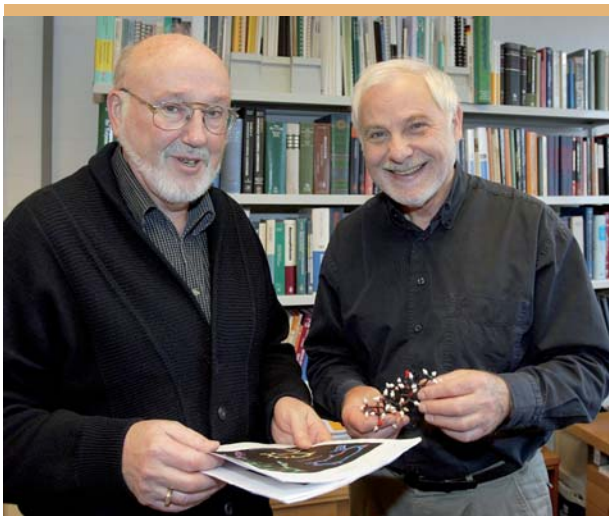
Highlights 2004/2005

AUTHOR | Manfred Braun | Department of Public Relations

Elite International Doctorate Candidates Highly qualified young researchers from around the world are getting the chance to polish their skills in Braunschweig. Since the autumn of 2004, the GBF has been coordinating the EU support programme "Miditrain". Together with the Hannover Medical School (MHH), the GBF is giving 12 doctoral candidates advanced training in pathogen research. The candidates were selected from 250 applicants. In the coming years, they will be working on various projects to study what molecular 'weaponry' pathogens use against humans, how our immune systems defend themselves against them and how these pathogens can be combated with vaccines or medicines. The EU has set aside some € 2 million from its scientific training programme "Marie Curie Actions" to finance "Miditrain" – which stands for "Molecular Interaction during Infection". *(Autumn 2004)*

Three GBF tutors for the Miditrain programme: Dr. Hansjörg Hauser (left), Dr. Siegfried Weiß(centre), and Dr. Andreas Lengling

Photo: Ammerpohl



Prof. Dr. Hans Reichenbach (left) and Prof. Dr. Gerhard Höfle (right) were honoured with the Karl Heinz Beckurts Prize.

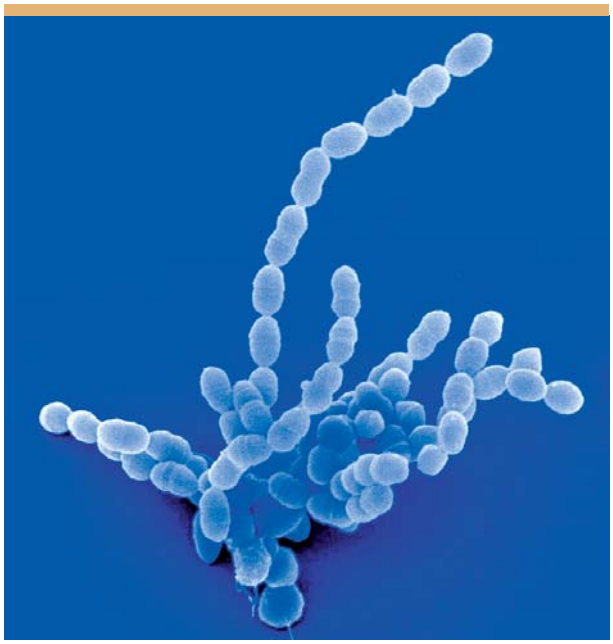
Photo: Hübner

Beckurts Prize for Cancer Drug Two GBF researchers were honored in December 2004 as the recipients of the prestigious Karl Heinz Beckurts Prize. Microbiologist Prof. Dr. Hans Reichenbach and chemist Prof. Dr. Gerhard Höfle received the award at a ceremony in Munich. The jury at the Karl Heinz Beckurts Foundation praised what it called "the excellent work in natural products research" contributed by Reichenbach and Höfle over the past few decades.

Since the 1970s, the two Braunschweig scientists have explored the biology and chemistry of myxobacteria. These soil-based bacteria have garnered the attention of the scientific community because they produce a wide variety of chemical compounds. Many of these substances possess interesting biological qualities, such as Epothilon, which has proved suitable for treating cancer and is currently undergoing clinical testing. *(December 2004)*

Fatal Tooth Decay Pathogens Bacteria known as oral streptococci, which live in the human oral cavity, not only cause tooth decay, but sometimes also far worse ailments, such as cardiacvalvulitis, or inflammation of the heart valves. A new research project, supported by the Helmholtz Association, is now taking a closer look at this pathogen to hopefully develop possible countermeasures. Involved in the project, along with the GBF, are the University of Kaiserslautern and the Leipzig University Hospital. The effort is being coordinated by Prof. Dr. Singh Chhatwal, head of the GBF department Microbial Pathogenicity and Vaccine Research.

Oral streptococci are primarily responsible for the creation of plaque and tooth decay. However, when they manage to enter the bloodstream – due to an injury for example – they can trigger blood poisoning. If they reach other parts of the body through the circulatory system they can cause abscesses in the throat, lungs and liver and even life-threatening cardiovalvulitis. *(November 2004)*



Oral streptococci

Photo: Dr. Rohde

Dr. Holger Ziehr, Head of the GMP unit, showing the GMP certification.

Photo: Hübner

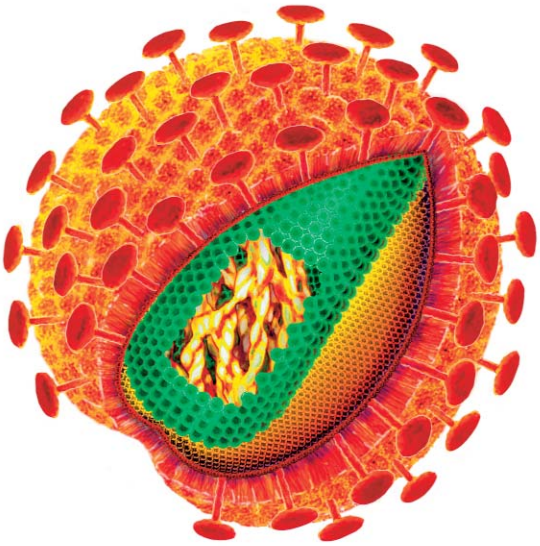
Broad GMP Certification Since the autumn of 2004, the GBF holds a broad certification for manufacturing pharmaceutical agents for clinical testing. The certification was authorized by the Federal Office for Pharmaceutical and Medical Products (BfArM) and the Braunschweig regional government. The certification allows the GBF to manufacture "recombinant nucleic acids and proteins from microorganisms and cell cultures", based on Good Manufacturing Practice standards (GMP). The permit includes most substances of medical interest that can be produced by biotechnological means and is independent of any application or mechanism of efficacy. Before obtaining this certification, the GBF had to apply for a manufacturing permit for each and every substance it wanted to produce according to GMP guidelines. *(Autumn 2004)*

GBF Acquires BioTec Start-up Centre The GBF has purchased the BioTec Start-up Centre on its campus from the City of Braunschweig. In an agreement between the Struktur-Förderung Braunschweig GmbH (SFB) and the GBF, the two partners secured the future support of start-up companies. The building will continue to be available to Biotech start-ups.

The innovation centre opened in September 2002, but only a few spaces were leased long-term. Now, the prospect of improved occupancy and a livelier campus offers advantages to the GBF: Several of the GBF buildings are in need of renovation, so the purchase of the startup centre spares the GBF from the costly and time-consuming development and construction of a new building. In addition to start-up companies, various GBF research groups will move into the start-up centre, including structural biology and instrumental analysis. *(Summer 2004)*

The BioTec Start-up Centre belongs now to the GBF.

Photo: Gabel GBF



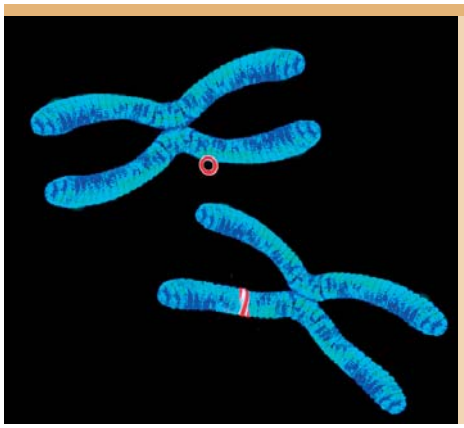
Schematic design of the HIV-virus.

Graphic design: B. Meyer, GBF

Combination-Vaccines against AIDS Scientists hope that with novel combination vaccines they will be able to combat the AIDS virus. Research laboratories in seven countries in Europe and Africa – among them a GBF group – have joined forces in the AIDS Vaccine Integrated Project, or, AVIP. The European Union is supporting the multinational effort with € 10 million. AVIP coordinator is Prof. Barbara Ensoli from the Istituto Superiore de Sanità in Italy.

AVIP researchers hope to tackle the HIV pathogen with a vaccine that contains both structural and regulatory virus components; in other words, HIV building blocks and molecules that control its reproductive cycle and the switching on and off of its genes. "The human body," says Prof. Dr. Carlos Alberto Guzmán, head of the vaccine research group at the GBF, "should then build up an immune reaction to all these components. Such a vaccine could be both preventative as well as therapeutic." *(July 2004)*

Gene Therapy: A Search for the Gentle Method (Episomes) Treating congenital disease by introducing functional genes into the human organism: with a new methodology, researchers from Germany, England and the Netherlands are aiming for a breakthrough in this process, known as gene therapy. The network of scientists, which also includes GBF research group leader Prof. Jürgen Bode, wants to advance the development of a certain type of DNA element – the so-called episomes. The European Union is contributing financial support for the Epi-Vector-Project.



On the outside instead of inside: The diagram shows a schematic representation of two chromosomes (blue). Inside the chromosomes the DNA is densely wound around protein bodies. Foreign DNA elements (red) can combine in various ways with a chromosome. Some of them become an integral component of the chromosome (lower half of the diagram), which is how many viruses smuggle themselves into the DNA of host cells. This is also how classic vectors (DNA transport elements) function in gene therapy. Episomes (upper half of diagram) are different, because these ring-like DNA molecules attach themselves to the outside of certain chromosome carrier structures. The advantage is that genes inside the host chromosome are not damaged and is the reason why scientists are now searching for ways to use episomes in gene therapy.

Graphic design: B. Meyer, GBF

Episomes are DNA elements that do not anchor themselves in the genetic material of host DNA and instead attach themselves only to certain support molecules in the nucleus of a cell. Their information is jointly read with that of the chromosomes and together they multiply with the chromosomes every time cell division occurs.

Researchers in the Epi-Vector-Project now want to find out whether episomes are suitable for a gentler form of gene therapy. *(Summer 2004)*

Human Genome Fully Deciphered Humans have fewer genes than previously thought. Instead of 30,000 to 40,000 as presumed in earlier estimates, the figure is now between 20,000 and 25,000. This surprising discovery was made by the 20 research institutes participating in the International Human Genome Project, which reported their findings on the nearly fully deciphered sequence of the human genome in the journal Nature. The article, published in October 2004, represented the completion of research with participation of the German Human Genome Project, headed by Dr. Helmut Blöcker from the GBF Department of Genome Analysis. An initial rough draft of the human genome sequence was first published in 2001.

In addition to the systematic analysis of further genomes, Dr. Blöcker's department in future will spend more time studying the function of individual genes, as well as bioinformatic methods for genome analysis. *(October 2004)*

International Mouse Genome Conference For four days in November 2003, experts in mammalian genome research from all over the world met in Braunschweig at the 17th International Mouse Genome Conference. The principal topic of the conference, mouse genetics, is becoming more and more important for medical developments as the mouse is now well established as a model organism for the analysis of diseases and the validation of new drug targets. The meeting was jointly organised by the International Mammalian Genome Society (IMGS) and the GBF, whose scientific director, Prof. Dr. R. Balling, was past-president of IMGS. *(November 2003)*

EHEC Experts Honored GBF scientist Dr. Andreas Matussek was awarded the doctorate prize of the Hannover Medical School (MHH) for his work on the dangers posed by the food germ EHEC.

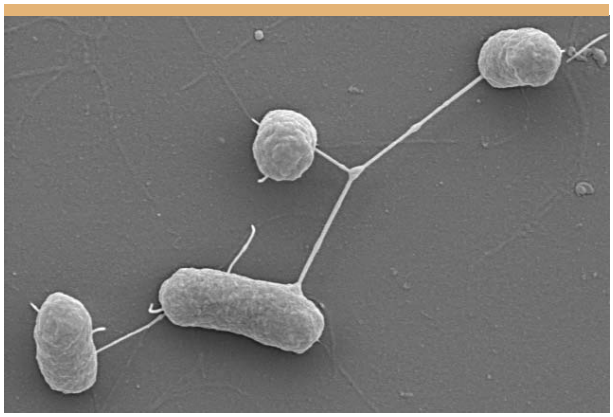
EHEC bacteria are generally ingested via impure foodstuffs. Children, in particular, frequently fall ill after an infection. The reason is a cell poison, called Shiga toxin, which exists in two variations. The EHEC bacteria pass this toxin to the intestine.

Using modern testing methods, Dr. Matussek investigated the influence of Shiga toxins on gene regulation in the human vascular walls for his doctoral thesis. He observed that the toxins apparently trigger a sequence of inflammatory reactions in human tissue cells that are so intense that they can severely damage the body. Dr. Matussek's thesis work was jointly supervised by the GBF and MHH. *(April 2004)*

Biotech Training for Researchers from ASEAN Countries Twenty-two young scientists from Southeast Asia – ranging from the medical biologist from Thailand, environmental biologist from Vietnam and the agro-biologist from Laos – took part in a six-week GBF course last May. The seminar, called "Modern Industrial Biotechnology", provided extensive information about current research methods. The programme included numerous lectures, laboratory visits, demonstrations and excursions.

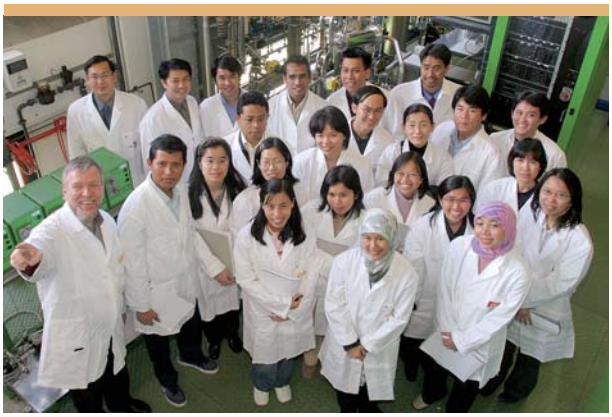
The course, held in Braunschweig, is an integral part of a one-year training programme designed to promote scientific and industrial cooperation between Germany and Southeast Asia.

Organizers, in addition to the GBF, are the German Federal Employment Office, the BioRegioN of Lower Saxony and Capacity Building International (InWent), Germany. *(May 2004)*



- *Electron-microscopic photograph of EHEC bacteria of the serotype O157:H7.*

Photo: Dr. Rohde



- *22 participants of the course "Introduction to Industrial Biotechnology" from ASEAN countries during the tour of the GBF together with the course coordinator Prof. Dr. R. Jonas.*

Photo: GBF

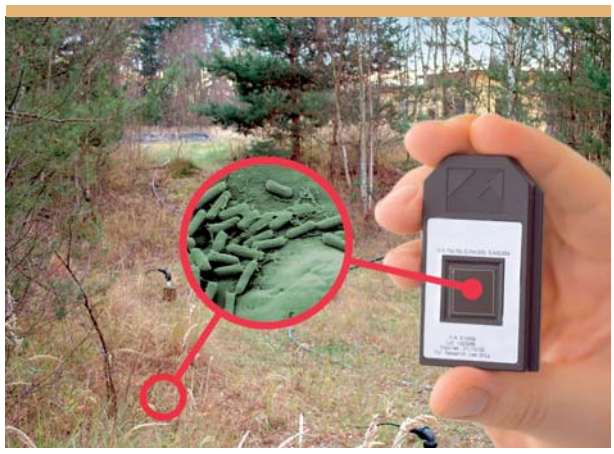
Büssing Award for B-Cell Researcher Like arrows in a quiver, specialized cells of our immune system maintain a regular cache of antibodies to defend against several typical bacteria and virus components. It is believed that, besides the well-known B2 lymphocytes, more "primitive" B cells represent a first line defense system. Signs that this is the case were discovered by Karsten Kretschmer, who wrote his PhD thesis at the GBF and now works in the USA. For his research work on B1 cells Dr. Kretschmer received the € 4,000 Heinrich-Büssing-Prize from the Foundation for the Advancement of Science at Braunschweig Technical University. *(June 2004)*

Dr. Karsten Kretschmer (3rd from the right side) together with Dr. Siegfried Weiß, his supervisor (2nd from the right side) after the receipt of the Heinrich-Büssing-Prize.

Photo: TU Braunschweig

A Fast-Track Diagnosis for Polluted Biotopes The analysis of bacterial genes and proteins could soon provide fast and reliable information about the self-cleansing activities of polluted biotopes. Developing the appropriate methods for these tests is the task of "BIOTOOL", a transnational research project involving nine laboratories from Germany, Spain, the Czech Republic, Denmark and Switzerland. Coordinator of the project is GBF researcher, Priv.-Doz. Dr. Dietmar Pieper. With the aid of molecular indicators the project partners are looking to answer one question early on: Will bacteria be able to degrade the toxins of a polluted biotope? The quick test procedure should help researchers develop a better understanding of the activities and adaptability of bacterial communities. The European Union is helping to finance the project with € 1.8 million. *(Autumn 2004)*

When Bacteria Stick Like Glue: The Biofilm Conference They can cause severe chronic infections, attach themselves to medical implantations, clog drains and pipes in factories – bacterial biofilms that install themselves on all kinds of surfaces are a phenomenon that science is only just beginning to understand. Experts from around the world exchanged their latest discoveries on this complex issue at the conference "Biofilms – Prevention of Microbial Adhesion". The meeting, held in Osnabrück from March 31 through April 2, 2004, was jointly sponsored by the GBF, the National Environment Foundation of Germany (DBU), and the University of Duisburg-Essen. The key, and very praxis-oriented, question at the conference was how to prevent biofilms from forming in the first place; for example, by using innovative surface materials that make bacterial adhesion more difficult. *(April 2004)*



Images of how the fast-track diagnosis may work after their development.

Photo and collage: Klimek

Four members (out of 7) of the managing committee of the newly founded "Federation of biological and biomedical societies" (vbbm): Prof. Dr. Harald Labischinski, Bayer AG, Wuppertal, member (left), Prof. Dr. Rudi Balling, GBF, chairman (2nd left), Prof. Dr. Angelika Noegel, Institute for Biochemistry, University of Köln, vice-chairwoman (2nd right), Prof. Dr. Walter Rosenthal, Research Institute for Molecular Pharmacology, Berlin-Buch, treasurer (right).

Photo: Gazlig

A Lobby for Life Sciences In the spring of 2004, thirteen societies involved in life science research joined forces to form a German life sciences umbrella organization. The Federation of biological and biomedical societies (vbbm) now represents mutual group interests in the political, economic and social spheres. The vbbm's first president is the scientific director of the GBF, Prof. Dr. Rudi Balling. As his deputies, the member societies selected Prof. Dr. Angelika Noegel and Prof. Dr. Ernst Rietschel at their founding conference in Kassel. vbbm founding members already represented some 17,000 bioscientists and that figure increased to about 25,000 by March 2005. Until now, bioscientists and medical biologists were scattered across more than 70 individual organizations, said Balling, making it "impossible to conduct effective lobbying for the life sciences in Germany." Prof. Balling pointed to the successful lobbying activities in other science sectors, such as physics and chemistry. "The example of the German Physics Society," says Balling, "demonstrates how much clout an association can have." *(March 2004)*

A Meeting of Microbiologists from Around the World Experts from many countries – from the USA and Japan to Ireland and Denmark – came together in Braunschweig in March to discuss bacteria research. Some 1,000 participants were greeted at the annual meeting of the Association for General and Applied Microbiology (VAAM) held at Braunschweig Technical University. The focus of the conference was on the application of computer models in biotechnology and infection research. VAAM is Germany's leading microbiology association with some 2,000 scientists involved in microbiology research. This year's meeting was organized by VAAM and five scientific institutes from the Braunschweig region, including the GBF and DSMZ. *(March 2004)*

Peptide Symposium at the GBF A highly versatile class of molecules was the subject of a conference held at the GBF in late February and early March of 2005. Experts from all over the world gathered in Braunschweig for the "7th German Peptide Symposium". Peptides are suited for a wide variety of medical applications because they are made of the same molecular building blocks as natural proteins. The German Peptide Symposium has been taking place every two years since 1993 and offers young researchers, in particular, a forum for presenting their work and for meeting the internationally recognized experts in this field. The 7th symposium, which included guests from the USA and several European countries, was organized by the GBF scientists Dr. Ronald Frank and Priv.-Doz. Dr. Jutta Eichler. *(February 2005)*

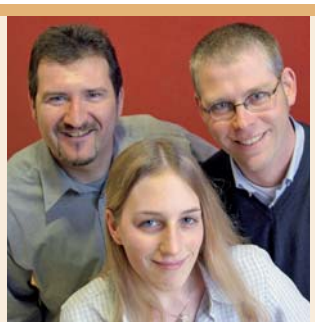
- *During a presentation at the Peptide Symposium at the GBF-FORUM, February 2005.*

Photo: GBF

2,000th Participant at School Laboratory The Biotechnology School Laboratory Braunschweig (BioS) greeted its 2,000th pupil at the beginning of the year. To celebrate the event, the BioS staff gave Roland Bunte, a student at Braunschweig's Ricarda-Huch-Gymnasium high school, and his classmates a chocolate marzipan cake shaped like an electrophorese gel used in DNA analysis. BioS has been established on the GBF campus in spring 2002 for the high school students in grades 10 through 13. The project is headed by the high school teachers Arntraud Meyer and Dr. Iris Eisenbeiser. Both have been released from teaching duties to concentrate on the experiment courses for the learning groups of 24 students. Pupils in the BioS courses can, for example, analyse foods using biotechnology methods or create a genetic fingerprint from a strand of hair. *(January 2004)*

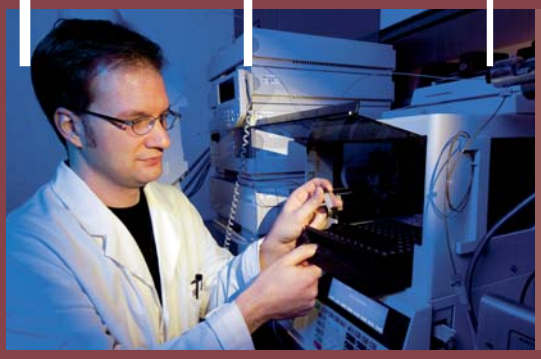
- *Roland Bunte (middle), Ricarda-Huch-Gymnasium, Braunschweig, is the 2000th course participant in the BioS course system. The teachers Dr. Iris Eisenbeiser and Arntraud Meyer (both on the right side) presented him and his classmates with a special chocolate marzipan cake, and Mr. Roland Willems (left) brought the best regards from the district government.*

Photo: Ammerpohl

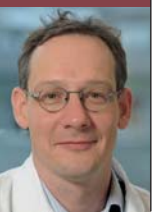


- *Hannes Schlender (right), biologist and journalist, is Head of the GBF Public Relations Department. Manfred Braun (left), biologist and journalist, is the GBF Press Spokesman. Anne Feldmann (middle) is the Secretary of the GBF Public Relations Department.*

Photo: GBF



Photos: left: Raimo Franke loads the autosampler of a LC/MS instrument for the analysis of synthetic peptides | centre: A C3H/HeN mouse | right: Phillip Hahn analysing a mouse mutant under the stereoscope. In the background Ivonne Wegener (le) and Stefanie Schiebe (ri) are discussing experimental results. Photos: Bierstedt



Gone but not Forgotten – the Impact of Apoptotic Cells on the Immune System

AUTHOR | **Dr. Andreas Lengeling | Research Group Infection Genetics | ale@gbf.de**

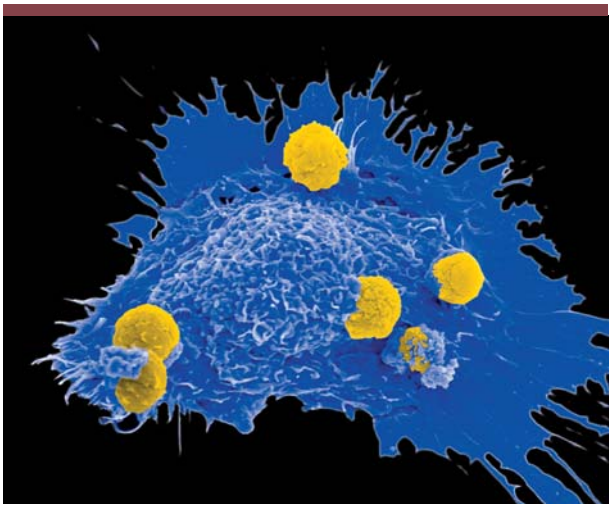
- Apoptosis, or programmed cell death, occurs in many tissues of the body throughout life. This process is important for the development and maintenance of cellular homeostasis, thereby ensuring that cells are eliminated, which are no longer needed, or, which may become harmful for the organism. The safe removal of apoptotic cells represents the final step, and perhaps the ultimate objective, of the apoptotic programme. Rapid engulfment of apoptotic cells ensures that dying cells are removed before they lose their membrane integrity and dispose their intracellular contents into the neighbouring tissues.
Macrophages represent one of the most important cell types involved in clearing apoptotic cells from the body. Most importantly, phagocytosis of apoptotic cells must not result in the induction of an inflammatory response and, therefore, this process is accompanied by powerful anti-inflammatory and immunosuppressive effects. Failure of apoptotic cell clearance can thus have serious consequences for the health status of an organism. Understanding the balance between pro- and anti-inflammatory responses of macrophages, in particular in the context of apoptotic cell removal, is important because it will provide new insights into the pathogenesis of infections, autoimmune disorders, persistent inflammatory diseases and cancer. Thus, unravelling the molecular mechanisms of apoptotic cell removal will have a significant impact on the development of future therapies of these diseases.

A historical perspective The seminal work of Ilya Ilyich Metchnikov in the late nineteenth century established a role for phagocytosis in the removal of senescent cells and in host defence. As a developmental biologist, Metchnikov worked in Messina (Sicily) with starfish larvae and described for the first time the complex cellular process of phagocytosis, by which particles are recognized, engulfed and eliminated. Through the observation of the uptake of rose thorns by mesodermally derived amoeboid cells in the starfish larvae, he realized that there is a conceptual link between the engulfment of effete cells as part of normal development and host defence. His work defined macrophages as gatekeepers in the first-line host defence and pinpointed the similarities in phagocytosis of microorganisms and of dying cells that need to be removed during development. For the discovery of phagocytosis, Ilya Metchnikov was awarded the Nobel Prize in 1908.

PAMPs and ACAMPs In recent years, studies in the laboratories of Jules Hoffmann at the University Louis-Pasteur in Strasbourg and of Charles Janeway and Ruslan Medzhitov at Yale University in New Haven have established the molecular concept of innate immune recognition of pathogens. It relies on a limited number of germ-line encoded receptors that recognize conserved products of microbial metabolism defined as "pathogen associated molecular patterns" (PAMPs). Activation of pattern recognition receptors by PAMPs results in the activation of signalling pathways that induce the phagocytotic removal of pathogens as well as protective inflammatory responses against the microbial invaders. This principle allows the immune system to distinguish between infectious non-self from non-infectious self.

Most strikingly, research on the molecular mechanisms of apoptotic cell clearance came up with a very similar concept. Apoptotic cells, like microbes, can present conserved molecular patterns to recognition receptors to mediate their removal from the body. These molecular patterns on apoptotic cells have been described as "eat me signals" for the phagocytosing cells and in analogy to the PAMPs of microorganisms they have been named "apoptotic-cell-associated molecular patterns" (ACAMPs). However, in sharp contrast to the PAMPs of micro-organisms, ACAMPs induce strong anti-inflammatory responses upon recognition by phagocytes. Clearly, such a process is required since it ensures an efficient and silent removal of apoptotic cells without the triggering of inflammatory reactions or the induction of immunity against self-antigens.

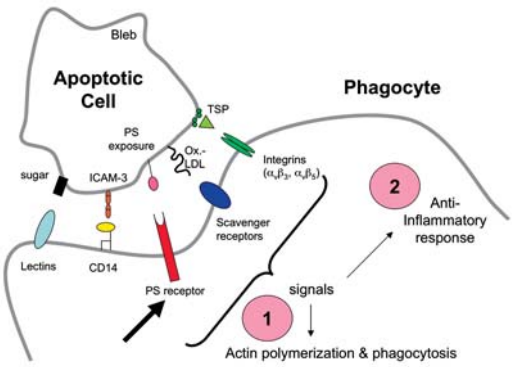
This mechanism is extremely important considering what phagocytes have to accomplish every given minute in our bodies to ensure a safe removal of apoptotic cells. It has been estimated that the total population of neutrophils in the human body is renewed two and a half times per day via apoptosis and replenished by the haematopoietic system. Therefore, it is essential for homeostasis of the organism that such high rates of apoptotic cell turnover are not associated with inflammatory responses. In addition, dead cell corpses have to be cleared quickly and efficiently before the cell integrity gets lost and potential cytotoxic and antigenic intracellular contents leak into the surrounding tissue.



- *Phagocytosis of apoptotic cells by macrophages. The scanning electron photomicrograph shows a macrophage (blue) that engulfs several apoptotic thymocytes (yellow). Different phases of the phagocytosis process are visible. In this particular experiment* phosphatidylserine receptor (Ptdsr)*-deficient mouse macrophages have been used, thus demonstrating that these macrophages are still capable to engulf apoptotic cells.*

Photo: Dr. Rohde

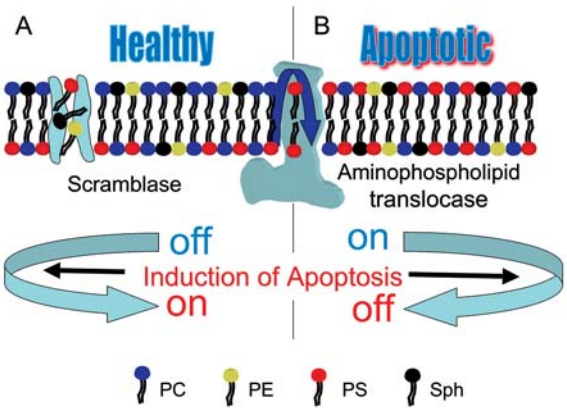
Who talks to whom? Several receptors on phagocytes have been implicated in the recognition of "eat me signals" on apoptotic cells in recent years. The molecular picture of the phagocytotic engulfment machinery has become remarkably complex. In analogy to the interactions that occur between antigen presenting cells and T cells at the immunological synapse, the molecular scanning process of apoptotic cell surfaces – which involves the coordinated interaction of several receptors on phagocytes – has been described as the engulfment synapse.



- *Schematic overview of the engulfment synapse. Different molecules are shown that interact at the apoptotic cell/phagocyte interface. One can differentiate between ACAMPs or "eat me signals" on apoptotic cells like thrombospondin binding sites (TSP), oxidized low-density lipoproteins (Ox.-LDL), phosphatidylserine (PS), ICAM-3 and sugar residues and their cognate receptors on the surface of the engulfing phagocyte. These are for example, integrins, scavenger receptors, CD14 and lectins. An arrow indicates the phosphatidylserine receptor, which has been particular implicated for the clearance of apoptotic cells. In this overview only selected molecules are shown that represent classes of molecules that have been identified to be important for apoptotic cell removal. The engulfment process is by far more complex, because many more molecules are involved that are not shown on this cartoon due to space limitations (for a recent review, see Devitt and Gregory, 2004).*

Among the identified receptors of the engulfment synapse are classical PAMP receptors like CD36, scavenger receptor A, CD14 and members of the collectin family of receptors, such as CD91 and calreticulin. Others include members of the integrin family, such as the vitronectin receptor $\alpha_V\beta_3$, the receptor tyrosine kinase Mer and the phosphatidylserine receptor. The latter has been described to specifically recognize the phospholipid phosphatidylserine on the surface of apoptotic cells. The exposure of phosphatidylserine (PS) in the outer leaflet of the plasma membrane of apoptotic cells is one of the hallmarks of the induction of apoptosis and is considered to be one of the most important signals required for apoptotic cell recognition and removal.

Besides the phosphatidylserine receptor, other molecules have been identified that can interact with exposed PS on the apoptotic cell surface. Among those are the serum proteins β2-glycoprotein 1, annexin 1, protein S, GAS-6 and the milk fat globule protein MFG-E8. It has been hypothesized that these molecules might serve as bridging molecules between apoptotic cells and the engulfing phagocytes. However, their cognate receptors on macrophages have not been well defined and the individual roles of these proteins in binding, phagocytosis and transduction of anti-inflammatory signals upon apoptotic cell recognition are currently not known. Working out why there are so many PS-binding molecules and how they all interact to accomplish a safe removal of apoptotic cells is a major preoccupation of researchers in the apoptosis field.

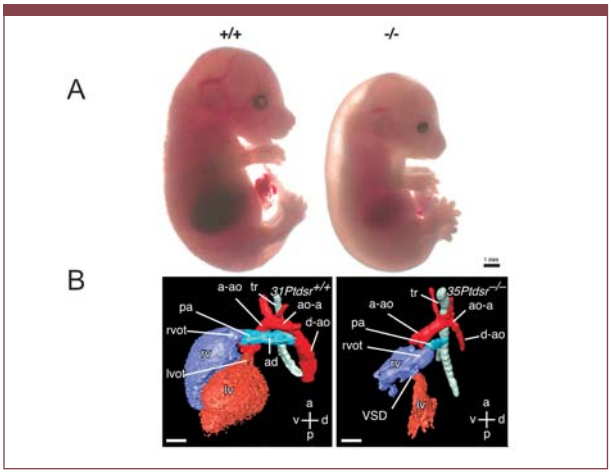


- *Model for the externalization of phosphatidylserine (PS) upon induction of apoptosis. Shown is the distribution of PS in the cytoplasma membrane of healthy (A) and apoptotic cells (B). In healthy cells PS is localized mainly in the inner leaflet of the cell membrane. The enzyme aminophospholipid translocase ensures that PS is pulled back from the outer leaflet to the inner leaflet of the cell membrane if it diffuses to the external lipid bi-layer. Upon induction of apoptosis the aminophospholipid translocase gets inactivated and an activated scramblase randomly distributes all phospholipids across the bi-layer. This results in PS exposure of the apoptotic cell. PC = phosphatidylcholine, PE = phosphatidylethanolamine, PS = phosphatidylserine, Sph = sphingomyelin.*

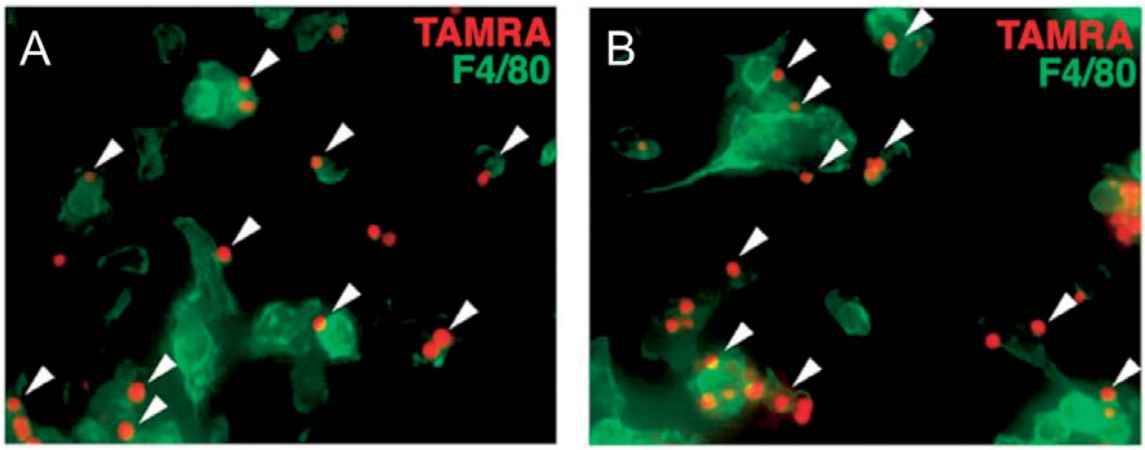
The phosphatidylserine receptor In 2000, a gene was cloned that was claimed – based on *in vitro* experiments – to encode a transmembrane receptor for PS on apoptotic cells. It was proposed that this phosphatidylserine receptor *(Ptdsr)* fulfils a crucial role as a signalling switch after the engagement of macrophages with apoptotic cells. This would trigger the release of anti-inflammatory mediators, including transforming growth factor-β1, platelet-activating factor (PAF), and prostaglandin E2. To investigate the biological function of the *Ptdsr in vivo*, we generated a mouse mutant line that carries an inactivated phosphatidylserine receptor gene.

The deletion of the *Ptdsr*-gene in the mouse turned out to be associated with severe developmental defects during embryogenesis that culminated in the death of homozygous knockout mice at birth. Mutant *Ptdsr*-mice are growth retarded and display a delay in the terminal differentiation of several important internal organs including the kidney, liver and lungs. In addition, eye development is compromised in *Ptdsr*-deficient mice, ranging from mild retinal differentiation defects to absence of eyes that is associated with induction of ectopic, rudimentary eye structures in nasal cavities.

Moreover, we could recently demonstrate together with the group of Shuomo Bhattacharya at the University of Oxford that *Ptdsr* is required for heart development. Using magnetic resonance imaging, we found that the septum which usually separates the right and left ventricle does not close properly in *Ptdsr* knockout mice. In addition, *Ptdsr*-deficient embryos displayed defects in the development of the heart outflow tract and the pulmonary artery. These cardiopulmonary malformations are most likely the reason why inactivation of *Ptdsr* function is associated with neonatal lethality.



- Ptdsr *is required for heart development. (A)* Ptdsr*-deficient embryos are frequently displaying massive subcutaneous edema (left: mutant embryo, right: wild-type littermate). This is due to the development of cardiac malformations in* Ptdsr *knockout mice. (B) Identification of cardiac malformations in* Ptdsr *knockout mice using magnetic resonance imaging (MRI). Shown is a 3D reconstruction of MRI data comparing wild-type (left) and* Ptdsr *mutant hearts (right). It can be recognized that the ventricle volumes are reduced in mutant embryos (rv = right ventricle, lv = left ventricle). In addition, a ventricular septal defect (VSD) is visible in the mutant heart.*



- *Phagocytosis of apoptotic cells is not impaired in* Ptdsr *knockout mice. Phagocytosis of apoptotic cells by fetal liver derived macrophages from wild-type (A) and* Ptdsr*-deficient mice (B). Apoptotic thymocytes (red) were stained with TAMRA and feed to F4/80 stained fetal liver derived macrophages (green). Quantification of phagocytosis by wild-type or* Ptdsr$^{-/-}$ *macrophages revealed no differences in the efficacy of apoptotic cell engulfment.*

Photo: Dr. Böse

The developmental defects in *Ptdsr* mutant mice point to an essential role of the gene during embryogenesis. It shows that *Ptdsr* has a novel, yet unrecognized, function as an important differentiation-promoting gene in early mouse development. Surprisingly, our group found no evidence that *Ptdsr* is important for apoptotic cell removal.

A comprehensive investigation of apoptotic cell clearance *in vivo* and in *vitro* demonstrated that engulfment of apoptotic cells was normal in *Ptdsr* knockout mice, challenging the hypothesis that the protein functions as a receptor for phosphatidylserine. Furthermore, work from our laboratory and from others could demonstrate that the *Ptdsr*-protein is located in the cell nucleus, raising further doubt that *Ptdsr* has functions as a classical transmembrane receptor.

However, *Ptdsr* seems to be very important for macrophage function in other respects. Stimulation of *Ptdsr*-deficient macrophages with PAMPs like lipopolysaccharide showed that pro-inflammatory cytokine secretion and signalling is compromised. These findings, together with the additional haematopoietic defects observed, suggest that *Ptdsr* still is important for immune function, even if the gene is not required for apoptotic cell removal as previously hypothesized.

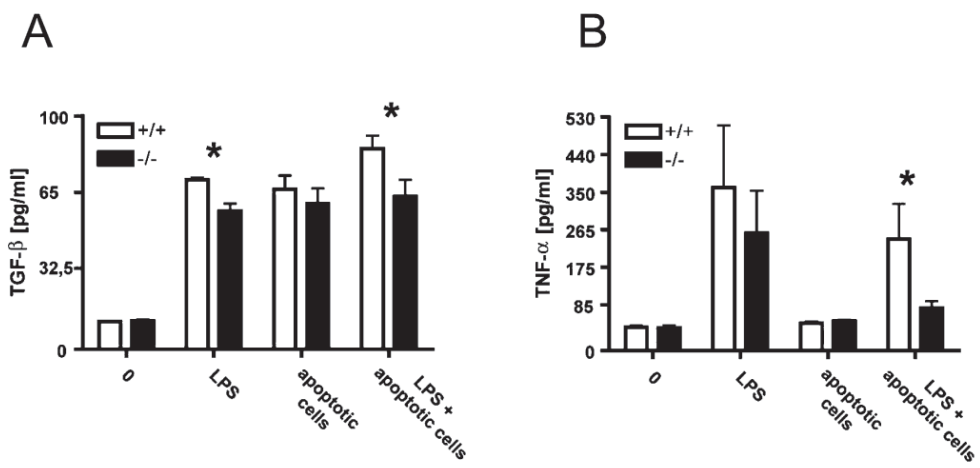
Autoimmune disease – An opening for new strategies

The tight linkage of apoptotic cell removal and anti-inflammatory signalling has a significant impact on the development of diseases. Ingested apoptotic cells are a source for antigens that can be presented via the MHC class-I-presentation pathway to T cells for immune tolerance or immune activation. The context in which the presentation of self-antigens occurs is important for the induction or suppression of adaptive immunity.

The process of phagocytosis of apoptotic cells by macrophages and immature dendritic cells actively suppresses inflammation and promotes peripheral tolerance through increased production of anti-inflammatory mediators and inhibition of pro-inflammatory cytokine release. This inhibits dendritic cell maturation and leads to a reduced capacity of the dendritic cells to directly stimulate and activate T cells. As a consequence, the tolerance against self-antigens is usually maintained.

However, the situation changes when the clearance of apoptotic cells becomes associated with danger signals for the immune system. Such signals could be tissue injury, inflammation or infections that can cause cellular apoptosis in affected tissue areas, as well as a release of inflammatory mediators. If, in such situations, the clearance of apoptotic cells is not occurring fast and efficiently, remaining apoptotic cells can undergo secondary necrosis and can become potent stimulators of antigen presenting cells. This can result in inappropriate presentation of self-antigens, activation of T cells and development of autoimmunity.

The importance of efficient mechanisms for apoptotic cell removal is supported by the observation that autoimmune responses can be provoked in mice when key molecules for apoptotic cell recognition and uptake are missing. Mice that lack the complement protein C1q, the Mer receptor, transglutaminase 2 or the PS-recognizing molecule MFG-E8 all display severe defects in apoptotic cell clearance and develop late onset autoimmunity diseases such as glomerulonephritis.



- *Pro- and anti-inflammatory cytokine production is impaired in* Ptdsr *knockout macrophages. Fetal liver derived macrophages from wild-type and* Ptdsr*-deficient embryos were incubated with medium (0), lipopolysaccharide (LPS, 10 ng/ml), apoptotic cells (ratio 1:10), or in combination with LPS and apoptotic cells. (A) Quantification of TGFß1 levels demonstrate that* Ptdsr$^{-/-}$ *macrophages are still able to secrete this anti-inflammatory cytokine upon recognition of apoptotic cells, although at a slightly lower level than wild-type macrophages. (B)* Ptdsr$^{-/-}$ *macrophages produce also significantly less of the pro-inflammatory cytokine TNF-α than wild-type macrophages upon single LPS stimulation or upon double stimulation with LPS and apoptotic cells.*

Defects in apoptotic cell removal can be associated with pathogenesis of autoimmunity. This has been demonstrated in patients with systemic *Lupus erythematosus* (SLE). Monocyte-derived macrophages isolated from SLE patients have been reported to have a reduced ability to phagocytose apoptotic cells and in some groups of patients free apoptotic cells were observed within lymph node germinal centres.

These observations clearly underline how important it is to understand the basic mechanisms of safe apoptotic cell removal. Key molecules involved in the process might be important targets for the design of future therapies. One could envision that manipulation of phagocyte clearance might shift the balance between pro- and anti-inflammatory responses. Influencing this process may thus allow the development of new therapeutic strategies for the treatment of inflammatory and autoimmune diseases on the one hand, and, improve vaccine strategies for infections and cancer on the other.

Strategies for immune evasion Many bacterial and viral pathogens have the capability to induce actively host cell apoptosis in the course of infection. At particular critical phases of infection, some pathogens make use of the induction of apoptosis as a strategy to overcome the host defence response. For example, many intracellular, bacterial pathogens like *Salmonella, Legionella, Shigella* and *Bordetella* can infect macrophages and subsequently induce their apoptosis as part of the regular infection programme.

To accomplish this, these pathogens express specialized virulence factors that modulate the survival of host cells. This allows pathogens to decide at which phase of infection it is most advantageous for them to eliminate macrophages – one of the most important first line defender cells of the host innate immune response. Macrophages have the ability to recruit appropriate types of immune cells to the site of infection. Therefore, it seems logical that many pathogens have evolved mechanisms to suppress or subvert the macrophage's ability to coordinate effective antimicrobial immune responses.

On the other hand, as already described, the clearance of apoptotic cells by phagocytes triggers the induction of strong anti-inflammatory responses. A pathogen that is able to activate this anti-inflammatory response can suppress an immune response and thereby avoid a further recruitment and activation of immune effector cells to the infection site. Therefore, induction of host cell apoptosis during infection might be associated with the generation of local, immunosuppressive environments that promotes pathogen survival and dissemination to other host tissues.

Apoptotic mimicry Very interestingly, recent reports about parasite infection cycles came up with additional and novel concepts on how pathogens might use the ACAMP phosphatidylserine (PS) to down-regulate the microbicidal activity of macrophages. The amastigote state of *Leishmania* spp. can invade macrophages by exposure of phosphatidylserine on the cell surface. PS exposure induces not only the internalization of the parasite into the host cell, it is also associated with secretion of the immunosuppressive cytokine TGFβ1 and the subsequent deactivation of the macrophage.

By using this mechanism, which has been described as "apoptotic mimicry", the parasite is able to evade the killing activity of macrophages and to thereby establish a persistent infection in the host. Similar mechanisms have been described for *Toxoplasma gondii* and *Trypanosoma cruzi*. Infections by these parasites are also associated with cell surface exposure of PS by the pathogen *(Toxoplasma)* or induction of intense lymphocyte apoptosis and subsequent macrophage deactivation by apoptotic cell clearance *(Trypanosoma)*. Both mechanisms allow the intracellular growth of these parasites by disabling the microbicidal effects of macrophages. It remains to be investigated if "apoptotic mimicry" might be also used as an immune evasion mechanism by other pathogens like bacteria and viruses.

The apoptosis puzzle In recent years, considerable progress has been made in the understanding of the molecular details of apoptotic cell clearance and the subsequent consequences that this process imposes on the immune system. It has been known since the early days of Ilya Metchnikov that macrophages play a pivotal role in the clearance of dead host cells as well as in the phagocytosis and elimination of infectious micro-organisms. However, the outcome of both macrophage encounters for the immune system can be very different, depending on the cellular context.

Clearance of apoptotic cells is associated with anti-inflammatory responses that are important to ensure the maintenance of cellular homeostasis. In contrast, macrophage mediated phagocytosis of pathogens triggers pro-inflammatory reactions crucial for host defence. If the balance between both macrophage effector mechanisms gets disturbed, diseases will develop.

Defects in apoptotic cell clearance may induce autoimmunity, and induction of apoptosis or apoptotic mimicry by pathogens during the infection process will modulate the immune response to the advantage of the pathogen.

The exposure of phosphatidylserine on the surface of apoptotic cells has been established as a hallmark of apoptosis induction and anti-inflammatory cell removal. However, a corresponding receptor for phosphatidylserine on the surface of phagocytes that is responsible for apoptotic cell engulfment and anti-inflammatory signalling has not been found so far. This leaves the possibility that there still might be an elusive orphan PS receptor on macrophages, which has yet to be identified and characterized, or it could mean the recognition of PS and the non-inflammatory removal of apoptotic cells are mainly mediated by PS bridging molecules and their cognate receptors.

Solving the central question of apoptosis and apoptotic cell removal will remain a challenge for the coming years. But one can expect the resolution of this puzzle to have a major impact on the design of future therapeutic targets that will efficiently treat diseases associated with disturbed clearance of apoptotic cells.

- *The ING-team from left to right: Bastian Pasche, Phillip Hahn, Andreas Lengeling, Laura Helming, Jens Böse, Ivonne Wegener, Stefanie Schiebe.*

Photo: Bierstedt

Andreas Lengeling born 1965, studies in Biology (University of Bielefeld). Diplomagraduation, 1993, PhD in Biology at the Department of Developmental Biology and Molecular Pathology (1997, University of Bielefeld). Post-Doctoral Research Fellow (1997-2000, University of Pennsylvania, Medical School, Department of Psychiatry and Genetics, Philadelphia, USA). Senior Research Scientist (2000-2001, Institute of Mammalian Genetics at the National Research Center for Environment and Health, GSF, Munich). Since 2001, Head of Junior Research Group at GBF.

LITERATURE Listed are our own publications relating to *apoptosis*, as well as selected papers from other groups.

- *Böse, J., Gruber, A.D., Helming, L., Schiebe, S., Wegener, I., Hafner, M., Beales, M., Köntgen, F., Lengeling, A. (2004). Essential functions of the phosphatidylserine receptor during embryogenesis, distinct from apoptotic cell removal. J. Biol. 3 (4): 15.*

- *Schneider, J.E., Böse, J., Bamforth, S.D., Gruber, A.D., Broadbent, C., Clarke, K., Neubauer, S., Lengeling, A., Bhattacharya, S. (2004). Identification of cardiac malformations in mice lacking Ptdsr using a novel high-throughput magnetic resonance imaging technique. BMC Developmental Biology 4:16*

- *De Freitas Balanco, J.M., Costa Moreira, M.E., Bonomo, A., Torres Bozza, P., Amarante-Mendes, G., Pirmez, C., Barcinski, M.A. (2001). Apoptotic mimicry by an obligate intracellular parasite downregulates macrophage microbicidal activity. Curr. Biol. 11: 1870-1873.*

- *Freire-de-Lima, C.G., Nascimento, D.O., Soares, M.B.P., Bozza, P.T., Gastro-Faria-Neto, H.C., de Mello, F.G., DosReis, G.A., Lopes, M.F. (2000). Uptake of apoptotic cells drives growth of a pathogenic trypanosome in macrophages. Nature 403: 199-203.*

- *Fadok, V.A., Savill, J.S., Haslett, C., Bratton, D.L., Dohtery, D.E., Campbell, P.A., Henson, P.M. (2000). A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature 405: 85-90.*

- *Franc, N.C., White, K., Ezekowitz, A.B. (1999). Phagocytosis and development: back to the future. Curr. Opin. Immunol. 11: 47-52.*

- *Gregory, C.D., Devitt, A. (2004). The macrophage and the apoptotic cell: an innate immune reaction viewed simplistically? Immunology 113: 1-14.*

- *Hoffmann, J.A. (2003). The immune response of Drosophila. Nature 426: 33-38.*

- *Janeway, C.A., Medzhitov, R. (2002). Innate immune recognition. Ann. Rev. Immunol. 20: 197-216.*

- *Kunisaki, Y., Masuko, S., Noda, M., Inayoshi, A., Sanui, T., Harada, M., Sasazuki, T., Fukui, Y. (2003). Defective fetal liver erythropoiesis and T lymphopoiesis in mice lacking the phosphatidylserine receptor. Blood 103: 3362-3364.*

- *Li, M.O., Sarkisian, M.R., Mehal, W.Z., Rakic, P., Flavell, R.A. (2003). Phosphatidylserine receptor is required for clearance of apoptotic cells. Science 302: 1560-1563.*

- *Seabra, S.H., de Souza, W., DaMatta, R.A. (2004). Toxoplasma gondii exposes phosphatidylserine inducing a TGFβ1 autocrine effect orchestrating macrophage evasion. BBRC 324: 744-752.*

- *Williamson, P., Schlegel, R.A. (2004). Hide and seek: the secret identity of the phosphatidylserine receptor. J. Biol. 3: 14.*

- *Weitzman, J.B. (2004). The curious world of apoptotic cell clearance. J. Biol. 3: 13.*

Synthetic Peptides as Inhibitors of Protein-Ligand Interactions

AUTHOR | **Priv.-Doz. Dr. Jutta Eichler | Research Group Conformational Protein-Ligand Interactions | jei@gbf.de**

- Almost all biological processes are initiated by specific binding events between bio-macromolecules, primarily proteins, and their ligands. Inhibition of these interactions between receptors, enzymes or antibodies on the one hand and receptor agonists/antagonists, enzyme substrates/inhibitors or antigens on the other hand, is a popular strategy for interfering with the resulting biological effects, *e.g.* in the prevention or interruption of pathological processes.

  The design and generation of molecules, which – due to their specific molecular architecture – are capable of mimicking protein binding sites, represents a promising strategy for the development of inhibitors of protein-ligand interactions. Such protein mimetics are candidates for a range of preventive and/or therapeutic strategies, including the inhibition of pathogen-host cell interactions – the first step in the infection process.

  The goal of our work is to establish a repertoire of methods for the design and generation of protein mimetics based on the mimicry of conformationally defined protein binding sites. We want to use these methods to develop inhibitors of biomedically relevant protein-ligand interactions. Our projects include the inhibition of bacterial entry into host cells through peptides mimicking the binding site of a host cell receptor for a bacterial virulence factor, as well as the mimicry of a viral envelope protein binding site for its host cell receptor.

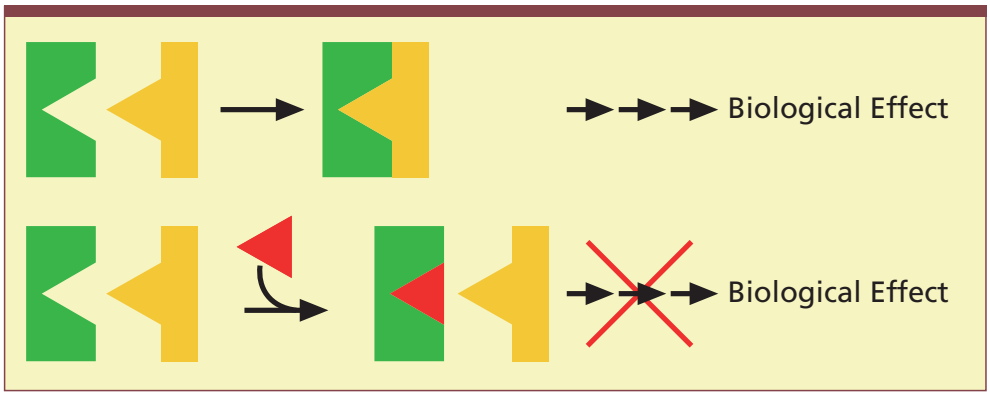
Synthetic peptides as proteinmimetics Proteins are spatially folded polypeptide chains, whose characteristics are determined by the amino acid sequence (primary structure), as well as the three-dimensional arrangement of the polypeptide chain (secondary and tertiary structure). The contact sites of proteins for their ligands, which are often proteins as well, are located in relatively small, defined regions of the molecules, called binding sites or epitopes. They can be reproduced in the shape of short amino acid sequences – peptides - and readily generated through chemical synthesis.

Using state-of the-art peptide synthesis instrumentation, up to several hundred peptides can be synthesized in parallel and in an automated, unattended fashion. Since synthetic peptides can be generated either as exact copies of protein fragments or as manifold sequence mutations, they are excellent and versatile tools for the mimicry of protein binding sites, and consequently, promising candidates for the inhibition of protein-ligand interactions.

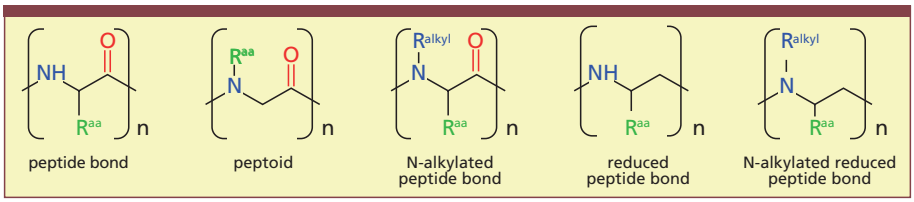
Moreover, protein binding sites can be identified through a process called peptide scanning, in which the protein sequence is chemically synthesized as a set of separate short peptides representing overlapping protein fragments which, as a whole, span the entire protein sequence. Testing each of the peptides individually for binding to the respective protein ligand yields information on the location of the binding site within the protein sequence.

Chemical modifications Like proteins, synthetic peptides composed of the 20 natural L-amino acids, are not stable in the gastrointestinal tract (GIT), where they are rapidly degraded by proteolytic enzymes. This fact, along with the poor resorption of peptides from the GIT into the blood circulation, largely compromises their use as potential drugs. Unlike in the ribosomal protein synthesis machinery, however, the amino acid repertoire to be used as building blocks for chemically synthesized peptides can be readily extended. The D-isomers of the proteinogenic L-amino acids can be included, as can a wide range of non-proteinogenic amino acids featuring chemical moieties that are not presented by the natural amino acids. This increases the chemical diversity presented by synthetic peptides, as well as their stability in biological fluids, since such non-proteinogenic amino acids are not recognized by proteolytic enzymes.

Furthermore, several strategies for the chemical modification of the amide backbone of synthetic peptides have been developed. They change the physico-chemical properties of the peptides and increase their metabolic stability, while at the same time maintaining the chemical diversity presented by the amino acid side chains. Such chemical modifications include N-alkylation and/or reduction of the backbone amide bonds, as well as the use of N-alkylglycine derivatives as building blocks for peptoids, in which the amino acid side chains are not attached to the C_α atom but to the amide nitrogen of the amino acids.



- *Prevention of a biological effect through inhibition of the initial interaction between a protein (yellow) and a ligand (green), by a protein binding site mimetic (red), which competes with the protein for binding to the ligand.*



- *Chemical modification of the amide backbone in synthetic peptides increases their metabolic stability.*

Rational versus random design Synthetic protein binding site mimetics can be created in two different ways: through the design of molecules, based on the three-dimensional structure of the protein, which are likely to adopt conformations that enable binding to the respective ligand in a manner similar to the protein-ligand interaction, or by having the protein ligand select the best-fitting structures from a large population, *e.g.*, a combinatorial library of chemically diverse molecules with common structural features that increase their propensity to mimic protein binding sites.

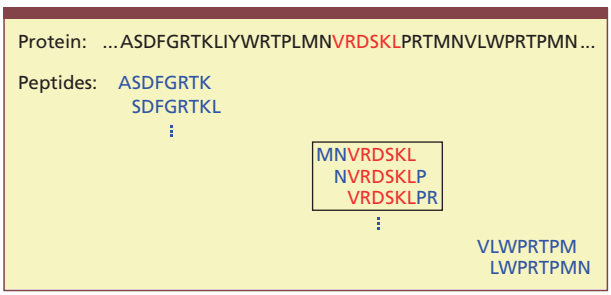
While the first approach is based on rational design and therefore more likely to yield true mimetics of the actual protein binding site, it requires detailed information on the three-dimensional protein structure, typically from NMR or X-ray crystal structure analysis. Such structural information, however, is not always readily available, in particular for newly discovered proteins.

The second approach does not rely on protein structure information and can be considered a random approach for the *de novo* development of molecules. While mimicking the binding and/or functional characteristics of the protein, these molecules do not necessarily resemble the structure of the protein binding site. Furthermore, such combinatorial libraries are valuable tools for the investigation and characterization of unknown binding specificities of protein-ligand interactions involving newly discovered proteins with potential biomedical relevance.

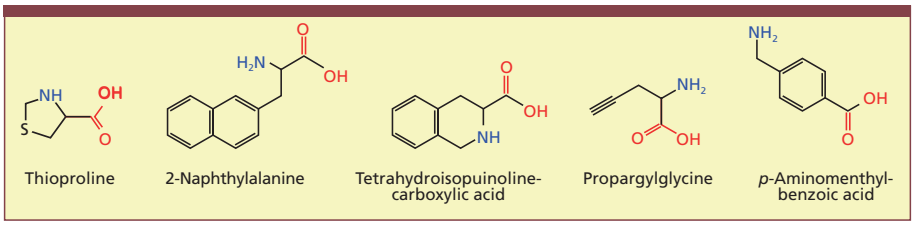
Protein binding site types Structurally, protein binding sites can be divided into three different types. Continuous, flexible binding sites are simply a stretch of consecutive amino acid residues in the protein sequence. They are typically on the surface of the molecule. Such binding sites can be readily mimicked by linear peptides covering this sequence stretch, and identified through peptide scanning.

The second type is also sequentially continuous, but constrained by its conformation, either through disulfide bridges between cysteine residues, or by a defined secondary structure like α-helices, or β-sheets. Such conformationally constrained binding sites often form loop-like structures, which can be mimicked by cyclic peptides.

The third and most complex type of protein binding sites is sequentially discontinuous, and consists of two or more protein fragments, which are far apart from each other in the protein sequence, but brought into spatial proximity by protein folding. Although the binding sites of many biomedically relevant proteins are sequentially discontinuous, systematic approaches, comparable to those used for the mimicry of continuous binding sites, are only beginning to be introduced into the tool box of peptide chemistry.



- *Peptide scanning for the identification of protein binding sites. Boxed peptide sequences bind to the respective protein ligand.*



● *Examples of non-proteinogenic animo acids as building blocks for chemical peptide synthesis.*

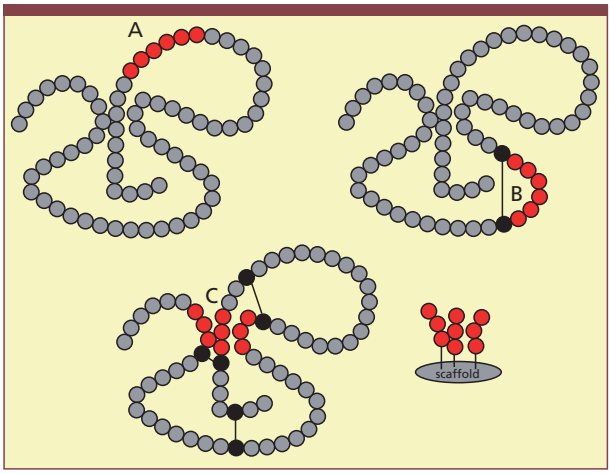
Mimicry of discontinuous binding sites It could be argued that a synthetic mimetic of a discontinuous protein binding site should present the protein-derived peptide fragments also in a sequentially discontinuous and conformationally constrained fashion – in order to increase the chances of the fragments to be presented in a spatial arrangement that resembles their structure within the protein. This consideration was the design rationale for scaffolded peptides, in which fragments of the sequence are presented through a molecular scaffold in a discontinuous and nonlinear fashion. The molecular architecture of the scaffold is a key element for such peptides, since it has to provide an appropriate spatial presentation of the attached protein-derived fragments. An important element in the design of such scaffolds is the possibility to selectively attach different peptide fragments to defined sites of the scaffolds. Amino groups are excellent attachment points for the site-selective introduction of peptide fragments, because they can be selectively protected and unprotected by a range of so-called orthogonal protecting groups.

We have developed versatile and robust synthesis strategies for the generation of a wide range of structurally diverse scaffolded peptides. This permits the creation of scaffolds covering a wide range of conformational flexibility using the same synthesis method. We expect this to be applicable to various protein targets with structurally unrelated binding sites.

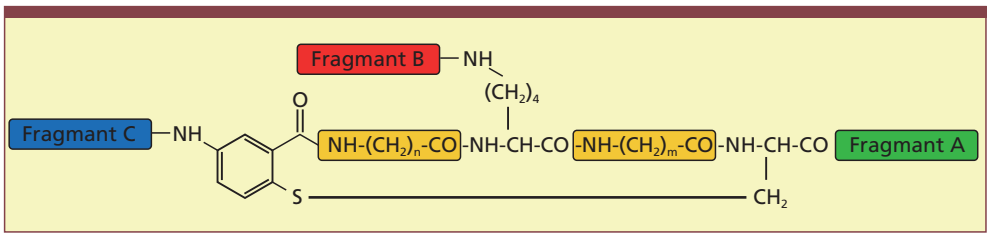
The HIV-1-gp120 binding site for CD4 gp120 is part of the exterior envelope protein of the human immunodeficiency virus (HIV-1). The interaction of gp120 with the receptor protein CD4 on T cells is the initial step in a cascade of molecular events that eventually enables virus entry into the T cell.

Most of the currently available anti-HIV-1 antibodies recognize highly variable regions of gp120, which render them poor vaccine candidates because of their narrow virus neutralizing capacity. On the other hand, the binding site of gp120 for CD4, which is sequentially discontinuous and located in three separate regions of the protein, is highly conserved among many virus strains. Furthermore, this binding site has been postulated – based on crystal structure analysis in conjunction with molecular modeling – to overlap with the epitope for an antibody that was found to neutralize a wide range of HIV-1 strains. Consequently, synthetic mimetics of the gp120 binding site for CD4 are promising candidates as immunogens for the generation of broadly neutralizing anti-HIV-1 antibodies.

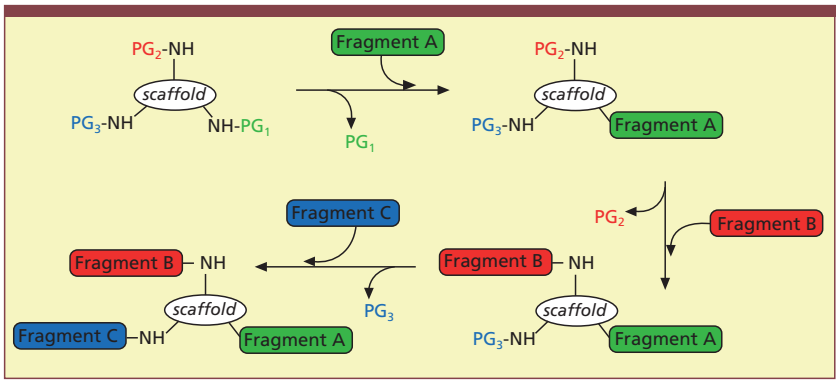
Our design blueprint for such molecules was the X-ray crystal structure of recombinant gp120 in complex with the gp120-binding, extracellular two-domain fragment of CD4. We have generated a range of gp120 mimetics in the form of scaffolded peptides presenting three peptide fragments that contain the primary contact residues of gp120 for its interaction with CD4. The affinity of some of these peptides to CD4 was found to be more than 200 times higher than the affinities of the three individual fragments, indicating a synergistic effect resulting from combining all three fragments into one molecule. Currently, antibodies against selected gp120 mimetics are being generated, which will subsequently be evaluated regarding their ability to recognize gp120, as well as to neutralize HIV-1.



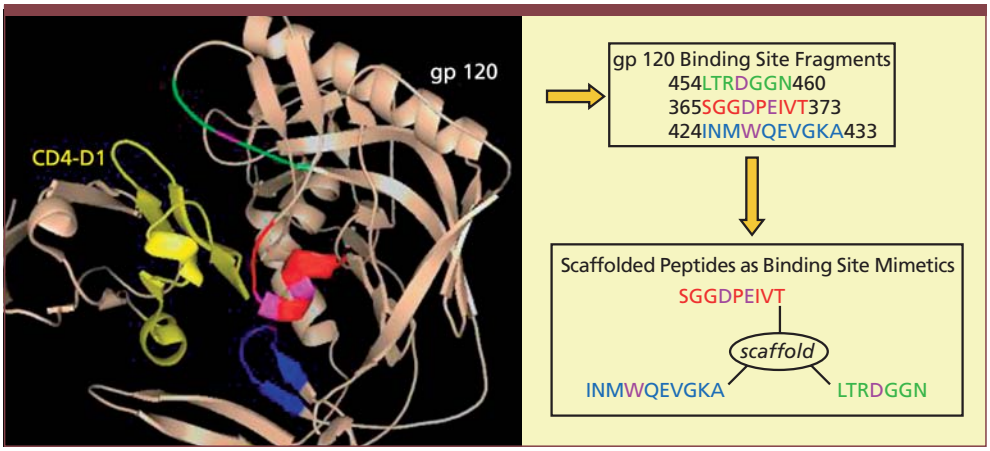
● *Structurally different types of protein binding sites (amino acids in red): A: Continuous, flexible. B: Continuous, constrained. C: Discontinuous. D: Scaffolded peptide mimicking a discontinuous protein binding site.*



- *Scaffold molecules for the presentation of protein-derived peptide fragments (A,B and C). Incorporation of spacer amino acids (orange) with varying backbone length (n,m = 1-5) yields scaffolds covering a wide range of conformational flexibility.*



- *Site-selective attachment of protein-derived peptide fragments to a scaffold. Attachment points (amino groups) on the scaffold can be individually addressed by using selectively removable "orthogonal" protecting groups (PG).*



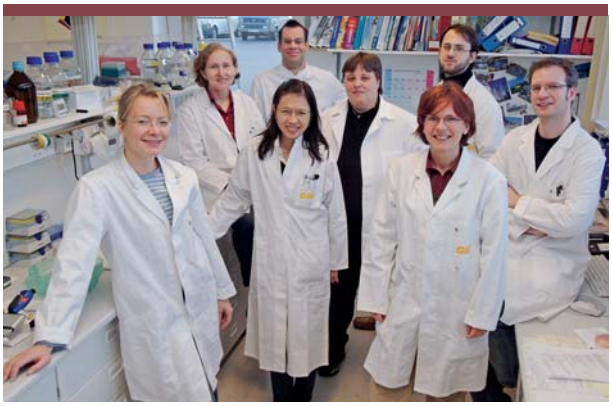
- *Design of gp120-mimetics based on the crystal structure of a gp120 – CD4 complex (left). Peptides covering the gp120 binding site fragments (green, red, blue), which contain the primary contact residues (pink) for the interaction with CD4 (yellow) are presented through a molecular scaffold (right).*

- *Numan Akyol controlling the synthesis robot for the automated, parallel synthesis of up to 192 peptides.*

Photo: Bierstedt

Perspective The high technical standard of current peptide chemistry, which has evolved over the past three decades, has profoundly facilitated the investigation of proteins and their interactions with other molecules at the level of individual amino acids. Using currently available peptide synthesis methods, sequentially continuous protein binding sites can be readily mapped, characterized, optimized, and used as lead compounds for inhibitors of protein-ligand interactions. For the mimicry of discontinuous protein binding sites, on the other hand, synthetic and recombinant strategies are just beginning to emerge. The ongoing development and elaboration of these strategies, in conjunction with their application to biomedically relevant proteins, is expected to improve our understanding of the molecular mechanisms of protein-ligand interactions. It will also enable new approaches to the design and generation of inhibitors of protein-ligand interactions.

Jutta Eichler born 1961, studies in Pharmacy (University of Greifswald). PhD in Bioorganic Chemistry (1991, Humboldt-University Berlin). Post-doctoral Fellow, Research Scientist and Assistant Member (1991-1998, Torrey Pines Institute for Molecular Studies, San Diego, USA). Director of Combinatorial Chemistry (1998-1999, Graffinity Pharmaceutical Design GmbH, Heidelberg). Since 2000 at GBF, and since 2001 Head of Junior Research Group (supported by the BMBF-BioFuture Programme). Habilitation in Bioorganic Chemistry (2004, Technical University of Braunschweig).

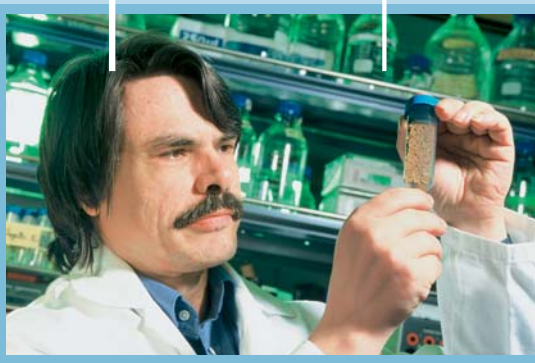


- *The KPLI-team. Back row, from left to right: Tatjana Hirsch, Ulf Strijowski, Cornelia Hunke, Numan Akyol, Raimo Franke. Front row, from left to right: Heike Overwin, Enge Sudarman, Jutta Eichler.*

Photo: Bierstedt

LITERATURE below are selected publications on the concept and chemistry of mimicking protein binding sites, as well as on the structure of HIV-1 gp120 as blueprint for vaccine design.

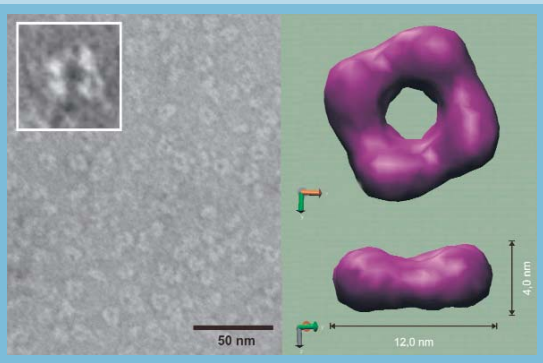
- *Eichler, J.(2004). Rational and Random Strategies for the Mimicry of Discontinuous Protein Binding Sites. Protein Peptide Lett. 11(4): 281-290.*
- *Franke, R., Doll, C., Wray V., Eichler, J. (2004). Loops on Loops: Generation of Complex Scaffolded Peptides Presenting Multiple Cyclic Fragments. Org.Biomol.Chem. 2: 2847-2851.*
- *Eichler, J. (2005). Synthetic Peptide Arrays and Peptide Combinatorial Libraries for the Exploration of Protein-Protein Interactions and the Design of Protein Inhibitors. Comb.Chem.High Throughput Screen. 8(2): 135-143.*
- *Kwong, P.D., Wyatt, R., Robinson, J., Sweet, R.W., Sodorski, J., Hendrickson, W.A.(1998). Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 393: 648-659.*
- *Burton, D.R., Desrosiers, R.C., Doms, R.W., Koff, W.C., Kwong, P.D., Moore, J.P. Nabel, G.J., Sodorski, J., Wilson, I.A., Wyatt, R.T. (2004). HIV vaccine design and the neutralizing antibody problem. Nature Immunol. 5: 233-236.*



Photos: left: A naturally grown biofilm from a lake was fixed in situ *and is now available for comparing analysis in the laboratory. After the transport of the biofilm into the laboratory, Dr. Wolf-Rainer Abraham is checking the biofilm for damages | centre: 3D-Reconstruction of negatively-stained FeS-Oxigenase molecules: (left part of the fig.) Uranyl-acetate negatively-stained enzyme particles, used for 3D-reconstruction analysis. The inset is a detailed view of an individual particle, shown in top-view projection. (right part of the fig.) 3D-model, seen from top (top) and side (bottom) | right: Dr. Manfred Rohde working at the electron microscope. Photos: GBF (le), Dr. Lünsdorf (ce), Bierstedt (ri)*

SCIENTIFIC REPORTS

FACTS AND FIGURES



Programme “Infection and Immunity”

PROGRAMME SPEAKER | Prof. Dr. Jürgen Wehland | Department of Cell Biology | jwe@gbf.de

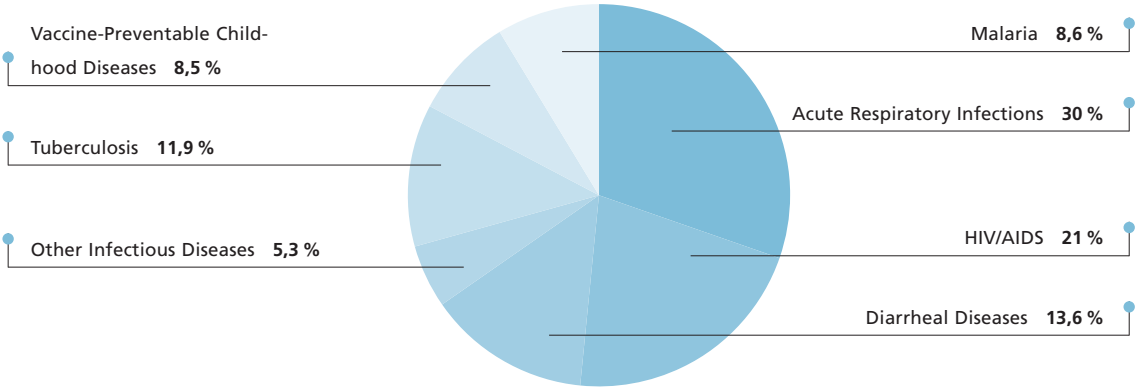
- Infections cause one third of all disease-related death cases worldwide. During the recent decades, improved hygiene and the availability of antibiotics and vaccines have led to a continuous reduction in such diseases. Today, however, we are confronted with the fact that many bacterial infectious diseases are not only re-emerging, but also becoming resistant to medication. Worldwide travel and the global exchange of goods have led to epidemic outbreaks of previously unknown diseases, like such as AIDS, and infections, that were thought to be eradicated, such as tuberculosis.

In Western industrial societies, infectious diseases have acquired a new, but onerous, impact due to modern high-tech medication: patients who have had a transplant, or are under intensive care, are highly susceptible to opportunistic infections as a result of immune-suppressive medication. Furthermore, even though clinics in general possess a good antibiotic management, new resistances continue to emerge and the chances of curing, not only acute diseases, but also chronic or persistent infections, are becoming greatly reduced. This situation emphasizes the urgent need for developing new strategies for diagnosis, prevention and therapy of infectious diseases. Therefore, it is imperative that basic research efforts concentrate on mechanisms underlying pathogen/host interactions during infection. In order to develop new, innovative vaccines we must examine how the host’s immune system reacts to the invasion of a pathogen and how an immune response is initiated. Last but not least, one has to know what influence the environment – *e. g.* food and pathogenic reservoirs –, has on the course of an infection and the defence against it. Opportunistic infections are a serious problem not only for immune-compromised patients, but also for the aging population.

Despite these alarming developments, the chances for establishing new diagnostic and effective therapeutic strategies are better than ever. Systematic genome analysis is providing information on potential drug targets, thus aiding the development of new anti-infectives. A better understanding of the functions of individual genes, combined with knowledge about the interactions of microbial genes with host cellular genes, is providing an excellent basis for the directed systematic design of chemotherapeutic strategies against microbial pathogens. Functional genome analysis also provides insight into the molecular basis of immune responses and the genetic susceptibility to infectious diseases.

Our increased knowledge of the molecular and cellular components of the immune system has opened up new possibilities of clinical intervention that will allow immunotherapies extending beyond prophylaxis and will include therapeutic intervention. Today, our understanding of immunity extends far beyond its protective role against infectious diseases. We know that the immune system not only

Deaths caused by infectious diseases, 2002



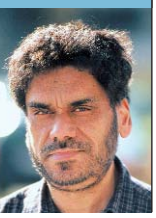
Source: WHO-Report 2004

protects the host from against microorganisms, but that it is also specialised in surveillance, detecting altered cellular antigens and thus monitoring and eliminating detrimental changes in the tissues and organs of the body. Nevertheless, the precise mechanisms by which the immune system is undermined by certain microorganisms, resulting in latent or chronic infections, are still only barely understood.

The GBF programme "Infection and Immunity" covers basic research in the area of infectious diseases and immunity. It is at the interface of these two fields where we expect the greatest potential for the development of new drugs and strategies to prevent and treat disease. The main objective of the programme is to understand the principle mechanisms that underlie the development of infectious diseases. This involves basic research on model microorganisms and their pathogenicity as well as a detailed analysis of the mechanisms of immunity. The aim is to understand the individual molecular and cellular steps that occur during the process of infection, the mechanisms of how selected micro-organisms cause disease and the basic principles of defence mechanisms that are used by the host to resist and control infections. This knowledge will be used to develop new strategies and tools to prevent and treat infectious diseases.

The topics of the research programme

- Microorganisms
- Pathogenesis
- Immunobiology
- Prevention and Therapy



Topic 01 – Microorganisms

TOPIC SPEAKER | **Prof. Dr. G. Singh Chhatwal | Department of Microbial Pathogenicity and Vaccine Research | gsc@gbf.de**

- Microbial infections remain a serious hazard for human health. A prerequisite for developing novel combat strategies is a complete understanding of the process by which pathogenic microorganisms cause disease. Therefore, in this topic, the objective is to study the biology and the molecular biology of microorganisms with the goal to identify and characterize virulence factors in bacteria, to elucidate the structure-function relationships of these factors, to analyse cellular networks and microbial communications and to understand mechanisms of antibiotic resistance.

This topic will employ different genetic and molecular approaches on a number of disease causing microorganisms. The current choice for the bacterial pathogens streptococci, pneumococci, *Listeria monocytogenes*, *Pseudomonas aeruginosa* is also made on the basis that they stand for specific biologic features as model organisms and that sufficient information and expertise has been accumulated. Group A streptococci cause a wide spectrum of diseases in humans, including invasive diseases and sequelae, such as rheumatic fever. Pneumococci are responsible for life-threatening diseases, such as pneumonia and meningitis. *Listeria monocytogenes* is the best studied pathogenic bacterium and provides an excellent system to identify the bacterial components involved in the disease system. The importance of *Pseudomonas aeruginosa*, as well as oral streptococci, as health relevant pathogens has been recognized in the last few years.

Complete genome sequences are now available for many microorganisms allowing holistic approaches to predict the function of every gene. This topic also deals with the sequence analysis and subsequent comparisons to determine the functions of genes in the bacterial species which have been chosen as model systems. Together with sequence comparisons of non-pathogenic, weakly and highly pathogenic variants or related species, it will be possible to identify potential virulence factors. These analyses also will elucidate the normal function of a bacterial virulence factor outside the context of the infected host, thus providing a better understanding of why certain strains cause diseases, whereas other highly related strains do not. Also, the knowledge of the function of genetic pathways, for example those implicated in essential metabolic pathways, can be used to identify molecular targets for the design of novel antimicrobial drugs.

To achieve these objectives, this topic constitutes six different projects. These projects, although different in expertise, show a high degree of synergy and bring together various efforts to reach the goals of this topic. The projects include:

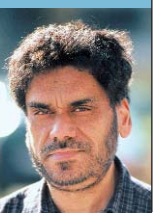
Identification and characterization of virulence factors of group A, C and G streptococci, *Listeria monocytogenes* and *Pseudomonas aeruginosa*. High resolution to 2D-gel electrophoresis, automated nano HPLC-Q-TOF mass spectrometry and DNA virulence arrays are being employed for this purpose. In addition, molecular epidemiology is being studied with a large number of clinical isolates using DNA arrays.

The 3D structure of identified potential virulence factors, alone or in complex with specific ligands, is being elucidated to identify the critical interfaces for rational design of therapeutics.

Understanding the genetic basis that leads to antibiotic resistance will be a major challenge in the coming years. Microorganisms have developed a number of escape mechanisms that may be exerted either at the individual level or at the level of a population. Again, whole genome analysis, as well as expression profiling, should allow us to elucidate these mechanisms and to design more effective drugs. This topic would lead to a better understanding of how pathogenic bacteria communicate with each other, as well as with host cells, to cause diseases. The identified factors involved in this process represent promising targets for vaccines and therapeutics.

Professor Chhatwal preparing for microscopic analysis.

Photo: Ammerpohl



01.1 Virulence Factors of Streptococci and Pneumococci

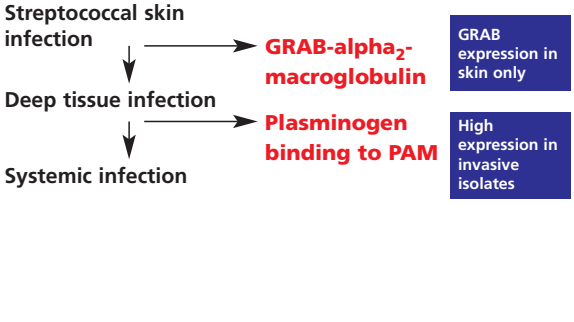
PROJECT LEADER | **Prof. Dr. G. Singh Chhatwal** | **Department of Microbial Pathogenicity and Vaccine Research** | **gsc@gbf.de**

PROJECT MEMBERS | Maike Bolm | Dr. Katrin Dinkla | Dr. Antonia Godehardt | Dr. Manfred Rohde | Inka Sastalla | Patricia Wegmeyer

Streptococci and pneumococci remain a serious hazard for human health. Despite the availability of antibiotics, it has not been possible to control the diseases caused by these microorganisms. High genetic diversity and a complex pathogenesis have hampered efforts to develop suitable strategies against them. The main focus of this project is the identification and characterization of factors that contribute to the virulence of these microorganisms. This information will lead to a better understanding of pathogenesis and to the identification of novel and promising targets for vaccine and therapy.

Interaction of streptococci with host proteins
More in-depth analysis was conducted on the relationship between the plasminogen-binding capacity of group A streptococci, plasminogen-binding protein PAM variants and the invasive capacity of the streptococcal isolates. We were able to show that in the presence of fibrinogen and streptokinase, invasive isolates bound more plasminogen than isolates from uncomplicated infections.
The biological role of the interaction between group A streptococcal surface protein GRAB and host alpha$_2$-macroglobulin was elucidated. The binding of alpha$_2$-macroglobulin to GRAB was shown to be mediated by two charged motifs in the delta A region of GRAB protein. A streptococcal mutant without surface GRAB was highly attenuated in a mouse skin model of infection.
We have previously shown that binding of collagen to group A streptococci forms the basis of rheumatic fever, an autoimmune disease induced by streptococci. This finding was further confirmed by the ability of rheumatogenic group C and group G streptococci – from Australia's Northern Territory – to directly bind collagen. A fibronectin binding protein was identified from group G streptococci and was named GfbA. Despite a large degree of homology, GfbA showed some unique features whose functions are now being evaluated.

New virulence factors Hydrogen peroxide was identified as an important virulence factor of streptococci. By using *C. elegans* as an infection model, we were able to show that hydrogen peroxide mediated killing is a common feature of different streptococcus species.
We have previously described a pneumococcal surface protein, SpsA, which interacts with the human polymeric Ig receptor, thereby mediating adherence and internalisation of the bacteria into epithelial cells. A unique feature of this interaction is that SpsA is highly specific for the human Ig receptor. This was confirmed by using chimeric human mouse receptors. It was further shown that the ecto-domains three and four of the human polymeric Ig receptor were involved in this interaction, as well as in pneumococcal invasion.
Further characterization of the identified virulence factors and the identification of new factors will contribute to solving the complex molecular events in the diseases caused by streptococci and pneumococci.



Biological consequences of streptococcal proteins GRAB and PAM with alpha$_2$- macroglobulin and plasminogen, respectively.

01.2 Identification and Characterization of Bacterial Virulence Factors

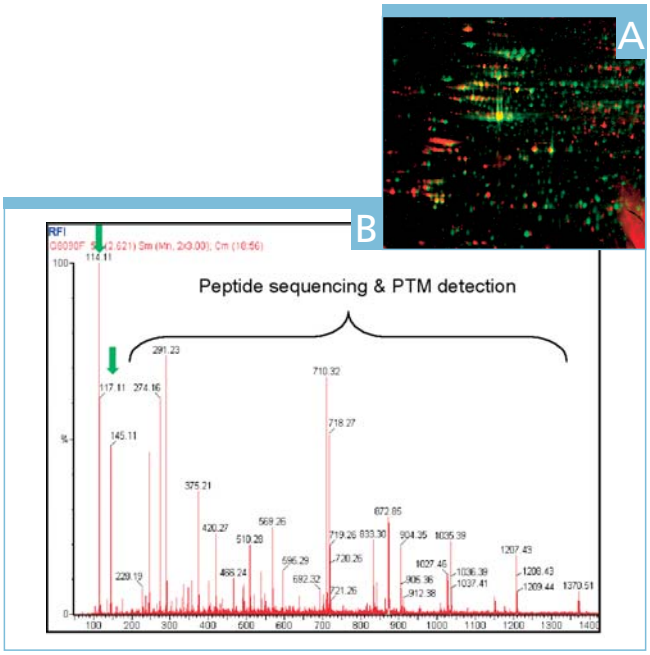
PROJECT LEADER | **Dr. Lothar Jänsch | Department of Cell Biology | lja@gbf.de**

PROJECT MEMBERS | Maja Baumgärtner | Roman Fischer | Dr. Uwe Kärst | Tobias Reinl | Kathrin Thedieck | Matthias Trost | Prof. Dr. Jürgen Wehland | Dr. Dirk Wehmhöhner

Listeria monocytogenes is a Gram-positive, facultative intracellular human pathogen that can cause food-borne infections like meningo-encephalitis, meningitis and prenatal infections, especially in immuno-compromised patients. Using *Listeria monocytogenes* as a model system, an interdisciplinary approach of proteome research, genomics and bioinformatics is used to define the interactions between bacterial pathogens and its host cells at the molecular level. This includes protein interaction- and signaling-analyses of virulence factors, as well as a search for hitherto uncharacterized proteins directly involved in the infection cycle.

New virulence factors Since the interactions of pathogens with host cells are mediated through their external, *i.e.* secreted, cell-wall-associated and membrane proteins, we have established methods to investigate all known bacterial surface proteomes. Comprehensive comparative proteome analyses between different mutants of the pathogenic species *L. monocytogenes* and the non-pathogenic species *L. innocua* have resulted in the identification of about 1,300 different bacterial proteins based on high resolution 2D-gel electrophoresis and automated nano HPLC-Q-TOF mass spectrometry. Several bioinformatic strategies were developed in cooperation with the Division of Structural Biology for systematic data storage and data mining. A newly established correlation database, called LEGER, contains information on: the predicted subcellular localization and regulation of all proteins; the applied biochemical strategies regarding the identified proteins; and a number of experimental conditions. Thus, LEGER supports the detection of protein expression profiles that correlate with the level of pathogenicity of the investigated species and genetic variants. Other bioinformatic strategies focus on data integration and visualization to promote the generation of new functional hypotheses.

Functional characterization of virulence factors Pathogenic bacteria use a variety of strategies to exploit the host environment to their advantage. The initial interaction between the host cell and the pathogen is supposed to be the decisive step in the course of pathogenicity. For the functional analysis of known virulence factors and the identification of new putative virulence factors, we focus on protein-protein interaction studies and the identification of affected proteins from different host cell lines. Different technologies for comparative phosphoproteome analyses of the infected host cells were established. Non-gel-based methods allow the systematic characterization of phosphorylation sites and are complemented with labeling strategies for the detection of differentially regulated kinase activities. These studies – aiming at the time-resolved characterization of signal-transduction processes during host cell invasion by Listeria – already revealed about 200 different phosphorylation sites of host proteins. Many of them have presently unknown upstream kinase activities.



Comparative proteome analyses can be carried out by gel- and non-gel-based methods. (A) Host cell response after listerial infection. Total cell lysates from infected HeLa cells were separated by 2D-PAGE and the resolved eukaryotic proteins were detected both by expression staining (green) using SYPRO Ruby and by specific detection of phosphorylation (red) using Pro-Q Diamond. (B) Peptide sequencing based on mass spectrometry. The combination of nano HPLC and ESI-Q-TOF mass spectrometry allows the automatic separation and fragmentation of peptides. A typical fragmentation pattern provides information about the peptide sequence, post-translational modified (PTM) amino acids and the relative abundance of that particular peptide as part of two different samples (iTRAQ labelled sample, using the 114Da and 117Da reagent, indicated by green arrows).

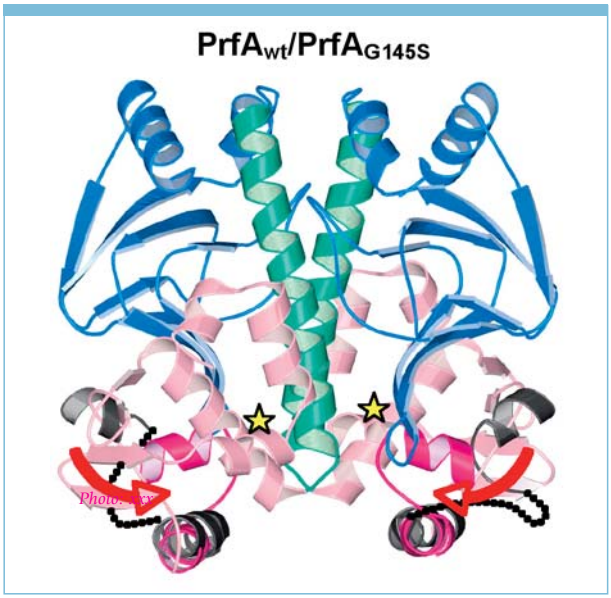
01.3 Structural Analysis of Virulence Factors

PROJECT LEADER | **Prof. Dr. Dirk Heinz | Department of Structural Biology | dih@gbf.de**

PROJECT MEMBERS | Maike Bublitz | Carina Büttner | Marina Eiting | Davide Ferraris | Susanne Freese | Gregor Hagelüken | Dr. Hans-Jürgen Hecht | Dr. Joop van den Heuvel | Dr. Birgit Hofmann | Dr. Hartmut Niemann | Dr. Wolf-Dieter Schubert | Thomas Wollert | Dr. Victor Wray

Bacteria, viruses and other pathogens interact with host cells via dedicated molecules presented on their surfaces. In this project, we aim to analyze bacterial molecules in atomic detail to understand the mechanisms through which the microbial pathogens outflank their human hosts.

Expression-Regulating of virulence factors The bacterium *Listeria monocytogenes* gives rise to listeriosis, a disease which leads to symptoms of meningitis and miscarriage, coupled to a significant mortality rate in the elderly or immuno-compromised individuals.
The mechanisms by which *L. monocytogenes* breaches host cell barriers and spreads to neighbouring cells and tissues have been investigated intensively over the last few years. They all involve individual proteins produced by the bacterium to exploit fundamental host processes. The principal transcriptional regulator for these virulence factors of *L. monocytogenes* is PrfA, a DNA-binding effector which induces their production under suitable environmental conditions.
We have solved the crystal structure of the transcriptional regulator PrfA and that of a constitutively active mutant $PrfA_{G145S}$. In this mutant, residue 145, a glycine has been replaced by a serine.
PrfA is a symmetric dimer of two monomers. Each monomer consists of three domains, an N-terminal domain (labeled blue in the figure), a central α-helix (green) that is responsible for forming the dimer, and a C-terminal, DNA-binding domain (pink). The C-terminal domain contains a helix-turn-helix (HTH) motif, which recognizes and binds to DNA.
The mutation G145S (yellow), located in the C-terminal domain, causes a major rearrangement of the HTH-motif. In the wild-type protein (black helices), the turn or loop connecting the α-helices of the HTH-motif is disordered. In the mutated protein (red helices), the loop is ordered and visible, indicating that the mutation leads to a stabilization.



Structure of PrfA from Listeria monocytogenes.

In addition, the first α-helix of the HTH-motif moves dramatically as a result of the mutation (red arrows), adopting a typical position of DNA-binding proteins. The replacement of Gly145 by serine is thus able to stabilize a conformation that is more likely to bind DNA. This explains why bacterial strains carrying this mutation constitutively express high amounts of virulence factors – independently of the environmental signals. The crystal structure also reveals a tunnel near the mutation site, which probably binds a hitherto unknown host-derived signal factor.

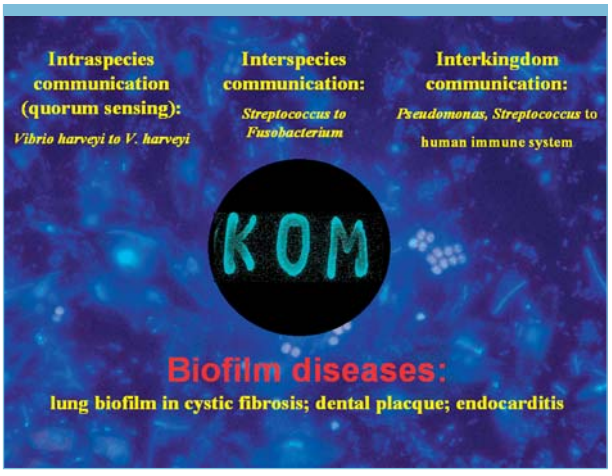
Virulence factors from *Mycobacterium tuberculosis*
Tuberculosis, caused by *Mycobacterium tuberculosis*, affects about one third of the world's population. The structure of the *M. tuberculosis* thiol peroxidase Tpx was solved in an EU-funded project on enzymes related to persistent tuberculosis. Tpx is an atypical peroxiredoxin, participating in the pathogen's antioxidant defence system which is implicated as a virulence-determining mechanism in isoniazid resistant KatG(-) strains. The inactive C60S variant has acetate bound in the active site.

01.4 Microbial Communication

PROJECT LEADER | **Priv.-Doz. Dr. Irene Wagner-Döbler | Research Group Microbial Communication | iwd@gbf.de**

PROJECT MEMBER | Dr. Hanno Biebl | Agnes Bodor | Dr. Ingrid Brümmer | Bettina Elxnat | Birte Engelhardt | Dr. Brigitte Kunze | Dr. Helena Sztajer | Dr. Roland Weller

Bacteria produce small diffusible signal molecules called autoinducers, which regulate the expression of fundamental physiological capabilities in a concentration dependent way. Thus, *Vibrio* cells start to luminesce and produce toxins only if their numbers are above a certain value, the so-called quorum. This type of communication, known as quorum sensing, controls many important traits, including the expression of virulence factors, production of antibiotics and the formation of biofilms. Understanding and interfering with microbial communication therefore opens up new opportunities for fighting infectious diseases and developing potential alternatives to antibiotics.



- *"KOM" for German "Kommunikation" is written on an agar plate by streaking out a culture of the bioluminescent* Vibrio harveyi *strain, mutants of which serve as biosensor for detecting the postulated universal signal molecule autoinducer-2. Cell-to-cell communication takes place (1) within a population of one single species to control action in a density dependent way (quorum sensing); (2) between species through universal signal molecules (e.g. autoinducer-2); (3) between bacteria and their eukaryotic hosts. Biofilms (background: complex aquatic biofilm) are a hot spot of communication and responsible for diseases which resist standard treatment.*

New communication signals in marine bacteria We used reporter strains with *luxR-gfp* transcriptional fusions to screen culture supernatants for signal molecules. The investigated bacteria belonged mainly to the *Roseobacter* clade and were isolated from dinoflagellates – marine algae which are responsible for toxic algal blooms in the sea – but also from the open water or marine sediments. A large variety of acylated homoserine lactone (AHL) signal molecules was discovered in over 50 % of the strains tested. This shows that this type of gene regulation is much more widespread than previously thought. Often whole arrays of AHLs with different chain lengths were found in the same organism and, in some cases, identical AHLs were found in distantly related bacteria. Thus, complex signalling cascades must be present, as well as crosstalk between genera. Some strains produced AHLs with side chains of 18 carbon atoms length, the longest AHL side chain discovered until now. The phenotypes regulated by these compounds in bacteria, as well as their potential effect on the eukaryotic host, are unknown today.

The postulated universal signal AI-2 in *Streptococcus mutans* While most bacterial signal molecules are species specific, one autoinducer is widespread in both Gram-negative and Gram-positive bacteria and thus has the potential to act as a universal signal molecule. It is produced as a by-product during the detoxification of SAM (*S*-adenosyl-methionine) by the luxS enzyme. It has been termed autoinducer 2 (AI-2) and has been shown to modulate the expression of virulence factors and biofilm formation, often in combination with other quorum sensing signals. Using the *Vibrio harveyi* reporter strain, we studied the production of AI-2 in *Streptococcus mutans*, an important constituent of dental biofilms. *S. mutans* is one of the major causes of tooth decay and can also cause infective endocarditis. We showed that the production of AI-2 in *S. mutans* is strongly dependent on the cultivation conditions, including the growth phase and medium composition, as well as on the mode of growth – planktonic vs. biofilm. Thus, these factors must be taken into account when analysing the transcriptional response of *S. mutans* to AI-2.

01.5 Molecular Characterisation of *Pseudomonas aeruginosa* Small Colony Variants

PROJECT LEADER | **Dr. Susanne Häußler | Research Group Chronic Pseudomonas Diseases | sus@gbf.de**

PROJECT MEMBERS | Florian Bredenbruch | Andree Meissner | Caroline Zaoui

Slow-growing bacterial subpopulations have been described for more than 80 years. However, it was only in the last 10 years that these morphological variants, producing small colonies on nutrient agar, have gained increasing attention. Since then small colony variants (SCVs) of various bacterial species – usually known to be the source of acute infections – have been implicated in persistent and recurrent infections.

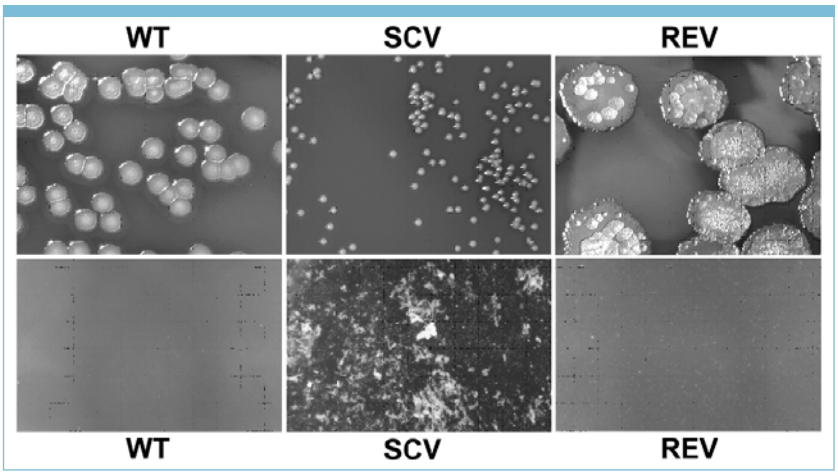
Pseudomonas aeruginosa has emerged as a major human opportunistic pathogen and represents a significant source of life-threatening nosocomial infections. It is also the most dominant bacterial pathogen that can be recovered from the chronically infected lungs in patients suffering from cystic fibrosis. Over the last few decades much effort has been undertaken to study the mechanisms that allow *P. aeruginosa* to persist during chronic infection – despite the immune responses of the host and despite antimicrobial therapy.

Besides adaptation caused by regulatory processes, adaptation to changing environmental conditions has been shown to be facilitated by the emergence of variant subpopulations in *Pseudomonas*. When introduced into a spatially structured microcosm, the ancestral genotype rapidly diversifies and produces a range of morphological distinct niche-specific genotypes with a functional gain, which are maintained by selection. The evolution of *P. aeruginosa* SCVs within the heterogeneous habitat of the chronically infected CF lung, may significantly contribute to bacterial persistence because they are especially adapted to their particular environment *e. g.* due to their increased capability to form biofilms.

Switch to a *P. aeruginosa* Biofilm-Phenotype Using transposon mutagenesis of clinical *P. aeruginosa* strains, we were able to identify several genes that are involved in the switch of a *P. aeruginosa* wild-type to a biofilm forming SCV phenotype and vice versa. Analysis of the global transcriptional pattern, expression of *P. aeruginosa* fimbria by Western blot and electron microscopic studies of selected mutants revealed that the expression of the so-called chaperon-usher pathway *(cupA)* gene cluster is linked to an autoaggregative biofilm-forming SCV phenotype.

This gene cluster is conserved among many Gram-negative bacteria and is responsible for the assembly of fimbria at the bacterial surface. The presence of *cupA* encoded fimbria at the bacterial surface of *P. aeruginosa* has not been described previously. There is growing evidence that the fimbria-mediated attachment of various bacteria to surfaces is regulated by intracellular cyclic di-GMP, a newly identified bacterial signal molecule.

We have started to dissect the molecular mechanisms involved in the regulation of the *cupA* genes and could show that over-expression of the EAL domain containing "phenotypic variant regulator" (PvrR) and a knock-out in diverse GGDEF containing membrane protein leads to a switch of an autoaggregative SCV to a wild-type phenotype. The modulation of intracellular cyclic di-GMP, seems to play a dominant role in the switch from planctonic growth to the biofilm-mode of growth and is currently the subject of our investigations.



- *Wildtype, SCV and* in vitro *generated revertants cultivated on Columbia agar plates (top) and in liquid culture (bottom).*

Photo: GBF

Topic 02 – Pathogenesis

TOPIC SPEAKER | **Prof. Dr. Jürgen Wehland | Department of Cell Biology | jwe@gbf.de**

- A prerequisite for the development of new diagnostic and therapeutic strategies is a detailed knowledge of the particular infection process and of how the disease progresses. The projects comprising this topic aim at analysing and elucidating the mechanisms of pathogenicity, not only in respect to the pathogen, but also in respect to the host system, focussing on host-pathogen interactions, especially the adhesion and invasion mechanisms of Streptococci, *Listeria* and pathogenic *E. coli*. Equally important are the reactions of the host during infection with respect to its immune defence system.

Here, pathogens have developed sophisticated virulence mechanisms which enable them to circumvent host defence systems and to survive long enough in the host for establishing an infection. In addition, the establishment of animal infection models is essential for the analysis of pathogenicity mechanisms.

- *Transferring bacterial colonies from an agar plate.*

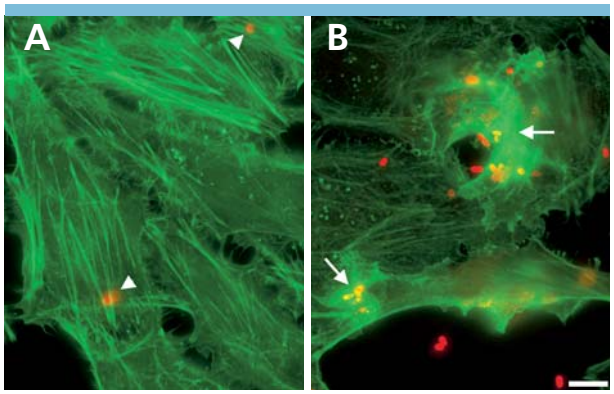
Photo: Bierstedt

02.1 Molecular Mechanisms of Host-Cell/Pathogen Interactions

PROJECT LEADER | **Dr. Klemens Rottner | Research Group Cytoskeleton Dynamics | kro@gbf.de**

PROJECT MEMBERS | Tanja Bosse | Julia Ehinger | Anke Fabian | Dr. Frank P. Lai | Dr. Silvia Lommel

This project focuses on the characterisation of the signalling pathways driving actin reorganization during different types of pathogen-interaction with their host cells. These studies are based on the observation that certain bacterial pathogens like *Listeria monocytogenes*, *Shigella flexneri* or *Burkholderia pseudomallei* are capable of directly recruiting parts of the actin polymerisation machinery. They recruit them to actively move within the cytoplasm of an individual host cell and to spread into neighbouring cells. Certain host cell factors have appeared essential for these processes, such as the actin nucleation promoting factor N-WASP.
N-WASP is the ubiquitously expressed relative of the haematopoietic Wiskott Aldrich Syndrome protein, the causative agent of a severe X-linked immunodeficiency. The generation and characterisation of N-WASP-defective cell lines has significantly contributed to revealing the multi-facetted functions of N-WASP-driven actin rearrangements in bacterial pathogenesis.



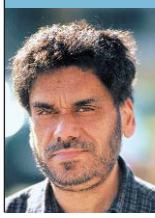
- *Actin rearrangements accompanying* Salmonella typhimurium *invasion: Fluorescence microscopy of tissue culture cells infected with a non-invasive mutant strain incapable of assembling the type III secretion system (kindly provided by D. Bumann, MHH) (A) or wildtype* Salmonella typhimurium *(strain SL1344) (B). The actin cytoskeleton is stained with fluorescent phalloidin and displayed in green, bacteria are shown in red. Note the absence of any detectable actin rearrangements upon contact (arrowheads) of these cells with the non-invasive mutant (A) as compared to the dramatic actin rearrangements (arrows) induced by the wildtype strain (B). The host-cell factors directly mediating these actin reorganizations are largely uncharacterised. Bar equals 10 µm.*

Photo: Dr. Rottner

These functions include the requirement for another type of actin-driven process, which is the surfing of pathogenic *E. coli* (EPEC and EHEC) on infected epithelial cells. Therefore, current investigations are aimed at defining the molecular details of N-WASP recruitment and activation at the cell surface during EPEC and EHEC infections. Moreover, there are still ongoing efforts to characterise the molecular prerequisites of N-WASP activation leading to actin assembly on *Shigella* surfaces. These studies include reconstitution experiments in N-WASP-defective cells of *Shigella* motility in the presence and absence of key functional modules of the protein. These modules can directly affect movement velocity, and hence, *Shigella* virulence.

Physiological functions of N-WASP We have recently been able to get more insight into the potential cellular functions of the N-WASP protein. This can in turn help to interpret the reasons for the key role of this protein in the pathogenesis of certain bacteria. We could show that N-WASP, by directly activating the actin-nucleating enzyme Arp2/3-complex, increases the frequency of actin assembly at internalising clathrin-coated pits during receptor-mediated endocytosis. Thereby it is affecting the efficiency of clathrin-mediated endocytosis. This notion has important implications for understanding why N-WASP may have developed into a favourite target of pathogens as diverse as EPEC and EHEC, *Shigella flexneri* or Vaccinia virus.

Actin rearrangements during bacterial invasion
Our studies have been expanded now to characterising the molecular regulation of actin rearrangements, initiating the infection cycle of some pathogens like *L. monocytogenes* or *S. flexneri*. We have employed cell lines genetically deficient for, or suppressed in, their expression of key signalling factors for actin reorganization – such as Rho family GTPases. They dissect the signalling pathways driving the actin rearrangements required for the entry of Gram-positive *L. monocytogenes*, but also Gram-negative *S. flexneri* and the closely related invasive pathogen *Salmonella typhimurium*.

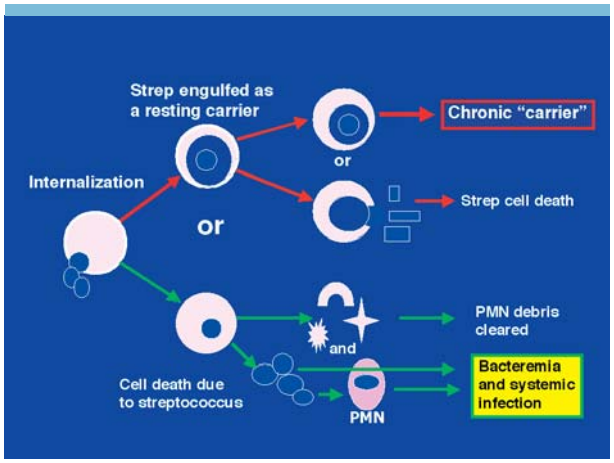


02.2 Molecular Mechanisms of Interactions between Streptococci and Host Cells

PROJECT LEADER | **Prof. Dr. G. Singh Chhatwal** | **Department of Microbial Pathogenesis and Vaccine Research** | **gsc@gbf.de** |

PROJECT MEMBERS | Prof. Edward Kaplan | Andreas Nerlich | Dr. Manfred Rohde

Adherence to host cells and invasion is the basic principle behind infections caused by many pathogenic microorganisms. Group A streptococci exploit the host's extracellular matrix components, such as collagen and fibronectin, for adherence and invasion and also for intracellular persistence and survival. The elucidation of molecular events underlying these processes is a prerequisite to understanding the complex nature of host-pathogen interactions of streptococcal infections. This project focuses on studying the interactions between streptococci and host cells at the molecular level. Besides studying adherence, invasion and intracellular survival, the project deals with signal transduction events in streptococcal invasion, as well as the mode of eradicating persistent streptococci.



Biological significance of persistence of streptococci in human cells.

Role of fibronectin and collagen We have previously shown that collagen and fibronectin play an important role in adherence and invasion of streptococci. A crucial pathogenicity factor is the SfbI protein of group A streptococci, which binds fibronectin. SfbI protein is also involved in the caveolae-dependent internalisation and intracellular survival of streptococci.
Because of the important biological function of the SfbI protein, we looked for its homologues in other streptococcal species and identified GfbA in group G streptococci. We obtained evidence of interspecies gene transfer between the important virulence factor genes *sfbI* and *gfbA*. It appears that homologous recombination is occurring between these species, because the identified group G streptococcus *gfbA* types possess DNA cassettes that can be identified in a number of group A streptococcus strains. SfbI dependent internalisation was shown to be rac1 dependent. Preliminary experiments were also carried out by studying the internalisation of M3 serotype streptococci with endothelial cells, which was shown to be mediated by Src-kinase.
The interaction of collagen contributed towards the rheumatogenic potential of streptococci. M3 protein and M18 capsule have so far been identified as collagen binding streptococcal factors. Collagen, therefore, represents an important autoantigen in rheumatic fever induced by streptococci.

Latent streptococcal infections Persistence of streptococci in host cells can have important biological consequences. It may lead to dissemination or latent infection. In addition, intracellular streptococci may evade the action of penicillin, which is the gold standard for treatment of streptococcal infections. We now have experimental evidence that intracellular streptococci can only be killed by antibiotics which can enter eukaryotic cells. Understanding of the molecular events underlying the persistence of streptococci is required to define the optimal management of latent streptococcal infections.

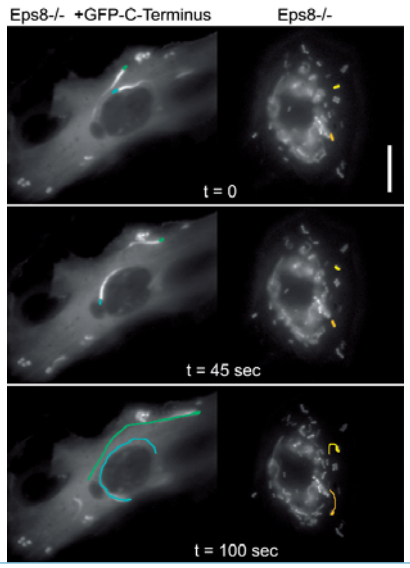
02.3 Signalling to Acting Dynamics

PROJECT LEADER | **Dr. Theresia Stradal | Research Group Signalling and Mobility | ths@gbf.de**

PROJECT MEMBERS | Andrea Jenzora | Kai Städing | Anika Steffen | Stefanie Weiss

Numerous cellular functions and processes depend on dynamic changes of the actin cytoskeleton. This includes morphogenetic movements during embryonic development, chemotactic movement of cells of the immune system and migration of fibroblasts during wound healing. A large number of actin binding proteins have been described, which regulate the dynamic reorganisation of the actin cytoskeleton. However, the exact signalling pathways and principles for the tight control of spatially and temporally regulated actin assembly remain largely enigmatic. We try to gain more insight into these pathways.

The discovery that similar dynamic changes of the actin cytoskeleton play an essential role in host-pathogen interactions had, and still has, an enormous impact on this field. One major player driving the *de novo* nucleation of actin filaments is the Arp2/3 complex, which is activated by so-called nucleation promoting factors (NPFs).



- *Eps8-null embryonic fibroblasts transfected with an empty vector (right) or a GFP–tagged C-terminal Eps8 fragment harbouring the capping activity (left) were infected with* Listeria monocytogenes. *After 3–4 h, cells were subjected to video microscopy and the velocity of individual bacteria was determined. Note that the speed of intracellular* Listeria *is increased several-fold after expression of the fragment as indicated by the length of representative paths of two bacteria in each cell (green, blue versus yellow, orange). The time between representative video frames is in seconds and the scale bar equals 15 µm.*

Photo: GBF

WAVE WAVE proteins, prominent members of the WASP/Scar family play an important role in Rac-mediated lamellipodia formation. However, the pathway leading to WAVE-activation is indirect, since the small GTPase Rac is not able to directly bind to, and activate, WAVE. This led us to search for the missing links between Rac and WAVE proteins. We have now established the ability of two novel proteins, Nck associated protein 1 – Nap1 – and specifically Rac associated protein 1 – Sra-1 – to interact with both Rac and WAVE proteins via the Abl interacting protein family, the Abi proteins.

We then targeted Nap1 or Sra-1 expression by RNA interference (RNAi) and found that both proteins are absolutely required for the transduction of Rac signals to WAVE-dependent lamellipodia formation. The role of Nap1 and Sra-1 for the formation of structures other than lamellipodia is currently being investigated.

Eps8 To gain more insight into the pathways leading to actin assembly upstream and downstream of Rac activation, we further analysed the role of EGF receptor substrate 8 – Eps8. Eps8 is a SH3 domain containing adaptor molecule, which has been identified as an interaction partner of the aforementioned Abi proteins. Eps8 has a well established function in receptor mediated Rac-activation, but is now also recognized to affect actin assembly directly. We have identified a novel actin filament capping activity in the C-terminus of Eps8, promoting localized actin assembly. This effect was quantified by employing the Rho-GTPase independent intracellular movement of *L. monocytogenes.*

PREL1/RIAM ActA, which is essential for the actin-based motility of *Listeria*, had contributed considerably to the understanding of how Ena/VASP proteins, important modulators of actin based motility, are recruited via their EVH1-domains to proline-rich ligands. Identification of novel interaction partners is believed to aid in understanding their mechanism of action. A screen for novel proline-rich EVH1-ligands revealed a hitherto unknown human protein very similar to *C. elegans* MIG10. We tentatively named this protein PREL1 and analysed its subcellular dynamics and binding partners. Besides interaction with the Ena/VASP family, PREL1 is able to bind to activated Ras GTPases in a lipid dependent fashion, providing the frequently predicted direct link between Ras signalling and actin rearrangements.

02.4 Immunogenetics of Group A Streptococcal Infections in the Mouse Model

PROJECT LEADER | **Dr. Eva Medina | Research Group Infection Immunology | eme@gbf.de**

PROJECT MEMBERS | Dr. Oliver Goldmann | Jeannette Siegert

Group A streptococci are common human pathogens capable of producing a variety of diseases, ranging from very mild infections – pharyngitis, tonsillitis or impetigo – to very severe diseases associated with high mortality rates – necrotizing fascitiis, streptococcal toxic shock syndrome. Although the molecular mechanisms underlying the different clinical manifestations of Group A streptococcal infections have not yet been clarified, several lines of evidences have suggested that individuals vary genetically in their susceptibility to infection with Group A streptococci.

Response of H-2 congenic BALB-mice to group A streptococcal infections Recent studies have shown that allelic variations of the major histocompatibility complex (MHC) class II antigens may contribute to susceptibility to, or protection from, severe streptococcal diseases by its ability to modulate the magnitude of the inflammatory cytokine response elicited to bacterial superantigens.

Mouse models have proved especially important in demonstrating the influence of genetic factors on host susceptibility and resistance to these Group A streptococcal infections. To gain more insight into the influence of the MHC class II on susceptibility to this pathogen, we have conducted comparative studies of the responses to infection of congenic BALB mice of the H-2^b (BALB/b), H-2^c (BALB/c), and H-2^k (BALB/k) haplotypes. Notable differences were observed among these strains after infection with Group A streptococcus. Whereas BALB/k was highly susceptible and succumbed to infection, BALB/b and BALB/c developed far less severe disease and survived infection with an effective bacterial clearance. Similarly to humans, the susceptible H-2 haplotype (H-2^k) produced higher levels of inflammatory cytokines in response to streptococcal superantigens than the more resistant H-2^b and H-2^d haplotypes. Therefore, our results support the human studies suggesting that allelic and haplotype variations of major histocompatibility complex class II antigens can influence the level of severity of an already invasive streptococcal infection.

Two possible scenarios of host predisposition

However, variations of the MHC class II cannot explain the establishment of invasive diseases such as necrotizing fasciitis or bacteremia, which arise due to the inability of the host to limit bacterial growth and dissemination. Thus, one could predict two major scenarios that can predispose the host to severity of streptococcal infections. First, the capacity of the innate immune system to control bacterial growth during the very early infection might be a decisive factor on whether infection is limited or progress towards invasive disease. Second, in patients with invasive infection the magnitude and quality of the downstream inflammatory response, defined by the strength of the interaction between streptococcal superantigens and the MHC region, might be a decisive parameter determining the progression towards streptococcal toxic shock syndrome.

Therefore, the possibility exists that polymorphisms in other immune system-related genes of the MHC region are responsible for the varying susceptibility of different individuals to streptococcal infections. The identification of these genes could have important diagnostic and therapeutic implications.

Dr. Eva Medina preparing the next analysis.

Photo: Bierstedt

Topic 03 – Immunobiology

TOPIC SPEAKER | Dr. Werner Müller | Department of Experimental Immunology | wmueller@gbf.de

- The focus of immunobiology is to better understand the development and function of the immune system. The immune system protects us from infections and is therefore essential for life. When the immune system is disturbed, we become susceptible to diseases, like cancer, and infections. Disturbed immune reactions can result in autoimmune diseases and allergies. The research focus of the GBF in immunobiology is the analysis of infections. Our specialty is the study of bacteria-host interactions. Recently, we established a specialized lab, unique in Germany, to study the infection of mice with different bacterial species. The key feature of this mouse infection laboratory is that the environment of the facility is carefully controlled. Using genetic tools, we specifically disturb gene function of the bacteria and the mice.

Furthermore, parts of the complex host defence mechanism are modelled in cell cultures, using cell biological and molecular biological methods. In these cultures we analyse signals used by individual cell types of the immune system. In addition, we directly analyse the interaction of different immune cell types before, during and after the infection processes.

The work on this topic will result in a better understanding of the interactions between bacteria and the host. Our aim is to continually search for new strategies that will help us fight existing and newly emerging infectious diseases.

- *Insight look at the infection unit S2 of the mouse facility. On the right site are the cages, on the left side are a cabin for changing mice, and laboratory coaches. Two animal care takers are doing their service.*

Photo: Bierstedt

03.1 Signal Transduction and Gene Regulation

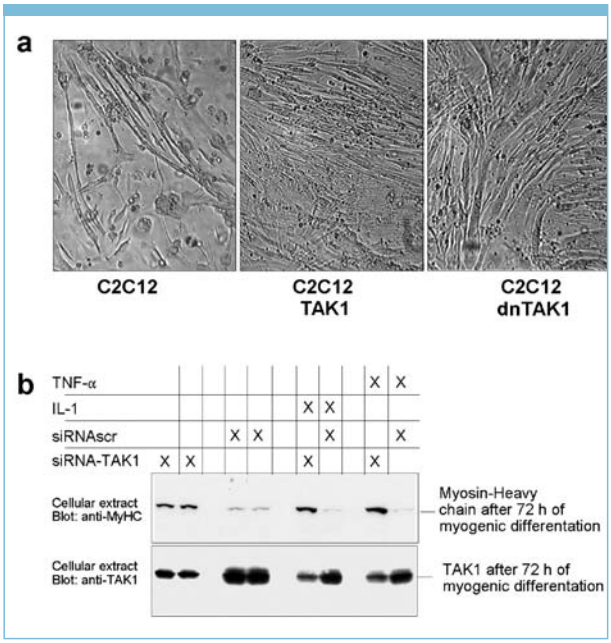
PROJECT LEADER | **Dr. Hansjörg Hauser | Department of Gene Regulation and Differentation | hha@gbf.de**

PROJECT MEMBERS | Dr. Thomas Böldicke | Thomas Frahm | Natali Froese | Dr. Gerhard Gross | Dr. Andrea Hoffmann | Katjana Klages | Dr. Mario Köster | Dr. Andrea Kröger | Sandra Shahab | Anja Wiese | Andreas Winkel

The attack of pathogens induces numerous activities in host cells, including innate immune responses and inflammation. One of our studies focuses on inflammation in tissues, which serves as an effective defence and as an initiator of tissue repair. TAK1, a serine/threonine kinase, is a central signalling mediator activated by proinflammatory cytokines, bacterial and viral components, and which also integrates factors influencing mesenchymal differentiation. We will address the question how the block of TAK1 signalling pathways is counteracting inflammatory diseases and simultaneously enhancing tissue regeneration. These studies may lead to new therapeutic approaches.

TAK1 interference TAK1 belongs to the mitogen-activated protein kinases. Because of its central signalling mediator activity, we are characterizing the cross-talk with different signalling pathways, such as STATs, Wnts, and NF-κB. Our aim is to understand the network of signalling mediator interactions.
We found that TAK1 interferes with nuclear translocation of Smad signalling mediators, which leads to severe biological consequences: The BMP-dependent tissue-regeneration seems entirely blocked by activated TAK1 during inflammation. Moreover, tissue differentiation and regeneration via ubiquitous bHLH transcription factors, such as E12, is also impaired by their TAK1-mediated rapid turnover. In addition, we found that TAK1 blocks tissue regeneration via tissue-specific bHLH transcription factors such as myoD. The activity of myoD is obstructed by TAK1-dependent rapid phosphorylation of a serine residue at position 200 which interferes with the nuclear localization of myoD and with myogenesis in mesenchymal progenitors.

Three novel factors Additionally, three novel factors that interact with TAK1 were isolated. Two of these contribute to the activation TAK1. The third is the tyrosine kinase receptor ROR2, which is involved in Robinow syndrome and brachydactyly B. TAK1 phosphorylates ROR2 in a p38-MAPK-like TGY-motif. This interaction seems to prevent surface presentation of ROR2. TAK1-binding of ROR2 is dependent on the Wnt-ligand: Wnts serving the canonical pathway displace TAK1 from ROR2, while Wnts serving the non-canonical pathway do not. We are now assessing the role of TAK1 in various inflammatory model systems for example, in a TNF-alpha/IL-1 dependent rheumatoid arthritis model – and we plan to investigate its role in inflammatory diseases like colitis.



TAK1 determines myogenic differentiation levels in myogenic progenitors C2C12. a. Dominant-negative TAK1 (dnTAK1) in C2C12 stimulates myogenic differentiation while active TAK1 interferes with myotube formation. b. Endogenous TAK1 levels determine the extent of myogenic differentiation in C2C12. TAK1 was knocked down by siRNA-TAK1 in comparison with a control siRNA (siRNAscr). The level of myogenic differentiation in the presence or absence of proinflamatory cytokines (IL-1, TNF-α) is exemplified by the expression of a myogenic marker gene: Myosin Heavy Chain (MyHC).

03.2 Epigenetic Principles of Gene Regulation

PROJECT LEADER | **Prof. Dr. Jürgen Bode | Research Group Epigenetic Regulation Mechanisms | jbo@gbf.de**

PROJECT MEMBERS | Dr. Kristina Nehlsen | Martin Klar | Dr. André Oumard | Junhua Qiao | Eric Stellamans | Silke Winkelmann | Dr. Manfred Wirth

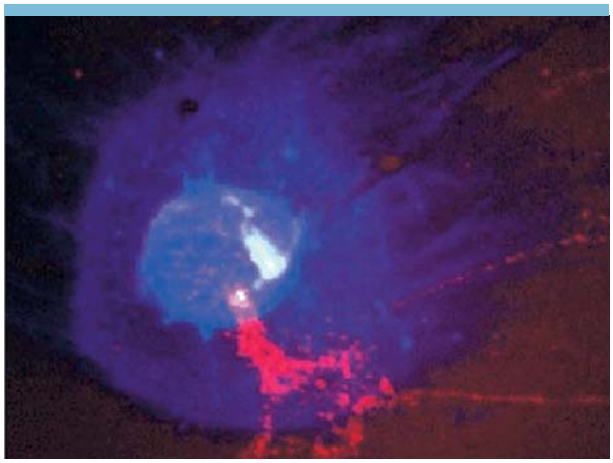
Only recently has the key role of epigenetic control for gene expression and DNA replication been fully recognized. Our research concerns the structure and function of chromatin domains exemplified by the type I interferon (IFN) gene clusters of humans and mice. We adapted the SIDD algorithm for a prediction of boundary elements and regulatory sites. Novel techniques have been developed and refined for the study of genomic insulators, such as scaffold/matrix attachment regions (S/MARs). The elucidation of S/MAR-functions has led to the development of nonviral episomes with a range of novel properties and applications.

SIDD Negative superhelical tension leads to DNA strand unpairing – stress-induced duplex destabilization (SIDD) – within active eukaryotic chromatin domains. Preferred sites become hypersensitive to the action of DNAse I and gain regulatory potential. We have localized three sites – HS1-3 – relative to the transcription start of the IFN-β gene and identified the binding factors. These studies revealed a striking regulatory potential of YY1 and YY2 transcription factors.

Halo FISH Recent modifications of the FISH procedure were applied to show that the loop organization of the IFN-locus is cell specific and depends on its expression capacity. They have also been used to explain the co-suppression phenomenon for multi-copy transgenes and to visualize individual domains of the type 1 interferon gene clusters.
Another major topic regards the mechanism of retroviral integration. It explains the transcriptional properties of proviruses and leads to understanding the pitfalls of gene therapy protocols based on retroviral vectors. We have also shown that functional human endogenous retroviruses (HERVs) localize to the nuclear matrix, while non-functional HERVs are part of the halo portions.
Current studies concern aspects of lentiviral persistence and the susceptibility to lentivirus infection. Parallel gene expression profiling experiments for acute and chronically SIV infected T cells show differences regarding the regulation of histone genes, the ubiquitinylation and glycosylation pathways and the activation of cytokine-/cytokine receptor genes.

RMCE The Flp-recombinase mediated cassette exchange (RMCE) strategy has become a key technology in a multitude of projects to generate production cell lines and identify silent-but-activatable genomic loci suited for inducible expression systems.
The most important potential of RMCE may lie in the predictable modification of ES cells. Such an approach has recently been realized. The Elk-1 locus has been tagged in a way permitting multiple subsequent replacements by gene mutants. The study generated viable mouse offspring and opened the way to a new variant of the ´tag-and-exchange´ approach.

Nonviral episomes We have developed a novel prototype episomal vector which remains extrachromosomal in the absence of continued selection pressure and independent of viral activator proteins. Site specific recombination technology has now been applied to remove nonessential vector parts. The resulting 4 kb mini-circles proved to be superior vehicles in that they behave as consistently high and stable expressers over extended periods of time.



Halo FISH visualization of an IFN-β transgene that has been integrated into a murine host cell at 100 copies (red signals). Only the active part of these copies colocalizes with the nuclear matrix (nuclear remnant in the centre) while the majority extends to the halo. The endogenous, functional IFN genes of the murine cells (bright blue signals) are firmly associated with the nuclear matrix.

Photo: GBF

03.3 Cellular Models for Infectious Diseases

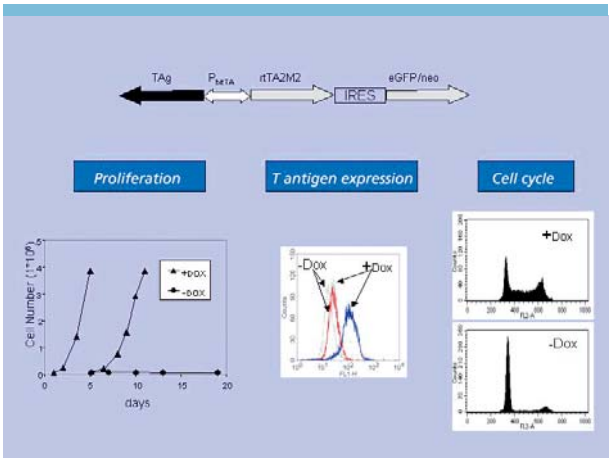
PROJECT LEADER | Dr. Hansjörg Hauser | Department of Gene Regulation and Differentation | hha@gbf.de

PROJECT MEMBER | Tobias May | Dr. Peter Müller | Roland Schucht | Dr. Herbert Weich | Dr. Dagmar Wirth

The understanding of infection related reactions of the host requires experimental studies of whole organisms, as well as of individual cell types. In order to analyse cells from transgenic mice, we have established standard methods to immortalize fibroblastoid and epithelial cells from ES cells, embryonic or adult tissues of mutant mice. Our work forms the basis of a broader approach that includes other cell types, in particular, those from the hematopoietic system.

Cell immortalization Established cell lines differ from the primary cells in the way they express immortalizing genes or oncogenes. This causes a multitude of changes in the gene expression pattern of cells. To minimize such influences we are establishing tools to reversibly immortalize cells. With this technique, specific cell populations can be expanded to achieve a sufficient number of homogenous cells for analysis. After the growth phase, the expression of the immortalizing gene is switched off and cells return to a quiescent state, similar to the status of the majority of primary cells in most adult tissues. Reversibly immortalized cells can be genetically manipulated and may eventually be reintroduced into the organism to monitor the cells fate and function *in vivo*.

Single-step transfection To achieve conditional immortalisation, an immortalizing gene was expressed from an autoregulated vector that can be transfected in a single step. This is an important requirement for the immortalization of primary cells that exhibit low transduction efficiency.
For proof of concept mouse primary fibroblasts were immortalized by SV40 large T antigen (TAg) that was expressed from a Doxycyline (Dox) dependent promoter. The resulting cell clones show strict Dox-dependent proliferation. Cells arrest uniformly in the G0/G1 phase of the cell cycle, but resume proliferation when Dox is added to the culture. Gene expression profiling revealed that TAg expression results in the differential expression of 10 % of all genes analysed.



After transduction by the conditional expression cassette (upper) of mouse embryonic fibroblasts, cell lines could isolated that showed strict Dox dependent but reversible growth properties (lower left). This regulation is due to the conditional expression of the immortalising gene (TAg) (lower middle) and is reflected by cell cycle profiles characteristic for growing and resting cells (lower right, upper and lower, respectively).

03.4 Genetic Mechanisms of Infection Susceptibility and Macrophage Functions

PROJECT LEADER | **Dr. Andreas Lengeling | Research Group Infection Genetics | ale@gbf.de**

PROJECT MEMBERS | Dr. Jens Böse | Lovet Eyongeta | Phillip Hahn | Laura Helming | Dr. Bastian Pasche

The focus of our group is on the development of new mouse models for human infectious diseases to identify host factors that determine the outcome of infections. The goal is to use mouse genetics to identify genes that are associated with susceptibility, or resistance, to different pathogens, *e.g. Streptoccus, Filaria, Listeria*. To accomplish this, we are analysing the immune status and infection susceptibility of different inbred and mutant strains of mice after a challenge with pathogens. In the second project, we try to understand macrophage functions. Macrophages are important effector cells of the innate immune system and provide a first line of defence against many microorganisms. They are essential for the control of common bacterial infections. We want to identify factors that can modulate macrophage effector functions and that are therefore important for innate immune responses.

Clearance of apoptotic cells Macrophages play important roles in the clearance of cells that have undergone programmed cell death. Removal of apoptotic cells is associated with powerful anti-inflammatory and immunosuppressive responses. Many bacterial and viral pathogens induce apoptosis of host cells in critical phases of infection. They are using this mechanism to efficiently evade the host immune system. Recently, we have generated and characterized a mouse knockout mutant of the phosphatidylserine receptor gene, a molecule that has been implicated in apoptotic cell clearance. We could show that this molecule is not essential for apoptotic cell removal, but for the control of important differentiation processes during development. And we found that the phosphatidylserine receptor is necessary for the regulation of macrophage cytokine responses.

Vitamin D Recently, evidence has accumulated that the vitamin D hormone has important functions in the immune system. Vitamin D3 can regulate adaptive immune responses by acting immunosuppressively on dendritic and T-helper cells. However, the role of vitamin D3 in regulating macrophage responses was largely unknown. We found that vitamin D3 is a potent suppressor of interferon-γ mediated macrophage activation and can down-regulate interferon-γ responsive genes in activated macrophages. This has major consequences on macrophage effector functions, such as bactericidal killing activity and regulation of inflammatory responses.

Host factors responsible for infection susceptibility It is well documented that sex-dependent factors affect susceptibility to infection with most mouse models demonstrating higher resistance in females. We have made the unexpected observation that infection of mice with the intracellular bacterium *Listeria monocytogenes* shows an opposite sex susceptibility pattern. Female mice are much more susceptible to *Listeria* infection than male mice and this correlates with higher levels of the immunosuppressive cytokine interleukin 10 in the serum. Our findings provide important new insights into underlying mechanisms of sex-determined susceptibility, since similar sex differences in infection susceptibility to intracellular pathogens have been reported in humans.

Phillip Hahn analysing a mouse mutant under the stereoscope. Stefanie Schiebe controls the coloration of cell preparations.

Photo: Bierstedt

03.5 T-Cell Development and Function

PROJECT LEADER | **Prof. Dr. Jan Buer | Research Group Mucosal Immunity | jab@gbf.de**

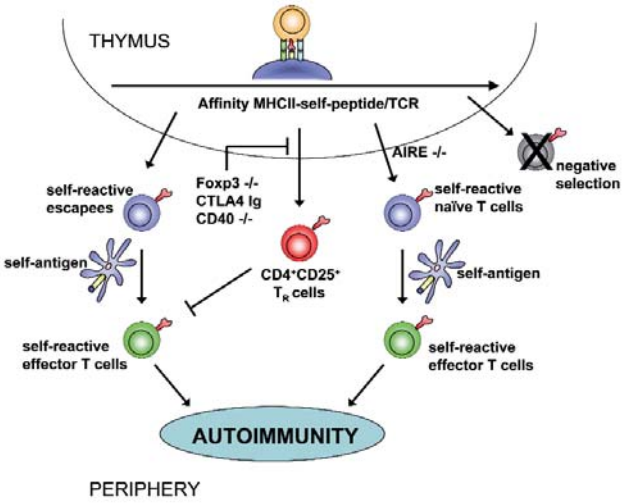
PROJECT MEMBERS | Dr. Dunja Bruder | Dr. Robert Geffers | Marcus Gereke | Ulrike Goelden | Dr. Lothar Gröbe | Dr. Wiebke Hansen | Susanne Pförtner | Simone Reinwald | Sya Ukena | Dr. Astrid Westendorf

The research undertaken in our group is divided into basic mechanisms of T-cell tolerance, mucosal immunity and inflammatory disease. Our goal is to develop new therapeutic approaches for patients suffering from immunopathology. A focus is here on diseases associated with mucosal inflammation (*e.g.* inflammatory bowel disease and asthma) and organ-specific autoimmunity (*e.g.* Diabetes).

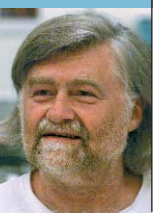
T-cell tolerance In a joint project with the Hannover Medical School (MHH), we were able to demonstrate the induction of GATA-3 in CD-4-T cells after G-CSF stimulation of peripheral blood stem cells from normal donors. This was associated with a Th2-shift in CD4-T cells. By using single cell RT-PCR we also were able to illustrate that CD4-T cells express the G-CSF receptor, and hence, that it is possible to directly influence CD4-T-cell function by G-CSF *in vivo*. In collaboration with the Pasteur Institute, we could further elucidate some new aspects of GATA-3 in the development of lymphocytes. Very recently – in collaboration with the MHH and the Deutsches Rheumaforschungszentrum – we were able to identify and characterize two new potential markers of regulatory T cells that might help to elucidate their role in inflammation.
Current work focusing on the development of autoimmunity has been able to detect up-regulation of MHC class II expression by beta cells of the pancreas during disease progression. This cooperation with the Dana-Farber Cancer Institute in Boston provides a missing link to explain how an infectious agent might initiate autoimmunity.

Mucosal immunity and inflammatory disease The central task of the mucosal immune system is the development and maintenance of immunological tolerance against a large number of antigens from the environment. To this end, a TCR transgenic murine model was established for the analysis of mucosal dysregulation in the bowel, based on selective expression of a model antigen in the gastrointestinal tract. It provides a focused starting point for the further elucidation of genetic and mechanistic aspects of intestinal inflammation and immune regulation.
A novel murine model was recently developed to study disruptions in the mucosal-associated immune system of the lung. Results from this work – in cooperation with scientists at Yale University – provide evidence that regulatory T cells can be induced in the lung at sides of inflammation counteracting disease progression.

An additional project focus is on the molecular characterization of the complex cross-talk of pathogenic bacteria with host cells. Here we could unravel some of the basic mechanisms of EHEC disease based on a genome-wide scan of the human umbilical cord.
Additionally, we are interested in the modulation of the mucosal barrier by probiotic bacteria. To this end, we generated recombinant *E. coli* NISSLE 1917 and exploited its therapeutic potential *in vitro* and *in vivo*.
And, finally, we investigated basic mechanisms of cellular regeneration in liver cells – one of the key targets of human viral diseases.



- *Immunologic self-tolerance. Naturally occurring CD4+CD25+ regulatory T cells develop in the thymus, and migrate into the periphery to regulate immune response and maintain self-tolerance. High avidity self-reactive T-cell clones are typically deleted during development in the thymus by negative selection, but this mechanism is known to be incomplete. In cooperation with other mechanisms CD4+CD25+ TR with relatively high avidity (but to low to be deleted) control the balance between immunity and tolerance. Deletion or blocking of molecules like Foxp3, CTLA4 or CD40 abrogates the development of regulatory T cells from the thymus eventually leading to multiple manifestations of autoimmunity. It is assumed, that the transcriptional activator AIRE is critical for the expression of tissue-specific antigens in medullary thymus epithelial cells (mTECs). Therefore, mutations or deletion of AIRE impede negative selection of high affinity self-reactive T-cell clones leading to autoimmunity in humans (APECED) and mice.*



03.6 B-Cell Subpopulations

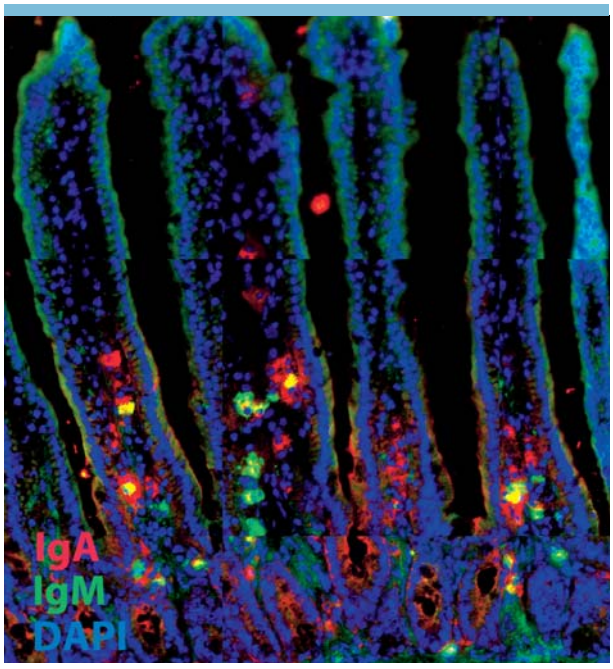
PROJECT LEADER | **Dr. Siegfried Weiss | Research Group Molecular Immunology | siw@gbf.de**

PROJECT MEMBERS | Sandra Düber | Karsten Kretschmer | Bishnudeo Roy | Britta Störmann

Antibody producing B cells can be classified into three subpopulations. The major population consists of so called B2 cells displaying exquisite specificity. The other two populations consist of B1 cells that dominate the body cavities, but are also found in the spleen and marginal zone (MZ) and B cells distributed around lymphoid follicles. Both are able to respond quickly after an infection. In addition, B1 cells are the source of natural antibodies, *i.e.* antibodies found even in germ-free or antigen-free mice. We have been concentrating on the physiology of B1 cells.

Splenic and peritoneal B1 cells A transgenic mouse was employed expressing high levels of a λ 2 light (L) chain. For unknown reasons, a complete absence of B2 cells in the spleen and the peritoneum of these mice has been observed. In single cell RT-PCR, the antibody repertoire of B1 cells from these mice was established. In the peritoneum the repertoire was dominated by three sequences. Such sequences were found only rarely amongst the B1 cells in the spleen. Thus, splenic and the peritoneal B1 cells are distinct populations with different tasks in the immune system. Similar indications were obtained from expression arrays, and point to the existence of two independent cell lineages giving raise to them. Interestingly, the spleen is required for the maintenance of the B1-cell subpopulations in the peritoneum. After a spleenectomy, the dominant sequences can no longer be found in the peritoneum. Thus, a factor is produced in the spleen, possibly even an autoantigen, which allows the survival of certain peritoneal B1-cell clones.
Still controversial is whether B1 cells can take part in gut associated humoral immunity. We have used our transgenic mice to study whether B cells would be present in gut-associated tissue. In the lamina propria, IgM producing plasma cells could be found besides the expected IgA producing plasma cells. However, in Peyer's Patches a large fraction of the B cells expressed an endogenous κ chain and no longer the transgenic λ chain. This could be due to attraction and selection of such B cells. Alternatively, such B cells might have undergone receptor editing. This could have caused a change in the phenotype, from B1 to B2, and correlates with the fact that this phenomenon does not occur in germ-free transgenic mice or in hybrids between the λ transgenic mouse and a mouse not containing a functional κ locus. In the first case, the antigenic pressure would be lost; in the second, the possibility to edit the B-cell receptor would be drastically diminished.

A new mouse-model To extend studies on the origin of B1 cells, which are claimed to be exclusively fetally and neonatally derived, we now have generated a recombinant mouse in which the B-cell ontogeny can be switched on at will after birth. This was achieved by inverse floxing of the Rag1 gene, which is essential for the rearrangement of immunoglobulin genes. The new mouse is presently bred with mice that contain inducible cre. Injection of recombinant cre protein will also be employed. These experiments should answer the question of whether the adult bone marrow is still able to generate B1 cells – a question that so far has not been answered definitively.



Immune histology of the lamina propria from transgenic L2 mice. Clearly IgM and IgA expressing cells can be distinguished. This is in clear contrast to normal control mice where mainly IgA producing plasma cells can be found. Histology was carried out in collaboration with Dr. O. Pabst, Hanover.

Photo: GBF

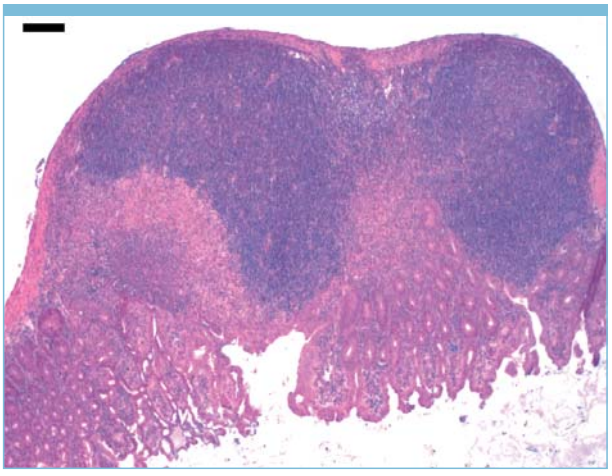
03.7 Biology of the Host Defense

PROJECT LEADER | **Dr. Werner Müller | Department of Experimental Immunology | wmueller@gbf.de**

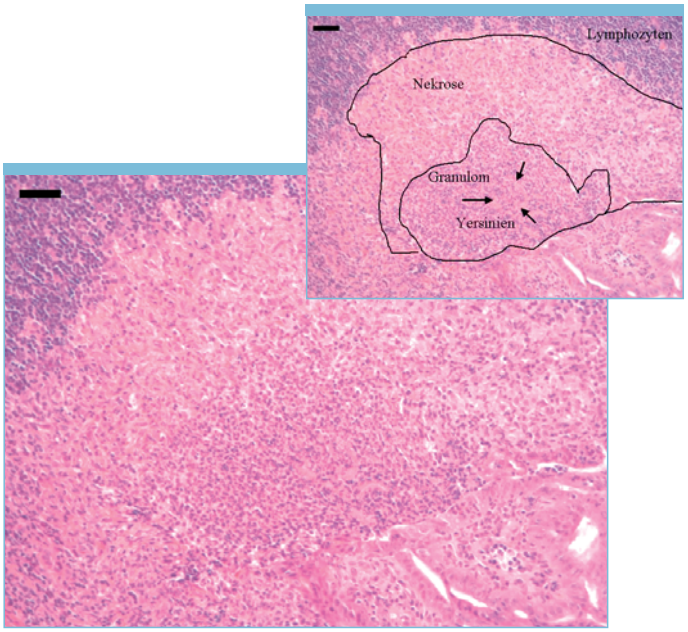
PROJECT MEMBERS | Dr. Mariela R. Bollati Fogolin | Nicolas Fasnacht | Anne Fleige | Dr. Ursula Frischmann | Marina Greweling | Dr. Martin Hafner | Annika Kochut | Karina Nawrath | Dr. Stephan Paxian | Ida Retter | Gudrun Schatt | Dr. Angela Schippers

Our immune system has many functions. In particular, it is protecting us very efficiently from infections. However, the immune system is very complex and because of its complexity only part of it can be studied in cell culture. The immune system of the mouse is very similar to the human immune system, both in its functions and its genes. In the mouse we can specifically inactivate genes responsible for the development and function of the immune system. By this method, we are analysing genes responsible for the cytokine network and for the development of specialised lymphoid organs.

The gut-associated immune system Most of our immune system is associated with the gut. In the gut lumen, many bacteria help to digest food. On the other hand, the body must be protected from these same bacteria in order to prevent infections. The immune system has therefore evolved mechanisms to keep our bacteria flora in check. In case the immune system gets out of control, chronic inflammatory bowl diseases like *Morbus Crohn* and *Colitis ulcerosa* may develop.



- *The picture depicts a Peyer's patch from a mouse, infected with* Yersinia enterocolitica *(dark region on the left site of the picture). Peyer's patches are specialised lymphoid organs associated with the gut. These structures protect the organism from infections with gut bacteria. The actual bacteria present in the Peyer's patch are hard to see on the picture. The picture was taken by Prof. Gruber, University of Vetenary Medicine Hannover.*



- *The picture depicts a region of a Peyer's patch infected with* Yersinia enterocolitica. *The bacterial colony can be recognized as light red stained areas (indicated by arrows). The Yersinia colonies are surrounded by a granulomatous tissue. This tissue protects the body from the bacterial colonies. At the border of the granulamotous tissue an area of necrotic tissue is visible. Cells in this area died due to the bacterial infection. The area altered by the infection is surrounded by lymphocytes. The picture was taken by Prof. Gruber, University of Vetenary Medicine Hannover.*

In our department, we develop methods to generate mouse mutants for the analysis and function of the gut-associated immune system. For this, we are utilising a very complex method of conditional gene targeting. This method allows us to activate, or inactivate, genes in individual cell types. Using this method, we were able to show that Interleukin-10, when produced by T cells, will protect the organism from a chronic inflammatory bowel disease. Using the human pathogen *Yersinia enterocolitica* as an infection model, we will probe whether the genetic changes introduced into the mouse will lead to a disturbed host defence in the gut-associated immune system.

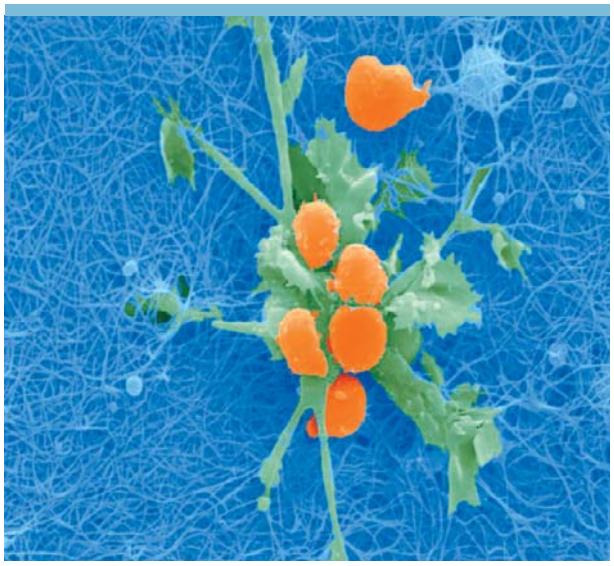
03.8 Imaging Cellular Dynamics of Immunological Processes

PROJECT LEADER | **Dr. Matthias Gunzer | Research Group Immunodynamics | mgu@gbf.de**

PROJECT MEMBERS | Bastian Dornbach | Mike Hasenberg | Anja Hillmer | Priyanka Narang | Dr. Peter Reichardt | Sarah Schröter

Immunity can be broadly divided into a humoral arm – mediated by soluble factors, such as antibodies or complement – and a cellular arm – mediated by whole cells, *e.g.* T cells, B cells or dendritic cells (DC). While humoral immunity is only indirectly observable, cells can be directly visualized while being "at work". Antigen presenting cells (APC) are at the beginning of every new cellular immune response. They reside in the periphery of the body, where they take up invading pathogens and transport them to draining lymph nodes to present peptide fragments (antigens) of pathogen to T cells. Although central to cellular immunity, this transportation process has not been observed directly, until now, and so far nothing is known about its *in vivo* dynamics. There are numerous situations where a defect in the generation of immune responses might be explained by disturbed DC migration. For example, immunotherapy of cancer tries to make use of DC as carriers of tumour antigens. But the major unsolved problem is what the optimum DC application in a patient might be without disturbing the inherent migration potential. Being able to visualize normal and defective DC migration *in vivo* would provide a useful tool for getting insights into this basic process, for optimizing protocols for vaccination programmes, or for understanding disease processes.

Another aspect of cellular immunity, which is currently being studied intensively, is the physical interaction of T cells with APC during antigen presentation. Most of the work underlying current theories for T-APC interaction has been performed *in vitro*. Only very recently imaging in explanted lymphatic tissue has shed light on the very dynamic migration processes going on in lymphatic tissue. Such studies may lead to an entirely new thinking of how T-cell activation is achieved *in vivo* and what goes wrong in the case of disease or lethal infection.



A dendritic cell engaging antigen specific T cells during the process of T-cell activation. The "spaghetti" structures around are fibres of artificial extracellular matrix consisting of 3-D-collagen fibres.

Photo: Dr. Rohde

High resolution images By using state of the art microscopy techniques, we visualize cellular immunity as it takes place within its natural environment. We want to get a comprehensive insight into the biophysical dynamics underlying cellular immune processes. Analysis of cellular dynamics within artificial extracellular matrices is a major tool for generating and testing our working hypotheses. In parallel, we have established imaging within explanted lymph nodes, as well as lymph nodes in living mice, by using time-lapse confocal and two-photon microscopy. The latter technique is able to generate high resolution images deep within vital tissue. To get a complete picture, imaging must be established both at sites of immune induction as well as immune intervention. To this end, a DFG-funded project was started recently to investigate the immune responses against an infection with the pathogenic fungus *Aspergillus fumigatus* in the lung of living animals. From this project, we hope to gain a better understanding of immune processes as they evolve, how they successfully function, and when or why they fail.

Topic 04 – Prevention and Therapy

TOPIC SPEAKER | **Prof. Dr. Dr. Carlos A. Guzmán | Research Group Vaccine Research | cag@gbf.de**

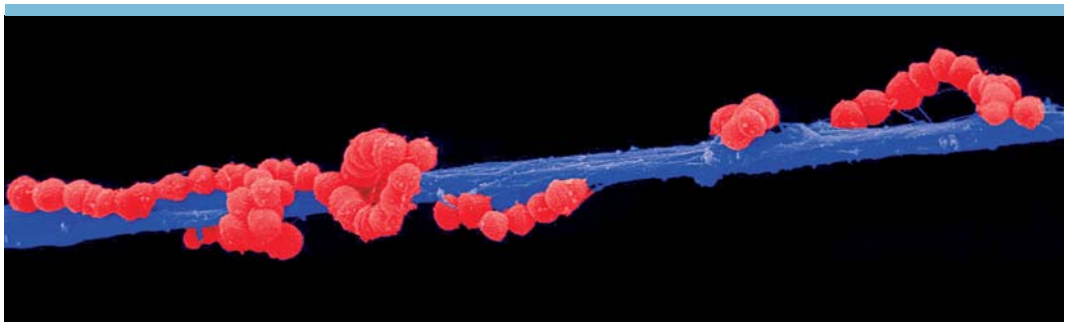
One third of all deaths occurring each year worldwide are directly caused by infectious agents. In addition, microorganisms are responsible for at least 15 % of new cancers and they are also involved in the pathogenesis of many chronic non-infectious diseases. The problems associated with infections are rendered more dramatic by the global emergence of multi-drug resistances. Thus, it is critical to develop new approaches to fight microbial pathogens. The main objective of this topic is to develop new tools and strategies to prevent and treat infectious diseases.

The anti-infective discovery project is focused on the identification and structure/function analysis of active compounds, as well as in the elucidation of their mechanisms of action. To this aim, microbial extracts and combinatorial chemical libraries are employed to search for small molecules with anti-infective activity. The structure and stereochemical properties of tubulysin, spirangien, cruentaren and elansolid were investigated. The antifungal metabolite cruentaren A turned out to be a new member of the salicylihalamide/apicularen family. Based on the structural information obtained for the new polyketide elansolid B, libraries of analogs will be generated. Two trifluorodiazirino-substituted epothilones were also synthesized, which retained their activity on tubulin polymerisation. In addition, a novel process was developed for the manufacturing of peptide/compound microarrays, which allows preparing thousands to millions of identical copies from only one parallel synthesis experiment. Systematic screening of peptide and small molecule libraries for binding to a large panel of proteins from proteomic studies is now feasible at very low cost.

In the antigen delivery systems and vaccines project, we focus on the development of new tools to optimize the delivery of antigens or DNA vaccines. These systems are then exploited to generate vaccine candidates against specific diseases. It was demonstrated that MALP-2, a synthetic agonist of the Toll-like receptor heterodimer 2/6, is an optimal mucosal adjuvant for HIV antigens. Additional work showed that MALP-2 promotes the activation and maturation of dendritic cells (DC), thereby increasing their stimulatory activity on naïve and antigen-specific T cells. Further studies established that bacterial ghosts constitute a promising delivery platform for targeting DNA vaccines to antigen presenting cells. Finally, the use of mice lacking the TGF-ß receptor in B cells demonstrated the critical role played by TGF-signaling in antigen-driven stimulation of secretory IgA responses after mucosal vaccination.

→

The therapeutic cellular vaccines project aims to develop DC-based vaccines to treat chronic diseases and tumors. To break immune escape mechanisms operating in chronic diseases, adenoviral vectors were constructed that code for tumor associated antigens and immunomodulatory molecules. Enhanced tumor-specific immune responses were observed after vaccination with adenovirally modified DC expressing HPV-E7 and co-stimulatory molecules of the TNF/TNF-receptor gene family. Additional work showed that 60 % of the genes affected by oncogenic transformation in different tumor cell lines are reverted after infection by IRF-1 expressing adenoviruses. Finally, cGMP-compliant production schemes were established for adenoviral vectors and modified DC, using an integrated bag and sterile docking system.



Electronmicroscopic photo from streptococci that are binding on collagen.

Photo: Dr. Rohde

04.1 Synthetic Combinatorial Molecular Repertoires

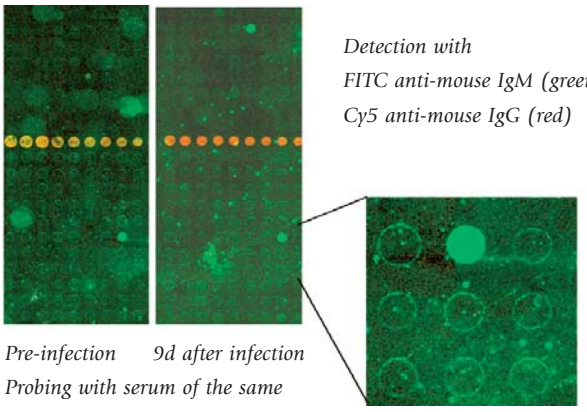
PROJECT LEADER | **Dr. Ronald Frank | Department of Chemical Biology | rfr@gbf.de**

PROJECT MEMBERS | Ulrike Beutling | Dr. Antonius Dikmans | Prof. Dr. Gerhard Höfle | Dr. Michael Morr | Dr. Jutta Niggemann | Dr. René Rübenhagen | Manuela Schüngerl | Dr. Werner Tegge | Valentina Wächter

An empirical search programme utilizing simultaneous and parallel chemical synthesis is carried out in this project as a complementary focus to the more classical natural product discovery programme at the GBF. The combinatorial synthesis and screening technologies for peptide and small molecule libraries that have been developed will continue to be applied in the systematic analysis and the selective inhibition of protein-protein-interactions involved in infectious processes. This will be further advanced and extended to search for new classes of compounds with antibiotic, chemotherapeutic and immunomodulatory activities.

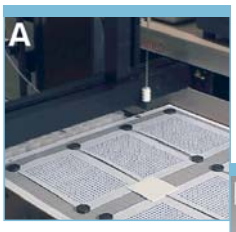
A new micro-array based screening tool One of our strong and original technology pillars is the miniaturized and highly parallel SPOT-synthesis on membrane supports. This method rapidly generates arrays of peptides and other synthetic compounds which are most advantageously utilized *in situ* as an immobilized array of probes to search for the selective binding of biological targets.
So far, one synthetic array has the dimension in the range of a microtiter plate. It requires some 10 ml of incubation medium with about 0.1 ml of sample volume. Re-using the array to analyse a series of different samples with the same compounds is cumbersome, as the stripping of bound proteins may not be complete. These drawbacks have now been overcome by employing a special membrane which can be solubilized after completion of all synthetic steps.

Solutions of compounds conjugated to soluble cellulose polymers obtained from individual spots can then be printed with an available contact-printing device (Micro-Grid II) in a further miniaturized format onto, for example, a microscope glass slide with up to 10,000 compound spots per 2x5 cm area. Only nanoliter volumes are required for this printing process. Thus, from one synthetic macro-array we can now easily produce millions of identical copies. Probing these micro-arrays also reduces the amount of sample needed by a factor of 100.

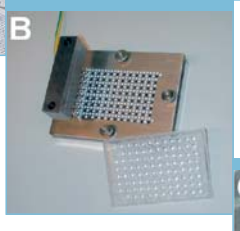


- *Peptide micro-arrays for serological screening: Immune profiling of mouse strains infected with* Y. enterocolytica, *a joint project with the department of Experimental Immunology (EI). Only microliter samples of blood can be taken from the mice during such studies. The figure shows a section of the whole array printed with three copies of 975 overlapping 15- mer peptides covering the amino acid sequences of the major virulence factors YadA, Invasin, YopE, YopH, YopM, YopP, YopT. The epitope peptide of mouse mAb 1D3 (IgG) is the control.*

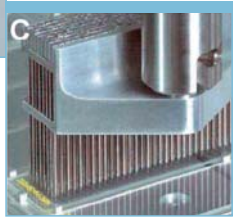
Photos: GBF



- *Synthesis of compounds on a cellulose solid support* e.g. *by the SPOT-method.*



- *Separation of the assembled solid supported compounds and distribution into microtiter plate wells; dissolving the cellulose-compound conjugates in a suitable solvent.*



- *Printing of multiple copies from the solutions onto* e.g. *glass slides.*

04.2 Biology of Microbial Bioactive Compounds

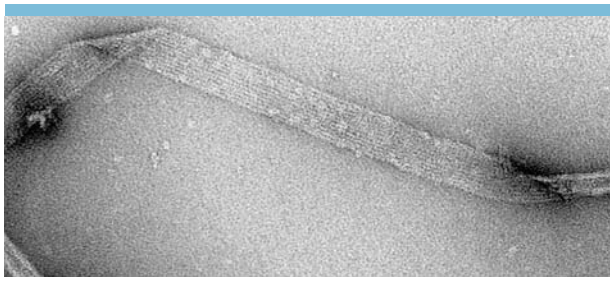
PROJECT LEADER | **Prof. Dr. Gerhard Höfle | Department of Natural Product Biology | gho@gbf.de**

PROJECT MEMBERS | Prof. Dr. Ursula Bilitewski | Ulrike Demuth | Dr. Yasser A. Elnakady | Dr. Meike Genrich | Dr. Klaus Gerth | Dr. Herbert Irschik | Sabine Kadow | Dr. Brigitte Kunze | Frank Lorenz | Bianca Lüderitz | Dr. Florenz Sasse | Dr. Jens Schumacher | Janine Wendler | Dr. Manfred Rohde

The search for novel natural products with biological activity in bacterial, fungal and mammalian cells based on our growing collection of myxobacteria and other gliding bacteria was continued. Thus, we succeeded in finding a new group of compounds, the elansolids, from a *Flexibacter spec.*, a gliding bacterium that does not belong to the myxobacteria.
An increasingly important group of myxobacterial compounds are those that interfere with tubulin polymerisation. Their mechanism of action was further investigated in greater detail. Preclinical development of tubulysin and disorazol was pursued in collaboration with industrial partners, while phase III clinical trials of the semisynthetic epothilone B-lactam started by Bristol-Myers Squibb are continuing.

Structure of epothilone-induced tubulin polymers

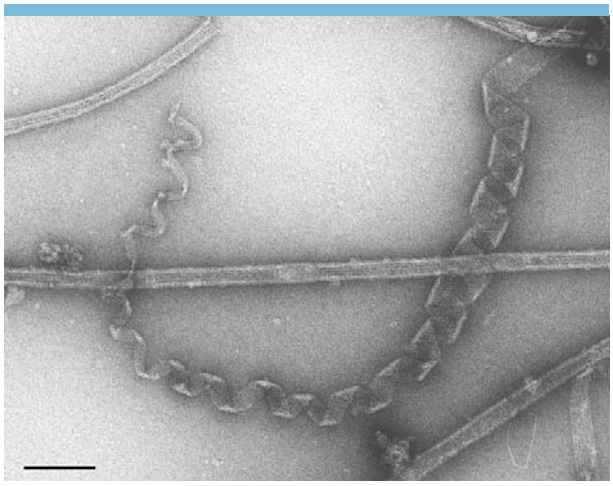
Epothilone interferes with the tubulin polymerisation of higher cells in a similar way as paclitaxel. During mitosis, epothilone and paclitaxel induce the formation of multiple asters instead of a normal bipolar mitotic spindle. At higher concentrations, microtubule bundles are formed in interphase cells. Whereas epothilone B and paclitaxel induce or accelerate polymerisation of purified tubulin *in vitro* at similar concentrations, epothilone B is 100 times more cytotoxic in cell cultures.
Electron microscopic investigations showed that epothilone induces short, sheet-like tubulin polymers when added to tubulin preparations. When epothilone B was added to an equilibrium mixture of tubulin and microtubules, we observed formation of microtubules that at one end continued as long ribbons which show protofilaments running in parallel (Fig. 1).



- *Transition of a microtubule to a straight running ribbon of 11 parallel protofilaments.*

Photo: GBF

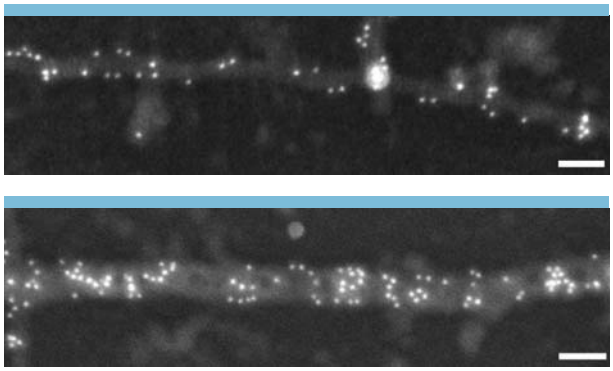
The ends of the ribbons formed left-handed helices (Fig. 2)- while the number of protofilaments is gradually decreased to a number of three. Helical ribbons were only observed with epothilone B, not with paclitaxel.



- *The ends of the epothilone-induced ribbons form curles, the number of protofilaments is decreasing. Scale bar 100 nm.*

Photo: GBF

Investigations on the polymeric tubulin structures within the cells using gold labelled anti-α-tubulin antibodies, showed that under the influence of epothilone B the cells also contain broader sheet-like tubulin polymers (Fig. 3). These run straight and are not curled.



- *In epothilone treated cells we observe normal microtubules which were labelled by gold particles (top). But we also find broader tubulin filaments (bottom) that seem to have a sheet-like structures. Scale bar 100 nm.*

Photo: GBF

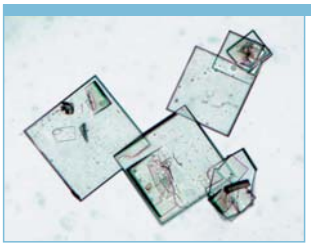
04.3 Chemistry of Microbial Bioactive Compounds

PROJECT LEADER | **Prof. Dr. Gerhard Höfle | Department of Natural Product Biology | gho@gbf.de**

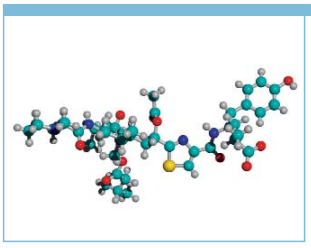
PROJECT MEMBERS | Dr. Nicole Glaser | Dr. Rolf Jansen | Dr. Dimitry Kashin | Dr. Jutta Niggemann | Heinrich Steinmetz | Dr. Larissa Vollbrecht | Dr. Peter Washausen

In the period covered by this report, work focused on our collection of myxobacterial metabolites which have been isolated over the past 25 years. Structure and stereochemical details of tubulysin, spirangien and two new metabolites, cruentaren and elansolid were investigated. Two trifluorodiazirino-substituted epothilones were synthesized which retained their activity for induction of tubulin polymerisation. According to MALDI-MS, they label covalently tubulin by irradiation at 365 nm.

Tubulysin After the structure and absolute configuration of this highly cytotoxic tubulin inhibitor had been elucidated by chemical means, crystals were obtained for the first time and an X-ray structure analysis could be performed which confirming the earlier results. NMR conformational analysis indicates that the solution conformation of tubulysin A is close to that in the crystal.

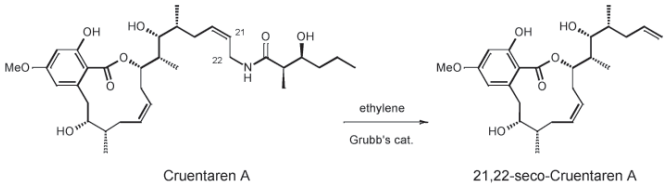


Crystals of Tubulysin A.



Crystal structure of Tubulysin A (E. Herdtweck, TU Munich)

Cruentaren A The highly cytotoxic and antifungal metabolite of *Byssophaga cruenta* turned out to be a new member of the salicylihalamide/apicularen family characterised by a 12-membered lactone and *N*-acylallylamine side chain. Its absolute configuration was determined by chemical degradation, and stereoselective synthesis of building blocks for reference. Olefin cross-metathesis with ethylene smoothly cleaved the side-chain double bond to give 21,22-seco-cruentaren A which crystallised. X-ray crystal structure analysis confirmed the absolute configuration established before.



Elansolid B Elansolid B is a novel polyketide isolated from a *Flexibacter* strain. According to ^{13}C labelling and biosynthetic considerations its unique bicyclic core structure is formed by a Diels-Alder reaction. The benzylic lactone is easily cleaved by base-induced fragmentation followed by addition of various nucleophiles to the intermediary quinone methide. Obviously, this reaction in combination with others may be applied for the generation of a library of elansolid analogs.

04.4 Antigen Delivery Systems and Vaccines

PROJECT LEADER | **Prof. Dr. Dr. Carlos A. Guzmán | Research Group Vaccine Research | cag@gbf.de**

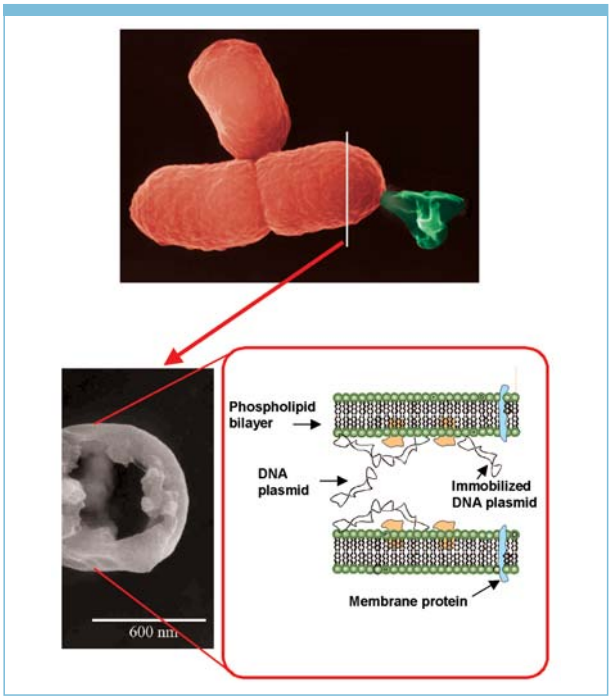
PROJECT MEMBERS | Pablo D. Becker | Dr. Stefan Borsutzky | Dr. Dunja Bruder | Prof. Dr. Jan Buer | Silvia I. Cazorla | Dr. Thomas Ebensen | Sandra Felk | Dr. Claudia Link | Dr. Faiza Rharbaoui | Dr. Kai Schulze | Dr. Astrid M. Westendorf | Beata Zygmunt

Vaccination is the most cost-effective strategy to prevent microbial infections. The main aim of this project is the development of tools to optimize the delivery of antigens or eukaryotic expression vectors, and their subsequent exploitation for the generation of vaccine candidates against specific diseases. Since mucosal territories are the main portals of entry for infectious agents, the optimization of mucosal vaccine formulations constitutes a major research focus.

Efficient delivery of the HIV-1 Tat protein A major goal of HIV/AIDS research is the development of a mucosal vaccine able to stimulate humoral and cell-mediated immunity, both at systemic and mucosal levels. This would allow the blocking of virus replication at the site of entry. Thus, a vaccine prototype was developed, based on the HIV-1 Tat protein as antigen and a synthetic derivative of the macrophage-activating lipopeptide MALP-2 as mucosal adjuvant. Intranasal administration to mice resulted in the stimulation of strong humoral and cellular anti-Tat responses, not only at systemic, but also at mucosal levels. These results suggest that MALP-2 represents an optimal mucosal adjuvant for HIV vaccines. Functional studies also demonstrated that MALP-2 stimulates the activation and maturation of dendritic cells (DC), thereby increasing their T cell stimulatory activities.

TGF-ß receptor signaling is critical for mucosal IgA responses We investigated adaptive immune responses in mice lacking the TGF-ß receptor in B cells (TßRII-B) after mucosal immunization using adjuvants acting on different molecular targets, namely the cholera toxin B subunit and MALP-2. Strong antigen-specific cellular and humoral responses were triggered in controls and TßRII-B mice. However, a significant reduction in antigen-specific IgG2b and increased levels of IgG3 were observed in TßRII-B mice. Antigen-specific IgA secreting cells, serum IgA and secretory IgA were also undetectable. These results demonstrate the critical role played by TGF-signaling in antigen-driven stimulation of secretory IgA responses and IgG class switch.

Bacterial ghosts Mass implementation of DNA vaccines is hindered by the requirement of high plasmid dosages and poor immunogenicity. We have evaluated bacterial ghosts as delivery systems for DNA vaccines. Bacterial ghosts are a non-living vaccination platform based on the conditional expression of the lethal lysis gene *E* from bacteriophage PhiX174 in Gram-negative bacteria. This leads to the formation of a trans-membrane tunnel through the bacterial envelope, through which the cytoplasm content is expelled. Plasmid-loaded bacterial ghosts were efficiently taken up by macrophages and DC, thereby leading to 52-60 % transfection rates. They target the DNA vaccine to APC and act as natural adjuvants, by providing a strong danger signal. Immunization studies demonstrated that ghost-mediated delivery of DNA vaccines stimulates more efficient immune responses than naked DNA. Vaccination with DC loaded *ex vivo* with plasmid-containing ghosts also resulted in strong antibody and CD8+ T-cell responses. Thus, bacterial ghosts constitute a promising technology platform for the development of more efficient DNA vaccines.



Bacterial ghost-mediated delivery of DNA vaccines. Scanning electron micrograph of a bacterial ghost generated by conditional expression of the gene E. A cross section reveals the ghost envelope complex, to which the loaded plasmid DNA remains associated.

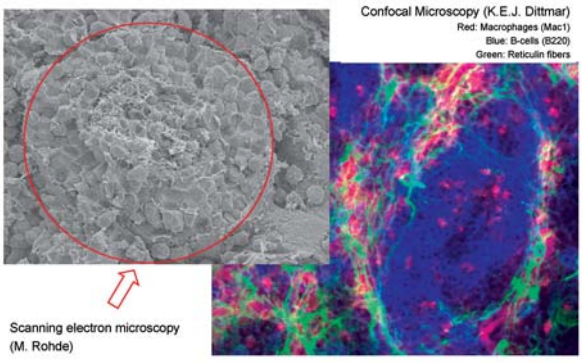
04.5 Therapeutic Cellular Vaccines

PROJECT LEADER | **Dr. Werner Lindenmaier | Department of Gene Regulation and Differentiation | wli@gbf.de**

PROJECT MEMBERS | Bin Ma | Dr. Kurt E. J. Dittmar | Dr. Andrea Kröger | Lars Macke | Wilhelm Meyring | Dr. Ute Pägelow | Carsten Wiethe

In cancer and in many persistent infections an effective antigen specific T-cell response is important to control the disease. One of the basic problems for immunotherapy of these diseases is that, despite an existing immune response, a therapeutic effect is difficult to achieve because of tolerogenic, anergy-inducing responses of the target and other immune escape mechanisms. In this context, the present project aims at a deeper understanding and further improvement of immuno-stimulatory functions of dendritic cells (DCs). We studied their interactions with effector cells and investigated the functional effects of genetic modification of antigen presenting cells. We also implemented prerequisites for the production of a therapeutic cellular vaccine.

Imaging of interactions Specific imaging techniques were developed to investigate the interactions of different cell types in lymphoid tissues, which determine the outcome of the immune response. Detailed structural information was gained by electron microscopy, whereas confocal microscopy was used to follow cell migration and dynamic interactions *in vitro*. Furthermore, the influence of infection and genetic modification on cell interactions in lymphoid organs was visualized in cooperation with other GBF research units.

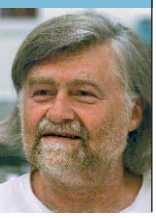


- *B-cell follicles in retroauricular lymph nodes.*

Modulation of the immune response For the modulation of the immune response, we constructed adenoviral vectors encoding tumour associated antigens, immuno-modulatory molecules and reporter genes. They allowed efficient transfer in primary cells and regulated expression of multiple genes. Analysis of several factors of modified human DCs confirmed that adenoviral gene transfer did not induce significant unspecific effects.
Adenoviral transfer of immuno-modulatory genes, however, allowed specific modification of DC properties. Functional properties of modified cells were monitored using murine model systems: the immune response to a tumour antigen (E7) encoded by a human papillomavirus was analysed. Enhancement of the E7-specific immune response was demonstrated after vaccination with adenovirally modified DCs.

Murine tumour model In a transplantable liver tumour model, activation of the transcriptional activator IRF-1 leads to the inhibition of tumour growth and induction of a specific immune response. To investigate this effect in other tumour systems an adenovirus expressing IRF-1 was established. Infection of different tumour cell lines with it showed the typical IRF-1 mediated phenotypes: inhibition of cell proliferation, increased MHC I expression, IFN-β secretion and reversion of the transformed phenotype. 60 % of the genes affected by oncogenic transformation are reverted as a consequence of IRF-1 activation.

GMP for recombinant adenoviral vectors and dendritic cells Adenovirally modified DCs are promising agents for immunotherapy of cancer and persistent infections. Translation of basic research into clinical trials and therapeutic application requires the development of cGMP-compliant production schemes for adenoviral vectors and modified cells. For safe and reproducible cGMP-compliant production of genetically modified DCs an integrated bag system is under development.



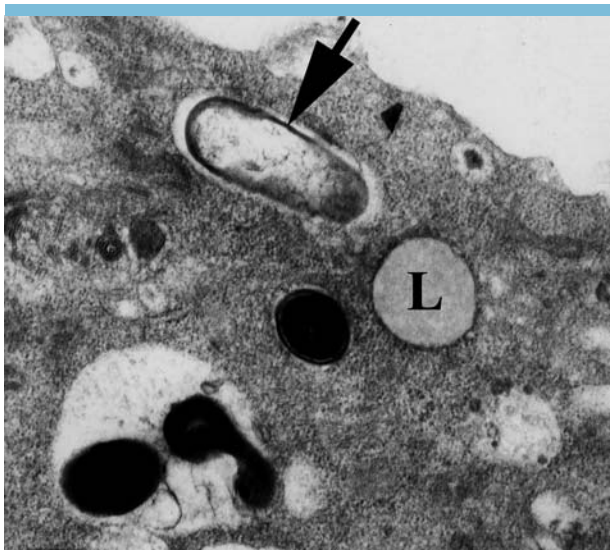
04.6 DNA Vaccination and Immune Modulation

PROJECT LEADER | **Dr. Siegfried Weiss | Research Group Molecular Immunology | siw@gbf.de**

PROJECT MEMBERS | Heike Bauer | Christian Becker | Anne Endmann | Jadwiga Jablonska | Stefan Lienenklaus | Holger Loessner | Christofer Samuelsson | Katrin Westfal | Andrea Zellmer

The use of bacteria as a transfer vehicle for eukaryotic expression plasmids into mammalian host cells is well accepted by now. We have used two species of bacteria as transfer vehicles. First, *Salmonella typhimurium* was used to administer DNA vaccines orally to mice. Secondly, *Lysteria monocytogenes* was studied as a vector for the delivery of therapeutic DNA.

Salmonella typhimurium In the past, this way of vaccination was often problematic because the original high copy plasmid was frequently unstable in culture, as well as in the mouse. We replaced the high copy number origin of replication (ori) by low copy number oris. This completely stabilized the Salmonella transformants *in vitro* and *in vivo*. More importantly, this led to a significant improvement of the immune responses after oral application. The newly developed plasmids will be the basis for further improvements of the oral DNA vaccination system.



- *Electronmicrograph of a host cell infected with plasmid carrying* Lysteria monocytogenes *and subsequently treated with ampicillin to initiate DNA transfer. After 24 hrs many bacteria are already lysed and either carcasses are observed (arrowhead) or bacteria which appear to empty their content into the cytosol. Some bacteria are still surrounded by membranes (arrowhead) although usually bacteria escape from phagocytic vacuoles within the first 4 hrs. Lysosomes (L) are fusing with the bacteria containing vacuoles. N (nucleus).*

Photo: GBF

Lysteria monocytogenes Here it became clear that high numbers of bacteria are required to achieve significant transfer rates. Rapid lysis of the bacteria and release of the bacterial content could be demonstrated by electron microscopy, but only extremely few plasmids apparently are transferred. Despite this, high numbers of plasmids could be recovered from the host cells after DNA transfer. These plasmids are associated with high molecular weight components of the carrier bacteria or the host cells. Further detailed molecular analysis of these complexes should allow a rational design of improvements for the DNA transfer using *Listeria* as a carrier.

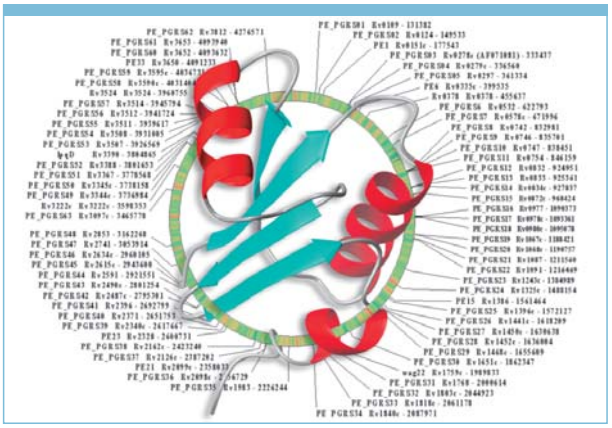
Bacteria-host interactions As an extension, bacteria-host interactions were studied. We investigated early events taking place in mice after infection with *Lysteria monocytogenes*. We could show that the inflammatory chemokine CCL2 dominates this phase. Interestingly, depletion of CCL2 resulted in a decreased bacterial load and immune histology revealed that the arrangement of the cells of the spleen was significantly altered. The normally observed composition of macrophage clusters is changed in such a way that now granulocytes can enter the clusters. Therefore, induction of CCL2 in the spleen of mice appears to be an immune escape mechanism of *Lysteria monocytogenes.*

IFNβ – An immune regulator Similarly, we are studying the biology of IFNβ. Using a recombinant mouse that is unable to express IFNβ, we could show that this cytokine has an important immune defence function. Thus, when mice were infected nasally with group A streptococci, a higher bacterial load was observed in IFNβ-deficient mice compared to normal mice. In contrast, when the mutant mice were exposed to Dextran sulfate to induce experimental colitis, the inflammation of the gut was significantly reduced. Thus, in this case the lack of IFNβ ameliorates the disease.
These two examples demonstrate the dichotomy of effects that is observed for this cytokine. The future goal of these studies will be to define the cells that are responsible for the production of IFNβ. To this end, a new recombinant mouse – based on a conditional knock-out mutant – has been established to facilitate the definition of the IFNβ producer cells.

Programme "Comparative Genome Research"

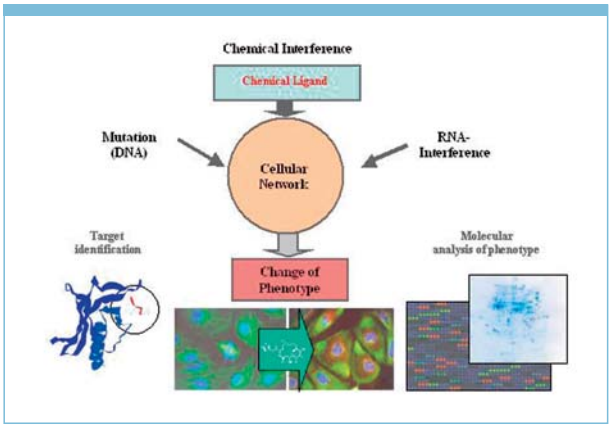
PROGRAMME SPEAKER | Dr. Helmut Blöcker | Department of Genome Analysis | hbl@gbf.de

- Pathogenesis results from a complex interaction of genotypic and phenotypic traits, including inherited genetic defects or dispositions, and factors such as age, lifestyle, host-pathogen interactions and environmental stress. The comparative analysis of genome information is an essential element in studying genotype–phenotype relationships for both prognostic and diagnostic aspects in health care. In addition, the role of individual genes within the cell and their interactions in cell complexes and networks, for example tissues, as well as their epigenetic, translational and post-translational regulation, still remain to be elucidated. Comparative genome research can combine model-driven experimental approaches with information-driven computational and theory-based data interpretation. Thus, this research programme combines the experimental functional characterization of genomes with comprehensive genome-based bioinformatics.



- *Novel drug and vaccine targets for combating TB: Genomic arrangement of a unique gene family (PE-PGRS) of Mycobacterium tuberculois.*

Collage: Klimek



- *Three complementary systematic approaches for the functional genome analysis.*

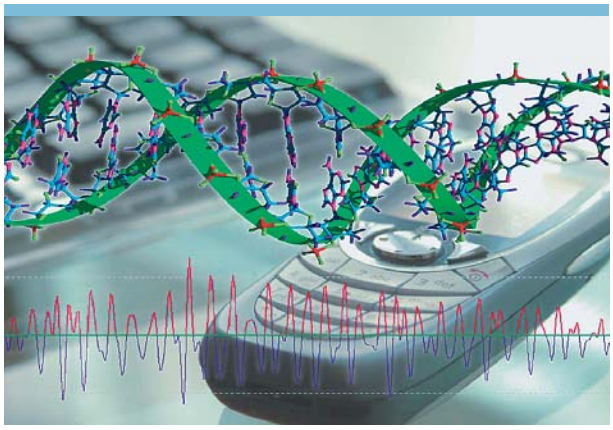
01 Generation and Exploitation of DNA Sequence Data

PROJECT LEADER | **Dr. Helmut Blöcker | Department of Genome Analysis | hbl@gbf.de**

PROJECT MEMBERS | Dr. Michael Böcher | Igor Deyneko | Frank Gößling | Michael Jarek | Yulia Kalybeava | Dr. Rosa Martínez | Bernard Neelen | Dr. Gabriele Nordsiek | Rosalila Peneido | Maren Scharfe | Dr. Oliver Schön | Prof. Dr. Mahavir Singh | Dr. Matthias Stehr | Harold Stiege | Dr. Maoyuang Yang

This project has a focus on the genome-wide study and in-depth analysis of genomic information. This involves high-throughput sequencing of DNA, as well as annotation, up to the level of metabolic and regulatory pathways. More than 50 percent of the department's activities are devoted to bioinformatical work. Future work also will include the comparative sequence analysis of selected genes in pathogens and patient cohorts, as well as of the methylation profile of selected genomic regions. As an adjunct to these activities further technology developments will be pursued.

Sequence analysis projects DNA sequencing is one of the key technologies in modern biological research and application. Comparative sequencing – resequencing – is the method of choice, once a reference genome is available. Accordingly, our future work will include the comparative sequence analysis of clinical isolates from pathogenic organisms such as *M. tuberculosis* with special emphasis on genes involved in virulence, persistence, antibiotic resistance and host preference. We shall also determine sequence polymorphisms in host genes and methylation profiles from selected genomic regions in patient cohorts and study their associations with disease susceptibility or resistance and pathogenicity.
Recently, we published the chimpanzee chromosome 22 sequence. We will now turn to sequencing and functional analysis on chimpanzee chromosome X. We are about to analyse selected regions from the horse, pig and cattle genomes, most of which are suspected to be disease-related. We have begun to analyse bacterial communities in the gut of mice. In a pilot project, we have taken measures to establish the infrastructure for large-scale methylation pattern analysis of entire chromosomes and comparative analysis of methylation patterns. After the completion of the sequencing phase of the analyses of the genomes of *Sorangium cellulosum*, *Bordetella petrii* and *E. coli* Nissle 1917, we are now working in different consortia to deep annotate these genomes.



Artwork representing the project of adapting principles of speech recognition to DNA sequence analysis.

Photo and Collage: Klimek

In 2004, a new activity has been initiated for performing comparative genome analysis of pathogenic mycobacteria, which cause major human and animal diseases like Tuberculosis, Paratuberculosis and Leprosy. The objectives are to identify and characterise drug targets and virulence factors of mycobacteria.

New technology The general advantage of our novel bioinformatics technology lies in the fact that it is based on physico-chemical/signal theoretical properties, rather than on letter code similarities or frequencies. For example, experiments are under way towards a new classification of promoter sequences from *E. coli* and other organisms which may reveal new insights in gene activity categories. All implementations will run on low-cost hardware. We are determined to pursue the further development of the technology and apply it to the comparative analysis of proteins and DNA, the pattern recognition in complex images and – as a long term project – the modeling of infection processes in near real-time.

02 Ligand-Based Target Discovery

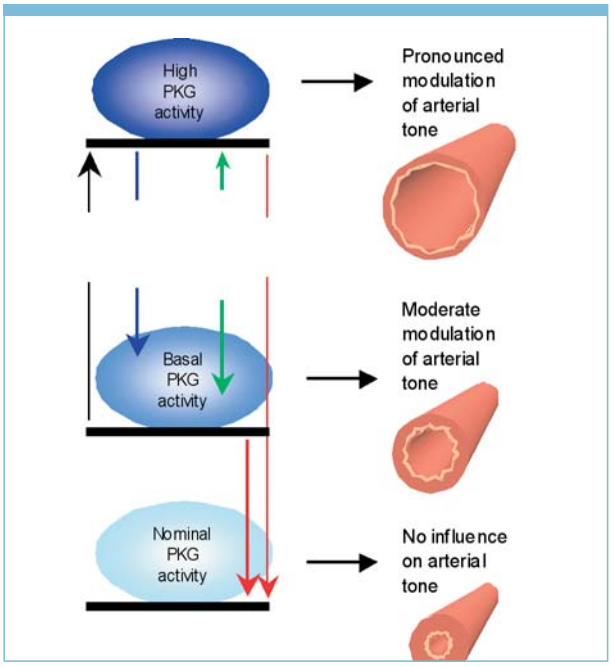
PROJECT LEADER | **Dr. Ronald Frank | Department of Chemical Biology | rfr@gbf.de**

PROJECT MEMBERS | Ulrike Beutling | Dr. Antonius Dikmans | Varsha Gupte | Dr. Jutta Niggemann | Dr. Rene Rübenhagen | Andrzej Swistowski | Dr. Werner Tegge

Both random and directed mutagenesis, as well as mRNA inactivation by antisense or RNAi methods, are experimental approaches of forward and reverse genetics to disturb the function of genes for the analysis of their phenotypic expression. An attractive and complementary new approach has been developed over the last years. This approach utilizes synthetic chemical compounds to act directly on the gene products – mostly proteins – as activating or inhibiting ligands. When applying diverse and competent synthetic compound repertoires, such a strategy of chemical interference is a global genomic and systematic approach.

Compound libraries The compound repertoires that we are applying primarily come from our combinatorial chemical synthesis and include peptides, peptidomimetics, as well as small organic compounds. Such libraries are prepared for internal screening projects. Supported by the "Nationales Genomforschungsnetz" (NGFN), the libraries also are applied in external projects of the Systematic Methodological Platforms (SMP) "Functional and Chemical Proteomics" and the "The Antibody Factory", as well as the disease-oriented genome networks. Thus, initiated by the GBF, we operate a central chemical synthesis unit for an NGFN Chemical Proteomics platform.

Membrane permeable specific inhibitors of cGMP-dependent protein kinase By screening peptide libraries, generated on SPOT membranes, a specific inhibitor of the cGMP-dependent protein kinase I ("alpha") was generated. Fusion of membrane permeable amino acid sequences to this peptide yielded highly potent and cell penetrating selective inhibitors. The sequence termed "DT-2" was used in its native and fluorescein-labelled form in experiments to elucidate the physiological functions of cGMP-dependent protein kinase (PKG) in the regulation of smooth muscle tone. Contrary to other known PKG inhibitors, DT-2 is able to lower the smooth muscle relaxing effect of the enzyme even below its basal activity. With DT-2 it is now possible for the first time to investigate the physiological function of this basal activity and thus obtain a better understanding of the regulation of smooth muscle tone. Currently, studies with mice are being carried out to corroborate the potential of these inhibitors to treat low blood pressure, an important condition, for example, in the therapy of systemic shock.



Model of vascular smooth muscle PKG activity and its impact on vascular tone. We propose that constitutive activity of PKG (basal PKG activity) exerts continuous modulation of vascular tone even at minimal cGMP levels. Increased cGMP further stimulates the kinase (high PKG activity), enhancing its modulatory influence. Available inhibitors, including KT-5823 (blue arrow) and the Rp-cGMPS derivatives (green arrow) such as Rp-8-pCPT-cGMPS and Rp-8-Br-PET-cGMPS reverse cGMP stimulated PKG activity to varying degrees, but only toward basal levels. Rp-cGMPS derivatives may also yield partial kinase stimulation as indicated by the double-headed arrow. DT-2 (red arrow), on the other hand, inhibits not only the cGMP-stimulated PKG activity but also a substantial portion of the basal PKG activity, essentially abolishing vasoregulation by the kinase.

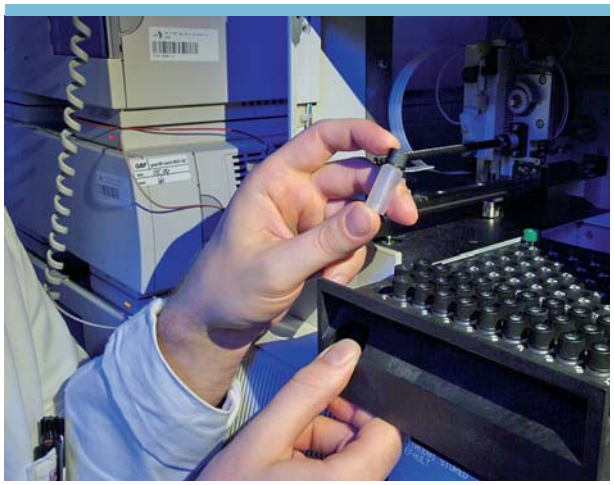
03 Conformational Protein-Ligand Interactions

PROJECT LEADER | **Priv.-Doz. Dr. Jutta Eichler | Research Group Conformational Protein-Ligand Interactions | jei@gbf.de**

PROJECT MEMBERS | Numan Akyol | Dr. Christian Doll | Raimo Franke | Cornelia Hunke | Dr. Ulf Strijowski | Enge Sudarman

The interactions of proteins with each other, or with other ligand molecules, are the molecular basis of all protein-mediated biological processes. The design and generation of synthetic molecules represents a promising strategy for the modulation of protein function through controlled interference with the underlying binding events – when they are capable of mimicking the binding and functional sites of proteins.

The binding sites of numerous biomedically relevant proteins are not located in continuous, consecutive stretches of the amino acid sequence. They are built into parts of the protein that are distant in the primary amino acid sequence and brought into spatial proximity by protein folding. The overall objective of our research is to develop and implement a general concept for the synthetic mimicry of such sequentially discontinuous protein binding sites.



Loading the autosampler of a LC/MS instrument for the analysis of synthetic peptides.

Photo: Bierstedt

Inhibitors of Protein-Ligand Interactions The synthesis concept for such inhibitors involves "rational design", which is based on the known structure of the binding site, in conjunction with the use of scaffolded or assembled peptides. In such peptides, protein-derived fragments making up the binding site are presented through a molecular scaffold in a nonlinear and discontinuous fashion. The repertoire of synthetic methods developed so far enables the synthesis of structurally diverse scaffold molecules with varying degrees of conformational flexibility.

Using the established synthesis methods, we were able to design and generate mimetics of the binding sites of several proteins. Our aim was to develop inhibitors of the interactions between them and their respective ligands. Synthetic mimetics of the binding sites of the Mena EVH1 domain and of gp120 of HIV-1, respectively, have already been shown to inhibit the interactions of these proteins with proline-rich peptide ligands and the T-cell receptor CD4, respectively. Additional projects focus on inhibiting the interaction of E-cadherin on epithelial cells with internalin A of *L. monocytogenes*, as well as the interaction of viral interleukin-6 with the receptor gp130.

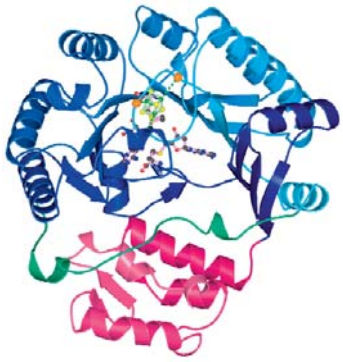
04 Comparative Structural Analysis of Metabolic Pathways

PROJECT LEADER | **Prof. Dr. Dirk Heinz | Department of Structural Biology | dih@gbf.de**

PROJECT MEMBERS | Isabel Astner | Dr. Hans-Jürgen Hecht | Dr. Wolf-Dieter Schubert | Jörg Schulze

Far-reaching similarities between human and bacterial metabolism support the Darwinian hypothesis of a common evolutionary origin of all forms of life. Studies into bacterial biochemistry reveal a simplified version of more complex human metabolic pathways. Specific differences often provide unique opportunities of exploiting bacterial vulnerabilities by developing antibiotics with potentially minimal side-effects for human patients.

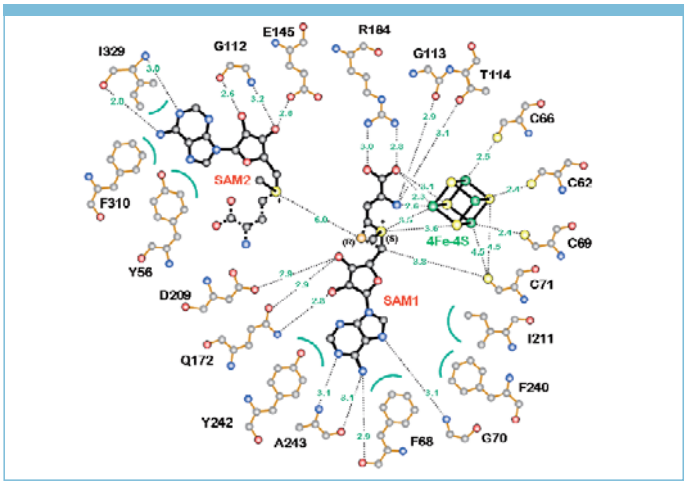
Enzymes of heme biosynthesis Heme and other tetrapyrroles, such as (bacterio-) chlorophylls and vitamin B12, are essential to all living cells. They constitute vital co-factors for numerous enzymes and participate in energy and electron transfer processes in photosynthesis and respiration. Another function unique to heme is to coordinate and transport oxygen and CO_2 in the blood of vertebrates.



The structure of HemN from E. coli. *The first structure of a radical SAM enzyme.*

In collaboration with the TU Braunschweig, we have solved the structure of oxygen-independent coproporphyrinogen III oxidase (HemN), an enzyme required by bacteria for heme-biosynthesis under anaerobic conditions. HemN catalyzes the prepenultimate step in heme biosynthesis. Prior to HemN, no structure of this family had been explained. HemN and other family members place a 4Fe-4S cluster and an S-adenosylmethionine (SAM) molecule right next to each other, so that the sulphur atom of SAM directly coordinates the fourth iron of the cluster. Previously, a similar arrangement of these co-factors had not been observed. The cluster, when reduced, transfers an electron onto SAM, breaking it up into a methionine and a 5′-adenosyl radical. The latter extracts a hydrogen atom from the substrate coproporphyrinogen III, converting it to the product protoporphyrinogen IX by the loss of a CO_2 group and an electron.
The structure of HemN also revealed that the enzyme binds two SAM molecules. This makes sense, because HemN actually needs to perform two oxidative decarboxylation reactions on two separate side chains of coproporphyrinogen III to yield the final product.

Enzymes of molybdopterin biosynthesis Molybdenum enzymes are important for diverse metabolic processes, such as sulphur detoxification and purine catabolism in mammals, as well as nitrogen assimilation and phytohormone synthesis in plants. Deficiency of the molybdenum co-factor biosynthesis in humans results in neurological abnormalities and early childhood death.
In collaboration with the TU Braunschweig, we investigated the structure of the G domain of the plant molybdopterin biosynthesis protein Cnx1. The high resolution crystal structure revealed adenylated molybdopterin bound to a mechanistically impaired mutant protein. This allowed the identification of adenylated molybdopterin as the reaction product of Cnx1G. The amino-terminal E domain of Cnx1 subsequently processes this in a magnesium-dependent reaction into an active molybdenum co-factor. X-ray anomalous dispersion identified copper bound to the molybdopterin dithiolate sulphurs. Together with the observed copper inhibition of Cnx1G activity this provides a molecular link between molybdenum and copper metabolism.



All interactions in the active site of HemN, showing the cofactor binding by the protein.

05 Modelling and Analysis of Cellular Networks

PROJECT LEADER | **Prof. Dr. An-Ping Zeng** | **Research Group Systems Biology** | **aze@gbf.de**

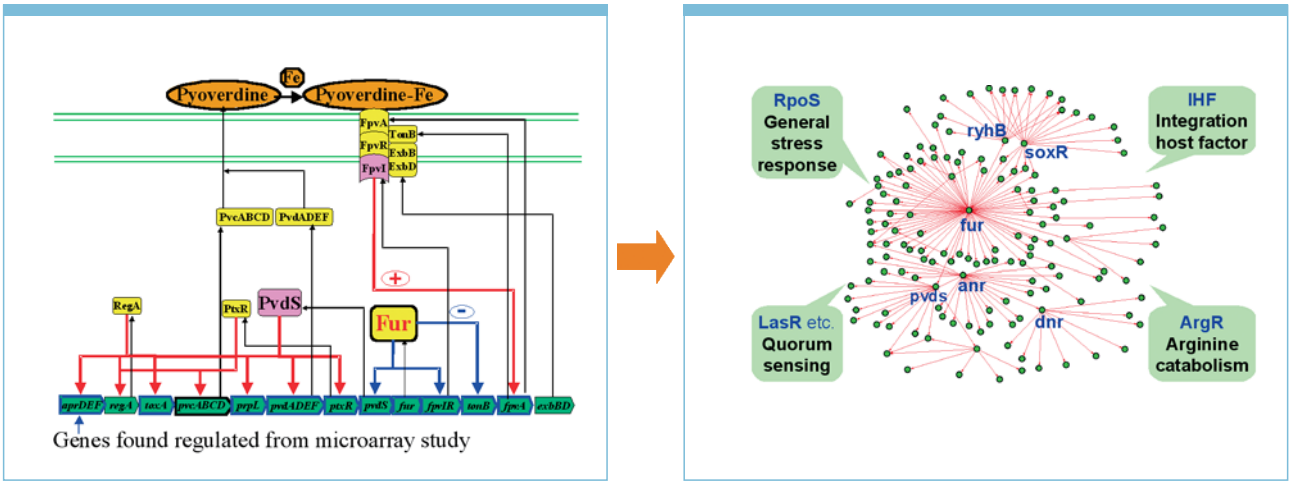
PROJECT MEMBERS | Eun-Jin Kim | Bharani Kumar | Dr. Hongwu Ma | Marcio Rosa da Silva | Dr. Jibin Sun | Ping Zheng

The research focus of this project is on the reconstruction, analysis and modelling of metabolic and regulatory networks of selected biological systems. We have approached the system-biology by integrating the use of genomic and functional genomic data, bioinformatic tools and mathematical modelling. To this end, we are developing new algorithms and methods for the reconstruction and analysis of metabolic and regulatory networks.

E. coli The algorithm IdentiCS developed last year by our group was applied to identify the coding sequences (CDSs) from raw genome sequences of the probiotic *E. coli* strain Nissle 1917 (O6:K5:H1) and to establish its potential metabolic network. The CDSs predicted for *E. coli* Nissle 1917 were compared with those of all five other sequenced *E. coli* strains published to date, leading to the identification of 108 CDSs unique for this isolate.
We were able to identify motifs and functional modules in transcriptional regulation networks of *E. coli* and yeast by a newly developed method. We found a hierarchical modular structure which is useful in identifying global regulators and for analysing micro-array data, especially for inferring the regulation mechanisms from expression profiling data.

Communication signal A comparative and phylogenetic analysis of 138 completed genomes was conducted regarding the synthesis and signal transduction pathways for autoinducer-2, which according to the literature has stipulated was supposed to be a universal signal for interspecies communication. It was found that the LuxS enzyme required for AI-2 synthesis is widespread in bacteria, while the periplasmic binding protein LuxP is only present in *Vibrio* strains.

"Intergenomics" Within the BMBF project "Intergenomics" we have studied the metabolic and regulatory networks involved in the formation of virulence factors and stress responses of *Pseudomonas aeruginosa* during lung infection. We focused on the availability of iron and oxygen and their interaction with the generation of virulence factors. Experimental studies, like pathophysiology, genetic perturbation or omics analysis, were combined with bioinformatic tools to establish the regulatory pathways and network involved and to understand the complexity of the interactions.



- *From pathways to gene network for iron regulation and stress response in* P. aeruginosa.

Programme "Sustainable Use of Landscapes"

PROGRAMME SPEAKER | **Prof. Dr. Kenneth N. Timmis | Department of Environmental Microbiology | kti@gbf.de**

Microorganisms are ubiquitous and, because they can tolerate environmental conditions far too extreme for higher organisms, their habitats define the biosphere. Microbial activities profoundly influence global processes, like the carbon cycle and global warming, and local ones, such as animal and plant disease. They also provide essential nutrients for plants and animals. Microbes have a critical impact on human beings and their activities, in a multitude of both positive and negative ways: some are responsible for the greater portion of human disease and mortality, whereas others provide us with antibiotics to treat disease, and yet others play a critical role in cleansing our environment of organic waste. Much of biotechnology is based on microbes and their products. Our ability to influence microbial activities – to obtain greater benefit from the positive aspects and diminish the effects of the negative repercussions – requires an understanding of how microbes live and function in their habitats, and how their activities are regulated.

Classical microbiology focuses on the study of pure cultures growing under laboratory conditions. However, microbes in nature grow as complex, diverse and dynamic communities, the members of which interact and share available resources in complex ways. It is these interactions, and interactions with other biotic and abiotic components of their environment, that determine community activities. At present, we have no general understanding of such interactions.

The goals of the Environmental Biotechnology research programme are to understand microbial communities as functional units, to elucidate the critical interactions that regulate community activities, to develop and validate interventions that result in substantive increases in activities of biotechnological interest, and to discover new microbial products and metabolic activities by exploring microbial diversity. The research programme is characterised by a multi-scale approach – gene, organism, community; test tube, chemostat, natural habitat – and a multi-disciplinary one – microbial ecology, physiology, phylogeny, biochemistry, analytical chemistry, genetics/genomics, bioinformatics, and modelling. Though the results obtained will be generally applicable to most types of microbial communities, our research focuses on microbial communities that either cause disease in humans or metabolise environmental pollutants. An important goal of the programme is to make key contributions to the sustainable development of our society.

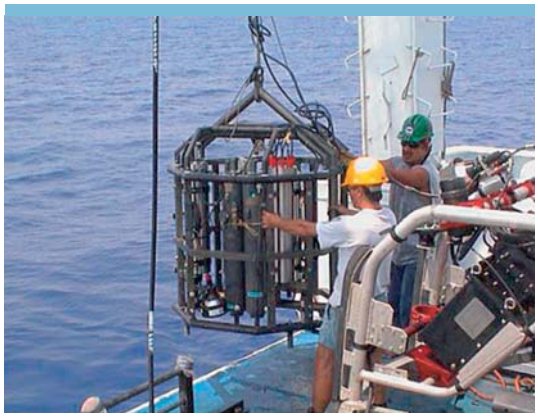
01 Functional Genomics and Niche Specificities

PROJECT LEADER | **Prof. Dr. Kenneth N. Timmis | Department of Environmental Microbiology | kti@gbf.de**

PROJECT MEMBERS | Angelika Arnscheidt | Dr. T. Chernikova | Christoph Gertler | Dr. Peter N. Golyshin | Olga Golyshina | Dr. Oleg R. Kotsyurbenko | Taras Nechitaylo | Julia Sabirova

Question central to understanding microbial diversity and exploitation of diverse niches, many of which are characterized by hostile conditions is: what are the ecophysiological mechanisms of habitat specificity, and what is the underlying basis of metabolic diversity?

Mining environmental genomes Environmental genomes of the microbial communities have been studied in two environments: the deep-sea hypersaline anoxic basins (DHABs) of the Mediterranean Sea and the bovine rumen. DHABs represent isolated extreme environments that may harbour uncharacterized forms of biodiversity. We retrieved five distinct types of enzymes. One – which exhibits no sequence homology to enzymes in any database – is a new, bi-functional, multi-catalytic site enzyme. It is tolerant of a wide range of salinities, hydrostatic pressure, polar solvents and reducing conditions. Majority rumen enzymes exhibited little similarity to known classes of enzymes and showed a number of unusual, and thus far unreported, properties, such as solvent resistance, substrate specificities, high specific activity to the number of substrates and in some cases high enantio-/regioselectivity.



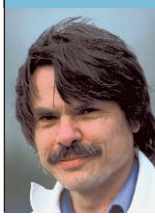
Sampling deep Mediterranean Sea.

Photo: GBF

A key factor for bacterial survival A new principle was experimentally evaluated for expression of heat-sensitive recombinant proteins in *E. coli* at temperatures close to 4°C. This principle was based on simultaneous expression of the target protein with chaperones (Cpn60 and Cpn10) from a psychrophilic bacterium, *Oleispira antarctica* RB8T, that allows *E. coli* to grow at high rates at 4°C. The expression of a temperature-sensitive esterase in this host at 4 to 10°C yielded enzyme activities that were almost 200-times higher than those obtained from the non-chaperon-producing *E. coli* strain grown at 37°C. We present evidence that the increased activity was due to the low growth temperature, which was beneficial to folding.

Anomaly of pH optima A wide range of acidophiles inhabit acidic environments and grow optimally at pH values between 0 and 3. The intracellular pH of these organisms is close to neutrality or slightly acidic. Enzymatic activities dedicated to extracellular functions would be adapted to the prevailing low pH of the environment, whereas intracellular enzymes would be optimally active at the near-neutral pH of the cytoplasm. Intracellular enzymes we have cloned from *Ferroplasma acidiphilum* function optimally and are stable in the pH range 1.7-4.0 and have pH optima up to 3 pH units lower than the intracellular pH of 5.6. This "anomaly" suggests the existence of as yet unknown selective forces operating in the cytoplasm.

Functional genomics of *Alcanivorax* An *in silico* analysis of the *Alcanivorax* genome has been undertaken to analyse cellular responses of this organism to pertinent environment factors. Multiple alkane oxidation gene clusters, multiple ammonium uptake systems, sodium-dependent transporters, compatible solute biosynthesis clusters, a low number of genes specifying signal transduction systems and the lack of nitrogen fixation pathways are consistent with an oligotrophic marine lifestyle, and explain the excellent hydrocarbon-degradation abilities and efficient nutrient scavenging capabilities of *Alcanivorax*.



02 Biofilm Communities in Environment and Health

PROJECT LEADER | **Dr. Wolf-Rainer Abraham | Research Group Chemical Microbiology | wab@gbf.de**

PROJECT MEMBERS | Elke Hase | Daniela Kobbe | Dr. Heinrich Lünsdorf | Alexandre Macedo | Sonja Pawelczyk | Jennifer Skerra | Esther Surges

Biofilm grown in an acidic mining lake in Lusatia. Note the minerals formed in the biofilms seen here as dark brown spots.

Photo: GBF

The goals of this project are to understand microbial communities as functional units and to discover new metabolic activities by exploring microbial diversity. Our research focuses on microbial communities that metabolise environmental pollutants and thus build the basis for bioremediation techniques.

Diversity of biofilm communities growing on PCB
The ability of microbial communities from non-polluted habitats to use xenobiotic compounds has been assessed and the microbial communities characterized. Soil samples have been taken from seven different sites in Germany. They were used as inocula for growing biofilms on polychlorinated biphenyls (PCB). We found that the microbial communities from six soil samples were able to form a biofilm and to degrade PCB congeners. The chemical analysis of the remaining PCB revealed small differences in the preferences of the biofilm communities for the individual PCB congeners. Surprisingly, the biofilm communities found on the PCB oil were both very diverse, displaying a high number of community members as well as being very different from the biofilm communities grown from the soil samples.

The results showed that there is a potential for microbes from non-polluted sites to use the highly hydrophobic substrate PCB. The ability to use this substrate is widespread in the bacterial world, resulting in very different microbial biofilm communities, which form the basis for enhancements by intrinsic bioremediation techniques.

Biofilms from acidic mining lakes The exposure of sulphides from open mining leads to their oxidation and the resulting sulphates are washed into the water filling the pits. These acidic mining lakes have a pH of 2.5 and often high concentrations of heavy metals. That makes them hostile to any higher organisms other than bacteria and fungi. To assess the potential of microbial communities for the neutralization of acidic mining lakes, biofilm formation on different substrata in one of these lakes has been investigated.
Wheat straw serving as a substrate for microbial communities and substrates serving only as support for the biofilms, like glass and the plastic Permanox™, have been exposed for several months to conditions in the lake. The resulting biofilm communities have been compared with biofilms on reed and birch leaves from the lake. Diverse biofilms of both bacteria and fungi were observed on the reed and wheat straw nutritive substrates. They were much denser and showed stronger mineral formation than those on the non-nutritive glass and Permanox™ substrata. The degradation of reed and wheat fibers seemed to require the interaction of fungi and bacteria because both groups occur together in these biofilms. This gives both fungi and bacteria a critical role in the bioremediation of these lakes.

03 Metabolic Diversity

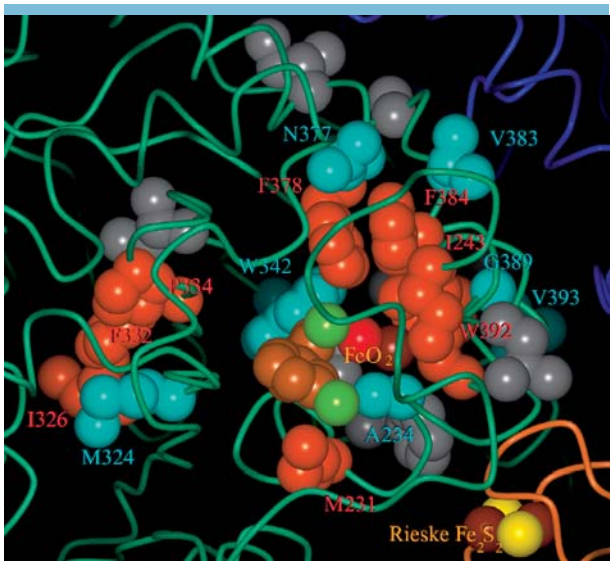
PROJECT LEADER | **Priv.-Doz. Dr. Dietmar Pieper | Research Group Biodegradation | dpi@gbf.de**

PROJECT MEMBERS | Hamdy Ali | Beatriz Camara | Carlos Cuel | Dr. Heike Gabriel-Jürgens | Dr. Bernd Hofer | Dr. Howard Junca | Anna-Maria Kicinska | Nguyen Ba Huu | Dr. Peter Rapp | Jens Schneider | Carsten Strömpl | Sabine Wittrock

The goal of the project is to quantify, predict and influence biodegradation processes under environmental conditions. To gain an understanding of the activities and adaptation abilities of bacterial communities *in situ*, a detailed analysis in pure cultures and model communities is performed and methods allowing analysis in complex systems are developed.

New biocatalysts Degradation of pollutants under environmental conditions through bioaugmentation is usually hampered by poor survival and *in situ* activity of the applied biocatalysts, or the poor bioavailability of pollutants. *Rhodococcus* sp. MS11, a psychrophilic bacterium can not only degrade an unexpectedly wide range of chlorinated and non-chlorinated hydrocarbons, including tetra-, tri-and dichlorobenzenes, as well as alkanes, but it also excretes biosurfactants during growth on n-alkanes. Strain MS11 may be well suited for bioremediation of soils and sediments contaminated with di-, tri- and tetrachlorobenzenes, as well as alkanes.

Tools to analyze community functions A detailed picture of the catabolic gene structure in environmental samples will increase our knowledge of the functional potential of microbial communities.
Diversity of catechol 2,3-dioxygenase (C23O) genes as key genes in aromatic hydrocarbon degradation was assessed by PCR-single-strand conformation polymorphism (PCR-SSCP). Site specific fingerprints were obtained, showing that gene diversity experienced shifts correlated to temporal changes and levels of contamination. To more rapidly determine diversity of functional genes, we developed an amplified functional DNA restriction analysis of C23O genes. This provided a quick assessment of functional gene diversity, insights into its gene phylogeny affiliation and determined the predominant polymorphism in environmental DNA extracts.
The above studies identified two predominant C23O sequences, differing only by a single amino acid. Even though the enzyme model does not suggest an influence of that variation on function, the proteins differed significantly in catalytic properties. This underscores the importance of enzyme function in amino acids that do not directly influence active site structures and proves the utility of recovering polymorphisms evolved and selected for special functions in natural ecosystems.



Structural model of a hydroxylase-substrate complex of biphenyl dioxygenase of LB400 in the region around the active site. A substrate molecule, 2,3'-dichlorobiphenyl, was placed into the active site cavity. Main chains of subunits are shown in wire type. a subunit A, green; a subunit B, brown; b subunit A, blue. The substrate molecule, the Rieske cluster, the mononuclear iron-dioxygen complex and side chains of AA residues that were replaced in this work are shown as CPK modules. Side chains are colored according to the effect of exchange upon substrate dioxygenation: (very) strong, orange; moderate, cyan; weak, grey. Substrate carbons, light brown; substrate chlorines, green; sulfur, yellow; iron, dark brown; oxygen, red.

Metabolic key enzymes Three regions of the biphenyl dioxygenase of *Burkholderia xenovorans* LB400 influence the interaction between enzymes and substrates. For a further discrimination within these regions, we investigated the effects of 23 individual amino acid exchanges. Eight residues influence the structure of the catalytic centre. Those lacking direct contact with the substrate had not been predicted to play an important role.
A three-dimensional structural model was constructed to characterize the active site of a glycosyltransferase. Kinetic characterization of directed mutants identified enzyme parts that are critical for substrate binding and turnover. This information was used to design schemes for random mutagenesis in order to obtain variants with altered specificities for the glycosyl donor.

04 Pathogen Ecology

PROJECT LEADER | **Priv.-Doz. Dr. Gerhard Höfle | Department of Environmental Microbiology | mho@gbf.de**

PROJECT MEMBERS | Dr. Ingrid Brettar | Dr. Stefan Eichler | Dr. Matthias Labrenz

The ecology of pathogens is a rather recent focus in microbial ecology. This new focus concentrates on environmental bacteria and viruses that cause infectious diseases and the ecological processes that control these pathogens. Natural or man-made environments are often important reservoirs of infectious bacteria. The ecological interactions with other microbes, or higher organisms, determine pathogen population levels and even virulence. The importance of such ecological interactions is underscored by the fact that some of the most effective measures taken to limit infectious diseases are not medical but environmental. An important example is the dramatic reduction of waterborne diseases due to wastewater treatment and the disinfection of drinking water.
The long-term aim of the project "Pathogen Ecology" is to assess and understand the distribution, activity, pathogenicity and reservoir size of major bacterial human pathogens in the environment which, in turn, would allow a prediction of threats to public health from these pathogens and help to promote measures against these threats.

The water reservoir of the Eckertalsperre in the Harz mountains.

Photo: Dr. Lange, Harzwasserwerke, Goslar, kind permission acknowledged

DNA-based detection The primary reason for our limited knowledge of bacterial pathogen ecologies is their unreliable detection by culture-based, conventional techniques. Almost all monitoring systems currently used in the public health sector are based on these conventional techniques. On the other hand, most pathogenic bacteria in the environment are in a so-called viable but non-culturable state and cannot be recovered with conventional procedures, although they are still infectious. Over the last decade, direct analysis of DNA extracted from environmental samples has been used to circumvent these limitations and detect bacteria using molecular methods without cultivation. The advent of genomics and DNA-chip technology will take this DNA-based detection technique one step further by allowing the assessment of activity and virulence of specifically targeted bacteria in the environment.

Waterborne pathogens A major human health hazard is the consumption of drinking water and bathing, if the water in question is contaminated with feces of human or animal origin.
As a first focus, a whole drinking water supply system (DWSS) was investigated from source to tap for its microflora, using a molecular fingerprint methodology. This particular DWSS provides most of the potable water for the city of Braunschweig and uses two large reservoirs in the Harz mountains as source water. The structure and composition of the bacterial microflora in both reservoirs was very different because their freshwater ecology differ substantially.
The first processing of the raw water by flocculation and sand filtration did not change the composition of the microflora. Major changes occurred, as expected, after chlorination. The drinking water from both reservoirs is mixed in a large storage container and delivered to Braunschweig through a roughly 40 km long pipeline system. Along the transport route no big changes in the drinking water microflora occurred and a rather stable bacterial community was detected in the tap water at the end of the supply system. This new molecular look at what we drink will help to optimize the processing of drinking water from natural raw water and help to maintain hygienic standards.

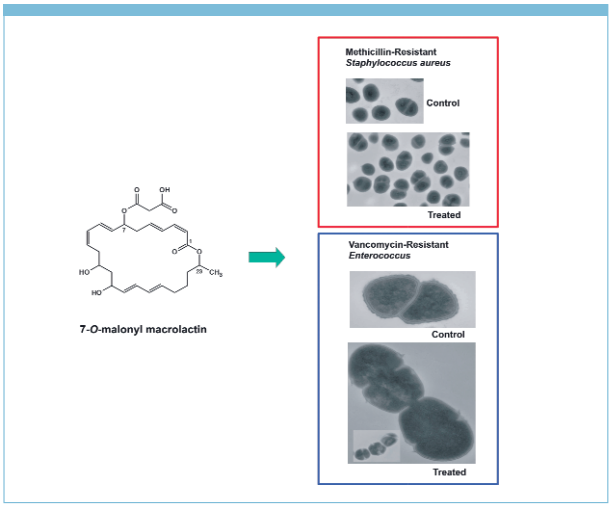
05 Microbial Diversity and Natural Products

PROJECT LEADER | **Prof. Dr. Kenneth N. Timmis** | **Department of Environmental Microbiology** | **kti@gbf.de**

PROJECT MEMBERS | Dr. Rolf Jansen | Dr. Gabriella Molinari | Magally Romero-Tabarez

There is a global threat of multi-drug resistant microorganisms, which renders increasingly difficult the clinical management of infected patients. The major objective of this project is to contribute to the solution of this problem by finding new drugs with novel mechanisms of action. To this aim, we are exploiting the huge untapped microbial diversity by screening environmental isolates from extreme environments for their production of bioactive compounds.

A new antibiotic active against multi-resistant bacteria A *Bacillus subtilis* strain isolated in Indonesia produced at least ten different compounds exhibiting antimicrobial activity, including three macrolactins: the previously characterized macrolactin A, 7-*O*-succinyl macrolactin A, and a new derivative, 7-*O*-malonyl macrolactin A. The C-7 substituted macrolactins showed higher antimicrobial activity and lower toxicity for mammalian cells than macrolactin A. The new 7-*O*-malonyl macrolactin A had high antibacterial activity against multi-resistant Gram-positive isolates, in particular methicillin-resistant *Staphylococus aureus* and vancomycin-resistant enterococci. Interestingly, it also inhibited slow growing small colony variants (SCV) of *Burkholderia cepacia* isolated from cystic fibrosis patients.



7-O-Macrolactin A and its effect on cell division.

New insights into drug action 7-*O*-malonyl macrolactin A is not bacteriocidal, but is strongly bacteriostatic, and not only inhibits bacterial growth, but also exerts a potent post antibiotic effect indicating that treated bacteria are seriously damaged. Transmission electron microscopy of treated methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus faecium* revealed multiple septation initiation sites and a lack of separation of daughter cells, suggesting that 7-*O*-malonyl macrolactin inhibits the process of cell division. Formation of abnormal buds at the points of cell division, and a smaller rounded morphology, was observed with treated SCV variants of *B. cepacia*. An explanation of the underlying mechanism of action of the 7-*O*-malonyl macrolactin is expected to provide new insights into drug action and reveal new cellular targets.

New screens Parallel work was carried out to establish new screens to identify secondary metabolites able to inhibit critical steps of the pathogenesis process, such as bacterial attachment and microbial capacity to produce biofilms. Compounds identified by these new screens are currently under scrutiny.

National and International Research Infrastructure – Biotech Facilities

HEAD | **Dr. Holger Ziehr | Biochemical Engineering Division | hzi@gbf.de**

SCIENTIFIC COLLABORATORS | Jörg Becker | Dr. Jochen Berlin | Christin Dangel | Andreas Düvel | Dr. Sevim Duvar | Thomas Gäbel | Joachim Hammer | Dr. Volker Hecht | Dr. Helmut Hustedt | Dr. Volker Jäger | Wolfgang Kessler | Stefan Kluger | Karl-Heinz Kroner | Reinhard Krützfeld | Holger Leptien | Dr. Ulrich Menge | Dr. Bernd G. Müller | Jürgen Nothnagel | Ramón Nuppenau | Dr. Neophytos Papamichael | Dr. Jens Ingwer Paulsen | Dr. Anton Roß | Matthias Patrick Weide

In 2003/2004, the Biochemical Engineering Division – Biotech Facilities – of the GBF continued to act as a technology service provider for clients both inside and outside the Helmholtz Association. These services comprised the development and scale-up of cultivation processes for microbial and animal cells, and purification processes for the isolation of biomolecules, such as proteins, nucleic acids and antibodies from cell mass and supernatant.
At the GBF, various biotechnological pilot plants are available for this purpose, housing numerous bioreactors, centrifuges, chromatographic and filtration systems. The facilities have been licensed since 1997 for the production of GMP-material, in accordance with the German Drug Act (AMG), thus enabling novel active pharmaceutical ingredients to be produced for clinical research. In compliance with the regulations, a highly compartmented clean room pilot plant (GMP I) was installed in 1999. In order to satisfy increasing demand for capacity and quality, an additional plant was installed in 2001 (GMP II). The commissioning and qualification of GMP II is currently ongoing. In May 2004, the Biochemical Engineering Division was successfully inspected by local (Bezirksregierung Braunschweig) and federal authorities (BfArM – Bundesamt für Arzneimittel und Medizinprodukte). The GBF is the first German institution to receive a general and non-product-specific GMP manufacturing license.
During 2003, about 240 microbial and mammalian cell cultivations were performed, of which 75 were for external clients. Of these, 15 came from academic institutes and 60 from industry.
Four projects dealing with the production of biopharmaceutical active proteins have been carried out for German pharmaceutical and Biotech companies. Three of these have already been successfully finalized with the manufacture and delivery of GMP-grade active pharmaceutical ingredients (APIs). The APIs, meanwhile, have been processed further to final dosage forms for use in several clinical trials.

Stefan Kluger controls a fermentor.

Photo: Bierstedt

Technological Platforms

A number of platform technologies essential for the research carried out at the GBF are made available to the scientific projects as centralized facilities. In the context of national and international research programmes, these platforms provide services not only to internal projects, but also to scientific collaborators from other Helmholtz research centres, German universities other public research institutes and industry. Below, some of the most important platforms are described in detail.

Dr. Stefan Matysiak (ri), Dr. Norbert Zander (mi) and Andrea Abrahamik (le) discussing the development of protecting groups for peptide synthesis.

Photo: Bierstedt

01 Central Animal Facility

HEAD | **Dr. David Monner | Central Animal Facility | dmo@gbf.de**

SCIENTIFIC COLLABORATOR | Dr. Werner Müller

The purpose of the Central Animal Facility is to care for and provide research animals – exclusively mice – for the scientists at the GBF and to monitor compliance to the guidelines of the federal Animal Welfare Act. All animals are kept under state of the art conditions in individually ventilated cages (IVC).
The facility consists of a building dedicated to breeding mice under specific pathogen free (SPF) conditions, a separate quarantine and a biosafety level 2 unit (BSL-2) for performing infection experiments under SPF-conditions.
In addition to caring for breeding colonies both under specific pathogen-free conditions and in quarantine, duties of the facility include performing back crosses and experimental breedings to create new mouse lines, routine health monitoring, the rederivation of strains by embryo transfer, archiving of strains by embryo cryopreservation, maintenance of nuclear breeding colonies, and the breeding and provision of donor animals and pseudo-pregnant females for the generation of new genetically-modified mouse lines by ES-cell blastocyst injection.

An animal core taker is moving the mice into new cages.

Photo: Bierstedt

Services In 2004, occupancy in the facility increased approximately 30 % to almost 3,600 cages by the end of the year. Currently, over 150 different mouse lines are housed in the facility. Besides providing standard animal care, the animal technicians carry out all experimental breeding with attendant data bank administration and perform a number of other services, including biopsies, blood sampling, immunisations and other manipulations. In 2004, rederivations were performed on 22 lines, while 30 lines were archived by embryo cryopreservation. *In vitro* fertilization techniques were also established in the facility this year.
The training programme for laboratory animal technicians continued to expand. Presently, one apprentice is in the third and final year of training, and two are in their second and first years, respectively. We plan to add five new apprentices to the programme in 2005.

Expansion of the infection platform In November, 2003, the Building D annex was put into service as a dedicated animal care unit for performing infection experiments at BSL-2. By the end of 2004 approximately 900 of the 1,700 available cages were occupied. Only SPF-certified animals are allowed into the unit and all activities are performed under correspondingly controlled conditions. The line of services provided here is the same as in the other units of the facility.

02 Gene Expression Analysis

HEAD | **Dr. Robert Geffers | Research Group Mucosal Immunity | rog@gbf.de**

SCIENTIFIC COLLABORATOR | Susanne Pförtner

The Array Facility is a central service unit at the GBF, performing micro array based expression analysis. Following the principle of high throughput analysis, a particular emphasis is based on automation of sample preparation and development of customer adapted micro arrays. This allows multiple screenings for gene sets of interest. The customized expression analyses are complemented by highly standardized GeneChip expression arrays manufactured by Affymetrix. The GeneChip system allows a whole genome expression analysis of approximately 40,000 genes on one chip. These chips are available for mammalian systems as well as for prokaryotes, *i.e. Pseudomonas aeruginosa* and *E. coli*.

Instrumentation of the Array Facility.

Services The Array Facility offers expression analysis services for GBF researchers and external collaborators. In 2004, a total of 500 expression arrays were performed, with 400 using the Affymetrix GeneChip technology. Out of these 400 expression arrays, 200 were requested by GBF researchers and approx. 200 by external research groups und collaborators. Some 100 experiments were performed on self-printed custom chips, so called theme arrays.
For the development and manufacturing of theme arrays, the array facility advises users on the optimum choice of materials and shipping conditions. The manufacture of the arrays is guaranteed by the Array Facility, and standardized quality controls are applied to each batch of arrays. Furthermore, optimized protocols for their application are made available to our customers.
In addition to array manufacturing and sample preparation, data mining, data housing and data interpretation also are available upon request. Standardized result reports are generated for over 80 % of the performed experiments.

Research… In the course of numerous internal and external collaboration, analyses were performed by the facility related to tumor growth and type, host-pathogen interactions – EHEC, Listeria, Yersinia, Pseudomonads and Mycobacteria – and immune responses.
A major aspect for expression profiling of the immune system was to gain more insight into regulatory mechanisms leading to peripheral T-cell tolerance. Research groups at the GBF, the DRFZ Berlin and the MHH were interested in a particular $CD4^+$ T-cell population preventing autoimmune diseases, such as *Diabetes mellitus*, *Morbus Crohn* and rheumatid arthritis. Expressions profiling identified new marker molecules for a more sophisticated isolation of these "regulatory T cells" (Treg). It was shown that the application of Treg cells to autoimmune mouse models prevented autoimmunity. Expression studies on human regulatory T-cell lines, in conjunction with bioinformatics approaches, should provide a better understanding of the function of these T cells. The Array Facility, therefore, has initiated a joint project with the bioinformatics company, BIOBASE, the MHH and GBF researchers to identify regulatory networks by bioinformatical methods. Additionally, we have developed a new micro array chip based on our knowledge of regulatory T cells. It allows expression profiling at a higher resolution and lower cost.

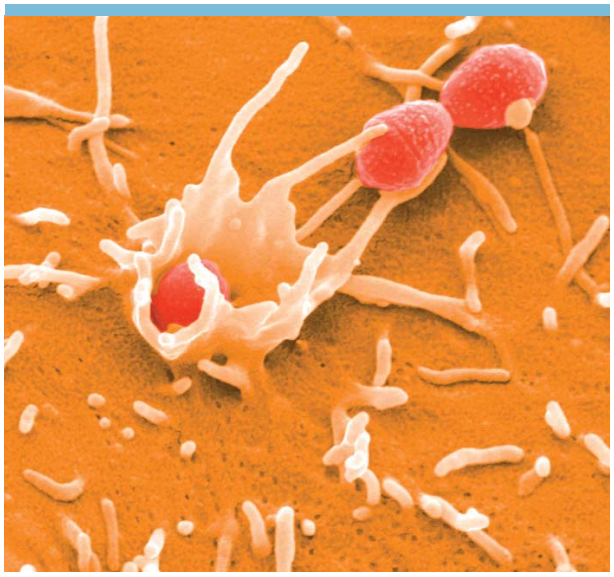
…and Development In the course of custom array development and improvement, three theme arrays were generated by researchers at the GBF. Further individual chip designs are intended for 2005.

03 Analytical Instruments

HEAD | **Dr. Victor Wray** | **Research Group Biophysical Analysis** | **vwr@gbf.de**

SCIENTIFIC COLLABORATORS | Dr. Heinrich Lünsdorf | Dr. Manfred Nimtz | Dr. Manfred Rohde

This platform is a facility for determining the three dimensional structure of all types of natural products and is equipped to carry out mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), X-ray crystallography, protein sequencing, electron microscopy and confocal laser microscopy. For the majority of low-molecular natural products, their structure can be elucidated in a routine manner using a combination of MS and NMR spectroscopy.
The direct analysis of large, intact biomolecules, such as proteins, oligonucleotides and complex carbohydrates is routinely carried out using MALDI- and ESI-MS. Mass spectrometry has the important advantage of providing information about very small amounts of compound.
The secondary and tertiary structure of peptides and proteins can be elucidated in solution using multidimensional NMR spectroscopy, when appropriately labelled material (^{15}N and ^{13}C) is available. Automated MS micro-techniques are used for the identification and characterization of proteins from 2D gels ("Proteomics") and from "gel-less" techniques, through the determination of the molecular weight of their proteolytic fragments using MALDI/TOF-MS/MS and HPLC-ESI-MS/MS.



Field emission scanning electron microscopic depiction of the cross-talk between serotype M1 Group A streptococci (red) and human epithelial cell (HEp-2) which results in the formation of membrane ruffles around the invading streptococcus.

Photo: Dr. Rohde

X-ray crystallography The main emphasis in X-ray crystallography is the structural analysis of proteins at the atomic level. A pipette-robot and a modern X-ray unit with an area detector and rotating anode are available for crystallisation and data collection. The measurement of high resolution data and phase determination using anomalous dispersion is available through the use of external synchrotron facilities.

Edman degradation N-terminal protein sequencing is performed by automated Edman degradation. Applications include the elucidation of new protein sequences, the identification of proteins in data bases, as well as checking the identity and purity of recombinant proteins. Samples, either in solution or bound to PVDF-membranes, may be analyzed in the low picomolar range.

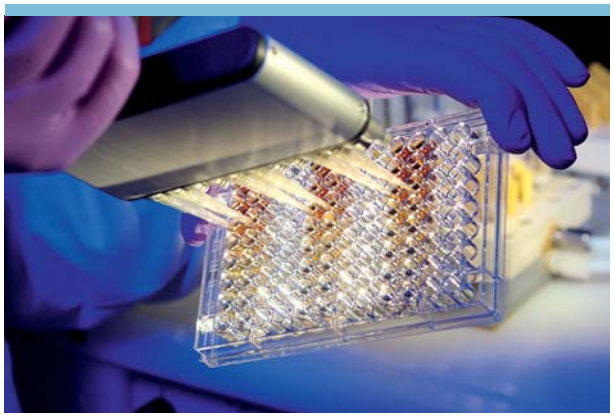
FESEM-techniques Electron microscopy is used to visualize the adherence to and invasion of host cells by a wide range of pathogens. Preparation protocols have been customized to undertake studies using high resolution field emission scanning electron microscopy (FESEM) revealing distinct pathways for invading the same host cell. In addition, a methodology has been developed to immuno-localize pathogenicity factors using FESEM, not only on the bacterial cell surface or the interface between bacterial and host cell membrane, but also inside the host cell using antibodies and colloidal gold-particles.

04 Peptide- and Chemical Synthesis

HEAD | Dr. Werner Tegge | Department Chemical Biology | wte@gbf.de
SCIENTIFIC COLLABORATORS | Dr. Ronald Frank | Dr. Michael Morr

The task of the platform is to generate synthetic peptides both in soluble form and immobilized in the form of arrays, as well as to produce special, commercially unavailable compounds. State-of-the-art equipment is employed for the synthesis. Soluble peptides are routinely characterized using HPLC and MALDI mass spectrometry. If necessary, further characterization is carried out by amino acid analysis, protein sequencing, special mass spectrometry techniques and NMR in the GBF Department of Structural Biology.
Depending on the intended usage and desired quality of the crude products, purifications are carried out, usually by preparative HPLC. For special investigations, the platform also routinely offers the following peptide modifications: phosphorylations, biotinylations, lipid additions, branched peptides and cyclizations.

SPOT-arrays In the platform, peptide arrays are generated to facilitate the systematic and empirical search for peptide ligands. For the successful design of such arrays, a thorough understanding of the biological problem is essential and attained by a close cooperation and collaboration with the users. The SPOT-arrays are currently generated semi-automatically on paper sheets or other polymeric supports. In the near future, however, this will be done fully automatically with the help of new synthesis robots. Each year, approximately 15,000 peptides and peptide mixtures are generated in an array format and utilized for the investigation of protein-protein interactions and enzyme-substrate recognition.



Preparing for the peptide synthesizer for the automated, parallel generation of up to 96 peptides.

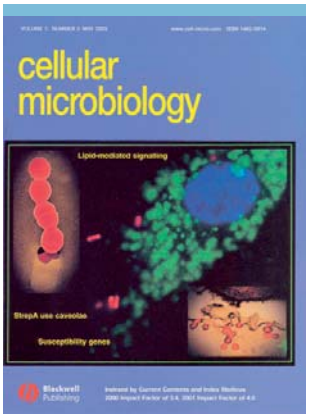
Photo: Bierstedt

Chemical Synthesis In a 20-step synthesis, galactosyl ceramide was synthesized and coupled to a synthetic peptide. Also, macrophage activating lipopeptide (MALP) and a polyethylene glycol derivative thereof (MALP-MPEG) were synthesized in multi-step syntheses. These classes of compounds are used by the GBF group Vaccine Research for the development of mucosal vaccines.
Other special compounds that were synthesized include chinolone derivatives, cyclic-di-GMP and cyclic-di-AMP, benzamido-adenine dinucleotide (BAD).

Publications 2003

Infection and Immunity – 2003

- *Alter-Koltunoff, M.; Ehrlich, S.; Dror, N.; Azriel, A.; Eilers, M.; Hauser, H.; Bowen, H.; Barton, C.-H.; Tamura, T.; Ozato, K., and Levi, B.-Z. Nramp 1 mediated innate resistance to intraphagosomal pathogens is regulated by IRF-8, PU.1 and Miz-1.* ***Journal of Biological Chemistry****. 2003;* ***278****:44025-44032.*
- *Balling, R. Die Maus als Modellsystem für Säugetierentwicklung. In: Lehrbuch der Genetik (W. Seyffert, ed.) 2. Auflage Heidelberg – Berlin: Spektrum Akademischer Verlag; 2003; pp. 669-679.*
- *Balling, R. Maus. In: Lehrbuch der Genetik (W. Seyffert ed.) 2. Auflage Heidelberg – Berlin: Spektrum Adakemischer Verlag; 2003; pp. 1011-1025.*
- *Barthel, M.; Hapfelmeier, S.; Quintanilla-Martinez, L.; Kremer, L.; Rohde, M.; Hogardt, M.; Pfeffer, K.; Rüssmann, H., and Hardt, W.-D. Pretreatment of mice with streptomycin provides a* Salmonella enterica *serovar* Typhimurium colitis *model that allows analysis of both pathogen and host.* ***Infection and Immunity****. 2003;* ***71****:2839-2858.*
- *Barthold, M.; Fargali, S.; Majore, I.; Zghoul, N.; Stahl, F.; Rohde, M.; Mayer, H., and Jäger, V. Proliferation and osteogenic differentiation of mesenchymal stem cells on calcium phosphate scaffolds in fixes bed bioreactors.* ***Tissue Engineering****. 2003;* ***9****:848.*
- *Baumann, S.; Hess, J.; Eichhorst, S. T.; Krueger, A.; Angel, P.; Krammer, P. H., and Kirchhoff, S. An unexpected role for FosB in activation-induced cell death of T cells.* ***Oncogene****. 2003;* ***22****:1333-1339.*
- *Beer, C.; Buhr, P.; Hahn, H.; Laubner, D., and Wirth, M. Gene expression analysis of murine cells producing amphotropic mouse leukemia virus at a cultivation temperature of 32 and 37º C.* ***Journal of General Virology****. 2003;* ***84****:1677-1686.*
- *Beer, C.; Meyer, A.; Müller, K., and Wirth, M. The temperature stability of mouse retroviruses depends on the cholesterol levels of viral lipid shell and cellular plasma membrane.* ***Virology****. 2003;* ***308****:137-146.*
- *Bergmann, S.; Wild, D.; Diekmann, O.; Frank, R.; Bracht, D., and Hammerschmidt, S. Binding of human plasmin(ogen) to surface displayed -enolase is mediated via two binding sites in Eno of* Streptococcus pneumoniae. ***Molecular Microbiology****. 2003;* ***49(2)****:411-423.*
- *Bilitewski, U.; Genrich, M.; Mersal, G., and Kadow, S. Biochemical analysis in microfluidic systems.* ***Analytical and Bioanalytical Chemistry****. 2003;* ***377(3)****:556-569.*
- *Bode, H. B.; Irschik, H.; Wenzel, S. C.; Reichenbach, H.; Müller, R., and Höfle, G. The leupyrrins: a structurally unique family of secondary metabolites from the Myxobacterium* Sorangium cellulosum. ***Journal of Natural Products****. 2003;* ***66****:1203-1206.*
- *Bode, H. B. and Müller, R. Possibility of bacterial recruitment of plant genes associated with the biosynthesis of secondary metabolites.* ***Plant Physiology****. 2003;* ***132****:1153-1161.*
- *Bode, H. B.; Zeggel, B.; Silakowski, B.; Wenzel, S. C.; Reichenbach, H., and Müller, R. Steroid biosynthesis in procaryotes: identification of myxobacterial steroids and cloning of the first bacterial 2,3(s)-oxidosqualen cyclase from the myxobacterium* Stigmatella aurantiaca. ***Molecular Microbiology****. 2003;* ***47****:471-481.*
- *Bode, J.; Goetze, S.; Heng, H.; Krawetz, S. A., and Benham, C. From DNA structure to gene expression: Mediators of nuclear compartmentalization and -dynamics.* ***Chromosome Research****. 2003;* ***11(5)****:435-445.*
- *Bode, J.; Götze, S.; Ernst, E.; Hüsemann, Y.; Baer, A.; Seibler, J., and Mielke, C. Architecture and utilization of highly-expressed genomic sites. (Makrides, S., editor). In: New Comprehensive Biochemistry 38 – Gene Transfer and Expression in Mammalian Cells: Elsevier; 2003; Chapter 20 pp. 551-572.*
- *Borsutzky, S.; Fiorelli, V.; Ebensen, T.; Tripiciano, A.; Rharbaoui, F.; Scoglio, A.; Link, C.; Nappi, F.; Morr, M.; Buttó, S.; Cafaro, A.; Mühlradt, P. F.; Ensoli, B., and Guzmán, C. A. Efficient mucosal delivery of the HIV-1 Tat protein using the synthetic lipopeptide MALP-2 as adjuvant.* ***European Journal of Immunology****. 2003;* ***33****:1548-1556.*
- *Breitbach, K.; Rottner, K.; Klocke, S.; Rohde, M.; Jenzora, A.; Wehland, J., and Steinmetz, I. Actin-based motility of* Burkholderia pseudomallei *involves the Arp2/3 complex, but not N-WASP and Ena/VASP proteins.* ***Cellular Microbiology****. 2003;* ***5(6)****:385-393.*
- *Bruns, K.; Fossen, T.; Wray, V.; Henklein, P.; Tessmer, U., and Schubert, U. Structural characterization of the HIV-1 Vpr N-terminus: Evidence of* cis/trans *proline isomerism.* ***Journal of Biological Chemistry****. 2003;* ***278****:43188-43201.*
- *Buchrieser, C.; Rusniok, C.; Kunst, F.; Cossart, P.; Glaser, P.; The European* Listeria *Genome Consortium (GBF: H. Blöcker; P. Brandt; U. Kärst; G. Nordsiek, and J. Wehland). Comparison of the genome sequences of* Listeria monocytogenes *and* Listeria innocua*: clues for evolution and pathogenicity.* ***FEMS Immunology and Medical Microbiology****. 2003;* ***35****:207-213.*
- *Budde, H.; Flohé, L.; Hecht, H.-J.; Hofmann, B.; Stehr, M.; Wissing, J., and Lünsdorf, H. Kinetics and redox-sensitive oligomerisation reveal negative subunit cooperativity in tryparedoxin peroxidase of* Trypanosoma brucei brucei. ***Biological Chemistry****. 2003;* ***384****:619-633.*
- *Budde, H. Flohé L.; Hofmann, B., and Nimtz, M. Verification of the interaction of a tryparedoxin peroxidase with tryparedoxin by ESI-MS/MS.* ***Biological Chemistry****. 2003;* ***384****:1305-1309.*
- *Buer, J. and Balling, R. Mice, microbes and models of infection.* ***Nature Reviews Genetics****. 2003;* ***4****:195-205.*
- *Buttó, S. ; Fiorelli, V.; Tripiciano, A.; Ruiz-Alverez, M. J.; Scoglio, A.; Ensoli, F.; Ciccozzi, M.; Collacchi, B.; Sabbatucci, M.; Cafaro, A.; Guzmán, C. A.; Borsetti, A.; Caputo, A.; Vardas, E.; Colvin, M.; Lukwija, M.; Rezza, G.; Ensoli, B., and and the Tat Multicentric Study Group. Sequence conservation and antibody cross-recognition of the Clade B HIV-1 Tat protein vaccine candidate in HIV-1-infected Italian, Ugandan and South African individuals.* ***Journal of Infectious Diseases****. 2003;* ***188****:1171-1180.*



- *Cover picture of the journal Cellular Microbiology, Vol. 5 (5), 2003, on the occasion of the publication of the article by Rohde, M.; Müller, E.; Chhatwal, G. S., and Talay, S. R. Host cell caveolae act as an entry-port for Group A streptococci.* ***Cellular Microbiology****. 2003;* ***5****:323-342. The permission of Blackwell Publishing is gratefully acknowledged.*

- *Clauss, M.; Pipp, F.; Issbrücker, K.; Weich, H.; Heil, M., and Schaper, W. Dissection of monocyte and endothelial activities by using VEGF-receptor specific ligands.* ***Advances in Experimental Medicine and Biology****. 2003;* ***522****:75-82.*

- *Deckert, M.; Lutjen, S.; Leuker, C. E.; Kwok, L.-Y.; Strack, A.; Müller, W.; Wagner, N., and Schlüter, D. Mice with neonatally induced inactivation of the vascular cell adhesion molecule-1 fail to control the parasite in* Toxoplasma encephalitis. ***European Journal of Immunology****. 2003;* ***33(5)****:1418-1428.*

- *Deiters, U.; Gumenschneider, M.; Galanos, C., and Mühlradt, P. F. TLR2/6-mediated stimulation by the lipopeptide MALP-2 induces LPS-cross tolerance in mice, resulting in protection from TNF-α but in only partial protection from lethal LPS doses.* ***Infection and Immunity****. 2003;* ***71****:4456-4462.*

- *Dietrich, G.; Spreng, S.; Favre, D.; Viret, J.-F., and Guzmán, C. A. Delivery of cancer DNA vaccines by live attenuated bacteria.* ***Enhancer-Immunotherapy of Cancer****. 2003;* ***1****:16-18.*

- *Dietrich, G.; Spreng, S.; Favre, D.; Viret, J.-F., and Guzmán, C. A. Live attenuated bacteria as vectors to deliver plasmid DNA vaccines.* ***Current Opinion in Molecular Therapeutics****. 2003;* ***5****:10-19.*

- *Dietz-Pfeilstetter, A.; Arndt, N.; Kay, V., and Bode, J. Molecular structure and regulatory potential of a T-DNA integration site in petunia.* ***Transgenic Research****. 2003;* ***12****:83-99.*

- *Ding, H.; Griesel, C.; Nimtz, M.; Conradt, H. S.; Weich, H. A., and Jäger, V. Molecular cloning, expression, purification, and characterization of soluble full-length, human interleukin-3 with a baculovirus-insect cell expression system.* ***Protein Expression and Purification****. 2003;* ***31****:34-41.*

- *Dinkla, K.; Rohde, M.; Jansen, W. T. M.; Carapetis, J. R.; Chhatwal, G. S., and Talay, S. R.* Streptococcus pyogenes *recrutis collagen via surface bound fibronectin: a novel colonisation and immune evasion mechanism.* ***Molecular Microbiology****. 2003;* ***47****:861-869.*

- *Dinkla, K.; Rohde, M.; Jansen, W. T. M.; Kaplan, E. L.; Chhatwal, G. S., and Talay, S. R. Rheumatic fever associated* Streptococcus pyogenes *isolates aggregate collagen.* ***Journal of Clinical Investigation****. 2003;* ***111(12)****:1905-1912.*

- *Duvar, S.; Berlin, J.; Ziehr, H., and Conradt, H. S. Modulation of the glycosylation repertoire of recombiant human EPO expressing model cell lines under different culture conditions. In: Proceedings of the 18th ESACT-Meeting-Granada. Granada; 2003.*

- *Düber, S. ; Engel, H.; Rolink, A.; Kretschmer, K., and Weiss, S. Germline transcripts of immunoglobulin light chain variable regions are structurally divers and differentially expressed.* ***Molecular Immunology****. 2003;* ***40(8)****:509-516.*

- *Ebensen, T.; Link, C., and Guzmán, C. A. Classical bacterial vaccines. (Kaufman, S. H. E., editor). In: Novel Vaccination Strategies. Weinheim, Germany: Wiley-VCH Verlag GmbH; 2003; Chapter 11 pp. 221-242.*

- *Eberhardt, M. O.; Frank, R.; Kratje, R., and Etcheverrigaray, M. Identification of two potential receptor-binding sites for hGM-CSF.* ***Brazilian Journal of Chemical Engineering****. 2003;* ***20****:15-20.*

- *Erck, C.; McLeod, R., and Wehland, J. Cloning and genomic organisation of the TTL gene on mouse chromosome 2 and human chromosome 2q13.* ***Cytogenetic and Genome Research****. 2003;* ***101****:47-53.*

- *Eubel, H.; Jänsch, L., and Braun, H. P. New insights into the respiratory chain of plant mitochondria: supercomplexes and a unique composition of complex II.* ***Plant Physiology****. 2003;* ***133(1)****:274-286.*

- *Fingerle-Rowson, G.; Petrenko, O.; Metz, C. N.; Forsthuber, T. G.; Mitchell, R.; Huss, R.; Moll, U.; Müller, W., and Bucala, R. The p53-dependent effects of macrophage migration inhibitory factor revealed by gene targeting.* ***Proceedings of the National Academy of Sciences, USA****. 2003;* ***100****:9354-9359.*

- *Focke, M.; Gieringer, E.; Schwan, S.; Jänsch, L.; Binder, S., and Braun, H. P. Fatty acid biosynthesis in mitochondria of grasses: malonlyl-CoA is integrated by a mitochondrial-localized acetyl-CoA carboxylase.* ***Plant Physiology****. 2003;* ***133(2)****:875-884.*

- *Franke, D.; Lorbach, V.; Esser, S.; Dose, C.; Sprenger, G. A.; Halfar, M.; Thömmes, J.; MüllerR.; Takors, R., and Müller, M. (S,S)-2,3-Dihydroxy-2,3-dihydrobenzoic acid: Microbial access with engineered cells of* Escherichia coli *and applicability as starting material in natural-product synthesis.* ***Chemisty – A European Journal****. 2003;* ***9****:4188-4196.*

- *Franzke, A.; Piao, W.; Lauber, J.; Gatzlaff, P.; Könecke, C.; Hansen W.; Schmitt-Thomsen, A.; Herstenstein, B.; Buer, J., and Ganser, A. G-CSF as immune regulator in T-cells expressing the G-CSF receptor: implications for transplantation and autoimmune diseases.* ***Blood****. 2003;* ***102****:734-739.*

- *Gardan, R.; Cossart, P.; Labadie, J.; The European* Listeria *Genome Consortium (GBF: H. Blöcker; P. Brandt; U. Kärst; G. Nordsiek, and J. Wehland). Identification of* Listeria monocytogenes *genes involved in salt and alkaline-pH tolerance.* ***Applied and Environmental Microbiology****. 2003;* ***69****:3137-3143.*

- *Gardan, R.; Duché, O.; Leroy-Setrin, S.; Labadie, J.; The European* Listeria *Genome Consortium (GBF: H. Blöcker; P. Brandt; U. Kärst; G. Nordsiek, and J. Wehland). Role of ctc from* Listeria monocytogenes *in osmotolerance.* ***Applied and Environmental Microbiology****. 2003;* ***69****:154-161.*

- *Gerth, K.; Pradella, S.; Perlova, O.; Beyer, S., and Müller, R. Myxobacteria: Proficient producers of novel natural products with various biological activities past and future biotechnological aspects with the focus on the genus* Sorangium. ***Journal of Biotechnology****. 2003;* ***106****:233-253.*

- *Goldmann, O.; Chhatwal, G. S., and Medina, E. Immune mechanisms underlying host susceptibility to group A streptococcal infections.* ***Journal of Infectious Diseases****. 2003;* ***187****:854-861.*

- *Grenklo, S.; Geese, M.; Lindberg, U.; Wehland, J.; Karlsson, R., and Sechi, A. S. Critical role for profilin: actin in the intracellular motility of* Listeria monocytogenes. ***EMBO Reports****. 2003;* ***4****:1-7.*

- *Götze, S. ; Gluch, A.; Benham, C., and Bode, J. Computational and* in vitro *analysis of destabilized DNA regions in the interferon gene cluster: the potential of predicting functional gene domains.* ***Biochemistry****. 2003;* ***42****:154-166.*

- *Götze, S.; Huesemann, Y.; Baer, A., and Bode, J. Functional characterization of transgene integration patterns by halo fluorescence* in situ *hybridization: electroporation versus retroviral infection.* ***Biochemistry****. 2003;* ***42(23)****:7035-7043.*

- *Heesemann, J.; Heinz, D. W.; Rüssmann, H.; Wehland, J.; Goebel, W., and Kuhn, M. Lektionen aus der Bakterienwelt: Ausnutzung von Wirtszellprozessen durch pathogene Mikroben.* ***BioSpektrum****. 2003;* ***9****:486-489.*

- *Heilmann, C.; Thumm, G.; Chhatwal, G. S.; Hartleib, J.; Uekotter, A., and Peters, G. Identification and characterization of a novel autolysin (Aae) with adhesive properties from* Staphylococcus epidermidis. ***Microbiology-SGM****. 2003;* ***149****:2769-2778.*

- *Helloin, E.; Jänsch, L., and Phan-Thanh, L. Carbon starvation survival of* Listeria monocytogenes *in planktonic state and in biofilm: a proteomic study.* ***Proteomics****. 2003;* ***3(10)****:2052-2064.*

- *Hollister, J.; Conradt, H. S., and Jarvis, D. L. Evidence for a sialic acid salvaging pathway in lepidopterian insect cells.* ***Glycobiology****. 2003;* ***13(6)****:487-495.*

- *Häußler, S. Chronic infections by small colony variants of pathogenic bacteria.* ***Biomedical Progress****. 2003;* ***16****:47-51.*

- *Häußler, S. Morphologische Varianten bakterieller Erreger und chronische Infektionen.* ***Gelbe Hefte****. 2003;* ***42****:17-24.*

- *Häußler, S.; Lehmann, C.; Breselge, C.; Rohde, M.; Claßen, M.; Tümmler, B.; Vandamme, P., and Steinmetz, I. Fatal outcome of lung transplantation in cystic fibrosis due to serum resistant* Burkholderia cepacia *comlex small colony variants.* ***European Journal of Clinical Microbiology and Infectious Diseases****. 2003;* ***22****:249-253.*

- *Häußler, S.; Rohde, M.; von Neuhoff, N.; Nimtz, M., and Steinmetz, I. Structural and functional cellular changes induced by* Burholderia pseudomallei. ***Infection and Immunity***. *2003;* ***71****:2970-2975.*

- *Häußler, S.; Ziegler, I.; Löttel, A.; Von Götz, F.; Rohde, M.; Wehmhöner, D.; Saravanamuthu, S.; Tümmler, B., and Steinmetz, I. Highly adherent small colony variants of* Pseudomonas aeruginosa *in cystic fibrosis lung infection.* ***Journal of Medical Microbiology***. *2003;* ***52****:295-301.*

- *Häußler, S.; Ziesing, S.; Rademacher, G.; Hoy, L., and Weißbrodt, H. Evaluation of the MERLIN, micronaut system for automated antimicrobial susceptibility testing of* Pseudomonas aeruginosa *and* Burkholderia *species isolated from cystic fribrosis patients.* ***European Journal of Clinical Microbiology Infectious Diseases***. *2003;* ***22****:496-500.*

- *Höfle, G.; Glaser, N.; Karama, U.; Leibold, T.; Sasse, F., and Steinmetz, H. Semisynthesis and degradation of the tubulin inhibitors epothilone and tubulysin.* ***Pure and Applied Chemistry***. *2003;* ***75****:167-178.*

- *Jansen, R.; Kunze, B.; Reichenbach, H., and Höfle, G. Chondrochloren A and B, new ß-amino styrenes from* Chondromyces crocatus *(Myxobacteria).* ***European Journal of Organic Chemistry***. ***2003****; 2684-2689.*

- *Jäger, V.; Barthold, M.; Fargali, S.; Majore, I.; Zghoul, N.; Stahl, F.; Rohde, M., and Mayer, H. 3-D cultivation and monitoring of bone precursor cells on calcium phosphate scaffolds in fixed bed bioreactors.* ***Artificial Organs***. *2003;* ***26****: 829.*

- *Karama, U. and Höfle, G. Synthesis of epothilone 16,17-alkyne analogs by replacement of the C13-C15(O)-ring segment of natural epothilone C.* ***European Journal of Organic Chemistry***. ***2003****;* ***(6)****:1042-1049.*

- *Kaverina, I.; Stradal, T. E. B., and Gimona, M. Prodosome formation in cultured A7r5 vascular smooth muscle cells requires Arp2/3-dependent* de-novo *actin polymerization at discrete microdomains.* ***Journal of Cell Science***. *2003;* ***116****:4915-4924.*

- *Kim, E.-J.; Sabra, W., and Zeng, A.-P. Iron deficiency leads to inhibition of oxygen transfer and enhanced formation of virulence factors in* Pseudomonas aeruginosa *cultures.* ***Microbiology-SGM***. *2003;* ***149****:2627-2634.*

- *Kresse, A. U.; Dinesh, S. D.; Larbig, K., and Römling, U. Impact of large chromosomal inversions on the adaption and evolution of* Pseudomonas aeruginosa *chronically colonizing cystic fibrosis lungs.* ***Molecular Microbiology***. *2003;* ***47(1)****:145-158.*

- *Kretschmer, K.; Jungebloud, A.; Stopkowicz, J.; Kleinke, T.; Hoffmann, R., and Weiss, S. The selection of marginal zone B cells differs from that of B-1a cells.* ***Journal of Immunology***. *2003;* ***171(12)****:6495-6501.*

- *Kretschmer, K.; Jungebloud, A.; Stopkowicz, J.; Stoermann, B.; Hoffmann, R., and Weiss, S. Antibody repertoire and gene expression profile: implications for different developmental and functional traits of splenic and peritioneal B-1 lymphocytes.* ***Journal of Immunology***. *2003;* ***171(3)****:1192-1201.*

- *Krumme, D.; Budde, H.; Hecht, H.-J.; Menge, U.; Ohlenschläger, O.; Roß, A.; Wissing, J.; Wray, V., and Flohé, L. NMR studies of the interaction of tryparedoxin with redox-inactive substrate homologs.* ***Biochemistry***. *2003;* ***42****:14720-14728.*

- *Kröger, A.; Dallügge, A.; Kirchhoff, S., and Hauser, H. IRF-1 reverts the transformed phenotype of oncogenically transformed cells* in vitro *an* in vivo. ***Oncogene***. *2003;* ***22(7)****:1045-1056.*

- *Kuhlmeier, D.; Rodda, E.; Kolarik, L. O.; Furlong, D. N., and Bilitewski, U. Application of atomic force microscopy and grating coupler for the characterization of biosensor surfaces.* ***Biosensors & Bioelectronics***. *2003;* ***18****:925-936.*

- *Kwissa, M.; Kröger, A.; Hauser, H.; Reimann, J., and Schirmbeck, R. Defining conditions for cytokine-facilitated priming of CD8+ T cell responses by DNA vaccination.* ***Journal of Molecular Medicine***. *2003;* ***81****:91-101.*

- *Köster, M.; Lykke-Andersen, S.; Elnakady, Y. A.; Gerth, K.; Washausen, P.; Höfle, G.; Sasse, F.; Kjems, J., and Hauser, H. Ratjadones inhibit nuclear export by blocking DRM1/exportin 1.* ***Experimental Cell Research***. *2003;* ***286****:321-331.*

- *Lavirk, I.; Krueger, A.; Schmitz, I.; Baumann, S.; Weyd, H.; Krammer, P. H., and Kirchhoff, S. The active caspase-8 heterotetramere is formed at the CD95 DISC.* ***Cell Death and Differentiation***. *2003;* ***10****:144-145.*

- *Lindenmaier, W. Gentechnik und Schöpfungsglaube – Plädoyer für eine konkrete Diskussion. (Babke, H.-G., Fritsche A., editors). In: Gerechtigkeit – ein globaler Wert. München; 2003; pp. 163-177.*

- *Lipps, H. J.; Jenke, A. C. W.; Nehlsen, K.; Scinteie, M.; Stehle, I. M., and Bode, J. Chromosome-based vectors for gene therapy.* ***Gene***. *2003;* ***304****:23-33.*

- *Lommel, S.; Benesch, S.; Rohde, M., and Wehland, J. Pedestal formation by pathogenic* E. coli: *A model system for studying signal transduction to the actin cytoskeleton. In: Cell Biology, A laboratory handbook. 3rd edition, 2003.*

- *Machens, H. G.; Jasmund, I.; Spanholtz, T.; Maichle, A. ; Niedworok, C.; Lindenmaier, W.; Herbort-Brand, S.; Görg, S.; Kropf, K.; Stöckelhuber, B. M.; Hellwig-Bürgel, T.; Krüger, S.; Reichert, B.; Siemers, F.; Krapohl, B. D., and Mailänder, P. A new model to induce angiogenesis by* ex vivo *transfected isogenic fibroblasts based on gene transfer. In: Proceedings of the 2nd Congress for Reconstructive Microsurgery (Germann, G., editor): Monduzzi Editione; 2003; pp. 213-216.*

- *Machens, H. G.; Salehi, J.; Weich, H.; Munch, S.; Siemers, S.; Krapohl, B. D.; Herter, K. H.; Krüger, S.; Reichert, B.; Berger, A.; Vogt, P., and Mailänder, P. Angiogenic effects of injected VEGF165 and sVEGFR.1 (sFLT-1) in a rat flap model.* ***Journal of Surgical Research***. *2003;* ***111****:136-142.*

- *Machens, H. G.; Spanholtz, T.; Maichle, A.; Niedworok, C.; Lindenmaier, W.; Herbort-Brand, U.; Görg, G.; Kropf, K.; Stöcklhuber, B.; Reichert, B.; Siemers, F.; Krapohl, B. D., and Mailänder, P. Ein neues gentechnologisches Modell zur Angiogeneseinduktion mittels* ex vivo *transfizierter isogener Fibroblasten. In: Chirurgisches Forum 2003 für experimentelle und klinische Forschung (Haas, N. P. Neugebauer E. Bauer H., editors): Springer-Verlag; 2003; Vol. 32, pp. 237-240.*

- *Machner, M. P.; Frese, S.; Schubert, W.-D.; Orian-Rousseau, V.; Gherardi, E.; Wehland, J.; Niemann, H. H., and Heinz, D. W. Aromatic amino acids at the surface of In1B are essential for host cell invasion by* Lysteria monocytogenes. ***Molecular Microbiology***. *2003;* ***48****:1525-1536.*

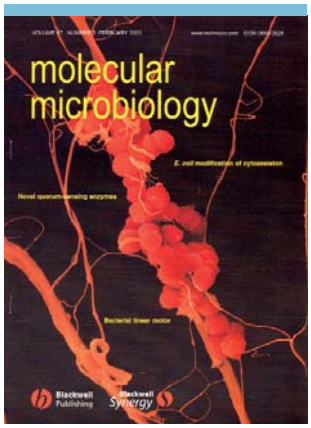
- *Maiorino, M.; Bosello, V.; Ursini, F.; Foresta, C.; Garolla, A.; Scapin, M.; Sztajer, H., and Flohé, L. Genetic variations of* gpx-4 *and male infertility in humans.* ***Biology of Reproduction***. *2003;* ***68****:1134-1141.*

- *Majore, I.; Barthold, M.; Stahl, F.; Schulz, R.; Loose, S.; Mayer, H.; Fargali, S., and Jäger, V. Optimization of cell culture conditions and comparative analysis of the proliferation and differentiation of human osteoprogenitor cells by DNA microarray analysis and non-invasive methods.* ***International Journal of Artificial Organs***. *2003;* ***26****:869.*

- *Manitz, M. P.; Horst, B.; Seeliger, S.; Strey, A.; Skryabin, B. V.; Gunzer, M.; Frings, W.; Schonlau, F.; Roth, J.; Sorg, C., and Nacken, W. Loss of S100A9 (MRP14) results in reduced interleukin-8-induced CD11b surface expression, a polarized microfilament system, and diminished responsiveness to chemoattractants* in vitro. ***Molecular and Cell Biology***. *2003;* ***23(3)****:1034-1043.*

- *Mascher, T; Zähner, D; Merai, M.; Balmelle, N.; de Saizieu, A. B., and Hakenbeck, R. The* Streptococcus pneumoniae *cia regulon: CiaR target sites and transcription profile analysis.* ***Journal of Bacteriology***. *2003;* ***185(1)****:60-70.*

- *Mataraza, J. M.; Briggs, M. W.; Li, Z.; Frank, R., and Sacks, D. B. Identification and characterization of the Cdc42-binding site of IQGAP1.* ***Biochemical and Biophysical Research Communications***. *2003;* ***305****:315-321.*



- *Cover picture of the journal Molecular Microbiology, Vol. 47 (3), 2003, on the occasion of the publication of the article by Dinkla, K.; Rohde, M.; Jansen, W. T. M.; Carapetis, J. R.; Chhatwal, G. S., and Talay, S. R.* Streptococcus pyogenes *recruits collagen via surface bound fibronectin: a novel colonisation and immune evasion mechanism.* ***Molecular Microbiology****. 2003;* ***47****:861-869. The permission of Blackwell Publishing is gratefully acknowledged.*

- *Matussek, A.; Lauber, J.; Bergau, A.; Hansen, W.; Rohde, M.; Dittmar, K. E. J.; Gunzer, M.; Mengel, M.; Gatzlaff, P.; Buer, J., and Gunzer, F. Molecular and functional analysis of Shiga toxin induced response patterns in human vascular endothelial cells.* ***Blood****. 2003;* ***102(4)****:1323-1332.*

- *Matys, V.; Fricke, E.; Geffers, R.; Gößling, E.; Haubrock, M.; Hehl, R.; Hornischer, K.; Karas, D.; Kel, A. E.; Kel-Margoulis, E. V.; Kloos, D. U.; Land, S.; Lewicki-Potapov, B.; Michael, H.; Münch, R.; Reuter, I.; Rotert, S.; Saxel, H.; Scheer, M.; Thiele, S., and Wingender, E. TRANSFAC®: transcriptional regulation, from patterns to profiles.* ***Nucleic Acids Research****. 2003;* ***31****:374-378.*

- *McArthur, J. D.; West, N. P.; Cole, J. N.; Jungnitz, H.; Guzmán, C. A.; Chin, J.; Lehrbach, P. R.; Djordjevic, S. P., and Walker, M. J. An aromatic amino acid auxotrophic mutant of* Bordetella bronchiseptica *is attenuated and immunogenic in a mouse model of infection.* ***FEMS Microbiology Letters****. 2003;* ***221****:7-16.*

- *McKay, F. C. ; McArthur, J. D.; Sanderson-Smit, M. L.; Gardam, S.; Currie, B. J.; Sriprakash, K. S.; Fagan, P. K.; Towers, R. J.; Batzloff, M. R.; Chhatwal, G. S.; Ranso, M., and Walker, M. J. Plasminogen binding by group A streptococcal isolates from tropical region with hyperendemic streptococcal skin infection and a high-incidence of invasive infection.* ***Infection and Immunity****. 2003;* ***72****:364-370.*

- *Medina, E.; Goldmann, O.; Toppel, A.; Rohde, M., and Chhatwal, G. S. Survival of* Streptococcus pyogenes *within host phagocytic cell: a pathogenic mechanism for persistence and systematic invasion.* ***Journal of Infectious Diseases****. 2003;* ***187****:597-603.*

- *Medina, E.; Rohde, M., and Chhatwal, G. S. Intracellular survival of* Streptococcus pyogenes *in polymorphonuclear cells results in increased bacterial virulence.* ***Infection and Immunity****. 2003;* ***71****:5376-5380.*

- *Monciardini, P.; Cavaletti, L.; Schuhmann, P.; Rohde, M., and Donadio, S.* Conexibacter woesei, *gen. nov., sp. nov., a novel representative of a deep evolutionary line of descent within the class* Actinobacteria. ***International Journal of Systematic and Evolutionary Micobiology****. 2003;* ***53****:569-576.*

- *Mothana, R. A. A.; Awadh Ali, N. A.; Jansen, R.; Wegner, U.; Mentel, R., and Lindequist, U. Antiviral lanostanoid triterpenes from the fungus* Ganoderma pfeifferi. ***Fitoterapia****. 2003;* ***74(1-2)****:177-180.*

- *Mundt, S.; Kreitlow, S., and Jansen, R. Fatty acids with antibacterial activity from the cyanobacterium* Oscillatoria redekei *HUB 051 .* ***Journal of Applied Phycology****. 2003;* ***15(2)****:63-267.*

- *Müller, P. P.; Wirth, D.; Unsinger, U., and Hauser, H. Genetic approaches to recombinant protein production in mammalian cells. (Vinci, V. A. and Parekh, S. R., editors). In: Handbook of Industrial Cell Culture: Mammalian, Microbial and Plant Cells: Humana Press; 2003; pp. 21-50.*

- *Müthing, J.; Kemminer, S. E.; Conradt, H. S.; Sagi, D.; Nimtz, M.; Kärst, U., and Peter-Katalinic, J. Effects of buffering conditions and culture pH on production rates and glycosylation of clinical phase I anti-melanoma mouse IgG3 monoclonal antibody R24.* ***Biotechnology and Bioengineering****. 2003;* ***83(5)****:321-334.*

- *Nakagawa, H.; Miki, H.; Nozumi, M.; Takenawa, T.; Miyamoto, S.; Wehland, J., and Small, J. V. IRSp53 is co-localised with WAVE2 at the tips of protruding lamellipodia and filopodia independently of Mena.* ***Journal of Cell Science****. 2003;* ***116****:2577-2583.*

- *Nedashkovskaya, O. I.; Kim, S. B.; Han, S. K.; Lysenko, A. M.; Rohde, M.; Zhukova, N. V.; Falsen, E.; Frolova, G. M.; Mikhailov, V. V., and Bae, K. S.* Mesonia algae *gen. nov., sp. nov., a novel marine bacterium of the family Flavobacteriaceae isolated from the green alga* Acrosiphonia sonderi *(Kütz) Kornm.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:1967-1971.*

- *Noguera-Obenza, M.; Ochoa, T. J.; Gomez, H. F.; Guerrero, M. L.; Herrera-Insua, I.; Morrow, A. L.; Ruiz-Palacios, G.; Pickering, L. K.; Guzmán, C. A., and Cleary, T. G. Human milk secretory antibodies against attching and effacing* Escherchia coli *antigens.* ***Emerging Infectious Diseases (United States)****. 2003;* ***9****:545-551.*

- *Ochoa, T. J.; Noguera-Obenza, M.; Ebel, F.; Guzmán, C. A., Gomez, H. F. and Cleary T. G. Lactoferrin impairs Type III secretory system function in enteropathogenic* Escherichia coli. ***Infection and Immunity****. 2003;* ***71****:5149-5155.*

- *Pancholi, V. and Chhatwal, G. S. Housekeeping enzymes as virulence factors for pathogens.* ***International Journal of Medicinal Microbiology****. 2003;* ***293****:1--11.*

- *Panthel, K.; Jechlinger, W.; Matis, A.; Rohde, M.; Szostak, M.; Lubitz, W., and Haas, R. Generation of* Helicobacter pylori *ghosts by PhiX protein E-mediated inactivation and their evaluation as vaccine candidates.* ***Infection and Immunity****. 2003;* ***71****:109-116.*

- *Pulz, M.; Matussek, A.; Monazahian, M.; Tittel, A.; Nikolic, E.; Hartmann, M.; Bellin, T.; Buer, J., and Gunzer, F. Comparision of a shiga toxin enzyme-linked immunosorbent assay and two different types of PCR for detection of shiga toxin-producing* Escherichia coli *in human stool specimens.* ***Journal of Clinical Microbiology****. 2003;* ***41(10)****:4671-4675.*

- *Ramnath, M.; Rechinger, K. B.; Jänsch, L.; Hastings, J. W.; Knochel, S., and Gravesen, A. The development of a* Listeria monocytogenes *EGDe proteome reference map and comparison with food isolates.* ***Applied and Environmental Microbiology****. 2003;* ***69(6)****:3368-3376.*

- *Ranson, T.; Vosshenrich, C. A.; Corcuff, E.; Richard, O.; Müller, W., and Di Santo, J. P. IL-15 is essential mediator of peripheral NK cell homeostasis.* ***Blood****. 2003;* ***101****:4887-4893.*

- *Reinscheid, D. J.; Ehlert, K.; Chhatwal, G. S., and Eikmanns, B. J. Functional analysis of a Pcs-deficient mutant of group B streptococcus.* ***FEMS Microbiology Letters****. 2003;* ***221****:73-79.*

- *Retter, I.; Blöcker, H.; Hühne, R.; Scharfe, M.; Riblet, R., and Müller, W. Characterisation of the immunoglobulin heavy chain locus of the mouse.* ***Immunobiology****. 2003;* ***208(1-3)****:103.*

- *Retter, I.; Hühne, R., and Müller, W. Automatic generation of a germ-line encoded immunoglobulin heavy chain variable gene database of the mouse. Proceedings of the German Conference on Bioinformatics; 2003; Vol. 2 pp. 70-72.*

- *Rodrigo, I.; Hill, R. E.; Balling, R.; Münsterberg, A., and Imai, K. Pax1 and Pax9 active Bapx1 to induce chondrogenic differentiation in the sclerotome.* ***Development****. 2003;* ***130****:473-482.*

- *Rohde, M.; Müller, E.; Chhatwal, G. S., and Talay, S. R. Host cell caveolae act as an entry-port for Group A streptococci.* ***Cellular Microbiology****. 2003;* ***5****:323-342.*
- *Rohde, M.; Püls, J.; Buhrdorf, R.; Fischer, W., and Haas, R. A novel sheathed surface organelle of the* Heliobacter pylori *cag type IV secretion system.* ***Molecular Biology****. 2003;* ***49****:219-234.*
- *Romanenko, L. A.; Schumann, P.; Zhukova, N. V.; Rohde, M.; Mikhailov, V. V., and Stackebrandt, E.* Oceanisphera litoralis *gen. nov., sp. nov., a novel halophilic bacterium from marine bottom sediment.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:1885-1888.*
- *Romanenko, L. A.; Zhukova, N. V.; Rohde, M.; Lysenko, A. M.; Mikhailov, V., and Stackebrandt, E.* Glaciecola mesophila *sp. nov., a novel marine agar-digesting bacterium.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:647-651.*
- *Romanenko, L. A.; Zhukova, N. V.; Rohde, M.; Lysenko, A. M.; Mikhailov, V. V., and Stackebrandt, E.* Pseudoalteromonas agarivorans *sp. nov., a novel marine agarlytic bacterium.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:125-131.*
- *Rubio-Aliaga, I.; Frey, I.; Boll, B.; Gronjeberg, D. A.; Eichinger, H. M.; Balling, R., and Daniel, H. Targeted disruption of the peptide transporter Pept2 gene in mice defines its physiological role in the kidney.* ***Molecular and Cellular Biology****. 2003;* ***23(9)****:3247-3252.*
- *Römling, U.; Bokranz, W.; Rabsch, W.; Zogaj, X.; Nimtz, M., and Tschäpe, H. Occurence and regulation of the multicellular morphotype in* Salmonella servovars *important in human disease.* ***International Journal of Medical Microbiology****. 2003;* ***293****:273-285.*
- *Samson, I.; Richard, O.; Tavian, M.; Ranson, T.; Vosshenrich, C. A. J.; Colucci, F.; Buer, J.; Grosveld, F.; Godin, I., and DiSanto, J. P. GATA-3 promotes maturation, IFN-γ production and liver-specific homing of NK cells.* ***Immunity****. 2003;* ***19****:701-711.*
- *Sasse, F.; Leibold, T.; Kunze, B.; Höfle, G., and Reichenbach, H. Cyrmenins, new β-methoxyacrylate of the respiratory chain isolated from Myxobacteria.* ***Journal of Antibiotics****. 2003;* ***56****:827-831.*
- *Sasse, F.; Steinmetz, H.; Höfle, G., and Reichenbach, H. Archazolid A, new cytotoxic macrolactones from* Archangium gephyra *(Myxobacteria).* ***Journal of Antibiotics****. 2003;* ***56(6)****:520-525.*
- *Satyanarayana, A.; Wiemann, S. U.; Buer, J.; Lauber, J.; Wüstefeld, T.; Blasco, M.; Manns, M. P., and Rudolph, K. L. Telomere shortening impairs organ regeneration by inhibition of cell cycle re- entry in a sub-population of cells with critical short telomeres.* ***EMBO Journal****. 2003;* ***22****:4003-4013.*
- *Scheele, U. ; Alves, J.; Frank, R.; Duwel, M.; Kalthoff, C., and Ungewickell, E. J. Molecular and functional characterization of clathrin and AP-2 binding determinants within a disordered domain of auxilin.* ***Journal of Biological Chemistry****. 2003;* ***278(28)****:25357-25368.*
- *Schmitz, I. ; Krueger, A.; Baumann, S.; Schulze-Bergkamen, H.; Krammer, P. H., and Kirchhoff, S. An IL-2 dependent switch between CD95 signaling pathways sensitizes primary human T cells towards CD95-mediated activation-induced cell death.* ***Journal of Immunology****. 2003;* ***171****:2930-2936.*
- *Schomburg, L.; Schweizer, U.; Holtmann, B.; Flohé, L.; Sendtner, M., and Köhrle, J. Gene disruption discloses role of selenoprotein P in selenium delivery to target tissues.* ***Biochemical Journal****. 2003;* ***370(2)****:397-402.*
- *Schrader, A. J.; Lauber, J.; Lechner, O.; Heidenreich, A.; Hofmann, R., and Buer, J. Application of real-time RT-PCR in urologic oncology.* ***Journal of Urology****. 2003;* ***169****:1858-1864.*
- *Schubert, W.-D. and Heinz, D. W. Details der Wechselwirkung zwischen Bakterium und Mensch.* ***Laborwelt****. 2003;* ***(1)****:16.*
- *Schubert, W.-D. and Heinz, D. W. Structural aspects of adhesion and invasion of host cells by the human pathogen* Listeria monocytogenes. ***ChemBioChem****. 2003;* ***4****:1285-1291.*
- *Schughart, K. and Accart, N. Use of adenovirus vectors for functional gene analysis in the chicken chorioallantoic membrane.* ***Biotechniques****. 2003;* ***34****:178-183.*
- *Schulze, K. and Guzmán, C. A. Identification of the domains of the fibronectin-binding protein I of* Streptococcus pyogenes *responsible for adjuvanticity.* ***FEMS Immunology and Medical Microbiology****. 2003;* ***37****:173-177.*
- *Schulze, K.; Medina, E.; Chhatwal, G. S., and Guzmán, C. A. Identification of B- and T-cell epitopes within the fibronectin-binding domain of the SfbI protein of* Streptococcus pyogenes. ***Infection and Immunity****. 2003;* ***71(12)****:7197-7201.*
- *Schulze, K.; Medina, E.; Chhatwal, G. S., and Guzmán, C. A. Stimulation of long-lasting protection against* Streptococcus pyogenes *after intranasal vaccination with non adjuvanted fibronectin-binding domain of the SfbI protein.* ***Vaccine****. 2003;* ***21(17-18)****:1958-1964.*
- *Sechi, A. S. and Wehland, J. Molecular basis of* Listeria monocytogenes *host cell invasion and intracellular motility: Implications for understanding actin-based processes.* ***Recent Research Development Infection and Immunity****. 2003;* ***1****:261-276.*

- *Cover picture of the journal The Journal of Clinical Investigation, Vol. 111(12), 2003, on the occasion of the publication of the article by Dinkla, K.; Rohde, M.; Jansen, W.T.M.; Kaplan, E.L.; Chhatwal, G.S. and Talay, S.R. Rheumatic fever-associated* Streptococcus pyogenes *isolates aggregate collagen.* ***The Journal of Clinical Investigation****. 2003;* ***111****:1905-1912. The permission of Blackwell Publishing is gratefully acknowledged.*

- *Sobrado, V. R.; Montemartini-Kalisz, M.; Kalisz, H.; De la Fuente, M. C.; Hecht, H.-J., and Nowicki, C. Involvement of conserved asparagine and arginine residues from the N-terminal region in the catalytic mechanism of rat liver.* ***Protein Science****. 2003;* ***12****:1039-1050.*
- *Spitzer, D.; Dittmar, K. E.; Rohde, M.; Hauser, H., and Wirth, D. Green fluorescent protein-tagged retroviral envelope protein for analysis of virus-cell interactions.* ***Journal of Virology****. 2003;* ***77****:6070-6075.*
- *Stock, M.; Schäfer, H.; Stricker, S.; Gross, G.; Mundlos, S., and Otto, F. Expression of galectin-3 in skeletal tissues is controlled by Runx2.* ***Journal of Biological Chemistry****. 2003;* ***278****:17360-17367.*
- *Stoeger, T.; Proetzel, G. E.; Welzel, H.; Papadimitriou, A.; Dony, C.; Balling, R., and Hofmann, C.* In situ *gene expression analysis during BMP2-induced ectopic bone formation in mice shows simultaneous endochondral and intramembraneous ossification.* ***Growth Factors****. 2003;* ***20****:197-210.*

- *Stradal, T. B.; Sechi, A. S.; Wehland, J., and Rottner, K. The cytoskeleton. In: Essential Cell Biology Vol. 1- Cell Structure: Practical Approach Series: Oxford University Press; 2003.*

- *Streetz, K. ; Wüstefeld, T.; Klein, C.; Kallen, K. J.; Tronche, F.; Betz, U.; Schütz, G.; Manns, M. P.; Müller, W., and Trautwein, C. Lack of gp 130 expression in hepatocytes promotes liver injury.* ***Gastroenterology****. 2003;* ***125(2)****:532-543.*

- *Streetz, K. L.; Tacke, F.; Leifeld, L.; Wüstefeld, T.; Graw, A.; Klein, C.; Kamino, K.; Spengler, U.; Kreipe, H.; Kubicka, S.; Müller, W.; Manns, M. P., and Trautwein, C. Interleukin 6/gp130-dependent pathways are protective during chronic liver diseases.* ***Hepatology****. 2003;* ***38****:218-229.*

- *Söker, U.; Kunze, B.; Reichenbach, H., and Höfle, G. Dawenol, a new polyene metabolite from the myxobacterium* Stigmatella aurantiaca. ***Zeitschrift für Naturforschung B****. 2003;* ***58b****:1024-1026.*

- *Toppel, A.; Rasmussen, M.; Rohde, M.; Medina, E., and Chhatwal, G. S. Contribution of protein G-related α2-macroglobulin binding protein to bacterial virulence in a mouse skin model of group A streptococcal infection.* ***Journal of Infectious Diseases****. 2003;* ***187****:1694-1703.*

- *Towers, R. J.; Fagan, P. K.; Talay, S. R.; Currie, B. J.; Sriprakash, K. S.; Walker, M. J., and Chhatwal. G. S. Evolution of sfbl encoding streptococcal fibronectinbinding protein I: horizontal genetic transfer and gene mosaic structure.* ***Journal of Clinical Microbiology****. 2003;* ***41****:5398-5406.*

- *Van Trappen P.O.; Steele, D.; Lowe, D. G.; Baithun, S.; Beasley, N.; Thiele, W.; Weich, H. A.; Krishnan, J.; Shepherd, J. H.; Pepper, M. S.; Jackson, D. G., and Sleeman, J. P. Expression of vascular endothelial growth factor (VEGF)-C and VEGF-D, and their receptor VEGFR-3, during different stages of cervical carcinogenesis.* ***Journal of Pathology****. 2003;* ***201****:544-554.*

- *Viret, J.-F.; Moser, C.; Rharbaoui, F.; Metcalfe, I. C., and Guzmán, C. A. Virosomal technology and mucosal adjuvants. (Kaufmann, S. H. E., ed.). Novel Vaccination Strategies. Weinhaim, Germany: Wiley-VCH Verlag GmbH; 2003; Chapter 10, pp. 197-217.*

- *Voigt, J. and Frank, R. 14-3-3 proteins are constituents of the insoluble glycoprotein framework of the clamydomonas cell wall.* ***Plant Cell****. 2003;* ***15(6)****:1399-1413.*

- *Vosshenrich, C.; Cumano, A.; Müller, W.; Di Santo, J. P., and Vieira, P. Thymic stromal-derived lymphopoietin distinguishes fetal from adult B cell development.* ***Nature Immunology****. 2003;* ***4(8)****:773-779.*

- *Walter, U.; Toepfer, T.; Dittmar, K. E. J.; Kretschmer, K.; Lauber, J.; Weiss, S.; Servos, G.; Lechner, O.; Scherbaum, W. A.; Bornstein, S. R.; von Boehmer, H., and Buer, J. Pancreatic NOD beta-cells express MHC class II protein and frequency of I-A(g7) mRNA expressing beta-cells strongly increase during progression to autoimmune diabetes.* ***Diabetologia****. 2003;* ***46(8)****:1106-1114.*

- *Wehmhöner, D.; Häussler, S.; Tümmler, B.; Jänsch, L.; Wehland, J., and Steinmetz. I. Inter – and intraclonal diversity of* Pseudomonas aeruginosa *proteome manifests within the secretome.* ***Journal of Bacteriology****. 2003;* ***185(19)****:5807-5814.*

- *Weinbauer, M. G.; Brettar, I., and Höfle, M. G. Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters.* ***Limnology and Oceanography****. 2003;* ***48****:1457-1465.*

- *Weinig, S.; Hecht, H.-J.; Mahmud, T., and Müller, R. Melithiazol biosynthesis: Further insights into myxobacterial PKS/NRPS systems and evidence for a new subclass of methyl transferase.* ***Chemistry and Biology****. 2003;* ***10****:939-952.*

- *Weinig, S.; Mahmud, T., and Müller, R. Markerless mutations in the myxothiazol biosynthetic gene cluster: A delicate megasynthetase with a superfluous nonribosomal peptide synthetase domain.* ***Chemistry and Biology****. 2003;* ***10****:953-960.*

- *Weiss, S. Transfer of eukayotic expression plasmids to mammalian hosts by attenuated* Salmonella *spp.* ***International Journal of Medical Microbiology****. 2003;* ***293****:95-106.*

- *Werhahn, W.; Jänsch, L., and Braun, H. P. Identification of novel subunits of the tom complex from* Arabidopsis thaliana. ***Plant Physiology****. 2003;* ***41****:407-416.*

- *Wiethe, C.; Dittmar, K. E. J.; Doan, T.; Lindenmaier, W., and Tindle, R. Enhanced effector and memory CTL responses generated by incorporation of receptor activator of NF-kappaB (RANK)/RANK ligand costimulatory molecules into dendritic cell immunogens expressing a human tumor-specific antigen.* ***Journal of Immunology****. 2003;* ***171****:4121-4130.*

- *Wiethe, C.; Dittmar, K. E. J.; Doan, T.; Lindenmaier, W., and Tindle, R. Provision of 4-1bb ligand enhances effector and memory ctl responses generated by immunization with dendritic cells a human tumor-associated antigen.* ***Journal of Immunology****. 2003;* ***170****:2912-2922.*

- *Wüstefeld, T.; Klein, C.; Streetz, K. L.; Betz, U.; Lauber, J.; Buer, J.; Manns, M. P.; Müller, W., and Trautwein, C. IL6/GP130-dependent pathways are protective during liver regeneration.* ***Journal of Biological Chemistry****. 2003;* ***278****:11281-11288.*

- *Zander, K.; Sherman, M. P.; Tessmer, U.; Bruns, K.; Wray, V.; Prechter, A. T.; Schubert, E.; Henklein, P.; Neidleman, J., Greene, W. C. and Schubert U. Cyclophilin A interacts with HIV-1 Vpr and is required for its functional expression.* ***Journal of Biological Chemistry****. 2003;* ***278****:43202-43213.*

- *Zander, N.; Gerhardt, J., and Frank, R. Polystyrylsulfonyl-3-nitro-1H-1,2,4-triazolide-resin: a new solid-supported reagent for the esterification of amino acids.* ***Tetrahedron Letters****. 2003;* ***44(35)****:6557-6560.*

- *Zeitz, U.; Weber, K.; Soegiarto, D. W.; Wolf, E.; Balling, R., and Erben, R. G. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor.* ***FASEB Journal****. 2003;* ***17****:509-511.*

- *Zogaj, X.; Bokranz, W.; Nimtz, M., and Römling, U. Production of cellulose and curli fimbriae by members of the family Enterobacteriaceae isolated from the human gastrointestinal tract.* ***Infection and Immunity****. 2003;* ***71(7)****:4151-4158.*

- *zur Lage, S.; Goethe, R.; Darji, A.; Valentin-Weigand, P., and Weiss, S. Activation of macrophages and interference with CD4+ T cell stimulation by* Mycobacterium avium *ssp.* paratuberculosis *and* Mycobacterium avium *ssp.* avium. ***Immunology****. 2003;* ***108(1)****:62-69.*

- *Zähner, D.; Kaminski, K.; van der Linden, L. M.; Mascher, T.; Merai, M., and Hakenbeck, R. The* ciaR/ciaH *regulatory system of* Streptococcus pneumoniae *is involved in beta-lactam resistance and genetic competence. In: Regulatory Networks in Prokaryotes. Norfolk (Dürre, P. and Friedrich, B. eds.): Horizon Press; 2003; pp. 41-46.*

Comparative Genome Research – 2003

- *Bi, J.-X.; Buhr, P.; Zeng, A. P., and Wirth, M. The human* c-fos *promotor mediates high-level, inducible expression in various mammalian cell lines.* ***Biotechnology and Bioengineering****. 2003;* ***81****:848-854.*

- *Bialek, K.; Swistowski, A., and Frank, R. Epitope-targeted proteome analysis: Towards a large-scale automated protein-protein-interaction mapping utilizing synthetic peptide arrays.* ***Analytical and Bioanalytical Chemistry****. 2003;* ***376(7)****:1006-1013.*

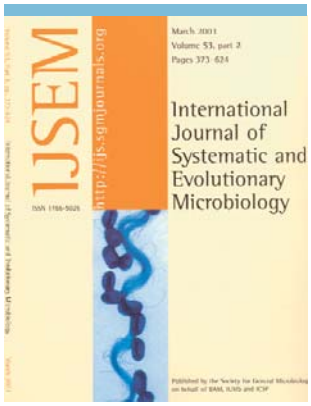
- *Black, G. F.; Weir, R. E.; Chaguluka, S. D.; Warndorff, D.; Crampin, A. C.; Mwaungulu, L.; Sichali, L.; Floyd, S.; Bliss, L.; Jarman, E.; Donovan, L.; Andersen, P.; Britton, W.; Hewinson, G.; Huygen, K.; Paulsen, J.; Singh, M.; Prestidge, R.; Fine, P. E., and Dockrell, H. M. Gamma interferon responses induced by a panel of recombinant and purified mycobacterial antigens in healthy, non-mycobacterium bovis BCG-vaccinated Malawian yound adults.* ***Clinical and Diagnostic Laboratory Immunology****. 2003;* ***10(4)****:602-611.*

- *Buchrieser, C.; Rusniok, C.; Kunst, F.; Cossart, P.; Glaser, P.; The European* Listeria *Genome Consortium (GBF: H. Blöcker; P. Brandt; U. Kärst; G. Nordsiek, and J. Wehland). Comparison of the genome sequences of* Listeria monocytogenes *and* Listeria innocua*: clues for evolution and pathogenicity.* ***FEMS Immunology and Medical Microbiology****. 2003;* ***35****:207-213.*

- *Eichler, J. Kombinatorische Chemie. Konzepte und Strategien. Teubner Studienbücher Chemie. Stuttgart: Teubner, B. G.; 2003.*
- *Eichler, J.; Hirsch, T., and Overwin, H. Synthetic mimicry of conformationally defind protein binding sites: hYAP-WW domain. (Benedetti, E. and Cedone, C., editors). In: Peptides 2002. Napoli, Italy; 2003; pp. 740-741.*
- *Franke, R.; Doll, C.; Wray, V., and Eichler, J. Solid-phase synthesis of structurally diverse scaffolded peptides for the mimicry of discontinuous protein binding sites.* ***Protein and Peptide Letters****. 2003;* ***10(6)****:531-539.*
- *Gardan, R.; Cossart, P.; Labadie, J.; The European* Listeria *Genome Consortium (GBF: H. Blöcker ; P. Brandt; U. Kärst; G. Nordsiek, and J. Wehland). Identification of* Listeria monocytogenes *genes involved in salt and alkaline-pH tolerance.* ***Applied and Environmental Microbiology****. 2003;* ***69****:3137-3143.*
- *Gardan, R.; Duché, O.; Leroy-Setrin, S.; Labadie, J.; The European* Listeria *Genome Consortium (GBF: H. Blöcker; P. Brandt; U. Kärst; G. Nordsiek, and J. Wehland). Role of ctc from* Listeria monocytogenes *in osmotolerance.* ***Applied and Environmental Microbiology****. 2003;* ***69****:154-161.*
- *Kauer, G. and Blöcker, H. Applying signal theory to the analysis of biomolecules.* ***Bioinformatics****. 2003;* ***19****:2016-2021.*
- *Krull, M.; Voss, N.; Choi, C.; Pistor, S.; Potapov, A., and Wingender, E. TRANSPATH®: an integrated database on signal transduction and a tool for array analysis.* ***Nucleic Acids Research****. 2003;* ***31****:97-100.*
- *Kuper, J.; Winking, J.; Hecht, H.-J.; Mendel, R. R., and Schwarz, G. The active site of molybdenum cofactorbiosynthetic protein domain Cnx 1.* ***Archives of Biochemistry and Biophysics****. 2003;* ***411****:36-46.*
- *Kämpfer, S.; Dalley, D.; Hewinson, R. G.; Chambers, M. A., and Singh, M. Multi-antigen ELISA for enhanced diagnosis of tuberculosis in badgers.* ***Veterinary Record****. 2003;* ***153****:403-404.*
- *Layer, G.; Moser, J.; Heinz, D. W.; Jahn, D., and Schubert, W.-D. Crystal structure of coproporphyrinogen III oxidase reveals cofactor geometry of radical SAM enzymes.* ***EMBO Journal****. 2003;* ***22****:6214-6224.*
- *Ma, H. and Zeng, A. P. The connectivity structure, giant strong component and centrality of metabolic networks.* ***Bioinformatics****. 2003;* ***19****:1423-1430.*
- *Ma, H. and Zeng, A.-P. Reconstruction of metabolic networks from genome data and analysis of their global structure for various organisms.* ***Bioinformatics****. 2003;* ***19****:270-277.*
- *Marcus, K.; Hultschig, C.; Frank, R.; Herberg, F. W.; Schuchhardt, J., and Seitz, H. Innovative Forschungsansätze im NGFN Verbund "The Human Brain Proteome Projekct HBPP".* ***GenomXPress****. 2003;* ***4****:5-8.*
- *Matys, V.; Fricke, E.; Geffers, R.; Gößling, E.; Haubrock, M.; Hehl, R.; Hornischer, K.; Karas, D.; Kel, A. E.; Kel-Margoulis, E. V.; Kloos, D. U.; Land, S.; Lewicki-Potapov, B.; Michael, H.; Münch, R.; Reuter, I.; Rotert, S.; Saxel, H.; Scheer, M.; Thiele, S., and Wingender, E. TRANSFAC®: transcriptional regulation, from patterns to profiles.* ***Nucleic Acids Research****. 2003;* ***31****:374-378.*
- *Moser, J.; Frere, F.; Heinz, D. W.; Jahn, D., and Schubert, W.-D. Die tRNA-abhängige Tetrapyrrol-Biosynthese.* ***BioSpektrum****. 2003;* ***(9)****:133-137.*
- *Münch, R.; Hiller, K.; Barg, H.; Heldt, D.; Linz, S.; Wingender, E., and Jahn, D. PRODORIC: prokaryotic datbase of gene regulation.* ***Nucleic Acids Research****. 2003;* ***31****:266-269.*
- *Qiao, J.-J.; Yuan, Y.-J.; Zhaol, H.; Wu, J.-C., and Zeng, A.-P. Apoptotic cell death in suspension cultures of* Taxus cuspidata *co-treated with sylicylic acid and hydrogen peroxide.* ***Biotechnology Letters****. 2003;* ***25****:387-390.*
- *Retter, I.; Blöcker, H.; Hühne, R.; Scharfe, M.; Riblet, R., and Müller, W. Characterisation of the immunoglobulin heavy chain locus of the mouse.* ***Immunobiology****. 2003;* ***208(1-3)****:103.*
- *Rodriguez, A.; Troye-Blomberg, M.; Lindroth, K.; Ivanyi, J.; Singh, M., and Fernandez, C. B- and C-cell responses to the mycobacterium surface antigen PstS-1 in the respiratory tract and adjacent tissues. Role of adjuvants and routes of immunization.* ***Vaccine****. 2003;* ***21****:458-467.*
- *Sabra, W.; Lünsdorf, H., and Zeng, A.-P. Alterations in the formation of lipopolysaccharide and membrane vesicle on the surface of* Pseudomonas aeruginosa *PAO1 under oxidative stress conditions.* ***Microbiology-SGM****. 2003;* ***149****:2789-2795.*
- *Shelest, E.; Kel, A. E.; Gößling, E., and Wingender, E. Prediction of potential C/EBP/NF-kappaB composite elements using the matrix-based search methods.* ***In Silico Biology****. 2003;* ***3(1-2)****:71-79.*
- *Sun, J.; van den Heuvel, J.; Soucaille, P.; Qu, Y., and Zeng, A. P. Comparative genomic analysis of dha regulon and related genes for anaerobic glycerol metabolism in microorganisms.* ***Biotechnology Progress****. 2003;* ***19****:263-272.*
- *Wang, W.; Sun, J.; Hartlep, M.; Deckwer, W.-D., and Zeng, A.-P. Combined use of proteomics analysis and enzyme activity assay for metabolic pathway analysis of glycerol fermentation by* Klebsiella pneumoniae. ***Biotechnology and Bioengineering****. 2003;* ***83****:525-536.*
- *Wang, W.; Sun, J.; Nimtz, M.; Zeng, A.-P., and Deckwer, W.-D. Protein identification from 2-dimentional gel electrophoresis analysis of* Klebsiella pneumoniae *by combined use of mass spectrometry data and unannotated genome sequences.* ***Proteome Science****. 2003;* ***1****:6.*
- *Zander, N.; Gerhardt, J., and Frank, R. Polystyrylsulfonyl-3-nitro-1H-1,2,4-triazolide-resin: a new solid-supported reagent for the esterification of amino acids.* ***Tetrahedron Letters****. 2003;* ***44(35)****:6557-6560.*

Sustainable Use of Landscape – 2003

- *Abraham, W.-R. and Hesse, C. Isotope fractionations in the biosynthesis of cell compounds by different fungi: A bais for environmental carbon flux studies.* ***FEMS Microbiology Ecology****. 2003;* ***46****:121-128.*
- *Abraham. W.-R.; Lünsdorf, H.; Strömpl, C.; Nogales, B.; Moore, E. R. B., and Timmis, K. N. Microbial communities in composite biofilms participating in the degradation of PCB.* ***Water, Air and Soil Pollution****:* ***Focus****. 2003;* ***3(3)****:57-64.*
- *Abraham, W.-R. and Wenderoth, D. F. Schicksal fakultativ pathogener Mikroorganismen während und nach dem Sommerhochwasser an Elbe und Mulde. (Geller, W.; Ockenfeld K.; Böhme M.; Voigt M.; editors). In: Schadstoffbelastung im Mulde- und Elbeeinzugsgebiet nach dem Augusthochwasser 2002. Magdeburg; 2003; pp. 6-9.*
- *Allgaier, M.; Uphoff, H.; Felske, A., and Wagner-Döbler, I. Aerobic anoxygenic photosynthesis in Roseobacter clade bacteria from diverse marine habitats.* ***Applied and Environmental Microbiology****. 2003;* ***69(9)****:5051-5059.*
- *Barreiros, L.; Nogales, B.; Manaia, C. M.; Ferreira, A. C. S.; Pieper, D. H.; Reis, A. M., and Nunes, O. C. A novel pathway for mineralization of the thiocarbamate herbicide molinate by a defined bacterial consortium.* ***Environmental Microbiology****. 2003;* ***5****:944-953.*
- *Bertini, I.; Provenzani, A.; Viezzoli, M. S.; Pieper, D. H., and Timmis, K. N. NMR spectroscopy as a tool to investigate the degradation of aromatic compounds by a* Pseudomonas putida *strain.* ***Magnetic Resonance Chemistry****. 2003;* ***41****:615-621.*
- *Brettar, I.; Christen, R., and Höfle, M. G.* Idiomarina baltica, *sp. nov. a marine bacterium with high temperature optimum isolated from surface water of the Central Baltic Sea.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:407-413.*
- *Brümmer, I. H. M.; Felske, A., and Wagner-Döbler, I. Diversity and seasonal variability of b-Proteobacteria in biofilms of polluted rivers analyzed by TGGE and cloning.* ***Applied and Environmental Microbiology****. 2003;* ***69(8)****:4463-4473.*



- *Cover picture of the journal International Journal of Systematic and Evolutionary Microbiology, Vol. 53, 2003, on the occasion of the publication of the article by Monciardini, P.; Cavaletti, L.; Schumann, P.; Rohde, M. and Donadio, S.* Conexibacter woesei *gen.nov., sp. nov., a novel representative of a deep evolutionary line of descent within the class* Actinobacteria. ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:569-576. The permission of Society for General Microbiology (SGM) is gratefully acknowledged.*

- *Budde, H.; Flohé, L.; Hecht, H.-J.; Hofmann, B.; Stehr, M.; Wissing, J., and Lünsdorf, H. Kinetics and redox-sensitive oligomerisation reveal negative subunit cooperativity in tryparedoxin peroxidase of* Trypanosoma brucei brucei. ***Biological Chemistry****. 2003;* ***384****:619-633.*

- *Demnerova, K.; Stiborova, H.; Leigh, M. B.; Pieper, D. H.; Pazlarova, J.; Brenner, V., and Macek, T. Bacteria degrading PCBs and CBs isolated from long-term PCB contaminated soil.* ***Water, Air and Soil Pollution****:* ***Focus****. 2003;* ***3(3)****:47-55.*

- *Felske, A.; Fehr, W.; Pauling, B. V.; von Canstein, H., and Wagner-Döbler, I. Functional profiling of mercuric reductase (*mer *A) genes in biofilm communities of a technical scale biocatalyzer.* ***BMC Microbiology****. 2003;* ***3****:22.*

- *Felske, A.; Vandieken, V.; Pauling, B. V.; von Canstein, H. F., and Wagner-Döbler, I. Molecular quantification of genes encoding for green fluorescent proteins.* ***Journal of Microbiological Methods****. 2003;* ***52****:297-304.*

- *Felske, A.; Heyrman, J.; Balcaen, A., and De Vos, P. Multiplex PCR screening of soil isolates for novel Bacillus-related lineages.* ***Journal of Microbiological Methods****. 2003;* ***53(2)****:447-458.*

- *Ferrer, M.; Chernikova, T. N.; Yakimov, M. M.; Golyshin, P. N., and Timmis, K. N. Chaperonins govern growth of* Escherichia coli *at low temperatures.* ***Nature Biotechnology****. 2003;* ***21****:1266-1267.*

- *Ferrer, M.; Golyshin, P., and Timmis, K. N. Novel maltotriose esters enhance biodegradation of Arochlor 1242 by* Burkholderia cepacia *LB400.* ***World Journal of Microbiology and Biotechnology****. 2003;* ***19****:637-643.*

- *Flohé, L. ; Jaeger, T.; Pilawa, S., and Sztajer, H. Thiol-dependent peroxidases care little about homology-based assignments of function.* ***Redox Report****. 2003;* ***8(5)****:1-10.*

- *Fry, J. C.; Giuliano, L., and Golyshin, P. N. New tools for elucidating the ecology of micro-organisms in aquatic environments.* ***FEMS Microbiology and Ecology****. 2003;* ***46****:231.*

- *Golyshin, P. N.; Martins Dos Santos, V. A. P.; Kaiser, O.; Ferrer, M.; Sabirova, Y. S.; Lünsdorf, H. Chernikova T. N.; Golyshina, O. V.; Yakimov, M. M.; Pühler, A., and Timmis, K. N. Genome sequence completed of* Alcanivorax borkumensis, *a hydrocarbon-degrading bacterium that plays a global role in oil removal from marine systems.* ***Journal of Biotechnology****. 2003;* ***106****:215-220.*

- *Hahn, M. W.; Lünsdorf, H.; Wu, Q.; Schauer, M.; Höfle, M. G.; Boenigk, J., and Stadler, P. Isolation of novel ultramicrobacteria classified as Actinobacteria from five freshwater habitats in Europe and Asia.* ***Applied and Environmental Microbiology****. 2003;* ***69****:1442-1451.*

- *Hallsworth, J. E.; Heim, S., and Timmis, K. N. Chaotropic solutes cause water stress in* Pseudomonas putida. ***Environmental Microbiology****. 2003;* ***5****:1270-1280.*

- *Heil, M.; Mittnacht-Krauss, R.; Issbrücker, K.; van den Heuvel, J.; Dehio, C.; Schaper, W.; Clauss, M., and Weich, H. A. An engineered heparin-binding form of VEGF-E: biological effects* in vitro *and mobilisation of precursor cells.* ***Angiogenesis****. 2003;* ***6(3)****:201-211.*

- *Heim, S.; Ferrer, M; Heuer, H.; Regenhardt, D.; .Nimtz, M., and Timmis, K. Proteome reference map of* Pseudomonas putida *strain KT2440 for gene profiling analysis: comparative whole genome analysis of iron deprivation in KT2440 and* P. aeruginosa *strain PAO1.* ***Environmental Microbiology****. 2003;* ***5****:1257-1269.*

- *Junca H. and Pieper, D. H. Amplified functional DNA restriction analysis to determine catechol 2,3-dioxygenase gene diversity in soil bacteria.* ***Journal of Microbiological Methods****. 2003;* ***55****:697-708.*

- *Kahl, S. and Hofer, B. A genetic system for the rapid isolation of aromatic-ring-hydroylating dioxygenase activities.* ***Microbiology-SGM****. 2003;* ***149****:1475-1481.*

- *Kämpfer, P.; Dreyer, U.; Neef, A.; Dott, W., and Busse, H.-J.* Chryseobacterium defluvii *sp. nov., isolated from wastewater.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:93-97.*

- *Labrenz, M.; Lawson, P. A.; Tindall, B. J.; Collins, M. D., and Hirsch, P.* Saccharospirillum impatiens *gen. nov., sp. nov., a novel gamma-Proteobacterium isolated from hypersaline Ekho Lake (East Antarctica).* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:653-660.*

- *Lucas, F.; Bertru, G., and Höfle, M. G. Characterization of free-living and attached bacteria in sediments colonized by* Hediste (Nereis) diversicolor. ***Aquatic Microbial Ecology****. 2003;* ***32****:165-174.*

- *Maiorino, M. ; Bosello, V.; Ursini, F.; Foresta, C.; Garolla, A.; Scapin, M.; Sztajer, H., and Flohé, L. Genetic variations of* gpx-4 *and male infertility in humans.* ***Biology of Reproduction****. 2003;* ***68****:1134-1141.*

- *Mauclaire, L.; Thullner, M.; Pelz, O.; Abraham, W.-R., and Zeyer, J. Assimilation of toluene carbon along a bacteria-protist food chain determined by* ^{13}*C-enrichment of biomarker fatty acids.* ***Journal of Microbiological Methods****. 2003;* ***55****:635-649.*

- *McKay, D.; Prucha, W.; Reineke, K.; Timmis, K. N., and Pieper, D. H. Substrate specificity and expression of three 2,3-dihydroxybiphenyl 1,2-dioxygenases from* Rhodococcus globerulus *strain P6.* ***Journal of Bacteriology****. 2003;* ***185****:2944-2951.*

- *Neef, A.; Schäfer, R.; Beimfohr, C., and Kämpfer, P. Fluorescence based rRNA sensors for whole cells of* Saccharomonospora *spp. and* Thermoactinomyces *spp.* ***Biosensors and Bioelectronics****. 2003;* ***18****:565-569.*

- *Nikodem, P.; Hecht, V.; Schlömann, M., and Pieper, D. H. A new bacterial pathway for 4- and 5-chlorosalicylate degradation via 4-chlorocatechol and maleylacetate in* Pseudomonas *sp. strain MT1.* ***Journal of Bacteriology****. 2003;* ***185****:6790-6800.*

- *Perez-Pantoja, D. T.; Ledger, D.; Pieper, D., and Gonzalez, B. Efficient turnover of chlorocatechols is essential for growth of* Ralstonia eutropha *JMP134(pJP4) in 3-chlorobenzoic acid.* ***Journal of Bacteriology****. 2003;* ***185****:1534-1542.*

- *Pollmann, K.; Wray, V.; Hecht, H.-J., and Pieper, D. H. Rational engineering of the regioselectivity of TecA tetrachlorobenzene dioxygenase for the transformation of chlorinated toluces.* ***Microbiology-SGM****. 2003;* ***149****:903-913.*

- *Rapp, P. and Gabriel-Jürgens, L. H. E. Degradation of alkanes, highly chlorinated benzenes and production of biosurfactans by a psychotrophic* Rhodococcus sp. *Genetic characterization of its chlorobenzene dioxygenase.* ***Microbiology-SGM****. 2003;* ***149****:2879-2890.*

- *Römling, U.; Bokranz, W.; Gerstel, U.; Lünsdorf, H.; Nimtz, M.; Rabsch, W.; Tschäpe, H., and Zogaj, X. Dissection of the genetic pathway leading to multicellular behaviour in* Salmonella enterica *serotype* typhimurium *and other Enterobacteriaceae. In: Medical Implications of Biofilms (Wilson, M.; Devine D., eds.). Cambridge: Cambridge University Press; 2003; pp. 231-261.*

- *Schmeisser, C.; Stöckigt, C.; Raasch, C.; Wingender, J.; Timmis, K. N.; Wenderoth, D. F.; Jaeger, K.-E.; Flemming, H.-C.; Liesegang, H.; Henne, A., and Streit, W. R. A metagenome survey of biofils grown on rubber-coated valves in drinking water networks.* ***Applied and Environmental Microbiology****. 2003;* ***69****:7298-7309.*

- *Simankova, M. V.; Kotsyurbenko, O. R.; Lueders, T.; Nozhevnikova, A. N.; Wagner, B.; Conrad, R., and Friedrich, M. W. Isolation and characterization of new strains of methanogens from cold terrestrial habitats.* ***Systematic and Applied Microbiology****. 2003;* ***26(2)****:312-318.*

- *Strömpl, C.; Hold, G. N.; Lündsorf, H.; Graham, J.; Gallacher, S.; Abraham, W.-R.; Moore, E. R. B., and Timmis, K. N.* Oceanicaulis alexandrii *gen. nov., sp. nov., a novel stalked bacterium isolated from a culture of the dinoflagellate* Alexandrium tamarense *(Lebour) Balech.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:1901-1906.*

- *Timmis, K. N. Forensische Methoden für die Nach-Koch'sche Ära.* ***Forschung und Diagnostik****. 2003;* ***1****:49.*

- *Torres, K. N.; Jaenecke, S.; Timmis, K. N.; Garcia, J., and Diaz, E. A dual lethal system to enhance predictability of recombiant microorganisms.* ***Microbiology-SGM****. 2003;* ***149****:3595-3601.*

- *Veluri, R.; Oka, I.; Wagner-Döbler, I., and Laatsch, H. New indole alkaloids from the North Sea bacterium* Vibrio parahaemolyticus *Bio 249.* ***Journal of Natural Products****. 2003;* ***66****:1520-1523.*

- *Von Canstein, H. F.; Li, Y.; Leonhäuser, J.; Deckwer, W.-D., and Wagner-Döbler, I. Bioremediation of mercury-polluted industrial wastewater by mercury-reducing biofilms.* ***Recent Research Developments in Biotechnology and Bioengineering****. 2003;* ***5****:147-171.*

- *Von Canstein, H. F.; Li, Y.; Leonhäuser, J.; Deckwer, W.-D., and Wagner-Döbler, I. Mikrobielle Reinigung von quecksilberhaltigen Industrieabwässern.* ***BioSpektrum****. 2003;* ***(2/3)****:150-152.*

- *Wagner-Döbler, I. Microbial inoculants – snake oil or panacea. (Singleton, M. G.; Milner I. M., eds.) Bioremediation – A Critical Review: Horizon Press; 2003; pp. 259-289.*

- *Wagner-Döbler, Minireview: Pilot plant for bioremediation of mercury contaminated industrial waste water.* ***Applied Microbiology and Biotechnology****. 2003;* ***62****:124-133.*

- *Wagner-Döbler, I.; Rheims, H.; Felske, A.; Pukall, R., and Tindall, B.* Jannaschia helgolandensis, *gen. nov., a novel abundant member of the marine Roseobacter clade from the North Sea.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53(3)****:731-738.*

- *Wagner-Döbler, I.; von Canstein, H. F.; Li, Y.; Leonhäuser, J., and Deckwer, W.-D. Process-integrated microbial mercury removal from wastewater of chlor-alkali electrolysis plants.* ***Engineering in Life Sciences****. 2003;* ***3(4)****:177-181.*

- *Witte, U.; Wenzhöfer, F.; Sommer, S.; Boetius, A.; Heinz, P.; Aberle, N.; Sand, M.; Cremer, A.; Abraham, W.-R.; Jörgensen, B. B., and Pfannkuche, O.* In situ *experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor.* ***Nature****. 2003;* ***424****:763-766.*

- *Yakimov, M. M.; Giuliano, L.; Gentile, G.; Cristafi, E.; Chernikova, T. N.; Abraham, W.-R.; Lünsdorf, H.; Timmis, K. N., and Golyshin, P. N.* Oleispira antarctica *gen. nov., sp. nov., a new hydrocarbonoclastic marine bacterium, isolated from an antarctic coastal seawater.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:779-785.*

- *Yakimov, M. M.; Lünsdorf, H., and Golyshin, P. N.* Thermoleophilum album *and* Thermoleophilum minutum *are culturable representatives of group 2 of the Rubrobacteidae (Actinobacteria).* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:377-380.*

- *Zielinski, M.; Kahl, S.; Hecht, H.-J., and Hofer, B. Pinpointing biphenyl dioxygenase residues that are crucial for substrate interaction.* ***Journal of Bacteriology****. 2003;* ***185****:6976-6980.*

Platforms – 2003

- *Barthold, M.; Fargali, S.; Majore, I.; Zghoul, N.; Stahl, F.; Rohde, M.; Mayer, H., and Jäger, V. Proliferation and osteogenic differentiation of mesenchymal stem cells on calcium phosphate scaffolds in fixes bed bioreactors.* ***Tissue Engineering****. 2003;* ***9****:848.*

- *Bringmann, G.; Mühlbacher, J.; Messer, K.; Deyer, M.; Ebel, R.; Nugroho, B. W.; Wray, V., and Proksch. P. Cyclorocaglamide, the first bridged cyclopentatetrahydrobenzofuran and a related "open chain" rocaglamide derivative from* Alaia oligophylla. ***Journal of Natural Products****. 2003;* ***66****:80-85.*

- *Ding, H.; Griesel, C.; Nimtz, M.; Conradt, H. S.; Weich, H. A., and Jäger, V. Molecular cloning, expression, purification, and characterization of soluble full-length, human interleukin-3 with a baculovirus-insect cell expression system.* ***Protein Expression and Purification****. 2003;* ***31****:34-41.*

- *Eckermann, C.; Matthes, B.; Nimtz, M.; Reiser, V.; Lederer, B.; Böger, P., and Schröder, J. Covalent binding of chloroacetamide herbicides to the active site cysteine of plant type II polyketide synthases.* ***Phytochemistry****. 2003;* ***64****:1045-1054.*

- *Fargali, S.; Barthold, M.; Majore, L., and Jäger, V. Three-dimensional cultivation of rabbit mesenchymal stem cells on bioresorbable, macroporous calcium phosphate scaffolds in vitro.* ***International Journal of Artificial Organs****. 2003;* ***26****: 859.*

- *Franke, R.; Doll, C.; Wray, V., and Eichler, J. Solid-phase synthesis of structurally diverse scaffolded peptides for the mimicry of discontinuous protein binding sites.* ***Protein and Peptide Letters****. 2003;* ***10(6)****:531-539.*

- *Heil, M.; Mittnacht-Krauss, R.; Issbrücker, K.; van den Heuvel, J.; Dehio, C.; Schaper, W.; Clauss, M., and Weich, H. A. An engineered heparin-binding form of VEGF-E: biological effects* in vitro *and mobilisation of precursor cells.* ***Angiogenesis****. 2003;* ***6(3)****:201-211.*

- *Häußler, S.; Rohde, M.; von Neuhoff, N.; Nimtz, M., and Steinmetz, I. Structural and functional cellular changes induced by* Burholderia pseudomallei. ***Infection and Immunity****. 2003;* ***71****:2970-2975.*

- *Jäger, V.; Barthold, M.; Fargali, S.; Majore, I.; Zghoul, N.; Stahl, F.; Rohde, M., and Mayer, H. 3-D cultivation and monitoring of bone precursor cells on calcium phosphate scaffolds in fixed bed bioreactors.* ***Artificial Organs****. 2003;* ***26****: 829.*

- *Lin, W.; Brauers, G.; Ebel, R.; Wray, V.; Berg, A.; Sudarsono, and Proksch, P. Novel chromone derivates from the fungus* Aspergillus versicolor *isolated from the marine sponge* Xestospongia exiqua. ***Journal of Natural Products***. *2003;* ***66****:57-61.*
- *Majore, I.; Barthold, M.; Stahl, F.; Schulz, R.; Loose, S.; Mayer, H.; Fargali, S., and Jäger, V. Optimization of cell culture conditions and comparative analysis of the proliferation and differentiation of human osteoprogenitor cells by DNA microarray analysis and non-invasive methods.* ***International Journal of Artificial Organs***. *2003;* ***26****:869.*
- *Marxen, J. C.; Nimtz, M.; Becker, W., and Mann, K. The major soluble 19.6 kda protein of the orgabnic shell matrix of the freshwater snail* Biomphalaria glabrata *is an N-glycosylated dermapontin.* ***Biochimica Et Biophysica Acta – Proteins & Proteomics***. *2003;* ***1650****:92-98.*
- *Münzenberger, B.; Hammer, E.; Wray, V.; Shauer, F.; Schmidt, J., and Strack, D. Detoxification of ferulic acid by ectomycorrhizal fungi.* ***Mycorrhiza***. *2003;* ***13****:117-121.*
- *Pipp, F.; Heil, M.; Issbrücker, K.; Ziegelhoeffer, T.; Martin, S.; van den Heuvel, J.; Weich, H.; Fernandez, B.; Colomb, G.; Caremeliet, P.; Schaper, W., and Claus, M. The VEGFR-1 selective VEGF-homologue PIGF is arteriogenic: evidence for a monocyte mediated mechanism.* ***Circulation Research***. *2003;* ***92****:378-385.*
- *Pollmann, K.; Wray, V.; Hecht, H.-J., and Pieper, D. H. Rational engineering of the regioselectivity of TecA tetrachlorobenzene dioxygenase for the transformation of chlorinated toluces.* ***Microbiology-SGM***. *2003;* ***149****:903-913.*
- *Proksch, P.; Ebel, R.; Erada, R. A.; Schupp, P.; Lin, W. H.; Sudarsono; Wray, V., and Steube, K. Detection of pharmacologically active natural products using ecology. Selected examples from Indopacific marine invertebrates and sponge-derived fungi.* ***Pure and Applied Chemistry***. *2003;* ***75****:337-346.*
- *Schultz, A.; Laschat, S.; Diele, S., and Nimtz, M. Tetraphenylethene-derived columnar crystals and their oxidative photocyclization.* ***European Journal of Organic Chemistry***. ***2003****; 2829-2839.*
- *Schupp, P.; Poehner, T.; Erada, R.; Ebel, R.; Wray, V., and Proksch, P. Eudistomins W and X, two new ß-carbolines from the Micronesian Tunicate* Eudistoma viride. ***Journal of Natural Products***. *2003;* ***66****:272-275.*
- *Wang, C.-Y.; Wang, B.-G.; Wiroyowidago, S.; Wray, V.; van Soest, R.; Steube, K. G.; Guan, H.-S.; Proksch, P., and Ebel, R. Melophins C-O, thirteen novel teramic acids from the marine sponge* Melophlus sarassinorum. ***Journal of Natural Products***. *2003;* ***66****:51-56.*
- *Wang, W.; Sun, J.; Nimtz, M.; Zeng, A.-P., and Deckwer, W.-D. Protein identification from 2-dimentional gel electrophoresis analysis of* Klebsiella pneumoniae *by combined use of mass spectrometry data and unannotated genome sequences.* ***Proteome Science***. *2003;* ***1****:6.*
- *Weinig, S.; Hecht, H.-J.; Mahmud, T., and Müller, R. Melithiazol biosynthesis: Further insights into myxobacterial PKS/NRPS systems and evidence for a new subclass of methyl transferase.* ***Chemistry and Biology***. *2003;* ***10****:939-952.*
- *Yasman; Edrada, R. A.; Wray, V., and Proksch, P. New 9-thiocyanatopupukeanane sesquiterpenes from the mudibranchs* Phyllidia varicosa *and its sponge-pres* Axinyssa aculeata *collected in Thousand Island National Park, Indonesia.* ***Journal of Natural Products***. *2003;* ***66****:1512-1514.*
- *Zander, K.; Sherman, M. P.; Tessmer, U.; Bruns, K.; Wray, V.; Prechter, A. T.; Schubert, E.; Henklein, P.; Neidleman, J., Greene, W. C. and Schubert U. Cyclophilin A interacts with HIV-1 Vpr and is required for its functional expression.* ***Journal of Biological Chemistry***. *2003;* ***278****:43202-43213.*
- *Zielinski, M.; Kahl, S.; Hecht, H.-J., and Hofer, B. Pinpointing biphenyl dioxygenase residues that are crucial for substrate interaction.* ***Journal of Bacteriology***. *2003;* ***185****:6976-6980.*
- *Zorn, H.; Langhoff, S.; Scheibner, M.; Nimtz, M., and Berger, R. G. Cleavage of ß,ß-carotene to flavourcompounds by* Lepista irina *versatile peroxidase.* ***Biochemical Journal***. *2003;* ***384****:1049-1056.*

Biotech facilities – 2003

- *Duvar, S.; Berlin, J.; Ziehr, H., and Conradt, H. S. Modulation of the glycosylation repertoire of recombiant human EPO expressing model cell lines under different culture conditions. In: Proceedings of the 18th ESACT-Meeting-Granada. Granada; 2003.*
- *Elias, C. B.; Carpentier, E.; Durocher, Y.; Bisson, L.; Wagner, R., and Kamen, A. Improving glucose and glutamine metabolism of human HEK 293 and* Trichoplusia ni *insect cells engineered to express a cytosolic pyruvate carboxylase enzyme.* ***Biotechnology Progress***. *2003;* ***19****:90-97.*
- *Hesse, F.; Ebel, M.; Konisch, N.; Sterlinski, R.; Kessler, W., and Wagner, R. Comparison of a production process in a membrane-aerated stirred tank and up to 1000 L airlift bioreactors using BHK-21 cells and chemically defined protein-free medium.* ***Biotechnology Progress***. *2003;* ***19****:833-843.*
- *Horn, H.; Cordes, C.; Krull, R.; Hempel, D. C.; Jahn, D., and Rinas, U. Einfluss der Pelletmorphologie von* Aspergillus niger *auf Stoffumsatz und Produktbildung – Experimentelle Untersuchungen und Modellierung.* ***Chemie Ingenieur Technik***. *2003;* ***75****:1070-1071.*
- *Krumme, D.; Budde, H.; Hecht, H.-J.; Menge, U.; Ohlenschläger, O.; Roß, A.; Wissing, J.; Wray, V., and Flohé, L. NMR studies of the interaction of tryparedoxin with redox-inactive substrate homologs.* ***Biochemistry***. *2003;* ***42****:14720-14728.*
- *Marten, E.; Müller, R.-J., and Deckwer, W.-D. Studies on the enzymatic hydrolysis of polyesters: I. Low molecular mass model esters and aliphatic polyesters.* ***Polymer Degradation and Stability***. *2003;* ***80(3)****:485-501.*
- *Müller, C. ; Richter, S., and Rinas, U. Kinetic control preferential heterodimer formation of platelet-derived growth factor from unfolded A- and B-chains.* ***Journal of Biological Chemistry***. *2003;* ***278****:18330-18335.*
- *Müller, R.-J. Biodegradability of polymers: regulations and methods for testing. (A. Steinbüchel, ed.) Biopolymers. Weinheim, Germany: Wiley-VCH; 2003; 10, Chapter 1 pp. 365-391.*
- *Priesner, C.; Elmahouhoub, A., and Wagner, R. Immortale Hepatocyten zur Analyse leberspezifischer Funktionen.* ***Bioforum***. *2003;* ***(1-2)****:35-37.*
- *Wang, W.; Sun, J.; Hartlep, M.; Deckwer, W.-D., and Zeng, A.-P. Combined use of proteomics analysis and enzyme activity assay for metabolic pathway analysis of glycerol fermentation by* Klebsiella pneumoniae. ***Biotechnology and Bioengineering***. *2003;* ***83****:525-536.*
- *Wang, W.; Sun, J.; Nimtz, M.; Zeng, A.-P., and Deckwer, W.-D. Protein identification from 2-dimentional gel electrophoresis analysis of* Klebsiella pneumoniae *by combined use of mass spectrometry data and unannotated genome sequences.* ***Proteome Science***. *2003;* ***1****:6.*

Publications 2004

Infection and Immunity – 2004

- *Albrecht, P.; Bode, J.; Buiting, K.; Prashanth, A. K., and Lohmann, D. R. Recurrent deletion of a region containing exon 24 or the RB1 gene caused by nonhomologous recombination between a LINE 1hs and MER21B element.* ***Journal of Medical Genetics****. 2004;* ***41****:e122.*

- *Auwerx, J.; Avner, P.; Baldock, R.; Ballabio, A.; Balling, R.; Barbacid, M.; Berns, A.; Bradley, A.; Brown, S.; Carmeliet, P.; Chambon, P.; Cox, R.; Davidson, D.; Davies, K.; Duboule, D.; Forejt, J.; Granucci, F.; Hastie, N.; de Angelis, M. H.; Jackson, I.; Kioussis, D.; Kollias, G.; Lathrop, M.; Lendahl, U.; Malumbres, M.; von Melchner, H.; Muller, W.; Partanen, J.; Ricciardi-Castagnoli, P.; Rigby, P.; Rosen, B.; Rosenthal, N.; Skarnes, B.; Stewart, A. F.; Thornton, J.; Tocchini-Valentini, G.; Wagner, E.; Wahli, W., and Wurst, W. The european dimension for the mouse genome mutagenesis program.* ***Nature Genetics****. 2004;* ***36(9)****:925-927.*

- *Balke, B.; Hoy, L.; Weissbrodt, H., and Häußler, S. Comparison of standard agar dilution with the Merlin, Micronaut System for automated broth microtiter antimicrobial susceptibility testing of mucoid and non-mucoid* Pseudomonas aeruginosa *isolates from cystic fibrosis patients.* ***European Journal of Clinical Microbiology Infectious Diseases****. 2004;* ***23(10)****:765-771.*

- *Balling, R. and Schlender, H. Nichts bleibt wie es ist! Die Biowissenschaften müssen sich der Globalisierung stellen.* ***Zeitschrift für Biopolitik****. 2004;* ***3(2)****:43-47.*

- *Bando, H.; Brokelmann, M.; Toi, M.; Alitalo, K.; Sleeman, J.; Sipos, B.; Groene, H. J., and Weich, H. A. Immunodetection and quantification of vascular endothelial growth factor receptor-3 in human malignant tumour tissue.* ***International Journal of Cancer****. 2004;* ***111****:184-191.*

- *Becker, P. D. and Guzmán, C. A. Rational design of vaccination strategies to promote antigen entry into the MHC class I-restricted presentation pathway.* ***Transfusion Medicine and Hemotherapy****. 2004;* ***31****:398-411.*

- *Benga, L.; Goethe, R.; Rohde, M., and Valentin-Weigand, P. Non-encapsulated strains reveal novel insights in invasion and survival of* Streptococcus suis *in epithelial cells.* ***Cellular Microbiology****. 2004;* ***6(9)****:867-881.*

- *Bergmann, S.; Rohde, M.; Chhatwal, G. S., and Hammerschmidt, S. Characterization of plasmin(ogen) binding to* Streptococcus pneumoniae. ***Indian Journal of Medical Research****. 2004;* ***119****:29-32.*

- *Bergmann, S.; Rohde, M., and Hammerschmidt, S. Glyceraldehyde-3-phosphate dehydrogenase of* Streptococcus pneumoniae *is a surface-displayed plasminogen-binding protein.* ***Infection and Immunity****. 2004;* ***72****:2416-2419.*

- *Bernhardt, H.; Brokelmann, M., and Weich, H. A. Hochempfindliches System zur Detektion von His-tag-Proteinen in HTS und Proteomics.* ***Laborwelt****. 2004;* ***5(2)****:15-16.*

- *Bertot, G. M.; Becker, P. D.; Guzmán, C. A., and Grinstein, S. Intranasal vaccination with recombinant P6 protein and adamantylamide dipeptide as mucosal adjuvant confers efficient protection against* Otitis media *and lung infection by nontypeable* Haemophilus influenzae. ***Journal of Infectious Diseases****. 2004;* ***189(7)****:1304-1312.*

- *Bode, H. B.; Wenzel, S. C.; Irschik, H.; Höfle, G., and Müller, R. An unusual biosynthesis leading to the unique leupyrrin class of natural products in the myxobacterium* Sorangium cellulosum. ***Angewandte Chemie International Edition****. 2004;* ***43****:4163-4167.*

- *Bohn, E.; Müller, S.; Lauber, J.; Geffers, R.; Speer, N.; Speer, C.; Krejci, J.; Manncke, B.; Buer, J.; Zell, A., and Autenrieth, I. B. Gene expression patterns of epithelial cells modulated by pathogenicity factors of* Yersinia enterocolitica. ***Cellular Microbiology****. 2003;* ***6(2)****:129-141.*

- *Bolm, M.; Chhatwal, G. S., and Jansen, W. T. Bacterial resistance of daf-2 mutants. (Letters to the Editor)* ***Science****. 2004;* ***303 (5666)****:1976.*

- *Bolm, M.; Jansen, W. T. M.; Schnabel, R., and Chhatwal, G. S. Hydrogen peroxide-mediated killing of* Caenorhabditis elegans: *a common feature of different streptococcal species.* ***Infection and Immunity****. 2004;* ***72(2)****:1192-1194.*

- *Bollati Fogolin, M.; Etcheverrigaray, M., and Kratje, R. Selection of the cell line and the appropriate vector system for bioactive RHGM-CSF expression. (Jonas, R.; Pandey, A., and Tharun, G., eds.) In: Biotechnological Advances and Applications in Bioconversion of Renewable Raw Materials. Braunschweig, Germany: Döring Druck; 2004; pp. 92-96.*

- *Borsutzky, S.; Cazac, B. B.; Roes, J., and Guzman, C. A. TGF-ß receptor signaling is critical for mucosal IgA responses.* ***Journal of Immunology****. 2004;* ***173****:3305-3309.*

- *Bruder, D.; Probst-Kepper, M.; Westendorf, A. M.; Geffes, R.; Beissert, S.; Loser, K.; Von Boehmer, H.; Buer, J., and Hansen, W. Neuropilin-1: a surface marker of regulatory T cells.* ***European Journal of Immunology****. 2004;* ***34(3)****:623-630.*

- *Bruder, D.; Westendorf, A. M.; Geffers, R.; Gruber, A. D.; Gereke, M.; Enelow, R. I., and Buer, J. CD4 T cell mediated lung disease: steady state between pathological and tolerogenic immune reactions.* ***American Journal of Respiratory and Critical Care Medicine****. 2004;* ***170****:1145-1152.*

- *Böse, J.; Gruber, A. D.; Helming, L.; Schiebe, S.; Wegener, I.; Hafner, M.; Beales, M.; Köntgen, F., and Lengeling, A. Essential functions of the phosphatidylserine receptor during embryogenesis, distinct from apoptotic cell removal.* ***The Journal of Biology****. 2004;* ***3****:15.*

- *Cesari, F.; Rennekampff, V.; Vintersten, K.; Vuong, L.; Bode, J.; Seibler, J.; Wiebel, F. F., and Nordheim, A. Elk-1 knock-out mice engineered by Flp recombinase-mediated cassette exchange genesis.* ***Genesis****. 2004;* ***38****:87-92.*

- *Chhatwal, G. S. Streptococcal research: new challenges ahead.* ***Indian Journal of Medical Research****. 2004;* ***119****:vii-viii.*

- *Dickschat, J. S.; Wenzel, S. C.; Bode, H. B.; Müller, R., and Schulz, S. Biosynthesis of volatiles by the mycobacterium* Myxococcus xanthus. ***ChemBioChem****. 2004;* ***5****:778-787.*

- *Dieterich, G.; Kvesic, M.; Schomburg, D.; Heinz, D. W. , and Reichelt, J. Integrating public databases into an existing protein visualization and modeling program BRAGI.* ***Lecture Notes in Informatics****. 2004;* ***P-53****:149-156.*

- *Dikopoulos, N.; Wegenka, U.; Kröger, A.; Hauser, H.; Schirmbeck, R., and Reimann, J. Recently primed CD8+ T cells entering the liver allow hepatocytes to interact with naive CD8+ T cell and downregulate their immune-suppressive effects.* ***Hepatology****. 2004;* ***39****:1256-1266.*

- *Disanza, A.; Carlier, M. F.; Strada, T. E. B.; Didry, D.; Frittoli, E.; Confalonieri, S.; Croce, A.; Wehland, J.; DiFiore, P. P., and Scita, G. Eps8 controls actin-based motility by capping the barbed ends of actin filaments.* ***Nature Cell Biology****. 2004;* ***5****:1180-1188.*

- *Dittmar, K. E. J.; Lindenmaier, W.; Hauser, H., and Pägelow, U. Flexibel einsetzbares System für zelluläre Impfstoffe.* ***Bioforum****. 2004;* ***5****:41-42.*

- *Dürfahrt, T.; Eppelmann, K.; Müller, R., and Marahiel, M. A. Rational design of a bimodular model system for the investigation of heterocyclization in nonribosomal peptide biosynthesis.* ***Chemistry and Biology****. 2004;* ***11****:261-271.*

- *Ebensen, T.; Guzman, C. A., and Schulze, K. Salmonellen – Feind, aber auch Freund.* ***Labor Praxis****. 2004; Oktober: 30.33.*

- *Ebensen, T.; Paukner, S.; Link, C.; Kudela, P.; de Domenico, C.; Lubitz, W., and Guzmán, C. A. Bacterial ghosts are an efficient delivery system for DNA vaccines.* ***Journal of Immunology****. 2004;* ***172****:6858-6865.*

- *Eberhardt, M. O.; Frank, R.; Etcheverrigaray, M., and Kratje, R. Defining the antigenic structure of human GM-CSF and its implication to receptor interaction and therapeutic treatments.* ***Molecular Diversity****. 2004;* ***8****:257-269.*

- *Eberhardt, M. O.; Frank, R.; Kratje, R., and Etcheverrigaray, M. GM-CSF epitope analysis using a panel of monoclonal antibodies. (Jonas, R.; Pandey A.; Tharun G., eds.) Biotechnological Advances and Applications in Bioconversion of Renewable Raw Materials. Braunschweig, Germany: Döring Druck; 2004; pp. 250-254.*

- *Elm, C.; Braathen, R.; Bergmann, S.; Frank, R.; Vaerman, J.-P.; Kaetzel, C. S.; Chhatwal, G. S.; Johansen, F.-E., and Hammerschmidt, S. Ectodomains 3 and 4 of human polymeric immunoglobulin receptor (hplgR) mediate invasion of* Streptococcus pneumoniae *into the epithelium.* ***Journal of Biological Chemistry****. 2004;* ***279(8)****:6296-6304.*

- *Elm, C.; Rohde, M.; Vaerman, J. P.; Chhatwal, G. S., and Hammerschmidt, S. Characterization of the interaction of the pneumococcal surface protein SpsA with the human polymeric immunoglobulin receptor hplgR.* ***Indian Journal of Medical Research****. 2004;* ***119****:61-65.*

- *Elnakady, Y. A.; Sasse, F.; Lünsdorf, H., and Reichenbach, H. Disorazol A1, a highly effective antimitotic agent acting on tubulin polymerization and inducing apoptosis in mammalian cells.* ***Biochemical Pharmacology****. 2004;* ***67****:927-935.*

- *Eschbach, M; Schreiber, K.; Trunk, K.; Buer, J.; Jahn, D., and Schobert, M. Anaerobic long-term survival of the opportunistic pathogen* Pseudomonas aeruginosa *via pyruvate fermentation.* ***Journal of Bacteriology****. 2004;* ***186****:4596-4604.*

- *Fey, A.; Eichler, S.; Flavier, S.; Christen, R.; Höfle, M. G., and Guzmán, C. A. Development of a real-time PCR-based approach for accurate quantification of bacterial RNA targets in water, using* Salmonella *as a model organism.* ***Applied and Environmental Microbiology****. 2004;* ***70****:3618-3623.*

- *Fogolin, M. B.; Wagner, R.; Etcheverrigaray, M., and Kratje, R. Impact of temperature reduction and expression of yeast pyruvate carboxylase on hGM-CSF-producing CHO cells.* ***Journal of Biotechnology****. 2004;* ***109****:179-191.*

- *Forno, G.; Fogolin, M. B.; Oggero, M.; Kratje, R.; Etcheverrigaray, M.; Conradt, H. S., and Nimtz, M. N- and O-linked carbohydrates and glycosylation site occupancy in recombinant human granulocyte-macrophage colony-stimulating factor secreted by a chinese hamster ovary cell line.* ***European Journal of Biochemistry****. 2004;* ***271****:907-919.*

- *Freiberg, A. ; Machner, M-P.; Pfeil, W.; Schubert, W.-D.; Heinz, D. W., and Seckler, R. Folding and stability of the leucine-rich repeat domain of internalin B from* Listeria monocytogenes*.* ***Journal of Molecular Biology****. 2004;* ***337(2)****:453-461.*

- *Gismondi, M. I.; Staendner, L. H.; Grinstein, S.; Guzmán, C. A., and Preciado, M. V. Hepatitis C virus isolates from Argentina disclose a novel genotype 1-associated restriction pattern.* ***Journal of Clinical Microbiology****. 2004;* ***42(3)****:1298-1301.*

- *Godehardt, A.; Hammerschmidt, S.; Frank, R., and Chhatwal, G. S. Binding of alpha 2-macroglobulin to GRAB, an important virulence factor of group A streptococci, is mediated via two charged motifs in the Delta A region.* ***Biochemical Journal****. 2004;* ***381****:877-885.*

- *Goldmann, O.; Chhatwal, G. S., and Medina, E. The role of host genetic factors in susceptibility to group A streptococcal infections.* ***Indian Journal of Medical Research****. 2004;* ***119****:141-143.*

- *Goldmann, O.; Rohde, M.; Chhatwal, G. S., and Medina, E. Role of macrophages on host resistance to group A streptococci.* ***Infection and Immunity****. 2004;* ***72(5)****:2956-2963.*

- *Gunzer, M.; Weishaupt, C.; Hillmer, A.; Basoglu, Y.; Fiedl, P.; Dittmar, K. E.; Kolanus, W.; Varga, G., and Grabbe, S. A spectrum of biophysical interaction modes between T cells and different antigen presenting cells during priming in 3-D collagen and* in vivo*.* ***Blood****. 2004;* ***104****: 2801-2809.*

- *Guzman, C. A. and Feuerstein, G. Pharmaceutical Biotechnology – Editorial Overview.* ***Current Opinion in Biotechnology****. 2004;* ***15****:503-505.*

- *Hafner, M. and Müller, W. Generation of mouse mutants by sequence information driven and random mutagenesis. (H. J. Hedrich, G. Bullock eds.) The Laboratory Mouse (The handbook of experimental animals). Amsterdam: Elsevier Academic Press; 2004; pp. 85-95.*

- *Hafner, M.; Wenk, J.; Nenci, A.; Pasparakis, M.; Scharffetter-Kochanek, K.; Smyth, N.; Peters, T.; Kess, D.; Holtkötter, O.; Shephard, P.; Kudlow, J. E.; Smola, H.; Haase, I.; Schippers, A.; Krieg, T., and Müller, W. Keratin 14 Cre transgenic mice authenticate keratin 14 as an oocyte-expressed protein.* ***Genesis****. 2004;* ***38(4)****:176-181.*

- *Hansen, W. and Buer, J. Role of regulatory T cells for the outcome of allo- and autoimmune responses.* ***Transfusion Medicine and Hemotherapy****. 2004;* ***31****:322-331.*

- *Heinemeyer, J.; Eubel, H.; Wehmhöner, D.; Jänsch, L., and Braun, H. P. Proteomic approach to characterize the supramolecular organization of photosystems in higher plants.* ***Phytochemistry****. 2004;* ***65****:1683-1692.*

- *Heng, H. H. Q.; Goetze, S.; Ye, C. J.; Lu, W.; Liu, G.; Bremer, S.; Hughes, M.; Bode, J., and Krawetz, S. A. Chromatin loops are selectively anchored using scaffold/matrix-attachment regions.* ***Journal of Cell Science****. 2004;* ***117****:999-1008.*

- *Huehn, J.; Siegmund, K.; Lehmann, J. C. U.; Siewert, C.; Haubold, U.; Feuerer, M.; Debes, G. F.; Lauber, J.; Frey, O.; Przybylski, G. K.;, Niesner U.; de la Rosa, M.; Schmidt, C. A.; Bräuer, R.; Buer, J.; Scheffold, A., and Hamann, A. Developmental stage, phenotype and migration distinguish naive- and effector/memory-like CD4+ regulatory T cells.* ***Journal of Experimental Medicine****. 2004;* ***199****:303-313.*

- *Häußler, S. Biofilm formation by the small colony variant phenotype of* Pseudomonas aeruginosa*.* ***Environmental Microbiology****. 2004;* ***6****:546-551.*

- *Innocenti, M.; Zucconi, A.; Disanza, A.; Frittoli, E.; Areces, L.; Steffen, A.; Stradal, T. E. B.; Di Fiore, P. P.; Carlier, M. F., and Scita, G. Abil is essential for the formation and activation of a WAVE2 signaling complex mediating Rac-dependent actin remodeling.* ***Nature Cell Biology****. 2004;* ***6****:319-327.*

- *Jenke, A. W. ; Stehle, I. M.; Eisenberger, T.; Baiker, A.; Bode, J.; Fackelmayer, F. O., and Lipps, H. J. Nuclear scaffold/matrix attached region modules linked to a transcription unit are sufficient for replication and maintenance of a mammalian episome.* ***Proceedings of National Academy of Sciences USA****. 2004;* ***101****:11322-11327.*

- *Jomantaité, I.; Dikopoulos, N.; Kröger, A.; Leithäuser, F.; Hauser, H.; Schrimbeck, R., and Reimann, J. Hepatic dendritic cell subsets in the mouse.* ***European Journal of Immunology****. 2004;* ***34****:355-365.*

- *Juhas, M.; Wiehlmann, L.; Huber, B.; Jordan, D.; Lauber, J.; Salunkhe, P.; Limpert, A. S.; Von Götz, F.; Steinmetz, I.; Eberl, L., and Tümmler, B. Global regulation of quorum sensing and virulence by VqsR in* Pseudomonas aeruginosa*.* ***Microbiology-SGM****. 2004;* ***150****:831-841.*

- *Kaps, C.; Hoffmann, A.; Zilberman, Y.; Pelled, G.; Häupl, T.; Sittinger, M.; Burmester, G.; Gazit, G., and Gross, G. Distinct roles of BMP receptors type IA and IB in osteo-/chondrogenic differentiation in mesenchymal progenitors (C3H10T″).* ***BioFactors****. 2004;* ***20****:71-84.*

- *Keller, C.; Lauber, J.; Blumenthal, A.; Buer, J., and Ehlers, S. Resistance and susceptibility to tuberculosis analysed at the transcription level: lessons from mouse macrophages.* ***Tuberculosis****. 2004;* ***84****:144-158.*

- *Kopp, M.; Irschik, H.; Gross, F.; Perlova, O.; Sandmann, A.; Gerth, K., and Müller, R. Critical variations of conjugational DNA transfer into secondary metabolite multiproducing* Sorangium cellulosum *strains So ce12 and So ce56 development of a* mariner*-based transposon mutagenesis system.* ***Journal of Biotechnology****. 2004;* ***107****:29-40.*

- *Kossatz, U.; Dietrich, N.; Zender, L.; Buer, J.; Manns, M. P., and Malek, N. P. Skp2 dependent degradation of p27kip1 is essential for cell cycle progression.* ***Genes and Development****. 2004;* ***18****:2602-2607.*

- *Kretschmer, K.; Stopkowicz, J.; Scheffer, S.; Greten, T. F., and Weiss, S. Maintenance of peritoneal B-1a lymphocytes in the absence of the spleen.* ***Journal of Immunology****. 2004;* ***173(1)****:197-204.*

- *Kunze, B.; Jansen, R.; Höfle, G., and Reichenbach, H. Ajudazols, new inhibitor of the mitochondrial electron transport from* Chondromyces crocatus. *Production, antimicrobial activity and mechanism of action.* ***Journal of Antibiotics****. 2004;* ***57(2)****:151-155.*

- *Kuper, J.; Llamas, A.; Hecht, H. J.; Mendel, R. R., and Schwarz, G. Structure of the molybdopterin-bound Cnx1G domain links molybdenum and copper metabolism.* ***Nature****. 2004;* ***430****:803-806.*

- *Lai, F. P.; Cole-Sinclair, M.; Cheng, W. J.; Quinn, J. M. W.; Gillespie, M. T.; Sentry, J. W., and Schneider, H. G. Myeloma cells can directly contribute to the pool of RANKL in bone bypassing the classic stromal and osteoblast pathway of osteoclast stimulation.* ***British Journal of Haematology****. 2004;* ***126****:192-201.*

- *Leibold, T.; Sasse, F.; Reichenbach, H., and Höfle, G. Cyrmenins, novel antifungal peptides containing a nitrogen-linked ß-methoxyacrylate pharmacophore: Isolation and structure elucidation.* ***European Journal of Organic Chemistry****. 2004; (2):431-435.*

- *Link, C.; Gavioli, R.; Ebensen, T.; Canella, A.; Reinhard, E., and Guzmán, C. A. The toll-like receptor ligand MALP-2 stimulates dendritic cell maturation and modulates proteasome composition and activity.* ***European Journal of Immunology****. 2004;* ***34(3)****:899-907.*

- *Loessner, H. and Weiss, S. Bacteria-mediated DNA transfer in gene therapy and vaccination.* ***Expert Opinion in Biological Therapy****. 2004;* ***4(2)****:157-168.*

- *Lommel, S.; Benesch, S.; Rohde, M.; Wehland, J., and Rottner, C.* Enterohaemorrhagic and enteropathogenic Escherichia coli *use different mechanisms for actin pedestal formation that converge on N-WASP.* ***Cellular Microbiology****. 2004;* ***6****:243-254.*

- *Luckow, B.; Joergensen, J.; Chilla, S.; Li, J.-P.; Henger, A.; Kiss, E.; Wieczorek, G.; Roth, L.; Hartmann, N.; Hoffmann, R.; Kretzler, M.; Nelson, P. J.; Lema, G. P. de; Maier, H.; Wurst, W.; Balling, R.; Pfeffer, K.; Gröne, H.-J.; Schlöndorff, D., and Zerwes, H.-G. Reduced intragraft mRNA expression of matrix metalloproteinases Mmp3, Mmp12, Mmp13 and Adam8, and diminished transplant arteriosclerosis in Ccr5-deficient mice.* ***European Journal of Immunology****. 2004;* ***34****: 2568-2578*

- *Ma, H.-W.; Buer, J., and Zeng, A.-P. Hierarchical structure and modules in the* Escherichia coli *transcriptional regulatory network revealed by a new top-down approach.* ***BMC Bioinformatics****. 2004;* ***5****:199.*

- *Ma, H.-W.; Kumar, B.; Ditges, U.; Gunzer, F.; Buer, J., and Zeng, A. P. An extended transcriptional regulatory network of* Escherichia coli *and analysis of its hierarchical structure and network motifs.* ***Nucleic Acids Research****. 2004;* ***32****:6643-6649.*

- *Ma, H.-W. and Zeng, A.-P. Phylogenetic comparision of metabolic capacities of organisms at genome level.* ***Molecular Phylogenetics and Evolution****. 2004;* ***31****:204-213.*

- *May, T.; Hauser, H., and Wirth, D. Transcriptional control of SV40 expression allows a complete reversion of immortalization.* ***Nucleic Acids Research****. 2004;* ***32****:5529-5538.*

- *McArthur, J.; Medina, E.; Mueller, A.; Chin, J.; Currie, B. J.; Sriprakash, K. S.; Talay, S. R.; Chhatwal, G. S., and Walker, M. J. Intranasal vaccination with streptococcal fibronectin binding protein, Sfb 1, fails to prevent growth and dissemination of* Streptococcus pyogenes *in a murine skin infection model.* ***Infection and Immunity****. 2004;* ***72****:7342-7345.*

- *McArthur, J.; Schulze, K.; Chin, J.; Currie, B. J.; Sriprakash, K. S.; Talay, S. R.; Chhatwal, G. S., and , Guzmán C. A. Immune responses of a liposome/ISCOM vaccine adjuvant against streptococcal fibronectin binding protein 1 (Sfb1) in mice.* ***Indian Journal of Medical Research****. 2004;* ***119 (Suppl.)****:113-118.*

- *McKay, F. C.; McArthur, J. D.; Sanderson-Smit, M. L.; Gardam, S.; Currie, B. J.; Sriprakash, K. S.; Fagan, P. K.; Towers, R. J.; Batzloff, M. R.; Chhatwal, G. S.; Ranso, M., and Walker, M. J. Plasminogen binding by group A streptococcal isolates from tropical region with hyperendemic streptococcal skin infection and a highincidence of invasive infection.* ***Infection and Immunity****. 2004;* ***72****:364-370.*

- *Mersal, G. A. M.; Khodari, M., and Bilitewski, U. Optimisation of the composition of the enzyme layer with respect to an improved selectivity and stability of glucose electrodes.* ***Biosensors and Bioelectronics****. 2004;* ***20****:305-314.*

- *Milkereit, G.; Morr, M.; Thiem, J., and Vill, V. Thermotropic and lyotropic properties of long chain alkyl glycopyranosides.* ***Chemistry and Physics of Lipids****. 2004;* ***127****:47-63.*

- *Müller, R. Don't classify polyketide synthases.* ***Chemistry and Biology****. 2004;* ***11****:4-6.*

- *Niedick, I.; Froese, N.; Oumard, A.; Müller, P. P.; Nourbakhsh, M.; Hauser, H., and Köster, M. Nucleolar localization and mobility analysis of the NF-κB repressing factor NRF.* ***Journal of Cell Science****. 2004;* ***117****:3447-3458.*

- *Niemann, H.; Schubert, W.-D., and Heinz, D. Adhesins and invasions of pathogenic bacteria: a structural view.* ***Microbes and Infection****. 2004;* ***6****:101-112.*

- *Niggemann, J.; Herrmann, M.; Gerth, K.; Irschik, H.; Reichenbach, H., and Höfle, G. Tuscolid and Tuscoron A and B: Isolation, structural elucidation and studies on the biosynthesis of novel furan-3(2H)-one-containing metabolites from the myxobacterium* Sorangium cellulosum.* **European Journal of Organic Chemistry****. 2004****; 487-492.*

- *Nimtz, M.; Conradt, H. S., and Mann, K. LacdiNAc (GalNAACß1-44lcNAc) is a major motif in N-glycan structures of the chicken eggshell protein ovocleiding-116.* ***Biochimica Et Biophysica Acta****. 2004;* ***1675****:71-80.*

- *Oggero, M.; Frank, R.; Etcheverrigaray, M., and Kratje, R. Defining the antigenic structure of human GM-CSF and its implication to receptor interaction and therapeutic treatments.* ***Molecular Diversity****. 2004;* ***8****:257-269.*

- *Pacholsky, D.; Himmel, M.; Löw, T.; Stradal, T. E. B.; Rottner, K.; Fürst, D. O., and Van der Ven, P. F. Xin repeats define a novel actin binding motif.* ***Journal of Cell Science****. 2004;* ***117****:5257-5268.*

- *Papagrigoriou, E.; Gringas, A. R.; Barsukov, I. L.; Bate, N.; Fillingham, I. J.; Patel, B.; Frank, R.; Ziegler, W. H.; Roberts, G. C. K.; Critchley, D. R., and Emsley, J. Activation of a vinculin binding site in the talin rod involves re-arrangement of a five helix bundle.* ***EMBO Journal****. 2004;* ***23****:2942-2951.*

- *Probst-Kepper, M.; Hecht, H.-J.; Herrmann, H.; Janke, V.; Ocklenburg, F.; Klempnauer, J.; Van den Eynde, B. J., and Weiss, S. Conformational restraints and flexibility of 14-meric peptides in complex with HLA-B*3501.* ***Journal of Immunology****. 2004;* ***173****:5610-5616.*

- *Retter, I.; Althaus, H. H.; Münch, R., and Müller, W. VBASE2, an integrative V gene database.* ***Nucleic Acids Research****. 2004;* ***33****:D671-D674.*

- *Rharbaoui, F.; Westendorf, A.; Link, C.; Felk, S.; Buer, J.; Gunzer, M., and Guzmán, C. A. The mycoplasma-derived lipopeptide MALP-2 triggers a global immune activation on nasal associated lymphoid tissues.* ***Infection and Immunity****. 2004;* ***72****:6978-6986.*

- *Roers, A.; Siewe, L.; Strittmatter, E.; Deckert, M.; Schlüter, D.; Stenzel, W.; Gruber, A. D.; Krieg, T.; Rajewsky, K., and Müller, W. T cell-specific inactivation of the interleukin-10 gene in mice results in enhanced T cell responses but normal innate responses to LPS or skin irritation.* ***Journal of Experimental Medicine****. 2004;* ***200****:1289-1297.*

- *Romanenko, L. A.; Schumann, P.; Rohde, M.; Mikhailov, V. V., and Stackebrandt, E.* Reinekea marinisedimentorum *gen. nov., sp. nov., a novel gammaproteobacterium from marine coastal sediments.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:669-673.*

- *Ross, A.; Kessler, W.; Krumme, D.; Menge, U.; Wissing, J.; van den Heuvel, J., and Flohé, L. Optimised fermentation strategy for 13C/15N recombinant protein labelling in* Escherichia coli *for NMR-structure analysis.* ***Journal of Biotechnology****. 2004;* ***108****:31-39.*

- *Rottner, K.; Lommel, S.; Wehland, J., and Stradal, T. E. B. Pathogen-induced actin filament rearrangement in infectious diseases.* ***Journal of Pathology****. 2004;* ***204****:396-406.*

- *Sandmann, A.; Sasse, F., and Müller, R. Identification and analysis of the core biosynthetic machinery of tubulysin, a potent cytotoxin with potential anticancer activity.* ***Chemistry and Biology****. 2004;* ***(11)8****:1071-1079.*

- *Saravanamuthu, S. S.; von Götz, F.; Salunkhe, P.; Choshavendan, R.; Geffers, R.; Buer, J.; Tümmler, B., and Steinmetz, I. Evidence for polyadenylated mRNA in* Pseudomonas aeruginosa. ***Journal of Bacteriology****. 2004;* ***186****:7015-7018.*

- *Satyanarayana, A.; Geffers, R.; Manns, M. P.; Buer, J., and Rudolph, K. L. Gene expression profile at the G1/S transition of liver regeneration after partial hepatectomy in mice.* ***Cell Cycle****. 2004;* ***3(11)****:1405-1417.*

- *Satyanarayana, A.; Greenberg, R. A.; Schaetzlein, S.; Buer, J.; Masutomi, K.; Hahn, W. C.; Zimmermann, S.; Martens, U.; Manns, M. P., and Rudolph, K. L. Mitogen stimulation cooperates with telomere shortening to activate DNA-damage responses and senescence signalling.* ***Molecular and Cellular Biology****. 2004;* ***24****:5459-5474.*

- *Schaumburg, J.; Diekmann, O.; Hagendorff, P.; Bergmann, S.; Rohde, M.; Hammerschmidt, S.; Jänsch, L.; Wehland, J., and Kärst, U. The cell wall subproteome of* Listeria monocytogenes. ***Proteomics****. 2004;* ***4****:2991-3006.*

- *Schmitz, I.; Weyd, H.; Krueger, A.; Baumann, S.; Fas, S. C.; rammer, P. H., and Kirchhoff, S. Resistance of short term activated T cells to CD95-mediated apoptosis correlates with* de novo *protein synthesis of c-FLIP(short).* ***Journal of Immunology****. 2004;* ***172****:2194-2200.*

- *Schneider, J. E.; Böse, J.; Bamforth, S. D.; Gruber, A. D.; Broadbent, C.; Clarke, K.; Neubauer, S. ; Lengeling, A., and Bhattacharya, S. Identification of cardiac malformations in mice lacking Ptdsr using a novel high-throughput magnetic resonance imaging technique.* ***BMC Developmental Biology****. 2004;* ***4****:16(e).*

- *Schrader, A. J.; von Knobloch, R.; Heidenreich, A.; Buer, J., and Hoffmann, R. Application of retinoids in the treatment of renal cell carcinoma – a futile effort?* ***Anti-Cancer Drugs****. 2004;* ***15****:819-824.*

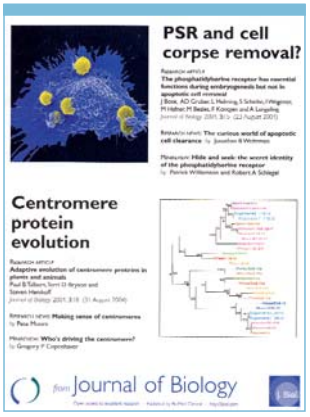
- *Sechi, A. S. and Wehland, J. ENA/VASP proteins: multifunctional regulators of actin cytoskeleton dynamics.* ***Frontiers in Bioscience****. 2004;* ***9****:1294-1310.*

- *Sechi, A. S. and Wehland,J. Interplay between TCR signalling and actin cytoskeleton dynamics.* ***Trends in Immunology****. 2004;* ***25****:257-265.*

- *Shpacovitch, V. M.; Varga, G.; Strey, A.; Gunzer, M.; Mooren, F.; Buddenkotte, J.; Vergnolle, N.; Sommerhoff, C. P.; Grabbe, S.; Gerke, V.; Homey, B.; Hollenberg, M.; Luger, T. A., and Steinhoff, M. Agonists of proteinase-activated receptor-2 modulate human neutrophil cytokine secretion, expression of cell adhesion molecules, and migration within 3-D collagen lattices.* ***Journal of Leukocyte Biology****. 2004;* ***76****:388-398.*

- *Spanholtz, T.; Niedworok, C.; Maichle, A.; Lindenmaier, W.; Herbort-Brand, U.; Krüger, S.; Stöckelhuber, B.; Krapohl, B. D.; Mailänder, P., and Machens, H. G. Synergistische therapeutische Effekte von bFGF und VEGF165 nach Transplantation isogener adenoviral transfizierter Fibroblasten im ischämischen Lappen-modell der Ratte. (Ulrich, B.; Jauch K. W.; Bauer H., editors). Chirurgisches Forum 2004 für experimentelle und klinische Forschung: Springer Verlag; 2004; Vol. 33; pp. 421-423.*

- *Steffen, A.; Rottner, K.; Ehinger, J.; Innocenti, M.; Scita, G.; Wehland, J., and Stradal, T. E. B. Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation.* ***EMBO Journal****. 2004;* ***23(4)****:749-759.*



- *Cover picture of the journal The Journal of Biology, Vol. 3, 2004, on the occasion of the publication of the article by Böse, J.; Gruber, A.D.; Helming, L.; Schiebe, S.; Wegener, I.; Hafner, M.; Beales, M.; Köntgen, F.; and Lengeling, A. Essential functions of the phosphatidylserine receptor during embryogenesis, distinct from apoptotic cell removal.* ***The Journal of Biology****. 2004;* ***3****:15. The permission of BioMed Central is gratefully acknowledged.*

- *Steinmetz, H.; Glaser, N.; Herdtweck, E.; Sasse, F.; Reichenbach, H., and Höfle, G. Isolation, crystal and solution structure determination, and biosynthesis of tubulysins – powerful inhibitors of tubulin polymerization from myxobacteria.* ***Angewandte Chemie International Edition****. 2004;* ***43****:4888-4892.*

- *Stradal, T. E. B.; Rottner, K.; Disanza, A.; Confalonieri, S.; Innocenti, M., and Scita, G. Regulation of actin dynamics by WASF and WAVE family proteins.* ***Trends in Cell Biology****. 2004;* ***14****:303-311.*

- *Towers, R. J.; Gal, D.; McMillan, D.; Sriprakash, K. S.; Currie, B. J.; Walker, M. J.; Chhatwal, G. S., and Fagan, P. K. Fibronectin-binding protein gene recombination and horizontal transfer between group A and G streptococci.* ***Journal of Clinical Microbiology****. 2004;* ***42(11)****:5357-5361.*

- *Unsinger, J.; Lindenmaier, W.; May, T.; Hauser, H., and Wirth, D. LTR – flanked autoregulated expression cassettesfor uniform and strictly controlled expression in adenoviral vectors.* ***Biochemical and Biophysical Research Communications****. 2004;* ***319****:879-887.*

- *Urzi, C.; Salamone, P.; Schumann, P.; Rohde, M., and Stackebrandt, E.* Blastococcus saxobsidens *sp. nov., and emended description of the genus* Blastococcus *Ahrens and Moll 1970 and* Blastococcus aggregatus *Ahrens and Moll 1970.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:253-259.*

- *Vidakovic, M.; Grdovic Quesada, P.; Bode, J., and Poznanovic, G. Poly(ADP-ribose) polymerase-1: association with nuclear lamins in rat hepatocytes.* ***Journal of Cellular Biochemistry****. 2004;* ***93****:1155-1168.*

- *Von Goetz, F.; Häußler, S.; Jordan, D.; Saravanamuthu, S.; Wehmhöner, D.; Strüßmann, A.; Lauber, J.; Attree, I.; Buer, J.; Tümmler, B., and Steinmetz, I. Expression analysis of a highly adherent and cytotoxic small colony variant of* Pseudomonas aeruginosa *isolated from a cystic fibrosis lung.* ***Journal of Bacteriology****. 2004;* ***186****:3837-3847.*

- *Vosshenrich, C. A. J.; Cumano, A.; Müller, W.; Di Santo, J. P., and Vieira, P. Pre-B cell receptor expression is necessary for thymic stromal lymphopoietin responsiveness in the bone marrow but not in the liver environment.* ***Proceedings of National Academy of Sciences USA****. 2004;* ***101(30)****:11070-11075.*

- *Walter, U.; Scherbaum, W. A., and Buer, J. MHC class II expression by beta cells in Type 1 diabetes: promoting or inhibiting the autoimmune process?* ***Diabetologia****. 2004;* ***47****:1641-1642.*

- *Wang, Y. L.; Burridge, K.; Dembo, M.; Gabbiani, G.; Hanks, S. K.; Hosoya, H.; Janmey, P.; Karlsson, R.; Lindberg, U.; Mabuchi, I.; Otey, C.; Rottner, K.; Small, J. V.; Wang, C. L., and Zigmond, S. Biomedical research publication system.* ***Science****. 2004;* ***303****:1974-1976.*

- *Weich, H. A.; Bando, H.; Brokelmann, M.; Baumann, P.; Toi, M.; Barleon, B.; Alitalo, K.; Sipos, B., and Sleeman, J. Quantification of vascular endothelial growth factor-C (VEGF-C) by a novel ELISA.* ***Journal of Immunological Methods****. 2004;* ***285****:145-155.*

- *Weig, M.; Jänsch, L.; Groß, U.; De Koster, C. G.; Klis, F. M., and De Groot, P. W. J. Systematic identification of covalently-bound cell wall proteins and analysis of protein-polysaccharide linkages of the human pathogen* Candida glabrata. ***Molecular Microbiology****. 2004;* ***150****:3129-3144.*

- *Weissenbach, M.; Weber, C.; Spitzer, D.; Wirth, D.; Heinrich, P. C., and Schaper, F. Interleukin-6-induced migration of human primary T-cells and T-cell derived cell lines.* ***European Journal of Immunology****. 2004;* ***34****:2895-2906.*

- *Wirth, D. and Hauser, H. Flp-mediated integration of expression cassettes into FRT tagged chromosomal loci. (Balbas, P. and Lorence, A., editors). Methods in Molecular Biology, Vol. 267: Recombinant Gene Expression Reviews and Protocols: Humana Press Inc.; 2004; pp. 467-476.*

- *Wirth, M. and Beer, C. Viroquant – Quantifizierung viraler Partikel mittels Fluoreszenzmikroskopie.* ***Bioforum****. 2004;* ***4****:53-55.*

- *Wollscheid-Lengeling, E.; Müller, R.-J.; Balling, R., and Schughart, K. Maintaining your immune-system – One method for enhanced longevity.* ***Science of Aging Knowledge Environment****. 2004;* ***2004(1)****:pe2.*

- *Zander, N. and Frank, R. Polystyrylsulfonyl-3-nitro-1H-1,2,4-triazolide-resin: a new reagent for the anchoring of Fmoc-amino acids to membrane supports as esters by the SPOT Technique. (Epton, R. ed.) Solid Phase Synthesis and Combinatorial Libraries. Kingswinford, UK: Mayflower Worldwide Ltd.; 2004; pp. 323-324.*

Comparative Genome Research – 2004

- *Bayle, J.; Letard, S.; Frank, R.; Dubreuil, P., and De Sepulveda, P. Suppressor of cytokine signaling 6 associates with KIT and regulates KIT receptor signaling.* ***Journal of Biological Chemistry****. 2004;* ***279****:12249-12259.*

- *Beutling, U.; Tegge, W.; Zander, N., and Frank, R. High-throughout synthesis and screening without wells and walls. (Epton, R., ed.) Solid Phase Synthesis and Combinatorial Libraries. Kingswinford, UK: Mayflower Worldwide Ltd.; 2004; pp. 191-194.*

- *Bialek, K.; Swistowski, A.; Beutling, U.; Mühle, L., and Frank, R. Development of a new biochip platform technology for the automated recognition and validation of peptide-protein-interactions. (Epton, R., ed.) Solid Phase Synthesis and Combinatorial Libraries. Kingswinford, UK: Mayflower Worldwide Ltd.; 2004; pp. 35-38.*

- *Bialek, K.; Swistowski, A., and Frank, R. Peptide and protein repertoires for global analysis of modules. (G. Cesareni, M. Gimona M. Sudol M. Yaffe eds.) Modular Protein Domains. Weinheim: Wiley-VCH Verlag; 2004; pp. 409-438.*

- *Buss, H.; Dörrie, A.; Schmitz, L. M.; Frank, R.; Livingstone, M.; Resch, K., and Kracht, M. Phosphorylation of serine 468 by GSK-3ß negatively regulates basal p65 NF-κB activity.* ***Journal of Biological Chemistry****. 2004;* ***279****:49571-49574.*

- *Dikmans, A. J.; Morr, M.; Zander, N.; Adler, F.; Türk, G., and Frank, R. A new compact disc format of high density array synthesis applied to peptide nucleic acids and* in situ *MALDI analysis.* ***Molecular Diversity****. 2004;* ***8****:197-207.*

- *Doll, C. and Eichler, J. Synthesis of scaffolded peptides by solid-phase fragment condensation. (Epton, R., ed.) Solid Phase Synthesis and Combinatorial Chemical Libraries. Birmingham, UK: Mayflower Scientific Ltd.; 2004; pp. 217-220.*

- *Doll, C.; Franke, R.; Wray, V., and Eichler, J. Loops on Loops: generation of complex scaffolded peptides: stepwise solid-phase synthesis vs. solid-phase fragment condensation.* ***Organic and Biomolecular Chemistry****. 2004;* ***2****:2847-2851.*

- *Eberhardt, M. O.; Frank, R.; Etcheverrigaray, M., and Kratje, R. Defining the antigenic structure of human GM-CSF and its implication to receptor interaction and therapeutic treatments.* ***Molecular Diversity****. 2004;* ***8****:257-269.*

- *Eberhardt, M. O.; Frank, R.; Kratje, R., and Etcheverrigaray, M. GM-CSF epitope analysis using a panel of monoclonal antibodies. (Jonas, R.; Pandey A.; Tharun G., eds.) Biotechnological Advances and Applications in Bioconversion of Renewable Raw Materials. Braunschweig, Germany: Döring Druck; 2004; pp. 250-254.*

- *Ehinger, S.; Schubert, W.-D.; Bergmann, S.; Hammerschmidt, S., and Heinz, D. W. Plasmin(ogen)-binding alpha-enolase from* Streptococcus pneumoniae*: crystal structure and evaluation of plasmin(ogen)-binding sites.* ***Journal of Molecular Biology****. 2004;* ***343****:997-1005.*

- *Eichler, R. Rational and random strategies for the mimicry of discontinuous protein binding sites.* ***Protein Peptide Letters****. 2004;* ***11(4)****:281-290.*

- *Frank, R. Funktionelle und chemische Genomik: Kleine Moleküle kommen ganz groß raus.* ***GenomXpress****. 2004;* ***2****:14-15.*

  Frank, R. High-density synthetic peptide arrays. ***Molecular Diversity****. 2004;* ***8****:175-176.*

- *Franke, R.; Doll, C.; Wray, V., and Eichler, J. Loops on loops: generation of complex scaffolded peptides presenting multiple cyclic fragments.* ***Organic and BiomolecularChemistry****. 2004;* ***2****:2847-2851.*

- *He, F.; Ma, H.; Zhao, X.-M.; Yuan, Y.-J., and Zeng, A.-P. Progress and perspective in the application of bioinformatics for the analysis of metabolic networks.* ***Journal of Chemical and Industrial Engineering (China)****. 2004;* ***55****:1593-1601.*

- *Hirsch, T.; Franke, R., and Eichler, J. Mimicking discontinuous protein binding sites: synthesis tools and protein targets. (Epton, R., ed.) Solid Phase Synthesis and Combinatorial Chemical Libraries. Birmingham, UK: Mayflower Scientific Ltd.; 2004; pp. 159-162.*

- *Hultschig, C. and Frank, R. Multiplexed sorting of libraries versus libraries empirical protein design by affinity-driven phage enrichment on synthetic peptide arrays.* ***Molecular Diversity****. 2004;* ***8****:231-245.*

- *Hultschig, C.; Hecht, H.-J., and Frank, R. Systematic delineation of a calmodulin peptide interaction.* ***Journal of Molecular Biology****. 2004;* ***343****:559-568.*

- *Humphray, S. J.; Oliver, K.; Hunt, A. R.; Plumb, R. W.; Loveland, J. E.; Howe, K. L.; Andrews, T. D.; Searle, S.; Hunt, S. E.; Scott, C. E.; Jones, M. C.; Ainscough, R.; Almeida, J. P.; Ambrose, K. D.; Ashwell, R. I. S.; Babbage, A. K.; Babbage, S.; Bagguley, C. L.; Bailey, J.; Banerjee, R.; Barker, D. J.; Barlow, K. F.; Bates, K.; Beasley, H.; Beasley, O.; Bird, C. P.; Bray-Allen, S.; Brown, A. J.; Brown, J. Y.; Burford, D.; Burrill, W.; Burton, J.; Carder, C.; Carter, N. P.; Chapman, J. C.; Chen, Y.; Clarke, G.; Clark, S. Y.; Clee, C. M.; Clegg, S.; Collier, R. E.; Corby, N.; Crosier, M.; Cummings, A. T.; Davies, J.; Dhami, P.; Dunn, M.; Dutta, I.; Dyer, L. W.; Earthrowl, M. E.; Faulkner, L.; Fleming, C. J.; Frankish, A.; Frankland, J. A.; French, L.; Fricker, D. G.; Garner, P.; Garnett, J.; Ghori, J.; Gilbert, J. G. R.; Glison, C.; Grafham, D. V.; Gribble, S.; Griffiths, C.; Griffiths-Jones, S.; Grocock, R.; Guy, J.; Hall, R. E.; Hammond, S.; Harley, J. L.; Harrison, E. S. I.; Hart, E. A.; Heath, P. D.; Henderson, C. D.; Hopkins, B. L.; Howard, P. J.; Howden, P. J.; Huckle, E.; Johnson, C.; Johnson, D.; Joy, A. A.; Kay, M.; Keenan, S.; Kershaw, J. K.; Kimberley, A. M.; King, A.; Knights, A.; Laird, G. K.; Langford, C.; Lawlor, S.; Leongamornlert, D. A.; Leversha, M.; Lloyd, C.; Lloyd, D. M.; Lovell, J.; Martin, S.; Mashreghi-Mohammadi, M.; Matthews, L.; McLaren, S.; McLay, K. E.; McMurray, A.; Milne, S.; Nickerson, T.; Nisbett, J.; Nordsiek, G.; Pearce, A. V.; Peck, A. I.; Porter, K. M.; Pandian, R.; Pelan, S.; Phillimore, B.; Povey, S.; Ramsey, Y.; Rand, V.; Scharfe, M.; Sehra, H. K.; Shownkeen, R.; Sims, S.; Skuce, C. D.; Smith, M.; Steward, C. A.; Swarbreck, D.; Sycamore, N.; Tester, J.; Thorpe, A.; Tracey, A.; Tromans, A.; Thomas, D. W.; Wall, M.; Wallis, J. M.; West, A. P.; Whitehead, S. L.; Willey, D. L.; Williams, S. A.; Wilming, L.; Wray, P. W.; Young, L.; Ashurst, J. L.; Coulson, A.; Blöcker, H.; Durbin, R. J.; Sulston, E.; Hubbard, T.; Jackson, M. J.; Bentley, D. R.; Beck, S.; Rogers, J., and Dunham, I. DNA sequence and analysis of human chromosome 9.* ***Nature****. 2004;* ***429****:369-374.*

- *Imaz, M. S.; Comini, M. A.; Zerbini, E.; Sequeira, M. D.; Latini, O.; Claus, J. D., and Singh, M. Evaluation of commercial enzyme-linked immunosorbent assay kits for detection of tuberculosis in Argentininean population.* ***Journal of Clinical Microbiology****. 2004;* ***42****:884-887.*

- *Jaeger, T.; Budde, H.; Flohé, L.; Menge, U.; Singh, M.; Trujillo, M., and Radi, R. Multiple thioredoxin-mediated routes to detoxify hydroperoxides in* Mycobacterium tuberculosis. ***Archives of Biochemistry and Biophysics****. 2004;* ***423(1)****:182-191.*

- *Jahn, D.; Moser, J.; Schubert, W.-D., and Heinz, D. W. Transfer RNA-dependent aminolevulinic acid formation: structure and function of glutamyl-tRNA synthase, reductase and glutamate-1-semialdehyde-2,1-aminomutase. Chlorophylls. Boca Raton: CRC Press; 2004; pp. 1257-1269.*

- *Kauer, G. and Blöcker, H. Analysis of disturbed images.* ***Bioinformatics****. 2004;* ***20****:1381-1387.*

- *Lara, M.; Sevin-Gonzalez, L.; Singh, M.; Moreno, C.; Cohen, I.; Nimtz, M., and Espitia, C. Expression, secretion, and glycosylation of the 45- and 47-kDa glycoprotein of* Mycobacterium tuberculosis *in* Streptomyces lividans. ***Applied and Environmental Microbiology****. 2004;* ***70****:679-685.*

- *Layer, G.; Heinz, D. W.; Jahn, D., and Schubert, W.-D. Structure and function of radical SAM enzymes.* ***Current Opinion in Chemical Biology****. 2004;* ***8****:468-476.*

- *Ma, H.-W.; Buer, J., and Zeng, A.-P. Hierarchical structure and modules in the* Escherichia coli *transcriptional regulatory network revealed by a new top-down approach.* ***BMC Bioinformatics****. 2004;* ***5****:199.*

- *Ma, H.-W.; Kumar, B.; Ditges, U.; Gunzer, F.; Buer, J., and Zeng, A. P. An extended transcriptional regulatory network of* Escherichia coli *and analysis of its hierarchical structure and network motifs.* ***Nucleic Acids Research****. 2004;* ***32****:6643-6649.*

- *Ma, H.-W.; Zhao, X.-M.; Yuan, Y.-J., and Zeng, A.-P. Decomposition of metabolic network based on the global connectivity structure of reaction graph.* ***Bioinformatics****. 2004;* ***20****:1870-1876.*

- *MacAry, P. A.; Javid, B.; Floto, R. A.; Smith, K. G.; Oehlmann, W.; Singh, M., and Lehner, P. J. HSP70 peptide binding mutants separate antigen delivery from dendritic cell stimulation.* ***Immunity****. 2004;* ***20****:95-106.*

- *Rampon, C.; Prandini, M.-H.; Bouillot, S.; Pointu, H.; Tillet, E.; Frank, R.; Vernet, M., and Huber, P. Protocadherin 12 (VE-cadherin) is expressed in endothelial trophoblast and mesangial cells and is dispensable for normal mouse development.* ***Experimental Cell Research****. 2004;* ***302****:48-60.*

- *Sänger, C.; Busche, A.; Bentien, G.; Spallek, R.; Jonas, F.; Böhle, A.; Singh, M., and Brandau, S. Immunodominant PstSI antigen of* Mycobacterium tuberculosis *is a potent biological response modifier for the treatment of bladder cancer.* ***BMC Cancer****. 2004;* ***4****:86(e).*

- *Scheller, J.; Kovaleva, M.; Rabe, B.; Eichler, J.; Kallen, K. J., and Rose-John, S. Development of a monoclonal antibody-based immunoabsorbent assay for the binding of gp130 to the IL-6/IL-6R complex and its competitive inhibition.* ***Journal of Immunological Methods****. 2004;* ***291(1-2)****:93-100.*

- *Schiller, D.; Rübenhagen, R.; Krämer, R., and Morbach, S. The C-terminal domain of the betaine carrier BetP of* Corynebacterium glutamicum *is directly involved in sensing K^+ as an osmotic stimulus.* ***Biochemistry****. 2004;* ***43****:5583-5591.*

- *Sun, J.; Daniel, R.; Wagner-Döbler, I., and Zeng, A.-P. Is auto-inducer-2 a universal signal for interspecies communication? A comparative and phylogenetic genomic analysis of the synthesis and signal transduction pathways.* ***BMC Evolutionary Biology****. 2004;* ***4****:36.*

- *Sun, J. and Zeng, A.-P. IdentiCS – Identification of coding sequence and* in silico *reconstruction of the metabolic network directly from unnanotated low-coverage bacterial genome sequence.* ***BMC Bioinformatics****. 2004;* ***5****:112.*

- *Taylor, M. S.; Okwuchukwuasanya, C.; Nickl, C. K.; Tegge, W.; Brayden, J. E., and Dostmann, W. R. G. Inhibition of cGMP-dependent protein kinase by the cell-permeable peptide DT-2 reveals a novel mechanism of vasoregulation.* ***Molecular Pharmacology****. 2004;* ***65(5)****:1111-1119.*

- *The International Chimpanzee Chromosome 22 Consortium: Watanabe, H.; Fujiyama, A.; Hattori, M.; Taylor, T. D.; Toyoda, A.; Kuroki, Y.; Noguchi, H.; BenKahla, A.; Lehrach, H.; Sudbrak, R.; Kube, M.; Taenzer, S.; Galgoczy, P.; Platzer, M.; Scharfe, M.; Nordsiek, G.; Blöcker, H.; Hellmann, I.; Khaitovich, P.; Pääbo, S.; Reinhardt, R. ; Zheng, H.-J.; Zhang, X.-L.; Zhu, G.-F.; Wang, B.-F.; Fu, G.; Ren, S.-X.; Zhao, G.-P.; Chen, Z.; Lee, Y.-S.; Cheong, J.-E.; Choi, S.-H.; Wu, K.-M.; Liu, T.-T.; Hsiao, K.-J.; Tsai, S.-F.; Kim, C.-G.; Oota, S.; Kitano, T.; Kohara, Y.; Saitou, N.; Park, H.-S.; Wang, S.-Y.; Yaspo, M.-L., and Sakaki, Y. DNA sequence and comparative analysis of chimpanzee chromosome 22.* ***Nature****. 2004;* ***429****:382-388.*

- *Varfolomeyev, S.; Efremenko, E.; Beletskaya, I.; Bertini, I.; Blackburn, G. M.; Bogdanov, A.; Cunin, R.; Eichler, J.; Galaev, I.; Gladyshev, V.; O'Hagan, D.; Haertle, T.; Jarv, J.; Karyakin, A.; Kurochkin, I.; Mikolajczyk, M.; Poroikov, V.; Sakharov, I.; Spener, F.; Voyer, N., and Wild, J. Post-genomic chemistry.* ***Pure and Applied Chemistry****. 2004;* ***76(11)****:1985-1999.*

- *Williams, A.; Reljic, R.; Naylor, I.; Clark, S. O.; Falero-Diaz, G.; Singh, M.; Challacombe, S.; Marsh, P. D., and Ivanyi, J. Passive protection with immunoglobulin A antibodies against tuberculous early infection of the lungs.* ***Immunology****. 2004;* ***111****:328-333.*

- *Xiu, Z.-L.; Song, B.-H.; Wang, Z.-T.; Sun, L.-H.; Feng, E.-M., and Zeng, A.-P. Optimization of dissimilation of glycerol to 1,3-propanediol by* Klebsiella pneumoniae *in one- and two-stage anaerobic cultures.* ***Biochemical Engineering Journal****. 2004;* ***19****:189-197.*

- *Zeng, A.-P. and Kim, E.-J. Iron availability, oxygen limitation,* Pseudomonas aeruginosa *and cystic fibrosis.* ***Microbiology Comment****. 2004;* ***150****:516-518.*

- *Zhou, J.; Schmid, T.; Frank, R., and Brüne, B. PI3K/Akt is required for heat shock proteins to protect hypoxiainducible factor 1alpha from pVHL-independent degradation.* ***The Journal of Biological Chemistry****. 2004;* ***279(14)****:13506-13513.*

Sustainable Use of Landscape – 2004

- *Abraham, W.-R.; Strömpl, C.; Vancanneyt, M.; Bennasar, A.; Swings, J.; Smit, J., and Moore, E. R. B.* Woodsholea maritima *gen. nov., sp. nov., a marine bacterium with a low diversity of polar lipids.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:1227-1234*

- *Abou-Zeid, D. M.; Biebl, H.; Spröer, C., and Müller, R.-J.* Propionispora hippei *sp. nov., a novel Gram-negative, spore-forming anaerobe that produces propionic acid.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:951-954.*

- *Asolkar, R. N.; Heckmann, R.; Lang, S.; Wagner-Döbler, I., and Laatsch, H. Marine bacteria, XXVII: Helquinoline, a new tetrahydroquinoline antibiotic from* Janibacter limosus *Hel 1.* ***Journal of Antibiotics****. 2004;* ***57****:17-23.*

- *Brettar, I.; Christen, R., and Höfle, M. G.* Aquiflexum balticum *gen. nov., sp. nov., a novel marine bacterium of the* Cytophaga-Flavobacterium-Bacteroides *group isolated from surface water of the central Baltic Sea.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:2335-2341.*

- *Brettar, I.; Christen, R., and Höfle, M. G.* Belliella baltica, *sp. nov., a novel marine bacterium of the Cythophage-Flavobacterium-Bacteroides group isolated from surface water of the central Baltic Sea.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:65-70.*

- *Bruemmer, I. H. M.; Felske, A. D. M., and Wagner-Döbler, I. Diversity and seasonal changes of uncultured Planctomycetales in river biofilms.* ***Applied and Environmental Microbiology****. 2004;* ***70(9)****:5094-5101.*

- *Chávez, F. P.; Lünsdorf, H., and Jerez, C. A. Growth of polychlorinated-biphenyl-degrading bacteria in the presence of biphenyl and chlorobiphenyls generates oxidative stress and massive accumulation of inorganic polyphosphate.* ***Applied and Environmental Microbiology****. 2004;* ***70(5)****:3064-3072.*

- *Comini, M. A.; Guerrero, S. A.; Haile, S.; Menge, U.; Lünsdorf, H., and Flohé, L. Validation of* Trypanosoma brucei *trypanothione synthetase as drug target.* ***Free Radicals in Biology and Medicine****. 2004;* ***36****:1289-1302.*

- *Cámara, B. ; Herrera, C.; González, M.; Couve, E.; Hofer, B., and Seeger, M. From PCBs to highly toxic metabolites by the biphenyl pathway.* ***Environmental Microbiology****. 2004;* ***6(8)****:842-850.*

- *Deckwer, W.-D.; Becker, F. U.; Ledakowicz, S., and Wagner-Döbler, I. Microbial removal of ionic mercury in a three-phase fluidized bed reactor.* ***Environmental Science and Technology****. 2004;* ***38****:1858-1865.*

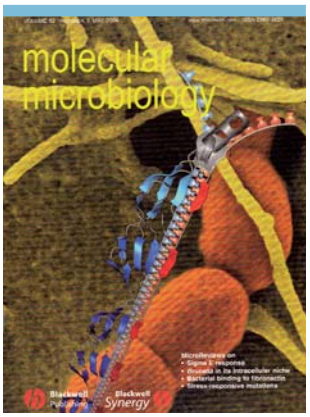
- *Eichler, S.; Weinbauer, M. G.; Dominik, D., and Höfle, M. G. Extraction of total RNA and DNA from bacterioplankton. (Akkermans, A. D. L., van Elsas J. D., de Bruijn F. J., eds.) Molecular Microbial Ecology Manual. 2nd edition Dordrecht, Netherlands: Kluwer Academic Publisher; 2004; pp. 103-120.*

- *Elnakady, Y. A.; Sasse, F.; Lünsdorf, H., and Reichenbach, H. Disorazol A1, a highly effective antimitotic agent acting on tubulin polymerization and inducing apoptosis in mammalian cells.* ***Biochemical Pharmacology****. 2004;* ***67****:927-935.*

- *Ferrer, M.; Chernikova, T. N.; Timmis, K. N., and Golyshin, P. N. Heterologous expression of heat-sensitive psychrophilic esterase in* E. coli *grown at low temperatures.* ***Applied and Environmental Microbiology****. 2004;* ***70(8)****:4499-4504.*

- *Ferrer, M.; Lünsdorf, H.; Chernikova, T. N.; Yakimov, M.; Golyshin, P., and Timmis, K. N. Functional consequences of single:double ring transitions in chaperonins: life in the cold.* ***Molecular Microbiology****. 2004;* ***53****:167-182.*

- *Fey, A.; Eichler, S.; Flavier, S.; Christen, R.; Höfle, M. G., and Guzmán, C. A. Development of a real-time PCR-based approach for accurate quantification of bacterial RNA targets in water, using* Salmonella *as a model organism.* ***Applied and Environmental Microbiology****. 2004;* ***70****:3618-3623.*



- *Cover picture of the journal Molecular Microbiology, Vol. 52(3), 2004, on the occasion of the publication of the article by Schwarz-Linek, U.; Hook, M. and Potts, J.R. The molecular basis of fibronectin-mediated bacterial adherence to host cells. Cover picture photographed by Dr. M. Rohde (GBF) is related to the mentioned article.* ***Molecular Microbiology****. 2004;* ***52****:631-641. The permission of Blackwell Publishing is gratefully acknowledged.*

- *Fritz, I.; Strömpl, C., and Abraham, W.-R. Phylogenetic relationships of the genera* Stella, Labrys *and* Angulomicrobium *within the "Alphaproteobacteria" and description of* Angulomicrobium amanitiforme *sp. nov.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:651-657.*

- *Göbel, M.; Kranz, O. H.; Kaschabek, S. R.; Schmidt, E.; Pieper, D. H., and Reineke, W. Microorganisms degrading chlorobenzene via* ameta*-cleavage pathway harbor highly similar chlorocatechol-2,3-dioxygenase-encoding gene clusters.* ***Archives of Microbiology****. 2004;* ***182****:147-156.*

- *Hahn, M. W.; Lünsdorf, H., and Janke, L. Exopolymer production and microcolony formation play an important role in protistan grazing defense of novel bacterial strains isolated from freshwater plankton.* ***Aquatic and Microbial Ecology****. 2004;* ***35****:297-308.*

- *Heyman, J.; Vanparys, B.; Logan, N. A.; Balcaen, A.; Rodriguez-Diaz, M.; Felske, A., and De Vos, P.* Bacillus novalis *sp. nov.* Bacillus vireti *sp. nov.* Bacillus soli *sp. nov.*, Bacillus bataviensis *sp nov. and* Bacillus drentensis *sp. nov. five new species isolate from the Drentse A grasslands.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:47-57.*

- *Höfle, M. G. Genotyping of bacterial isolates from the environment using low-molecular-weight RNA fingerprints. Akkermans, A. D. L. van Elsas J. D. de Bruijn F. J. Molecular Microbial Ecology Manual. 2nd ed. Dordrecht, Netherlands: Kluwer Academic Publisher; 2004; pp. 587-610.*

- *Ivanova, E. P.; Flavier, S., and Christen, R. Phylogenetic relationship among marine* Alteromonas*-like Proteobacteria: emended description of the family* Alteromonadaceae *and proposal of* Pseudoalteromonadaceae *fam. nov.*, Colwelliaceae *fam. nov.*, Shewanellaceae *fam. nov.*, Moritellaceae *fam. nov.*, Ferrimonadaceae *fam. nov.*, Idiomarinaceae *fam. nov., and* Psychromonadaceae *fam. nov.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:1773-1788.*

- *Junca, H. and Pieper, D. H. Functional gene diversity analysis in BTEX contaminated soils by means of PCR-SSCP DNA fingerprinting: comparative diversity assessment against bacterial isolates and PCR-DNA clone libraries.* ***Environmental Microbiology****. 2004;* ***6****:95-110.*

- *Junca, H.; Plumeier, I.; Hecht, H. J., and Pieper, D. H. Difference in kinetic behavior of catechol 2,3-dioxygenase variants from a polluted environment.* ***Microbiology-SGM****. 2004;* ***150****:4181-4187.*

- *Kotsyurbenko, O. R.; Chin, K. J.; Glagolev, M. V.; Stubner, S.; Simankova, M. V.; Nozhevnikova, A. N., and Conrad, R. Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog.* ***Environmental Microbiology****. 2004;* ***6(11)****:1159-1173.*

- *Labrenz, M.; Brettar, I.; Flavier, S.; Christen, R.; Bötel, J., and Höfle, M. G. Development and application of a real-time PCR approach for the detection and quantification of an uncultured* Thiomicrospira denitrificans*-like bacterium in the central Baltic Sea using environmental nucleic acids as external standards.* ***Applied and Environmental Microbiology****. 2004;* ***70****:4971-4979.*

- *Nikitin, D.; Strömpl, C.; Oranskaya, M. S., and Abraham, W.-R. Phylogeny of the ring-forming bacterium* Arcicella aquatica *gen. et sp. nov. (ex Nikitin et al. 1994) from a freshwater neuston.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:681-684.*

- *Ott, S. J.; Musfeldt, M.; Wenderoth, D.; Hampe, J.; Brant, O.; Filsch, U. R.; Timmis, K. N., and Schreiber, S. Reduction in diversity of the colonic mucosa-associated bacterial microflora in patients with active inflammatory bowel disease.* ***GUT****. 2004;* ***53****:685-693.*

- *Pauling, B. V.; Kroer, N., and Wagner-Döbler, I. Effect of selective pressure and GEM densities on mercury resistance (*mer*) operon transfer in Elbe river and estuarine sediments.* ***Environmental Practice****. 2004;* ***6****:68-82.*

- *Pieper, D. H.; Martins dos Santos, V. A. P., and Golyshin, P. N. Genomic and mechanistic insights into the biodegradation of organic pollutants.* ***Current Opinion in Biotechnology****. 2004;* ***15****:215-224.*

- *Pieper, D. H. and Reineke, W. Degradation of chloroaromatics by Pseudomona(d)s. (J. L. Ramos, ed.) The Pseudomonads Vol. III. Biosynthesis of Macromolecules and Molecular Metabolism. New York: Kluwer Academic/Plenum Publishers; 2004; pp. 509-574.*

- *Pradella, S.; Allgaier, M.; Hoch, C.; Päuker, O.; Stackebrandt, E., and Wagner-Döbler, I. The* pufLM *genes of the photosynthesis reaction centre may be located on linear/circular plasmids in marine Alphaproteobacteria.* ***Applied and Environmental Microbiology****. 2004;* ***70****:3360-3369.*

- *Ramm, W.; Schatton, W.; Wagner-Döbler, I.; Wray, V.; Nimtz, M.; Tokuda, H.; Enjyo, F.; Nishino, H.; Beil, W.; Heckamnn, R.; Lurtz, V., and Lang, S. Diglucosyl-glycerolipids from a marine sponge-associated* Bacillus pumilus *strain AAS3: their production, enzymatic modification and properties.* ***Applied Microbiology and Biotechnology****. 2004;* ***64****:497-504.*

- *Römling, U. and Lünsdorf, H. Characterization of cellulose produced by* Salmonella enterica *serovar* typhimurium. ***Cellulose****. 2004;* ***11****:413-418.*

- *Soler, L.; Yánez, M. A.; Chacon, M. R.; Aguilera-Arreola, M. G.; Catalan, V.; Figueras M.J., and Martinez-Murcia, A. J. Phylogenetic analysis of the genus* Aeromonas *based on two housekeeping genes.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:1511-1519.*

- *Sun, J.; Daniel, R.; Wagner-Döbler, I., and Zeng, A.-P. Is autoinducer-2 a universal signal for interspecies communication? A comparative and phylogenetic genomic analysis of the synthesis and signal transduction pathways.* ***BMC Evolutionary Biology****. 2004;* ***4****:36.*

- *Wagner-Döbler, I.; Rheims, H.; Felske, A.; El-Ghezal, A.; Laatsch, H.; Lang, S.; Pukall, R., and Tindall, B. J.* Oceanibulbus indolifex *gen. nov., sp. nov., a North sea* Alphaproteobacterium *producing bioactive metabolites.* ***International Journal of Systematic and Evolutionary Microbiology****. 2004;* ***54****:1177-1184.*

- *Yakimov, M. M.; Gentile, G.; Bruni, V.; Capello, S.; D'Auria, G.; Golyshin, P. N., and Giuliano, L. Crude oil-induced structural shift of coastal bacterial communities of Rod Bay (Terra Nova Bay, Ross Sea, Antartica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria.* ***FEMS Microbiology and Ecology****. 2004;* ***49(3)****:419-432.*

- *Yakimov, M. M.; Giuliano, L.; Denaro, R.; Cristafi, E.; Chernikova, T.; Abraham, W.-R.; Luensdorf, H.; Timmis, K. N., and Golyshin, P. N.* Thalassolituus oleivorans*, gen. nov. sp. nov., and new marine bacterium confined to the utilization of hydrocarbons.* ***International Journal of Systematic and Evolutionary Microbilogy****. 2004;* ***54****:141-148.*

Platforms – 2004

- *Aydogmus, Z.; Imre, S.; Ersoy, L., and Wray, V. Halogenated secondary metabolites from* Laurencia optusa. ***Natural Product Research****. 2004;* ***18(1)****:43-49.*

- *Duvar, S.; Hecht, V., and Ziehr, H. Biopharmazeutika-Produktion mit tierischen Zellen.* ***Laborwelt****. 2004;* ***5****:12-14.*

- *Ehrhardt, S.; Rittig, F.; Vogel, M.; Wray, V., and Skeries, B. Sucrosyl-(1⇒2)-ß-isomaltulose: Enzymatic synthesis and structure determination.* ***Journal of Carbohydrate Chemistry****. 2004;* ***23(2&3)****:163-168.*

- *Forno, G.; Fogolin, M. B.; Oggero, M.; Kratje, R.; Etcheverrigaray, M.; Conradt, H. S., and Nimtz, M. N- and O-linked carbohydrates and glycosylation site occupancy in recombinant human granulocyte-macrophage colony-stimulating factor secreted by a chinese hamster ovary cell line.* ***European Journal of Biochemistry****. 2004;* ***271****:907-919.*

- *Fouad, M.; Al-Traben, K.; Badran, M.; Wray, V.; Edrada, R.; Proksch, P., and Ebel, R. New steroidal saponins from the sponge* Erylus lendenfeldi. ***ARKIVOC****. 2004;* ***8****:17-27.*

- *Franke, R.; Doll, C.; Wray, V., and Eichler, J. Loops on loops: generation of complex scaffolded peptides presenting multiple cyclic fragments.* ***Organic and BiomolecularChemistry****. 2004;* ***2****:2847-2851.*

- *Hassan, W.; Edrada, R. A.; Ebel, R.; Berg, A.; Wray, V.; Steube, K.; van Soest, R.; Wiryowidagdo, S., and Proksch, P. New imidazole alkaloids from Indonesian sponge* Leucetta chagosensis. ***Journal of Natural Products****. 2004;* ***67****:817-822.*

- *Hassan, W.; Edrada, R. A. Ebel R.; Wray, V., and Proksch, P. New alkaloids from the mediterranean sponge* Hamigera hamigera. ***Marine Drugs****. 2004;* ***2****:88-100.*

- *Hiort, J.; Maksimenka, K.; Reichert, M.; Perovic-Ottstadt, S.; Lin, W. H.; Wray, V.; Steube, K.; Schaumann, K.; Weber, H.; Proksch, P.; Ebel, R.; Müller, W. E. G., and Bringmann, G. New natrual products from the sponge-derived fungus* Aspergillus niger. ***Journal of Natural Products****. 2004;* ***67****:1532-1543.*

- *Jadulco, R.; Edrada, R. A.; Ebel, R.; Berg, A.; Schaumann, K.; Wray, V.; Steube, K., and Proksch, P. New communesin derivatives from the fungus* Penicillium *sp. derived from the mediterranean sponge* Axinella verrucosa. ***Journal of Natural Products****. 2004;* ***67****:78-81.*

- *Lara, M.; Sevin-Gonzalez, L.; Singh, M.; Moreno, C.; Cohen, I.; Nimtz, M., and Espitia, C. Expression, secretion, and glycosylation of the 45- and 47-kDa glycoprotein of* Mycobacterium tuberculosis *in* Streptomyces lividans. ***Applied and Environmental Microbiology****. 2004;* ***70****:679-685.*

- *Pedpradab, S.; Edrada, R.-A.; Ebal, R.; Wray, V., and Proksch, P. New ß-carboline alkaloids from the Andaman sea sponge,* Dragmacidon *sp.* ***Journal of Natural Products****. 2004;* ***67****:2113-2116.*

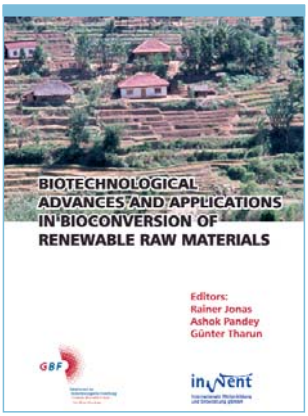
- *Ramm, W.; Schatton, W.; Wagner-Döbler, I.; Wray, V.; Nimtz, M.; Tokuda, H.; Enjyo, F.; Nishino, H.; Beil, W.; Heckamnn, R.; Lurtz, V., and Lang, S. Diglucosyl-glycerolipids from a marine sponge-associated* Bacillus pumilus *strain AAS3: their production, enzymatic modification and properties.* ***Applied Microbiology and Biotechnology****. 2004;* ***64****:497-504.*

- *Santamaria-Araujo, J. A.; Fischer, B.; Otte, T.; Nimtz, M.; Mendel, R. R.; Wray, V., and Schwarz, G. The tetrahydropyranopterin structure of the sulfur-free and metal-free molybdenum cofactor precursor.* ***The Journal of Biological Chemistry****. 2004;* ***279(16)****:15994-15999.*

- *Schultz, A.; Laschat, S.; Saipa, A.; Gießelmann, F.; Nimtz, M.; Schulte, J. L.; Baro, A., and Miehlich, B. Columnar liquid crystals with a central crown ether unit.* ***Advanced Functional Materials****. 2004;* ***14(2)****:163-168.*

- *Schwarz, M.; Wray, V., and Winterhalter, P. Isolation and identification of novel pyranoanthocyanins from black carrot (*Daucus carota *L.) juice.* ***Journal of Agricultural and Food Chemistry****. 2004;* ***52****:5095-5101.*

- *Wang, B.-G.; Ebel, R.; Wang, C.-Y.; Edrada, R. A.; Wray, V., and Proksch, P. Aglacins I-K, three highly methoxylated lignans form* Aglaia cordata. ***Journal of Natural Products****. 2004;* ***67****:682-685.*



- *Cover picture of the book Biotechnological Advances and Applications in Bioconversion of Renewable Raw Materials, editors: Rainer Jonas, Ashok Pandey and Günter Tharun. GBF/ InWent, Döring Druck Braunschweig, Germany, 2004,.312p. The permission of the editors is gratefully acknowledged.*

Biotech Facilities – 2004

- *Duvar, S.; Hecht, V., and Ziehr, H. Biopharmazeutika-Produktion mit tierischen Zellen.* ***Laborwelt****. 2004;* ***5****:12-14.*

- *Fogolin, M. B.; Wagner, R.; Etcheverrigaray, M., and Kratje, R. Impact of temperature reduction and expression of yeast pyruvate carboxylase on hGM-CSF-producing CHO cells.* ***Journal of Biotechnology****. 2004;* ***109****:179-191.*

- *Hoffmann, F. and Rinas, U. Roles of heat-shock chaperones in the production of recombinant proteins in* Escherichia coli. ***Advances in Biochemical Engineering and Biotechnology****. 2004;* ***89****:143-161.*

- *Hoffmann, F. and Rinas, U. Stress induced by recombiant protein production in* Escherichia coli. ***Advances in Biochemical Engineering and Biotechnology****. 2004;* ***89****:73-92.*

- *Hoffmann, F.; van den Heuvel, J.; Zidek, N., and Rinas, U. Minimizing inclusion body formation during recombiant protein production in* Escherichia coli *at bench and pilot plant scale.* ***Enzyme and Microbial Technology****. 2004;* ***34****:235-241.*

- *Lin, H.; Hoffmann, F.; Rozkov, A.; Enfors, S.-O.; Rinas, U., and Neubauer, P. Change of extracellular cAMP concentration is a sensitive reporter for bacterial fitness in high cell density cultures of* Escherichia coli. ***Biotechnology and Bioengineering****. 2004;* ***87****:602-613.*

- *Rinas, U. and Hoffmann, F. Selective leakage of host cell proteins during high-cell density cultivation of recombinant and non-recombinant* Escherichia coli. ***Biotechnology Progress****. 2004;* ***20****:679-687.*

- *Ross, A.; Kessler, W.; Krumme, D.; Menge, U.; Wissing, J.; van den Heuvel, J., and Flohé, L. Optimised fermentation strategy for 13C/15N recombinant protein labelling in* Escherichia coli *for NMR-structure analysis.* ***Journal of Biotechnology****. 2004;* ***108****:31-39.*

- *Sabra, W. A.; Ross, A., and Jonas, R. Alginate versus PHB production by the diazotrophic growing culture of* Azotobacter chroococcum. *(Jonas, R.; Pandey A., Tharun G., eds.) Biotechnological Advances and Applications of Renewable Raw Materials. Braunschweig, Germany: Döring Druck; 2004; pp. 309-311.*

- *Santiago, W. Jr.; Rosa da Silva, M.; Müller, M.; Hawumba, J. F.; Krützfeld, R., and Ross, A. Optimal control and downstream processing aspects of penicillin-G-acylase production using high cell density cultivation. (Jonas, R.; Pandey A., and Tharun, G., eds.) Biotechnological Advances and Applications in Bioconversion of Renewable Raw Materials. Braunschweig, Germany: Döring Druck; 2004; pp. 281-286.*

- *Vallejo, F. and Rinas, U. Optimized procedure for renaturation of recombiant human bone morphogenetic protein-2 at high protein concentration.* ***Biotechnology and Bioengineering****. 2004;* ***85****:601-609.*

- *Vallejo, L. F. and Rinas, U. Strategies for the recovery of active proteins through refolding of bacterial inclusion body proteins.* ***Microbial Cell Factories****. 2004;* ***3****:11:doi:10.1186/1475-2859-3-11.*

Other research areas – 2004

- *Carvalho-Jonas, M. F.; Schneider, A. L. S.; Furlan, S. A., and Jonas, R. Characterization of the ß-D-galactosidase of* Kluyveromyces marxianus *CDB 002. (Jonas, R.; Pandey A.; Tharun G.; eds.) Biotechnological Advances and Applications in Bioconversion of Renewable Raw Materials. Braunschweig, Germany: Döring Druck; 2004; pp. 139-146.*

- *Jonas, R.; Pandey, A., and Tharun, G., Editors. Biotechnological Advances and Applications in Bioconversion of Renewable Raw Materials. Braunschweig, Germany: Döring Druck; 2004; pp. 1-312.*

- *Jonas, R. and Silveira, M. M. Sorbitol can be produced not only chemically but also biotechnologically.* ***Applied Biochemistry and Biotechnology****. 2004;* ***118****:321-336.*

- *Kern, M. and Jonas, R. Aspects of the future of agrotechnologies. (Jonas, R.; Pandey A.; Tharun G.; eds.) Biotechnological Advances and Applications in Bioconversion of Renewable Raw Materials. Braunschweig, Germany: Döring Druck; 2004; pp. 38-50.*

- *Sabra, W. A.; Ross, A., and Jonas, R. Alginate versus PHB production by the diazotrophic growing culture of* Azotobacter chroococcum. *(Jonas, R.; Pandey A.; Tharun G.; eds.) Biotechnological Advances and Applications of Renewable Raw Materials. Braunschweig, Germany: Döring Druck; 2004; pp. 309-311.*

- *Schneider, A. L. S.; Gern, R. M. M.; Ninow, J. L.; Furlan, S. A., and Jonas, R. Characterization of microbial inulinases. (Jonas, R.; Pandey A.; Tharun G.; eds.) Biotechnological Advances and Applications in Bioconversion of Renewable Raw Materials. Braunschweig, Germany: Döring Druck; 2004; pp. 128-132.*

Publications 2005

Infection and Immunity – 2005

- *Bauer, H.; Darji, A.; Chakraborty, T., and Weiss, S. Salmonella-mediated oral DNA vaccination using stabilised eukaryotic expression plasmids.* ***Gene Therapy****. 2005;* ***12****:364-372.*
- *Bollati Fogolin, M.; Forno, G.; Nimtz, M.; Conradt, H.; Etcheverri-garay, M., and Kratje, R. Temperature reduction in cultures of hGM-CSF-expressing CHO cells: effect on productivity and product quality.* ***Biotechnology Progress****. 2005;* ***21(1)****:17-21.*
- *Dikopoulos, N.; Bertoletti, A.; Kröger, A.; Hauser, H.; Schirmbeck, R., and Reimann, J. Type I interferon negatively regulates CD8+ T cell responses through IL-10-producing CD4+ TR1 cells.* ***Journal of Immunology****. 2005;* ***174****:99-109.*
- *Gaitatzis, N.; Kunze, B., and Müller, R. Novel insights into side-rophore formation in myxobacteria.* ***ChemBioChem****. 2005;* ***6(2)****:365-374.*
- *Gerth, K. and Müller, R. Moderately thermophilic myxobacteria: novel potential for the production of natural products isolation and characterization.* ***Environmental Microbiology****. 2005; doi: 10.1111/j.1462-2920.2005.00761.x.*
- *Goetze, S.; Baer, A.; Winkelmann, S.; Nehlsen, K.; Seibler, J.; Maass, K., and Bode, J. Performance of genomic bordering elements at pre defined genomic loci.* ***Molecular and Cellular Biology****. 2005;* ***25****:2260-2272.*
- *Goldmann, O.; Chhatwal, G. S., and Medina, E. Contribution of NK cells to the pathogenesis of septic shock induced by* Strepto-coccus pyogenes *in mice.* ***Journal of Infectious Diseases****. 2005;* ***191****:1280-1286.*
- *Hansen, W.; Grabenhorst, E.; Nimtz, M.; Conradt, H. S., and Wirth, M. Generation of serum-stabilized mouse retroviruses: Reduction of alpha 1,3-galactosyltransferase synthesis in a murine packaging cell line by expression of chimeric glycosyltransferases.* ***Metabolic Engineering****. 2005; doi: 10.1016/j.ymben.2005.02.004.*
- *Heinz, D. W.; Schubert, W.-D., and Höfle, G. Lange gesucht – Die bioaktive Konformation von Epothilon und seine Bindung im Tubulin.* ***Angewandte Chemie****. 2005;* ***117(9)****:1324-1327.*
- *Henklein, P. ; Bruns, K.; Nimtz, M.; Wray, V.; Tessmer, U., and Schubert, W.-D. Influenza A virus protein PB1-F2: Synthesis and characterization of the biologically active full length protein and related peptides.* ***Journal of Peptide Science****. 2005; doi: 10.1002/psc.641.*
- *Jenzora, A.; Behrendt, B.; Small, J. V.; Wehland, J., and Stradal, T. E. B. PREL1 links Ras signalling to actin remodeling via Ena/VASP proteins.* ***FEBS Letters****. 2005;* ***579****:455-463.*
- *Juhas, M.; Wiehlmann, L.; Salunkhe, P.; Lauber, J.; Buer, J., and Tummler, B. Gene Chip expression analysis of the VqsR regulon of* Pseudomonas aeruginosa *TB.* ***FEMS Microbiology Letters****. 2005;* ***242****:287-295.*
- *Kayser, A.; Weber, J.; Hecht, V., and Rinas, U. Metabolic flux analysis of* Escherichia coli *in glucose-limited continuous culture: I. Growth rate dependent metabolic efficiency at steady state.* ***Microbiology****. 2005;* ***151****:693-706.*
- *Kuhlmeier, D.; Rodda, E.; Kolarik, L. O.; Furlong, D. N., and Bilitewski, U. Application of atomic force microscopy and grating coupler for the characterization of biosensor surfaces.* ***Biosensors & Bioelectronics****. 2005;* ***18(7)****:925-936.*
- *Lechel, A.; Satyanarayana, A.; Ju, Z.; Plentz, R.; Schaetzlein, S.; Rudolph, C.; Wilkens, L.; Wiemann, S. U.; Saretzki, G.; Malek, N. P.; Manns, M. P.; Buer, J., and Rudolph, K. L. The cellular level of telomere dysfunction determines induction of senescence or apoptosis* in vivo. ***EMBO Reports****. 2005;* ***6****:275-281.*
- *Lenz, T.; Gauer, s.; Weich, H. A.; Haak, T.; Bergner, R., and Gossmann, J. VEGF and Flt-1 are not correlated to EPO in diabetics with normal or reduced renal function.* ***Nephrology****. 2005;* ***10(1)****:84-89.*
- *McMillan, D. and Chhatwal, G. S. Prospects for a group A streptococcal vaccine.* ***Current Opinion in Molecular Therapeutics****. 2005;* ***7****:11-16.*
- *Michalzik, M.; Wendler, J.; Rabe, J.; Büttgenbach, S., and Bilitewski, U. Development and application of a miniaturised quartz crystal microbalance (QCM) resonator as immunosensor for bone morphogenic protein-2.* ***Sensors and Actuators B****: 2005;* ***105(2)****:508-515.*
- *Prabhakar, S.; Töpfer, T.; Buer, J., and Tümmler, B. Genome-wide transcriptional profiling of the steady state response of* Pseudomonas aeruginosa *to hydrogen peroxide.* ***Journal of Bacteriology****. 2005; 187:2565-2572.*
- *Pust, S.; Morrison, H.; Wehland, J.; Sechi, A., and Herrlich, P.* Listeria monocytogenes *exploits ERM protein functions to efficiently spread from cell to cell.* ***EMBO Journal****. 2005;* ***24****:1287-1300.*
- *Rasmussen, U.; Schreiber, V.; Schultz, H.; Mischler, F., and Schughart, K. Tumor cell targeting by phage displayed peptides.* ***Cancer Gene Therapy****. 2005;* ***9****:606-612.*
- *Reichelt, J.; Dieterich, G.; Kvesic, M.; Schomburg, D., and Heinz, W. D. BRAFI. Linking and visualization of database information in a 3D-viewer and modelling tool.* ***Bioinformatics****. 2005;* ***21****:1291-1293.*
- *Rharbaoui, F.; Bruder, D.; Vidakovic, M.; Ebensen, T.; Buer, J., and Guzman, C. A. Characterization of a B220+ lymphoid cell subpopulation with immune modulatory functions.* ***Journal of Immunology****. 2005;* ***174****:1317-1324.*
- *Schulze, K.; Goldmann, O.; Toppel, A.; Medina, E., and Guzmán, C. A. The FAI protein of group C streptococci targets B cells and exhibits adjuvant activity.* ***Vaccine****. 2005;* ***23****:1408-1413.*
- *Stradal, T. E. B.; Lommel, S.; Wehland, J., and Rottner, K. Host-pathogen interactions and cell motility: learning from bacteria. (Wedlich, D., ed.) Cell Migration in Development and Disease. Weinheim: Wiley VCH Verlag; 2005; pp. 205-248.*
- *Sun, J.; Gunzer, F.; Westendorf, A. M.; Buer, J.; Scharfe, M.; Gößling, F.; Blöcker, H., and Zeng, A. P. Genomic peculiarity of coding sequences and metabolic potential of probiotic* Escherichia coli *strain NISSLE 1917 inferred from raw genome data.* ***Journal of Biotechnology****. 2005; doi: 10.1016/j.jbiotec.2005.01.008.*
- *Weber, J.; Kayser, A., and Rinas, U. Metabolic flux analysis of* Escherichia coli *in glucose-limited continuous culture: II. Dynamic response to famine and feast, activation of the methylglyoxal pathway and oscillatory behavior.* ***Microbiology****. 2005;* ***151****:707-716.*
- *Wendler, J.; Vallejo, L. F.; Rinas, U., and Bilitewski, U. Application of an SPR-based receptor assay for the determination of biologically active recombinant bone morphogenetic protein-2.* ***Analytical and Bioanalytical Chemistry****. 2005;* ***381(5)****:1056-1064.*
- *Wenzel, S. C.; Kunze, B.; Höfle, G.; Silakowski, B.; Blöcker, H., and Müller, R. Structure and biosynthesis of myxochromides S in* Stigmatella aurantiaca*: Evidence for an iterative and stuttering bacterial type I polyketide synthase and for module skipping in nonribosomal peptide biosynthesis.* ***ChemBioChem****. 2005;* ***6(2)****:375-385.*
- *Westendorf, A. M.; Gunzer, F.; Deppenmeier, S.; Tapadar, D.; Hunger, J. K.; Schmidt, M.; Buer, J., and Bruder, D. Intestinal immunity of* E. coli *NISSLE 1917: A safe carrier for therapeutic molecules.* ***FEMS Immunology and Medical Microbiology****. 2005;* ***43(3)****:373-384.*

- *Westendorf, A. M.; Templin, M.; Geffers, R.; Deppenmeier, S.; Gruber, A. D.; Probst-Kepper, M.; Hansen, W.; Liblau, R. S.; Gunzer, F.; Bruder, D., and Buer, J. CD4+ T cell mediated instinal immunity: chronic inflammation versus immune regulation.* ***Gut****. 2005;* ***54****:60-69.*

- *Wiemann, S. U.; Satyanarayana, A.; Buer, J.; Kamino, K.; Manns, M. P., and Rudolph, K. L. Contrasting effects of telomere shortening on organ homeostasis, tumor suppression, and survival during chronic liver damage.* ***Oncogene****. 2005;* ***24****:1501-1509.*

Infection and immunity – 2005 – in press

- *Barthold, M.; Majore, I.; Fargali, S.; Stahl, F.; Schulz, R.; Lose, S.; Mayer, H., and Jäger, V. 3D-cultivation and characterisation of osteogenic cells for the production of highly viable bone tissue implants. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers; 2005.*

- *Bassani Molinas, M. M.; Nelving, A.; Beer, C.; Hesse, F.; Wirth, M.; Durocher, Y.; Kamen, A., and Wagner, R. Intracellular nucleotide pools for optimizing product-oriented transient tranfection of HEK293 cells in suspension. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers; 2005.*

- *Beer, C. and Wirth, M. A new method for the quantitative determination of enveloped viral particles. Animal Cell Technology Meets Genomics. Dordrecht, Niederlande: Kluver Academic Publishers; 2005.*

- *Beer, C. and Wirth, M. The role of cholesterol rich domains and cellular proteins in mouse retrovirus assembly.* ***Current Topics in Virology****. 2005.*

- *Bilitewski, U. Biosensors for Bioprocess Monitoring. (Gorton, L., ed.) Biosensors and Modern Biospecific Analytical Techniques: Elsevier; 2005.*

- *Bilitewski, U. Lab-on-a-chip Technologies. Encyclopedia of Analytical Science 2nd ed. Elsevier; 2005.*

- *Blumenthal, A.; Lauber, J.; Hoffmann, R.; Ernst, M.; Keller, C.; Buer, J.; Ehlers, S., and Reiling, N. Common and unique gene expression signatures of human macrophages in response to four strains of* Mycobacterium avium *differing in their growth and persistence characteristics.* ***Infection and Immunity****. 2005.*

- *Bollati Fogolin, M.; Irani, N.; Beccaria, A. J.; Schulz, C.; van den Heuvel, J.; Elias, C. B.; Carpentier, E.; Durocher, Y.; Bisson, L.; Etcheverrigaray, M.; Kratje, R. B.; Wirth, M.; Kamen, A., and Wagner, R. Impact of the expression of yeast pyruvate carboxylase on the productivity of different animal host cell lines. Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers; 2005.*

- *Borsutzky, S.; Kretschmer, K.; Becker, P. D.; Mühlradt, P. F.; Kirschning, C. J.; Weiss, S., and Guzman, C. A. The mucosal adjuvant MALP-2 directly stimulates B lymphocytes via the TLR2 without the need of accessory cells.* ***Journal of Immunology****. 2005.*

- *Brigelius-Flohé, R.; Maiorino, M.; Ursini, F., and Flohé, L. Selenium – an Antioxidant? (Cadenas, E. and Packer, L., editors). In: Handbook of Antioxidant. 2nd ed. New York: Marcel Dekker Inc.; 2005.*

- *Chhatwal, G. S. and McMillan, D. Uncovering the mysteries of invasive streptococcal diseases.* ***Trends in Molecular Medicine****. 2005.*

- *Deiters, U.; Barsig, J.; Tawil, B., and Mühlradt, P. F. The macrophage activating lipopeptide MALP-2 accelerates wound healing in diabetic mice.* ***Journal of Investigative Dermatology****. 2005.*

- *Dieterich, G.; Heinz, D. W., and Reichelt, J. Matching of PDB chain sequences to information in public databases. Journal of Integr.* ***Bioinformatics****. 2005.*

- *Dieterich, G.; Kärst, U.; Wehland, J., and Jänsch, L. MineBlast: A literature presentation service supporting protein annotation by data Mining of blast results. Bioinformatics. 2005.*

- *Disanza, A.; Steffen, A.; Hertzog, M.; Frittoli, E.; Rottner, K., and Scita, G. Actin polymerization machinery: the finish line of signalling networks, the starting point of cellular movement.* ***Cellular and Molecular Life Sciences****. 2005.*

- *Eiting, M.; Hagelüken, G.; Schubert, W.-D., and Heinz, W.-D. The mutation G145S in PrfA, a key virulence regulator of* Listeria monocytogenes*, increases DNA-binding affinity by stabilizing the HTH-motif.* ***Molecular Microbiology****. 2005.*

- *Eming, S.; Lauer, G.; Cole, M.; Jurk, S.; Christ, H.; Hornig, C.; Krieg, T., and Weich, H. A. Increased levels of the soluble variant of the vascular endothelial growth factor receptor VEGFR-1 are associated with a poor prognosis in wound healing.* ***Journal of Investigative Dermatology****. 2005.*

- *Frank, R. and Dübel, S. Analysis of protein interactions with immobilized peptide arrays synthesized on membrane supports. Cold Spring Harbor; 2005.*

- *Gekara, N. and Weiss, S. Lipid rafts clustering and signalling by listeriolysin O.* ***Biochemical Society****. 2005.*

- *Guzmán, C. A.; Borsutzky, S.; Favre, D., and Dietrich, G. Vaccines against infections caused by* Salmonella, Shigella *and pathogenic* Escherichia coli. *EcoSAL* Escherichia coli *and* Salmonella*: Cellular and Molecular Biology. Washington, DC: ASM Press; 2005; Chapter 8.8.14.*

- *Guzmán, C. A.; Borsutzky, S.; Griot-Wenk, M.; Metcalfe, I. C.; Pearman, J.; Collioud, A.; Favre, D., and Dietrich, G. Vaccines against Typhoid fever.* ***Vaccine****. 2005.*

- *Guzmán, C. A.; Cebolla, A.; Beltrametti, F.; Staendner, L. H., and de Lorenzo, V. Physiological state of intracellular* Shigella flexneri *probed with a metabolic sensor fused to a surface-reporter system.* ***FEBS Letters****. 2005.*

- *Günther, C.; Hause, B.; Heinz, D. W.; Krauzewicz, N.; Rudolph, R., and Lilie, H. Conbination of listeriolysin O and a tumor-specific antibody facilitate efficient cell-type specific gene delivery of conjugated DNA.* ***Cancer Gene Therapy****. 2005.*

- *Höfle, G. and Reichenbach, H. Epothilone, a myxobacterial metabolite with promising antitumor activity. (G. Cragg, ed.) Antitumor Agents from Natural Sources. Boca Raton, USA: CRC Press; 2005.*

- *Köster, M.; Frahm, T., and Hauser, H. Nucleocytoplasmic shuttling analysis of STAT1 by FRAP and FLIP.* ***Current Opinion in Biotechnology****. 2005.*

- *Lommel, S.; Benesch, S.; Rohde, M., and Wehland, J. Pedestal formation by pathogenic* E. coli*: a model system for studying signal transduction to the actin cystoskeleton. (Celis, J. E., ed.) Cell Biology: A Laboratory Handbook. 3rd ed. Academic Press; 2005.*

- *Lommel, S.; Benesch, S.; Rohde, M.; Wehland, J., and Rottner, K. Enterohaemorrhagic and enteropathogenic* Escherichia coli *use different mechanisms for actin pedestal formation that converge on N-WASP.* ***Cell Microbiology****. 2005.*

- *Mayer, H.; Bertram, H.; Lindenmaier, W.; Korff, T.; Weber, H., and Weich, H. A. Vascular endothelial growth fractor (VEGF-A) expression in Human mensenchymal stem cells: autocrine and paracrine role on osteoblastic and endothelial differentiation.* ***Journal of Cellular Biochemistry****. 2005.*

- *Medina, E. Models of group A streptococcal diseases: a review of current status. Drug Discovery Today: Disease Models. Review. 2005.*
- *Nedshkovskaya, O. I.; Vancanneyt, M.; Van Trappen, S.; Vandemeulebroecke, K.; Lysenko, A. M.; Rohde, M.; Falsen, E.; Frolova, G. M.; Mikhailov, V. V., and Swings,* J. Algoriphagus aquimarinus *sp. nov.*, Algoriphagus chordae *sp. nov. and* Algoriphagus winogradskyi *sp. nov., from seawater and algae. Transfer of* Hongiella halophila *Yi and Chun 2004 to the genus* Algoriphagus *as* Algoriphagus halophilus *comb. nov. emended description of the genera* Algoriphagus *Bowman et al. 2003 and* Hongiella *Yi and Chun 2004. **International Journal of Systematic and Evolutionary Microbiology. 2005**.*
- *Paschen, A.; Schadendorf, P., and Weiss, S. Bacteria as vector for gene therapy of cancer. (Templeton, N. S. and Lasic, D. D., eds.). In: Gene Therapy: Therapeutic mechanisms and strategies. New York, Basel: Marcel Dekker; 2005.*
- *Rharbaoui, F. and Guzmán, C. A. New generation of immune modulators based on toll-like receptor signaling. **Current Immunology Review**. 2005.*
- *Rinas, U.; El-Enshasy, H.; Emmler, M.; Hille, A.; Hempel, D. C., and Horn, H. Model-based prediction of substrate conversion and protein synthesis and excretion in recombinant* Aspergillus niger *biopellets. **Chemical Engineering Science**. 2005.*
- *Rottner, K.; Kaverina, I. N., and Stradal, T. E. B. Cytoskeleton proteins. (Celis, J. E., ed.) Cell Biology: A Laboratory Handbook. 3rd Edition ed. Academic Press; 2005.*
- *Schumacher, J. T.; Mersal, G. A. M., and Bilitewski, U. (Pandey, A., ed.) Immobilisation of enzymes; 2005.*
- *Trost, M.; Wehmhöner, D.; Kärst, U.; Dieterich, G.; Wehland, J., and Jänsch, L. Comparative proteome analysis of secretory proteins from pathogenic and non-pathogenic* Listeria *species. **Proteomics**. 2005.*
- *Wehmhöner, D.; Dieterich, G.; Fischer, E.; Baumgärtner, M.; Wehland, J., and Jänsch, L. "LaneSpector", a toll for membrane proteome profiling based on SDS-PAGE / LC-MS/MS analysis: Application to* Listeria monocytogenes *membrane proteins. **Electrophoresis**. 2005.*
- *Zhou, J.; Schmid, T.; Frank, R., and Brüne, B. Pl3K/Akt is required for heat shock proteins to protect HIF-1 from pVHL-independent degradation. **Journal of Biological Chemistry**. 2005.*

Comparative Genome Research – 2005

- *Frere, F.; Reents, H.; Schubert, W.-D., and Heinz, D. W. Tracking the evolution of porphobilinogen synthase metal dependence* in vitro. ***Journal of Molecular Biology**. 2005; **345(5)**:1059-1070.*
- *Gail, R.; Frank, R., and Wittinghofer, A. A systematic peptide-array based delineation of differential beta-Catenin interaction with Tcf4, E-Cadherin and APC. **Journal of Biological Chemistry**. 2005; **280**:7107-7117.*
- *Heinz, D. W.; Schubert, W.-D., and Höfle, G. Lange gesucht – Die bioaktive Konformation von Epothilon und seine Bindung im Tubulin. **Angewandte Chemie**. 2005; **117(9)**:1324-1327.*
- *Kaisermann, M. C.; Sardella, I. G.; Trajman, A.; Coelho, L. V.; Kämpfer, S.; Jonas, F.; Singh, M., and Saad, M. H. F. IgA antibody responses to* Mycobacterium tuberculosis *recombinant MPT-64 and MT-10.3 (Rv3019c) antigens in pleural fluid of patients with tuberculous pleurisy. **The International Journal of Tuberculosis and Lung Disease**. 2005; **9(4)**:461-466.*
- *Sun, J.; Gunzer, F.; Westendorf, A. M.; Buer, J.; Scharfe, M.; Gößling, F.; Blöcker, H., and Zeng, A. P. Genomic peculiarity of coding sequences and metabolic potential of probiotic* Escherichia coli *strain NISSLE 1917 inferred from raw genome data. **Journal of Biotechnology**. 2005; doi: 10.1016/j.jbiotec.2005.01.008.*
- *Wenzel, S. C. ; Kunze, B.; Höfle, G.; Silakowski, B.; Blöcker, H., and Müller, R. Structure and biosynthesis of myxochromides S in* Stigmatella aurantiaca*: Evidence for an iterative and stuttering bacterial type I polyketide synthase and for module skipping in nonribosomal peptide biosynthesis. **ChemBioChem**. 2005; **6(2)**:375-385.*
- *Zander, N. and Frank, R. The use of polystyrylsulfonyl chloride resin as a solid supported condensation reagent for the formation of esters: Synthesis of N-(9-fluorenylmethoxy)carbonyl)-L-aspartic acid; α-tert-butyl ester, ß-(2-ethyl[(1E)-4-nitrophenyl)azo]phenyl]amino]ethyl ester. Organic Syntheses: Wiley; 2005; Vol. 81; pp. 235-243. ISBN: 0-471-68257-8.*

Comparative Genome Research – 2005 – in press

- *Eichler, J. and Houghten, R. A. Combinatorial synthesis. (Goodman, M.; Felic, A.; Moroder, L., and Toniolo, C., Editors). In: Synthesis of Peptides – Houben-Weyl. Stuttgart: Thieme; 2005; E 22a.*
- *Hinssen, H.; Fock, U.; Schubert, W.-D., and Jochusch, B. Topological assignment of the* N-terminal *extension of plasma gelsolin to the gelsolin surface. **Biochemical Journal**. 2005.*
- *International Human Genome Sequencing (GBF: Blöcker, H.) Finished sequence of the human genome. **Nature**. 2005.*
- *Jahn, D. and Heinz, D. W. Biosynthesis of 5-aminolevulinic acid. (Warren, M. J.; Smith A., eds.) Tetrapyrroles: Their birth, life and death. Georgetown, USA: Landes Bioscience; 2005.*
- *Kim, E.-J.; Deckwer, W.-D.; Wang, W., and Zeng, A.-P. Expression of the quorum sensing regulator protein LasR is strongly affected by iron deficiency and oxygen concentration in* Pseudomonas aeruginosa *irrespective of cell density. **Microbiology**. 2005.*
- *Ma, H. and Zeng, A.-P. Reconstruction of metabolitc network from genome information and its structural and functional analysis (Eils, R.; Kriete A., eds.). Computational Systems Biology: Elsevier Inc., USA; 2005.*
- *Zeng, A.-P. and Bi, J., Cell culture kinetics and modelling (Ozturk, S. S.; Hu, W.-S., editors). In: Cell Culture Technology for Pharmaceutical and Cellular Therapies; 2005; Chapter 16.*
- *Zeng, A.-P.; Sun, J. Wang, W.; Ma, H., and Deckwer, W.-D. Application of genomic and proteomic data for bioprocess analysis and optimisation (Yang, S. T.; ed). Bioprocessing for Value-added Products from Renewable Resources: Elsevier; 2005.*

Sustainable Use of Landscape – 2005

- *Fritz, I.; Strömpl, C.; Nikitin, D. I.; Lysenko, A. M., and Abraham, W.-R.* Brevundimonas mediterranea *sp. nov., a non-stalked species from the Mediterranean Sea.* ***International Journal of Systematic and Evolutionary Microbiology****. 2005;* ***55****:479-486.*
- *Höfle, M. G.; Flavier, S.; Christen, R.; Bötel, J.; Labrenz, M., and Brettar, I. Retrieval of nearly complete 16S rRNA gene sequences from environmental DNA following 16S rRNA based community fingerprinting.* ***Environmental Microbiology****. 2005; doi: 10.1111/j.1462-2920.2005.00738.x.*
- *Katsivela, E. ; Moore, E.; Maroukli, D.; Strömpl, C.; Pieper, D. H., and Kalogerakis, N. Bacterial community dynamics during* in-situ *bioremediation of petroleum waste sludge in landfarming sites.* ***Biodegradation****. 2005;* ***16(2)****:169-180.*
- *Pelz, O.; Abraham, W.-R.; Saurer, M.; Siegwolf, R., and Zeyer, J. Microbial assimilation of plant-derived carbon in soil traced by isotope analysis.* ***Biology and Fertility of Soils****. 2005;* ***41(3)****:153-162.*
- *Pollmann, K.; Wray, V., and Pieper, D. H. Chloromethylmuconolactones as critical metabolites in the degradation of chloromethylcatechols: On the recalcitrance of 2-chlorotoluene.* ***Journal of Bacteriology****. 2005;* ***187****:2332-2340.*
- *Tillmann, S.; Strömpl, C.; Timmis, K. N., and Abraham, W.-R. Stable isotope probing reveals the dominant role of Burkholderia sp. in aerobic degradation of PCBs.* ***FEMS Microbiol Ecology****. 2005;* ***52(2)****:207-217.*

Sustainable Use of Landscape – 2005 – in press

- *Abraham, W.-R. Controlling Gram-negative pathogenic bacteria by interfering with their biofilm formation.* ***Drug Design Reviews-Online****. 2005.*
- *Abraham, W.-R.; Wenderoth, D. F., and Gläßer, W. Diversity of biphenyl degraders in a chlorobenzene polluted aquifer.* ***Chemosphere****. 2005.*
- *Druschel, G. K.; Labrenz, M.; Thomsen-Ebert, T.; Fowle, D. A., and Banfield, J. F. Geochemical modeling of ZnS in biofilms:An example of ore depositional processes.* ***Economic Geology and the Bulletin of the Society of Economic Geologists****. 2005.*
- *Golyshin, P. N.; Timmis, K. N., and Yakimov, M. M. Family* Alcanivoraceae. *In: Bergey's Manual of Systematic Bacteriology. 3rd ed. New York: Springer; 2005; 2.*
- *Hallworth, J. E.; Prior, B. A.; Nomura, Y., and Timmis, K. N. Compatible solutes protect against chaotrope-(ethanol-) induced, non-osmotic water stress.* ***Applied and Environmental Microbiology****. 2005.*
- *Junca, H. and Pieper, D. H. Diagnosing the biodegradation potential of soils. Trends in soil and sediment. IWA Publishing. 2005.*
- *Kotsyurbenko, O. R. Trophic interactions in the methanogenic microbial community of low-temperature terrestrial ecosystems.* ***FEMS Microbiology and Ecology****. 2005.*
- *Labrenz, M. and Hirsch, P. The Genus* Antarctobacter. *In: Bergey's Manual of Systematic Bacteriology: Bergey's Manual Trust; 2005.*
- *Labrenz, M. and Hirsch, P. The Genus* Roseovarius. *In: Bergey's Manual of Systematic Bacteriology: Bergey's Manual Trust; 2005.*
- *Labrenz, M. and Hirsch, P. The Genus* Staleya. *In: Bergey's Manual of Systematic Bacteriology: Bergey's Manual Trust; 2005.*
- *Martins dos Santos, V.; Heim, S.; Strätz, M.; Moore, E. R. B., and Timmis, K. N.* In silico *analysis of the* Pseudomonas putida *genome: niche specificity.* ***Environmental Microbiology****. 2005.*
- *Martins dos Santos, V. A. P.; Timmis, K. N.; Tümmler, B., and Weinel, C. Genomic features of* Pseudomonas putida *strain KT2440. (Ramos, J. L., ed.) Pseudomonas: Genomics life style and molecular architecture: Kluwer Academic/Plenum Publishers; 2005; 1.*
- *Pauling, B. V. and Wagner-Döbler, I. Stream microcosm for investigating GEM impact on the autochthonous bacterial community in river water and sediment.* ***Journal of Applied Microbiology****. 2005.*
- *Pieper, D. H. Aerobic degradation of polychlorinated biphenyls.* ***Applied Microbiology and Biotechnolology****. 2005.*
- *Simankova, M. V. and Kotsyurbenko, O. R. Genus II.* Aceto-bacterium. *Bergey's Manual of Systematic Bacteriology. 3rd ed. 2005: Springer Verlag; 2005.*
- *Vancanneyt, M.; Segers, P.; Abraham, W.-R., and de Vos, P. Genus* Brevundimonas *Segers, Vancanneyt, Pot, Torck, Hoste Dewettinck, Falsen, Kersters, de Vos 1994, 507VP emend. Abraham, Strömpl, Meyer, Lindholst, Moore, Christ, Vancanneyt, Tindall, Bennasar, Smit, Tesar 1999, 1070VP. (George M. Garrity, editor) In: Bergey's Manual of Systematic Bacteriology. 3rd ed. 2005: Springer Verlag; 2005; 2.*
- *Yakimov, M. M.; Denaro, R.; Genovese, M.; Cappello, S.; D'Auria, G.; Chernikova, T. N.; Timmis, K. N.; Golyshin, P. N., and Giuliano, L. Natural microbial diversity in superficial sediments of Milazzo harbor (Sicily) and community successions during microcosm enrichment with various hydrocarbons.* ***Environmental Microbiology****. 2005.*

Platforms – 2005

- *Pollmann, K.; Wray, V., and Pieper, D. H. Chloromethylmuconolactones as critical metabolites in the degradation of chloromethylcatechols: On the recalcitrance of 2-chlorotoluene.* ***Journal of Bacteriology****. 2005;* ***187****:2332-2340.*
- *Rau, U.; Nguyen, L. A.; Schulz, S.; Wray, V.; Nimtz, M.; Roeper, H.; Koch, H., and Lang, S. Formation and anlysis of mannosylerythritol lipids secreted by* Pseudozyma aphidis. ***Applied Microbiology and Biotechnology****. 2005;* ***66****:551-559.*

Platforms – 2005 – in press

- *Fargali, S.; Barthold, M.; Rohde, M.; Majore, I., and Jäger, V.* In vitro *cultivation of rabbit mesenchymal stromal cells on 3D bioresorbable calcium phosphate scaffolds for the generation of bone tissue implants. (Merten, O. W.; Griffiths J. B.; Godia F., eds.) Animal Cell Technology Meets Genomics: Kluwer Academic Publishers; 2005.*
- *Trost, M.; Wehmhöner, D.; Kärst, U.; Dieterich, G.; Wehland, J., and Jänsch, L. Comparative proteome analysis of secretory proteins from pathogenic and non-pathogenic* Listeria *species.* ***Proteomics****. 2005.*

Biotech Facilities – 2005

- *Kayser, A.; Weber, J.; Hecht, V., and Rinas, U. Metabolic flux analysis of* Escherichia coli *in glucose-limited continuous culture: I. Growth rate dependent metabolic efficiency at steady state.* ***Microbiology****. 2005;* ***151****:693-706.*

- *Weber, J.; Kayser, A., and Rinas, U. Metabolic flux analysis of* Escherichia coli *in glucose-limited continuous culture: II. Dynamic response to famine and feast, activation of the methylglyoxal pathway and oscillatory behavior.* ***Microbiology****. 2005;* ***151****:707-716.*

- *Wendler, J.; Vallejo, L. F.; Rinas, U., and Bilitewski, U. Application of an SPR-based receptor assay for the determination of biologically active recombinant bone morphogenetic protein-2.* ***Analytical and Bioanalytical Chemistry****. 2005;* ***381(5)****:1056-1064.*

Biotech Facilities – 2005 – in press

- *Ahmed Elsayed, A.; Piehl, G.-W.; Medronho, R. A.; Deckwer, W.-D., and Wagner, R. Use of a hydrocyclone as an efficient and easily scalable perfusion system for mammalian cell bioreactors. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers; 2005.*

- *Barthold, M.; Majore, I.; Fargali, S.; Stahl, F.; Schulz, R.; Lose, S.; Mayer, H., and Jäger, V. 3D-cultivation and characterisation of osteogenic cells for the production of highly viable bone tissue implants. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers; 2005.*

- *Bassani Molinas, M. M.; Nelving, A.; Beer, C.; Hesse, F.; Wirth, M.; Durocher, Y.; Kamen, A., and Wagner, R. Intracellular nucleotide pools for optimizing product-oriented transient tranfection of HEK293 cells in suspension. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers; 2005.*

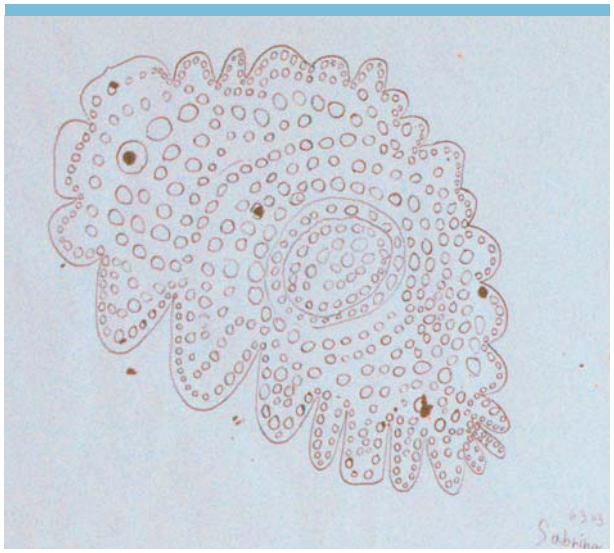
- *Bollati Fogolin, M.; Irani, N.; Beccaria, A. J.; Schulz, C.; van den Heuvel, J.; Elias, C. B.; Carpentier, E.; Durocher, Y.; Bisson, L.; Etcheverrigaray, M.; Kratje, R. B.; Wirth, M.; Kamen, A., and Wagner, R. Impact of the expression of yeast pyruvate carboxylase on the productivity of different animal host cell lines. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers; 2005.*

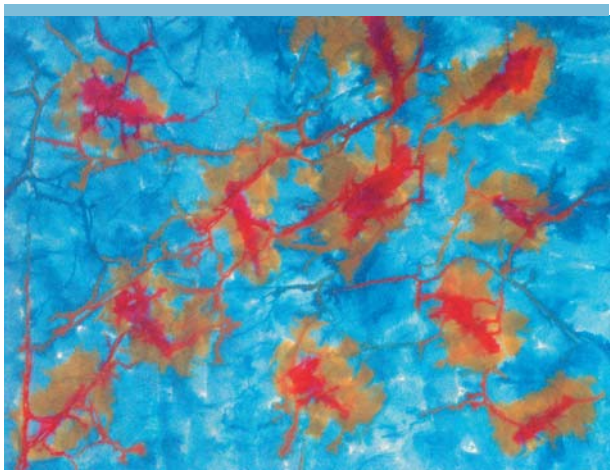
- *Hesse, F.; Nelving, A., and Wagner, R. Correlation of intracellular nucleotide pools to amino acid concentrations in culture media by the application of multivariate methods. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers; 2005.*

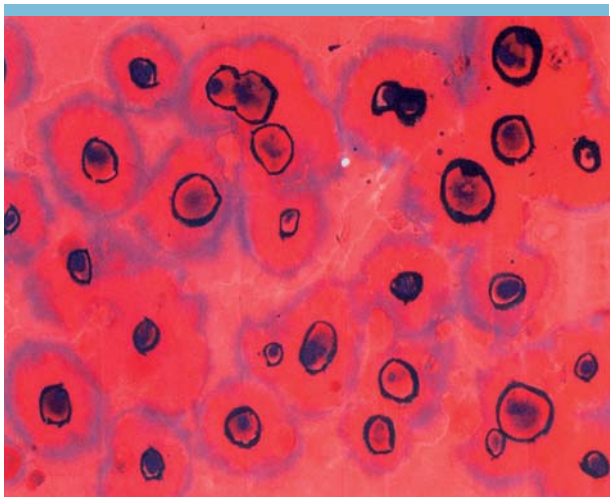
- *Rinas, U.; El-Enshasy, H.; Emmler, M.; Hille, A.; Hempel, D. C., and Horn, H. Model-based prediction of substrate conversion and protein synthesis and excretion in recombinant* Aspergillus niger *biopellets.* ***Chemical Engineering Science****. 2005.*

Microbial structures seen by children

In 2003 children from the "Peter-Räuber-Schule", a school for mentally handicapped children, located in Wolfenbüttel, visited the GBF to perform some experiments and to look at the cellular and microbial world through the microscope. They transferred their impressions into a remarkable series of paintings. These were arranged for an exhibition at the GBF FORUM. The project was supported by the GBF Förderverein. Below 4 selected paintings are shown.

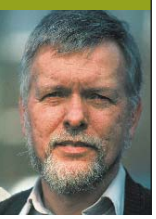






Photos: left: Aerial photo of GBF Campus (2004) | centre: During the scientific seminars of the Biotech Fair organized by Omnilab at the GBF FORUM and BioTec-Gründerzentrum in September 2004 | right: Participants of the Meeting where the first version of the virtual network was presented by Paul Charlton Coaching, InWEnt and GBF. Photos: GBF (le), Omnilab (ce), Dr. Charlton (ri)


German Asean Biotech Network
March 3, 2005
Rajpruek Club



FACTS and FIGURES

Prof. Dr. Rainer Jonas | Department of Scientific Information | rjo@gbf.de

- In 1965 the GBF was founded as "Centre for Molecular Biological Research" (GMBF) with financial support by the Volkswagen Foundation. In 1976 the Federal Government through the Ministry for Research and Technology (BMFT) together with the State of Lower Saxony took over the Centre, now called "German Research Centre for Biotechnology" (GBF). Since then the BMFT/BMBF as well as the State of Lower Saxony jointly finance the GBF. In 2002, the GBF took the decision to focus its research activities towards the understanding of basic mechanisms of infectious diseases.

Research Financing In 2004 the total costs of the GBF amounted to 48.8 Mio. € with more than half, 35.3 Mio. €, devoted to the programme "Infection and Immunity".

External Funding More than 70 % of the external funding came from national research programmes. About 11 % and 15 % were from EU programmes and industry, respectively.

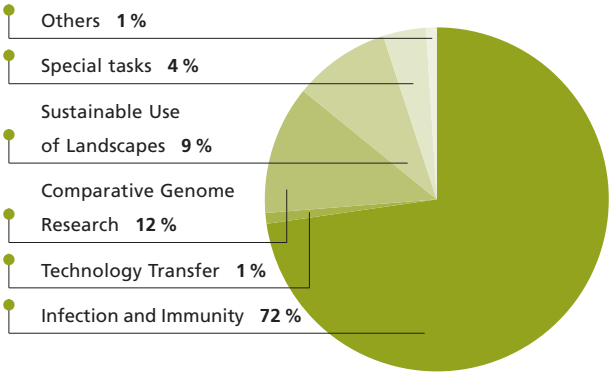
Costs per programme (in T€)

Research Area	Programme	Full Costs
Health	Infection and Immunity	35 253
	Comparative Genome Research	6 021
Earth and Environment	Earth and Environment Sustainable Use of Landscape	4 557
Technology Transfer		507
Special Tasks		1 944
Others		469
Total Sum		**48 751**

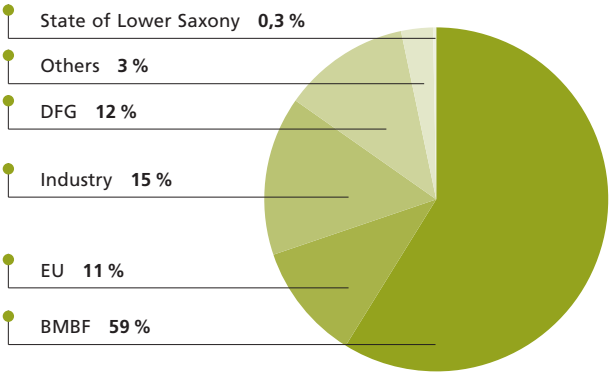
External financing (in T€)

Source	Sum
BMBF	7 656.79
DFG	1 535.62
EU	1 420.23
Industry	1 876.38
State of Lower Saxony	96.38
Others	342.46
Total Sum	**12 867.87**

Full costs 2004



External funding 2004 – by source



Property Rights / Licences In 2004, twelve patents were applied for, six each in Germany and Europe. Nine of these patents were originated in the research area "Health", the others in the research area "Environment".

Patents and property rights, licences, year 2004

	Total number	Germany	
Priority based applications (2004)	12	6	6
Priority based applications, total number	74	58	16
Granted patents (2004)	13	0	13
Total number of held property rights*	138	62	76
Licence agreements (total number)	56	40	16
Licence proceeds ** (in T€)	**732**	**555**	**177**

** This number of patents has been counted differently compared to previous reports. European patents were counted as one patent, and no longer counted for each of the countries.*

*** Including revenues from other "know-how"-transfer agreements*

Publications, Professorships, DFG-Programmes, and Guest Scientists A bibliometric analysis performed by the internationally highly recognized Center for Science and Technology Studies (CWTS), Leyden, The Netherlands, showed that the quality of the GBF-publications is above world average. The GBF has even further increased the impact of their scientific output in recent years. Several articles have been published in highly renowned journals of the Nature-group (for further details see under "Publications" in the section Scientific Reports).
Many GBF scientists are participating in important national and international research programmes.

Participation of GBF scientists in national and international research programmes (main activities)

DFG (German Research Foundation)	
SFB 566	Cytokin-Receptors
SFB 578	From Genome to the Product
SFB 599	Permanent Implantates
SFB 621	Pathobiology of the Intestinal Mucosa
SSP 1087	Selenoproteins
SSP 1089	New Vaccination Strategies
SSP 1150	Signal Pathways to the Cytoskeleton and Bacterial Pathogenicity
SSP 1160	Colonisation and Infection through Human-Pathogen Fungi
FOR 119	Hepatocellular Carcinoma
FOR 471	Cell Differentiation

NGFN II (National Genome Research Network)	
SMP	Mammalian Models
SMP	Protein
SMP	Antibody Factory
SMP	Infection and Inflammation

Genomic (National Microorganism Network)	
Sorangium cellulosum	*Streptococcus pyogenes*
Alcanivorax borkumensis	*Listeria monocytogenes*
Metagenome Research	*Bordetella*

EU 6th Frame Programme	
LSH	Genostem
LSH	Marine Genomics
GCE	BIOTOOL
NMP	BIOMERCURY
Marie Curie EST	MIDITRAIN
LSH	AVIP
LSH	EPI-VECTOR
LSH	FPLFLEX
LSH	EUROPATHOGENOMICS
LSH	MUGEN
Marie Curie RTN	IMDEMI

Graduate Schools	
International PhD Programme	"Infection Biology"
Marie-Curie Graduate School	"MIDITRAIN"
International Graduate School	"Molecular Complexes"
DFG-Graduate School GRK 653	"Pseudomonas"
DFG-Graduate School GRK 705	"Characterization of Patho-Physiological Animal Models"

Quantitative Parameters	Category	2002	2003	2004
Publications	Publications in ISI-listed journals	232	230	185
	Books and publications in other journals	33	25	38
	Total number	265	255	223
	Habilitations	3	0	1
	Dissertations	14	23	33
Calls for professorships	Calls for C3- and C4-professorships at universities	1	4	0
Special DFG-Programmes	Special fields of interest, Transregios	5	5	4
	DFG-Research Focus (SFB)	3	4	4
	Graduiertenkollegs	1	2	3
	Total number	9	11	11
Guest Scientists		107	102	93

Technology Transfer The GBF has a great potential for the development of innovative products, processes and services, especially in cooperation with industrial partners. Therefore, an important goal is to foster the transfer of research results into industrial applications through technology transfer. Thus, the establishment of spin-off and start-up biotech companies, licence agreements as well as service contracts with industrial partners are important elements for the transfer of R&D results. In order to further support technology transfer activities, the GBF is a member of the BioRegioN and the "Transferkolleg Biotechnologie e.V.". Furthermore, the GBF is an active partner in BioRegioN GmbH as well as in "*BioProfil Functional Genome Analysis*".

The GBF Biotech Campus In 2004 the GBF acquired the BioTec-Gründerzentrum from the City of Braunschweig (for more details see under "Highlights" in the section *Focus*). The GBF offers now 3600 sqm of laboratory and office space for spin-off and start-up companies in the Y-building as well as the "BioTec-Gründerzentrum" (list of the firms, see Table).

Intellectual Property Since 2002 the *Ascenion Ltd. Co.* offers services principally for the four Helmholtz Research Centres in the area of health care: GSF, GBF, MDC, and DKFZ. The headquarters are in Munich, but an office with two employees is working on the GBF-Campus.

Ascenion Ltd. Co. principally manages the following areas for the GBF:

- Acquisition and management of intellectual property
- Evaluation of the commercial potential of an invention before patent filing
- Development and employment of strategies for the exploitation of the GBF patent portfolio

Biotech Fair on the GBF-Campus For the 3rd time OMNILAB organized a small biotech-fair and symposium in the FORUM as well as the "BioTec-Gründerzentrum", which took place on 23 September 2004. About 70 enterprises presented their products and more than 600 visitors from the region Braunschweig – Hannover – Magdeburg attended the fair. Fifteen lecturers gave highly interesting seminars on new R&D items.

- *During the exhibition of the Biotech Fair organized by Omnilab at the GBF FORUM and BioTec Gründerzentrum in September 2004.*

Photo: OMNILAB

Biotech Network Southeast Asian Region – Germany The GBF together with InWEnt and Paul Charlton Coaching started to establish a virtual network in biotechnology for the Southeast-Asian region with links to Germany. For this purpose they elaborated, together with ex-participants from the InWEnt-courses, scientists from enterprises and R&D institutions, the basic programme during workshops in Singapore and Bangkok in October 2004. The aim is to get better scientific communications between the interested groups, looking for collaboration and joint organisation of symposia within Southeast Asia, but also together with German partners.

- *The participants of the Workshop on Biotechnology Networking between Southeast Asia and Germany, organized by InWEnt and GBF, financed by the Ministry of Economic Affairs of the State of Lower Saxony.*

Photo: Dr. Charlton

List of the firms on the GBF Biotech Campus, Status: 31.03.2005

Company	Contact person	Telephone/Fax	E-Mail Address	Homepage
Ascenion	Dr. Sabina Heim/ Tina Damm	0531-6181-961/-962; Fax: -963	she@ascenion.de tda@ascenion.de	www.ascenion.de
AIMS Scientific Products GmbH	Dr. Norbert Zander	0531-260-2865; 0177-7637299; Fax: 260-2866	nza@aims-scientific-products.de	www.aims-sci.de
AMODIA Biosciences GmbH	Dr. Sabine Peters/ Dr. Ulrich Krause/ Frank Schwieger	0531-260-1764; Fax: -1766	info@amodia.de	www.amodia.com
Cosmix molecular biologicals GmbH	Dr. Thomas Wagner/ Ute Heidrich (Secretary)	0531-12086-0; Fax: -99	info@cosmix.de	www.cosmix.de
Eugene GbR	Dr. Werner Müller	0531-6181-687	wmu@gbf.de	
Research Group Wound Healing of the TU Braunschweig	Prof. Dr. Peter Mühlradt	0531-1217-954; Fax: 0531-1217-958		
Glyco Thera GbR	Dr. Harald Conradt	0531-7996785/-6181-287	hco@gbf.de	www.glycothera.de
Hartmann Analytic GmbH	Dr. Ursula Hartmann	0531-26028-0; Fax: -28	hartmann@hartmann-analytic.de	www.hartmann-analytic.de
IBA Biologics GmbH	Dr. J. Bertram/ Dr. Bernd Müller	0551-50672118; 0531-6181-170		
Lionex GmbH	Dr. Ralf Spallek/ Dr. Eva Gebhardt-Singh	0531-260-12-66; Fax: -260-11-59	msi@lionex.de	www.lionex.de
RELIATech GmbH	Dr. Bernhard Barleon	0531-260-1831; Fax: -1833	info@reliatech.de	www.reliatech.de
Vakzine-Management GmbH	Dr. Albrecht Läufer/ Ingeborg Jakobi (Secr.)	0531-28504-0: Fax: -29	jacobi@vakzine-manager.de	www.vakzine-manager.de
BIOS- Biotechnologisches Schülerlabor	Dr. Iris Eisenbeiser/ Arntraud Meyer	0531-6181-945; Fax: -949	Bios.lab@gbf.de	

Personnel At the end of 2004, the GBF staff comprised 608 persons with full time and part time occupation. Additionally, 86 guests worked in various projects, receiving their payment from third parties. In total, 241 scientists were working at the GBF, including 74 postdocs and 79 PhD-students.

Boards and Assemblies of the GBF The boards and assemblies of the GBF are the Board of Trustees, the Supervisory Board, the Scientific Committee and the Managing Directors.

Board of Trustees The Board of Trustees is formed by the two trustees of the GBF, the Federal Republic of Germany and the State of Lower Saxony, represented by their respective departments, the Federal Ministry of Education and Research (BMBF) and the Lower Saxony Finance Ministry.

Supervisory Board The Supervisory Board oversees the legality, expedience and economy of the management. It decides on general research goals, the principal research policy and financial affairs of the centre. It consists of a maximum of 15 members..

Scientific Advisory Committee The Scientific Advisory Committee consists of members of the Supervisory Board and external scientific experts. It advises the Supervisory Boards with regard to the R&D programme as well as general research strategy of the GBF.

Members of the Supervisory Board (SB) and the Scientific Advisory Committee (SC), Status: 31.3.2005

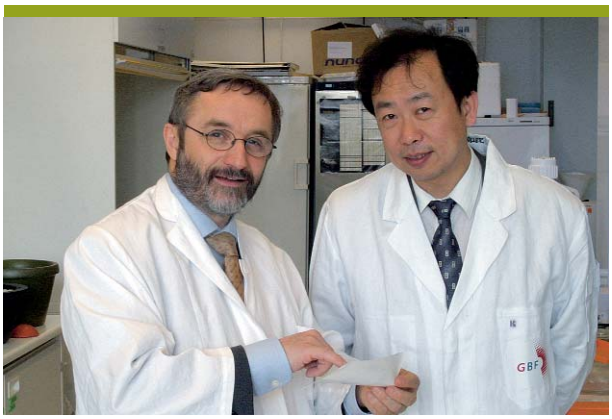
Function	Name, Title	Organisation	Locality
Chairman SB	Lange, MinDirig Dr. Peter	BMBF	Berlin
Vice-Chairman SB	Weise, MinDirig Dr. Dr. Christian	NMWK	Hannover
SB	Warmuth, MR Dr. Ekkehard	BMBF	Berlin
SB	Kuhny, Reg. Direktorin Corinna	Ministry of Finance State of Lower Saxony	Hannover
SB	Bilitewski, Prof. Dr. Ursula	GBF	Braunschweig
SB	Weiß, Dr. Siegfried	GBF	Braunschweig
SB + SC	Schiebler, Dr. Werner	Novartis Pharma AG	Basel/Schweiz
SB + SC	Bitter-Suermann, Prof. Dr. Dieter	MHH	Hannover
SB + SC	Müller-Goymann, Univ.-Prof. Dr. Christel	Technical University	Braunschweig
SB + SC Vice-Chairman SC	Jäckle, Prof. Dr. Herbert	MPI	Göttingen
SB + SC	Kurth, Dr. Bärbel-Maria	Robert-Koch-Institute	Berlin
SB + SC	Daniel, Prof. Dr. Hannelore	Wissenschaftszentrum Weihenstephan	Freising
SB + SC Chairman SC	Pfeffer, Prof. Dr. med. Klaus	Universitätsklinikum	Düsseldorf
SC	Hackler, Prof. Dr. Jörg	University	Würzburg
SC	Winterfeldt, Prof. Dr. Ekkehard	University	Hannover
SC	Apweiler, Dr. Rolf	EBI	Cambridge/MK
SC	Schendel, Prof. Dr. Dolores	GSF	München
SC	Birchmeier, Prof. Dr. Walter	MDC	Berlin-Buch
SC	Mann, Prof. Dr. Matthias	Protein Interaction Laboratory	Odense/Danmark
SC	Hämmerling, Prof. Dr. Günter	DKFZ	Heidelberg

Managing Directors The Managing Directors of the GBF:

- Research: Prof. Dr. Rudi Balling
- Administration: Dr. Georg Frischmann

- *Prof. Dr. Rudi Balling (le), Dr. Georg Frischmann (ri)*

Photo: Bierstedt



- *Prof. Dr. Rudi Balling together with Dr. Hong He, head of the Helmholtz Office in Beijing, China, during a visit at the GBF laboratories.*

Photo: Gazlig

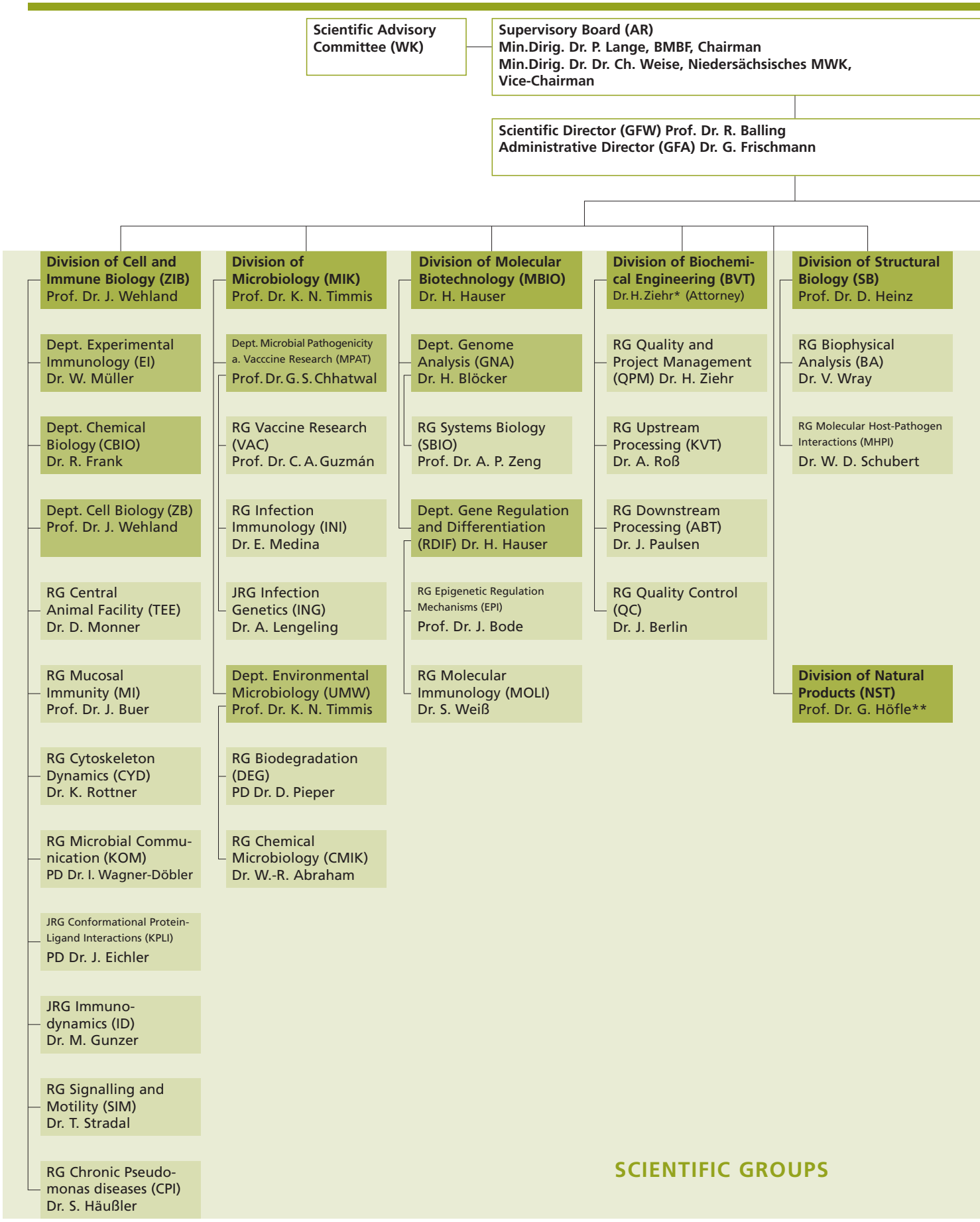
Scientists Assembly The scientists assembly of the GBF advises the Management in scientific matters. It consists of 22 elected scientists. The Managing Directors, the heads of the sections and junior research groups as well as a representative of the PhD-students are guests of the assembly. Chairman is Dr. Wolf-Rainer Abraham (since May 2003). Vice-chairman is Dr. Siegfried Weiß.

Direktorium The "Direktorium" advises the Managing Directors of the GBF in all important questions of the Centre. Members are the Managing Directors, the heads of the divisions, a representative of the junior research groups and the chairman of the Scientists Assembly.

Staff Council The Staff Council has certain consultation and co-determination rights in personnel and social questions. It consists of 11 members, elected by the GBF staff. Chairman is John Aubert.

Equal Opportunities Officer is Evelyn Rohn-Stenzel.

Chart of Organisation, Status 01 May 2005



Board of Scientific Directors (DR)

Staff Council (BR)
Chairman J. Aubert

Assembly of Scientists (WV)
Dr. W.-R. Abraham

Equal Opportunities Officer (GIB) E. Rohn-Stenzel

Scientific and Technical Services (WTD)
PD Dr. K. Schughart

- Scientific Information (WI) Prof. Dr. R. Jonas
- Library (BIB) A. Plähn
- Computer Centre (RZ) Dr. N. Bedorf
- Safety and Environmental Affairs (SU) Dr. E. Grund

Administration and Infrastrukture (VIN)
Dr. G. Frischmann

- Personnel Affairs (PA) M. Kaczmarek (Attorney)
- Financial Affairs (FA) G. D. Beutin
- Purchasing and Material Management (EM) B. J. Scherer
- Legal Affairs and Licenses (JUR) Dr. C. Kügler-Walkemeyer
- Patents (PS) D. Meseke
- Technical Services (TB) O. Rabe

Staff units

- Public Relations (ÖA) H. Schlender
- Controlling (CO) PD Dr. K. Schughart (PWC) U. Richter (BCO) Dr. M. Strätz (DMC)
- Organisation/SAP (ORG) H. Ohrdorf
- Internal Auditing (IR) R. Lomberg

Abbreviations:

Dept.	*Department*
RG	*Research Group*
JRG	*Junior Research Group*
PWC	*Scientific Controlling*
BCO	*Financial Controlling*
DMC	*External Funding Controlling*

* *temporarily acting*

** *this division was not continued after the retirement of Prof. Dr. G. Höfle in February 2005*

Annual Report 2004/2005

Published by :
German Research Centre for Biotechnology Ltd. Co. – GBF
Mascheroder Weg 1
D-38124 Braunschweig, Germany
Telephone (+49) 5 31/61 81-0
Telefax (+49) 5 31/61 81-515
info@gbf.de | www.gbf.de

Member of Helmholtz Association of
National Research Centres

Editors-in-Chief :
Prof. Dr. Rainer Jonas (V.i.S.d.P.) | rjo@gbf.de
Priv.-Doz. Dr. Klaus Schughart | kls@gbf.de
Dipl.-Journ. Dipl.-Biol. Hannes Schlender | has@gbf.de
Dipl.-Biol. Journalist Manfred Braun | mbn@gbf.de

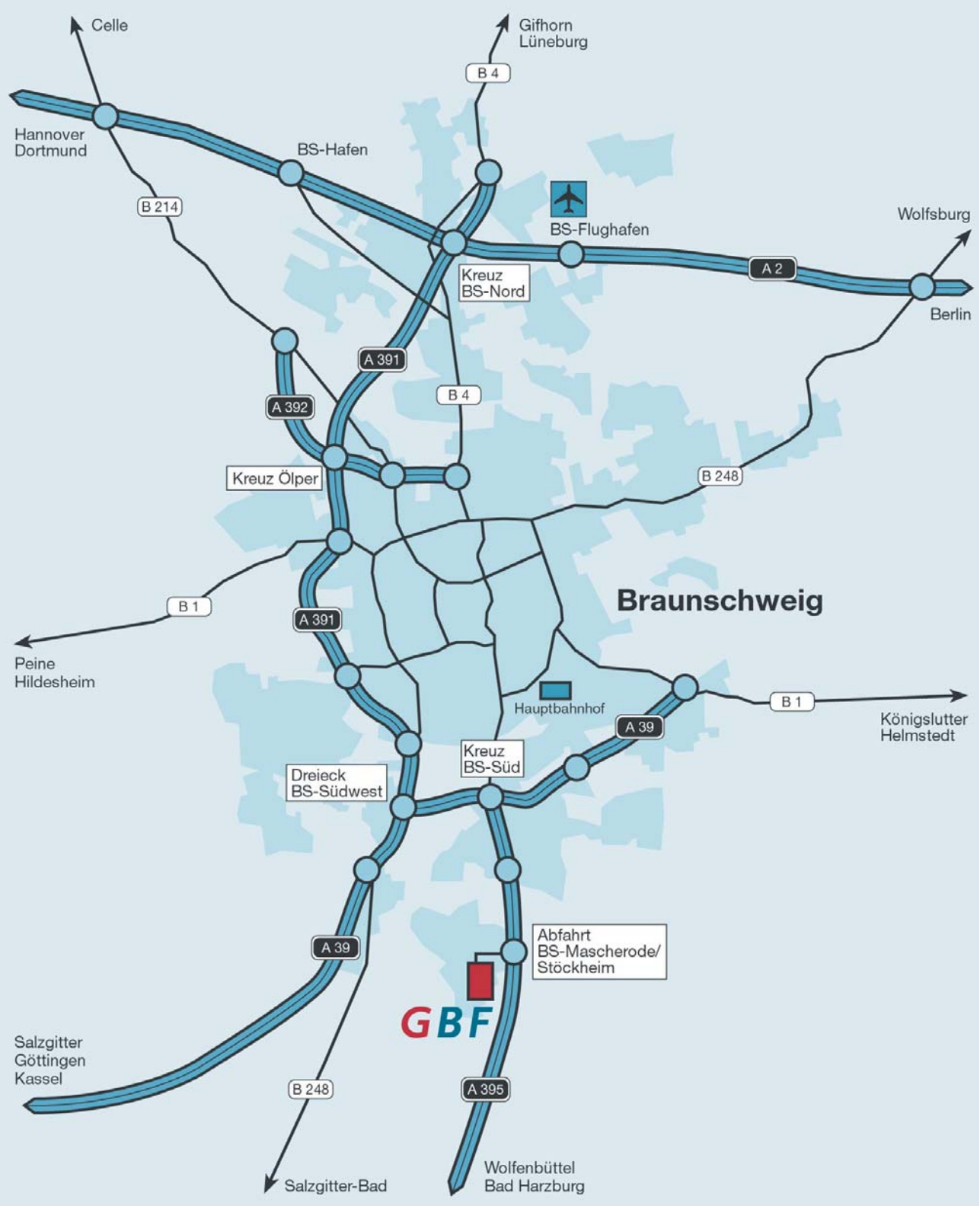
Editoral Assistance: Monica Kirchner, Sylvana Weidig

Text Editing and Realisation: Dr. Jo Schilling

Photographs:
The portraits were taken by
Thomas Ammerpohl: Pages 2, 54, 59, 61, 65, 73
Frank Bierstedt: Pages 3, 5, 22, 31, 40, 42, 44, 45, 46, 47, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 60, 62, 63, 66, 67, 68, 69, 70, 71, 72, 74, 75, 76, 77, 78, 79, 80, 81, 82, 85, 86, 88, 116, 117
Manfred Braun: Page 87
Susanne Hübner: Page 83
Dirk Hans: Page 52
For all other photographs, the photographers are mentioned below the legends.

Layout and design:
wir design GmbH, Braunschweig, Berlin
welcome@wir-design.de | www.wir-design.de

Printed by:
Döring Druck, Druckerei und Verlag GmbH,
Braunschweig
Printed on acid-free and chlorine-free paper
ISSN 0935-0497



- *Site plan of the GBF*

ISSN 0935-0497
German Research Centre for Biotechnology Ltd. Co.
Mascheroder Weg 1 | 38124 Braunschweig | Germany
http://www.gbf.de | e-mail: info@gbf.de