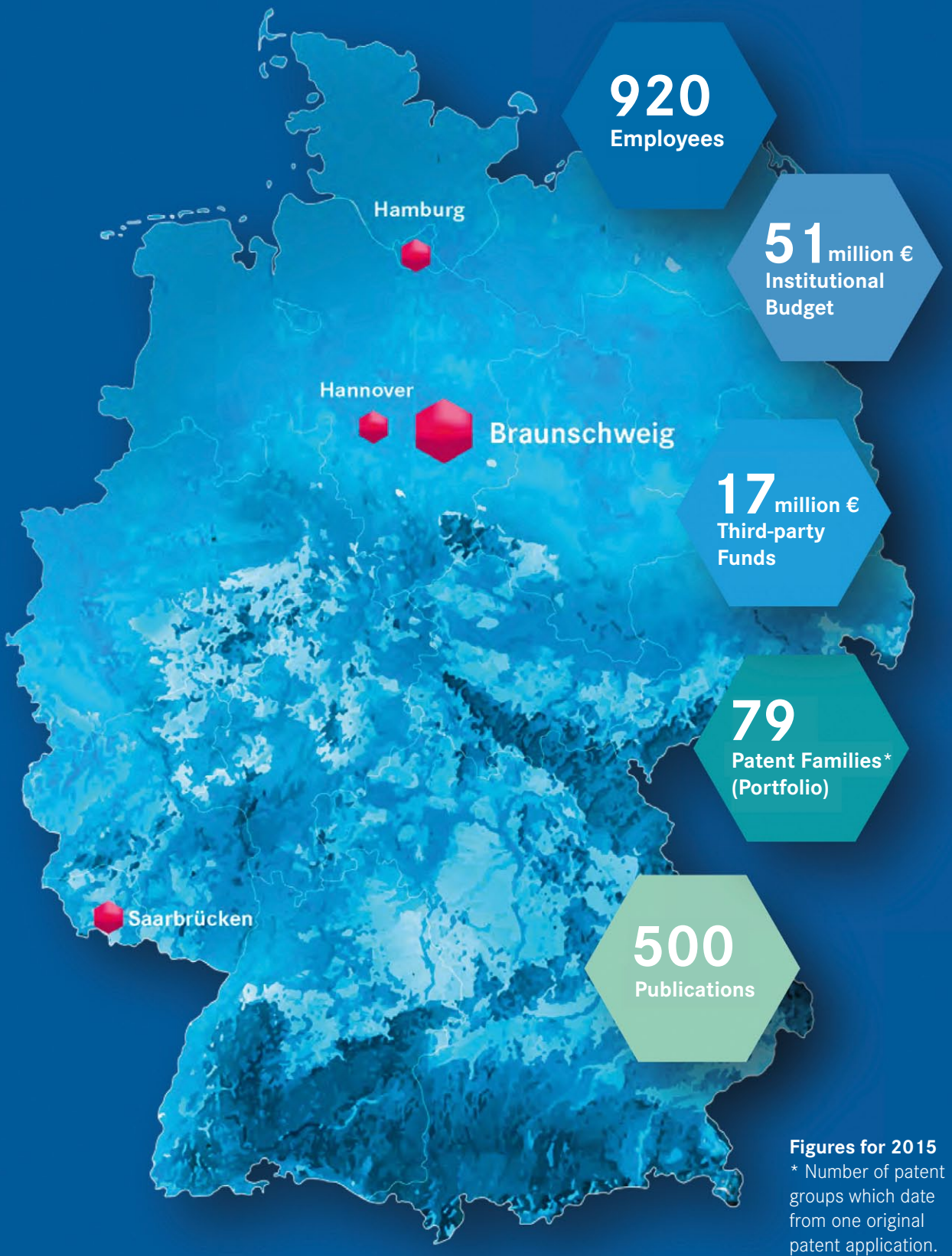
HELMHOLTZ
CENTRE FOR
INFECTION RESEARCH

RESEARCH REPORT

HZI 2014 2015

THE HELMHOLTZ CENTRE FOR INFECTION RESEARCH (HZI) AT A GLANCE



920
Employees

51 million €
Institutional Budget

17 million €
Third-party Funds

79
Patent Families* (Portfolio)

500
Publications

Figures for 2015
* Number of patent groups which date from one original patent application.

HZI Campus

TWINCORE

HIPS

CSSB

LOCATIONS

HZI Campus Braunschweig

- Headquarters of the HZI
- Central administration
- Research infrastructure
- Basic research on bacterial and viral infections, pathogen-host interactions, anti-infective agents, epidemiology
- Cooperation with the Technische Universität (TU) Braunschweig, in particular: cooperation within the Braunschweig Integrated Centre for Systems Biology (BRICS)

Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken

- Founded jointly by the HZI and Saarland University (UdS)
- Research into natural compounds, optimisation for pharmaceutical application
- Bridge between basic research and pharmaceutical industry

TWINCORE, Hannover

- Founded jointly by the HZI and Hannover Medical School (MHH)
- Translation, joint research projects of medical professionals and natural scientists
- Experimental and clinical infection research
- Bridge between basic research and clinical practice

Centre for Structural Systems Biology (CSSB), Hamburg

- Located on the campus of DESY (Deutsches Elektronensynchrotron)
- Jointly operated by several north German research establishments
- Elucidation of molecular processes in infections using highly specialised photon sources

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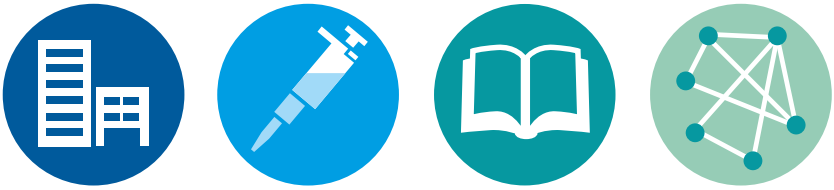


HIGHLIGHT PUBLICATIONS

PARTNERS AND NETWORKS

DEAR READER,

Prof. Dirk Heinz and Franziska Broer

In a field as dynamic as infection research, current needs have to be identified early on in order to react appropriately. To remain state-of-the-art in science and technology, a research institute needs to continually re-evaluate its activities and redefine them where necessary.

The HZI has already dealt with several changes in the past, taking new research directions and different names. A look at the eventful history of the centre and its predecessor institutions shows this very impressively. In the autumn of 2015, our 50th anniversary gave us the chance to pause for a moment and ponder this history and the repeated thematic realignments from which our centre has always emerged successfully. On the following pages, you will learn how we celebrated this anniversary and what interesting facets we discovered about the early years of the institute.

While remaining mindful of the past, in 2015 we also looked far ahead into the future. In our new strategic roadmap “HZI 2025”, completed in time for the anniversary, we point the way for the medium-term strategic and thematic development of the centre. This clear positioning will allow us to respond adeptly to the challenges that infectious diseases may present to society in the future. Comprehensive information about the roadmap and its strategic concepts is included in this Research Report.

Of course, we also give detailed accounts of our latest scientific highlights: recent developments in core research areas of the HZI, outstanding publications from our scientists, awards, partnerships and events that have left their mark over the past two years.

We are delighted about your continuing interest in our work and hope you will enjoy reading this report.

Prof. Dirk Heinz
Scientific Director

Franziska Broer
Administrative Director

ABOUT THE HZI

Portrait of the Research Centre

Around 950 employees at the Helmholtz Centre for Infection Research (HZI) share a common, overarching goal: to understand infections in order to better combat pathogens.

Infectious diseases still present a global threat to human health and are the cause of one fifth of all deaths worldwide. While the industrialised countries overcame many of these diseases during the 20th century with vaccinations, improved hygiene and antibiotics, new threats are already alarming health authorities. The rapid spread of newly emerging and resurgent pathogens, chronic infections, increasing resistance to antibiotics, as well as demographic and climate change are lending new urgency to the topic "infectious diseases".

In line with the mission of the Helmholtz Association to contribute to solving grand challenges facing society, science and industry, the HZI is investigating the basic principles underlying infection processes. It employs latest generation technologies and is developing new approaches to prevention, diagnosis and therapy of infectious diseases.

In addition to the main campus in Braunschweig, the HZI has several branches including the translation centre TWINCORE in Hannover, which is operated jointly with Hannover Medical School (MHH), and the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) in Saarbrücken. In Hamburg the HZI has a key role in the Centre for Structural Systems Biology (CSSB), a joint initiative of nine North German research partners. Soon the HZI will also be more strongly represented on the campus of the Technische Universität Braunschweig (TU-BS) by the new Braunschweig Integrated Centre of Systems Biology (BRICS), a research institution founded jointly by TU-BS and the HZI.

At these various locations, scientists from more than 40 countries are researching bacterial and viral pathogens of high clinical relevance. They are analysing both the infection strategies of pathogens and the defence mechanisms of host organisms, and identifying potential target structures for novel therapeutic interventions. Natural compounds are utilized as a unique source of novel anti-infectives. This requires collaboration across multiple scientific disciplines. Experts from life sciences, computer sciences, chemistry, pharmacy and medicine are therefore working closely together at the HZI and its partner institutes. The portfolio of their research activities spans all levels of an integrative approach, encompassing basic research, drug research and clinically related research. Researchers are investigating molecules and their interactions, genomes, cells and organisms, elucidating mechanisms of pathogen invasion and immune defences and mathematically modelling infection processes. Further activities include developing clinically oriented research projects, contributing to pre-clinical and clinical trials and researching epidemiological correlations in special patient and population cohorts.

Characteristic of the HZI, alongside its interdisciplinarity and integrative research approach, is its participation in excellent partnerships and networks. The HZI plays a prominent role in the German Centre for Infection Research (DZIF) - in science as well as administration. Along with seven other centres, it belongs to the research field “Health” within the Helmholtz Association. The Helmholtz centres involved in this field are investigating the causes and onset of major common diseases. They deploy synergies in expertise and infrastructure to conduct comprehensive research projects and thereby develop novel strategies for prevention, early diagnosis and effective therapies for the benefit of patients.

With the most important clinical cooperation partners, MHH and Otto von Guericke University Magdeburg (OVGU), the HZI is establishing close ties between basic research and clinical practice. Similarly intensive scientific cooperation exists with TU-BS, Saarland University (UdS), Leibniz University Hannover (LUH), University of Veterinary Medicine Hannover (TiHo), the Leibniz Institute German Collection of Microorganisms and Cell Cultures (DSMZ) and the Robert Koch Institute (RKI). This high-capacity partner network, in combination with the continuity and infrastructure of a publicly funded research centre, allows the HZI to bridge innovation gaps and pursue long-term research projects. The overall guiding principle is translation, the transferral of insights from basic research into clinical and industrial applications.

In the future the HZI will focus on particularly innovative fields in infection research, on the development of novel therapeutic interventions against infections and novel approaches towards individualised infection medicine.

The HZI’s research will thus help to keep pace with the rapidly evolving pathogens and establish the basis for reducing the burden of infectious diseases.

THE HELMHOLTZ PROGRAMME "INFECTION RESEARCH"

Prof. Dirk Heinz, Scientific Director and Programme Speaker

The HZI is a centre for innovation-driven infection research with a strong focus on translation. Its explicit aim is to contribute significantly towards overcoming the present and future challenges posed by infections.

In this spirit, scientists of the HZI and its partners have devised the internationally competitive Helmholtz programme "Infection Research". This programme defines the direction for the future development of the centre and its research priorities. Its objectives also meet major requirements in the field of health research as defined by the German government and the European Union.

The programme consists of the three topics "Bacterial and Viral Pathogens", "Immune Response and Interventions" and "Anti-Infectives".

Topic 1 "Bacterial and Viral Pathogens", spokesperson: Prof. Thomas Pietschmann

In collaboration with clinical partners, Topic 1 scientists investigate infections caused by bacteria and viruses. Their aims include understanding the molecular bases of virulence and resistance as well as determining risk factors for the spread of diseases, among them infectivity of and susceptibility to pathogens. They are also striving to identify new targets for antimicrobial therapies.

Topic 2: "Immune Response and Interventions", spokesperson: Prof. Carlos A. Guzmán

The focus of Topic 2 is on the host response to infections. Central themes are innate and adaptive immune responses, the transmission of pathogens and their elimination by the host's immune defences. Topic 2 scientists are furthermore studying mechanisms that allow pathogens to bypass the host's immune system or that even promote their further spread with the involvement of host factors. The insights gained will serve as the basis for new immune-focused strategies for diagnosis (biomarkers), prevention (vaccination) and treatment of infectious diseases.

From molecules to populations: The HZI is investigating infections at all levels of resolution. Molecular biology, structural biology, cell biology, animal models and clinically oriented research play a key role in this process; systems biologists and IT specialists model infection processes at all levels.

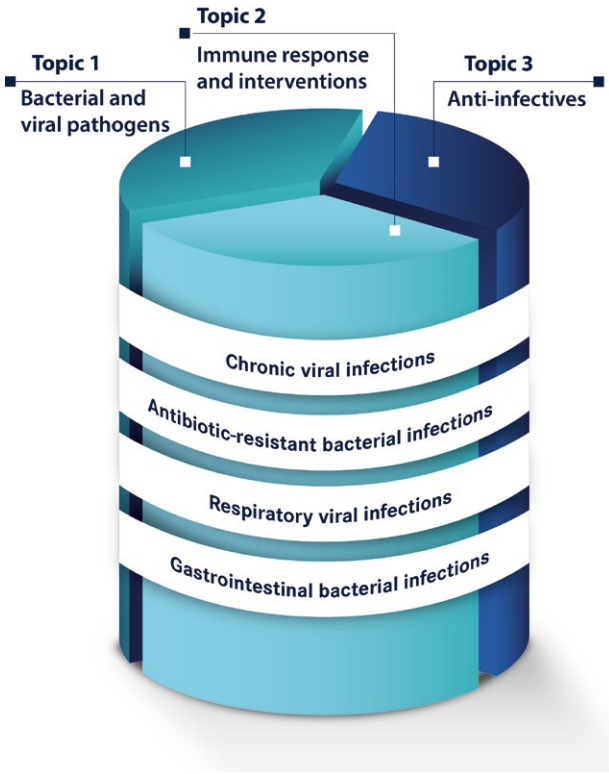
Topic 3: “Anti-Infectives”, spokesperson: Prof. Rolf Müller

In Topic 3, researchers are dedicated to discovering and developing new anti-infectives. Innovative screening methods, microbial genome mining and synthetic biotechnology make it possible to identify new natural compounds with antibacterial or antiviral properties. Working in close cooperation with partners in industry, the researchers study promising substances that intervene in the adhesion, invasion or communication of pathogens and optimise these compounds chemically and pharmaceutically. By means of medicinal chemistry and the development of suitable formulations for optimising drug delivery, the anti-infectives are prepared for preclinical and clinical trials.

Modern platforms and infrastructures support the research in each topic. These include sophisticated technologies for genome and proteome analysis, structural biology and imaging methods, animal experiment facilities and natural compound libraries.

Across all three topics, research activities are concentrated on selected clinically relevant pathogens (“focus pathogens”), which present particularly promising research subjects for the HZI and its partners. Focal themes are chronic viral infections, especially with hepatitis and herpes viruses, infections with antibiotic-resistant bacterial pathogens such as *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus*, gastrointestinal bacterial infections with Enterobacteriaceae and *Clostridium difficile* as well as respiratory viral infections, e.g. with influenza viruses and respiratory syncytial viruses (RSV).

Research foci are chosen depending on the clinical relevance of the underlying pathogens, existing expertise at the HZI and its partners (in particular MHH and DZIF), scientific excellence and optimal positioning within the German infection research community.



Three topics and various cross-topic activities: The HZI research programme.

Bacterial focus pathogens

Pathogen	Widespread or clinically relevant resistances
Staphylococcus aureus	Methicillin (MRSA)
Klebsiella pneumoniae	Carbapenem (metallo-beta-lactamase producers / MBL)
Pseudomonas aeruginosa	Constitutionally resistant to several penicillins and cephalosporins
Enterobacteriaceae	Extended-spectrum beta-lactamase producers (ESBL), resistant to penicillins and cephalosporins
Clostridium difficile	Erythromycin, clindamycin and others

Viral focus pathogens

Pathogen	Particular challenge
Hepatitis viruses	Acute and chronic symptoms, chronic persistence, triggering of further complications
Herpes viruses e.g. cytomegalovirus (CMV)	Widespread, chronic persistence, triggering of further complications, reactivation after immune suppression
Respiratory infection pathogens e.g. influenza viruses, human respiratory syncytial virus (RSV)	High pandemic potential, high variability, co-infections

The research programme is set up to promote intensive collaboration between all disciplines involved and thereby guarantee that results from excellent basic research are translated into practice as efficiently as possible. In the long term – as the aim of the programme – a profound understanding of the molecular and cellular mechanisms of infection processes will provide a basis for innovations in application. Novel antibiotics, biomarkers and other diagnostics, vaccines, immunotherapeutics and RNA-based agents discovered or optimised by the HZI and its partners will fill empty "development pipelines" and be made available for clinical applications.

The work of the HZI will provide a benefit to society extending beyond its research results and therapeutic approaches. As a driving force for infection research and as a mediator between basic research and clinical as well as industrial application, the centre is shaping the German research landscape and helping to secure its innovative power. As part of its research strategy, the HZI is developing new forms of cooperation with clinics and industry, stimulating research on anti-infectives and, together with its partners, training the next generation of outstanding infection researchers. Its research culture will set trends in and beyond the Hannover/Braunschweig area and will promote interdisciplinary and creative approaches in infection research and translation.

IN AND AROUND THE HZI

50 YEARS OF EXCELLENCE

From Molecular Biology via Biotechnology to Infection Research

Panel discussion on perspectives of the HZI and infection research in general with Prof. Otmar D. Wiestler, President of the Helmholtz Association, the moderator Lilo Berg, Franziska Broer, Administrative Director of the HZI, and Dr. Gabriele Heinen-Kljajić, Minister for Science and Culture in Lower Saxony (from left to right).

Retrospectives on the history of the centre were discussed by a panel consisting of former HZI employees Prof. Gerhard Höfle, Prof. Rudi Balling, Université du Luxembourg, and Prof. Jan Buer, Essen University Hospital, from left to right. The moderator was Lilo Berg (second from right).

2015 was a special year for the HZI – the centre on the research campus in the south of Braunschweig turned 50. Founded in 1965 as the Institute for Molecular Biology, Biochemistry and Biophysics (IMB), the establishment experienced exciting times: it changed its name three times and shifted its research focus twice.

In 1969, with substantial financial start-up aid by the foundation Stiftung Volkswagenwerk, the IMB became the Centre for Molecular Biological Research (GMBF). The institution was renamed the German Research Centre for Biotechnology (GBF) in 1975 and joined the Association of National Research Centers, which later became the Helmholtz Association. In the 1980s and 1990s, the GBF was a global reference centre for white biotechnology, i. e. the use of biotechnological methods to make industrial products. Since 2006, it has focused on infection research under its present name and has become an internationally renowned research institution in this field. The HZI celebrated its anniversary twice. A jubilee staff party in September 2015 with the traditional Helmholtz Mile relay, barbecue, quiz and music gave all employees the opportunity to come together. On October 6 guests from politics and partner institutions visited the official jubilee event, where panel discussions and historical reviews reflected past, present and future of the HZI and its research activities.

During the autumn of 2015, the public lectures series “Infection research over the course of time” took visitors on a journey from the beginnings of the scientific investigation of pathogens and immunity to today’s modern approaches.

HELMHOLTZ CENTRE FOR INFECTION RESEARCH
50 YEARS 1965 – 2015

Prof. Otmar D. Wiestler, President of the Helmholtz Association, welcomed the guests to the jubilee event.

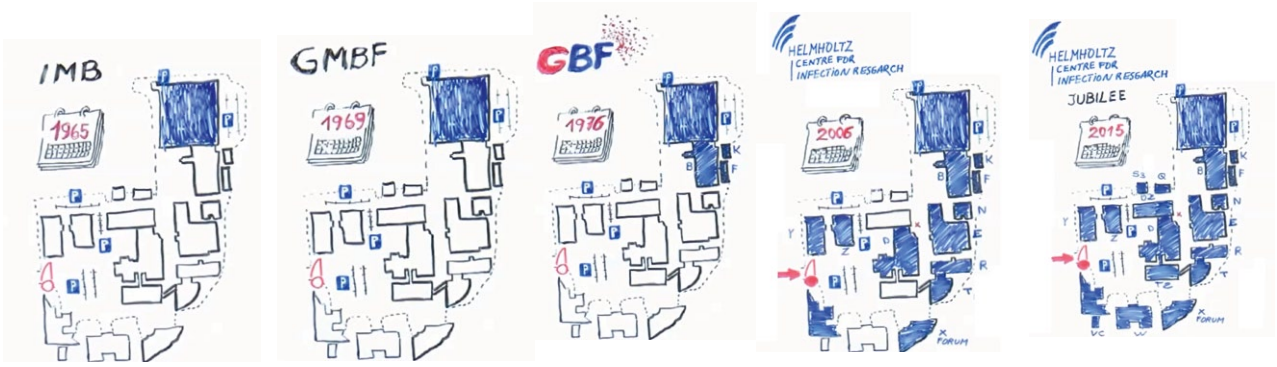
Employees of the HZI formed the institute's name in front of the administration building.

Prof. Lothar Jänsch was elected "Supervisor of the Year". He received the prize from Franziska Broer, Administrative Director of the HZI.

The winners of the "Helmholtz Mile" relay race: The runners of the team "Twincore I".

The award for the second place in the Helmholtz Mile went to the team "This is Sparta!". Scientific Director Prof. Dirk Heinz, right, presented the prizes to one of the team members.

HZI employees enjoyed the barbecue and a rich programme with quiz, live music and dance.



GROWING CAMPUS

Over the last 50 years, the campus of what today is the HZI has constantly evolved. Since 1965, several buildings have been constructed to support research on-site. Two animal facilities were built in 1997 and 2007, the newer building being one of the most modern and largest in Europe. Since 2013, a laboratory conforming to the S3 safety standard allows researchers to investigate pathogens that may cause serious diseases in humans. In addition, further laboratories and an administration building have been erected. In 2015, around 750 HZI employees worked on the campus that also houses the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures and one division of the Fraunhofer Institute for Toxicology and Experimental Medicine

(Marcia Duarte, PhD student at the HZI, drew this sketch for a presentation during the jubilee party).

For 50 years the research campus Braunschweig-Stöckheim has been involved in cutting-edge research in Braunschweig. As the town and the HZI belong together, the centre took the “blue 50” on a tour through Braunschweig.

1 Aerial image of the building of the photographic corporation Gevaert. In 1965, these premises were turned into a research institute, the "Institute of Molecular Biology, Biochemistry and Biophysics" (IMB). Picture: VolkswagenStiftung/Hans Steffens

2 Prof. Hans Herloff Inhoffen, founder and first Scientific Director of the IMB. Picture: Private/Joachim Klein

3 Transition to large-scale fermentation in biotechnology at the beginning of the 1970s. Picture: GMBF

4 Minister Hans Matthöfer visits the German Research Centre for Biotechnology (GBF). Picture: Private/Helmut Zeitträger

5 By 1984, the campus of the centre, which was named German Research Centre for Biotechnology (GBF) at the time, had started to grow.

6 Protein design on the computer.

7 The Human Genome Project: Gene sequencing at the GBF.

8 The development of the centre from its beginnings in 1965 until 2015 is told in the jubilee book "A history in stories": Interviews with former directors, researchers and politicians provide a good understanding of the exciting times the institute went through.

LOOKING BACK AND LOOKING AHEAD

Prof. Dirk Heinz (Scientific Director) and Franziska Broer (Administrative Director) about the History and Future Perspectives of the HZI

ANNIVERSARY YEAR 2015

Mrs. Broer, the 50th anniversary of the centre was a major event in 2015. How do you feel looking back on the centre's half a century of history?

Broer: There are two events that stand out for me, which have impressed me. One was the staff party, where the atmosphere was really fantastic. I had the impression that everyone enjoyed it and it was exactly the right type of event for this occasion. The other one was the ceremonial act with guests from politics and partner organisations. It offered a proper combination of retrospectives on history and glimpses into the future.

Professor Heinz, you have been at the centre for several years. Was there still anything new that you learnt from the retrospectives during the anniversary celebrations? Anything that surprised you?

Heinz: I had known, of course, that the centre had focused on different topics over its 50-year history and that there were several drastic realignments. But I was still surprised to learn how critical the situation had been at times – where the very existence of the centre was in question. In the seventies and eighties there was a lively debate on which location was most suitable as a hub for biotechnology research in Germany. The commitment to the GBF gave the centre a tremendous boost at the time. A second exciting phase then came at the end of the nineties.

STRATEGIC ALIGNMENT OF THE HZI

What were the outstanding events in the recent years from your point of view?

Heinz: An issue of key importance was how the centre handled the strategic recommendations we were given as a result of the review for programme-oriented funding. The reviewers assured us that the centre was on a good course. But they also told us that the HZI wasn't yet exploiting the

potential that strategic partnerships could offer – especially with clinics and in particular with Hannover Medical School, the MHH. And that we should focus ourselves accordingly in order to reach critical mass in important future fields of infection research. After hearing that, the centre repositioned itself, grew from this, and refined its strategy accordingly. It was a process supported and driven by the scientists, the administration and the scientific advisory committee of the centre, not just imposed from top down.

Broer: Looking back over the last three or four years – even if I have only been at the HZI for two of them myself – the deciding factor was that we established ourselves firmly as a centre for infection research. Infrastructures were created and things were set in stone. After the realignment years, the next steps followed logically: recruitment to key positions, expansion of the campus, new national and international partnerships. The HZI worked very determinedly on this and positioned itself successfully. Then came the decision of how to define the thematic priorities. The employees got very enthusiastically involved in this and helped shape the strategic process.

"Translation can only work on the basis of excellent basic research."

ROADMAP HZI 2025

On the subject of the strategic process: coinciding with the anniversary, you presented a strategic roadmap for the next ten years called "HZI 2025". Why does the centre need such a document?

Heinz: What is laid down in black and white carries much greater weight than informal meetings and talks. Now we have a document that has been drawn up together with experts inside and outside the centre, which outlines the strategy for the coming years in binding form.

Broer: Having set out the most important guidelines in writing offers security – for our employees who were perhaps sometimes worried by the upheavals in the past as well as for our partners and for the funding authorities.

Heinz: The roadmap clearly states what the HZI is and what it stands for. Innovative fields of infection research, novel therapeutic interventions and personalised infection medicine: these are our priority topics for the future and the core elements of our strategy. We will continue to align our research activities with them. Therefore, we have established partnerships and expertise, and we will stay on course.

FOCUS PATHOGENS

As a part of this roadmap, the HZI has also defined "focus pathogens", on which it will concentrate its research in future. Why is that?

Heinz: We cannot cover the entire field of infection research on our own. Some years ago, we already agreed on a rough prioritisation and stipulated that we will work on bacteria and viruses as pathogens, but not parasites or fungi. Still, there is tremendous diversity among bacteria and viruses. Experts have told us many times over: if the centre focused on only a few topics, the quality of its scientific output would increase significantly. And so we decided to concentrate on pathogens that, firstly, are of clinical relevance and, secondly, for which we have a "critical mass" of scientists and expertise – either within the HZI itself or among our cooperation partners. Thirdly, we asked ourselves: What are the best topics to position ourselves optimally in the German research community? These assessments finally led us towards the selection of our focus pathogens.

Some of the most dangerous bacteria and viruses in the world were not chosen as focus pathogens, such as *Mycobacterium tuberculosis*, the cause of tuberculosis. What was the reason for this decision?

Heinz: We are not a tuberculosis centre, and that was a conscious decision. Every prioritisation naturally also leads to a limitation. Nevertheless, I would like to add: we may not be working directly with mycobacteria, but our drug researchers are investigating new agents against many different pathogens. Together with partners from the industry, we did in fact discover a new antibiotic that acts selectively against the tuberculosis pathogen.

"It is impressive to see how greatly the topic of infection research has gained importance in the Braunschweig–Hannover region."

So does that mean no work will be done on pathogens that are outside the focus in future – apart from drug research?

Heinz: We have not ruled out that we may one day address topics that were not originally at the centre of focus. Epidemics like Ebola could be specific occasions for that. Such a decision, however, requires careful consideration and has to be discussed in our boards and committees. At the same time, scientists who are currently working on other organisms should be given enough time to realign to the priority topics in the long term. But for new recruitments and investment decisions, the chosen priorities are a helpful guideline.

Strategic guidelines for the upcoming years: The Roadmap HZI 2025, completed in October 2015.

BRANCHES AND PARTNERSHIPS

The HZI has established several branches – how do you cope with managing them over the considerable distances?

Broer: Even though the branches are 70 and 500 kilometres away from the main campus, the collaboration is – in my opinion – excellent. The administration at the HZI is very open and flexible. The employees approach matters constructively and don't try to force their schemes upon the branches, but rather work out individual solutions with them, especially in the development phase. That is very highly appreciated at the branches, too. Problems may occur here and there, but overall, the integration of our branches TWINCORE and HIPS is a real success story.

Heinz: There were fears at the beginning that TWINCORE and HIPS could weaken the core centre. But that proved not

to be the case at all. HIPS already has a pioneering position in drug research and enjoys international recognition. The same goes for TWINCORE in translational, clinically relevant research – and the main campus in Braunschweig benefits enormously from their renown and the interaction with these strong branches.

How is the HZI perceived from the outside, in your opinion?

Heinz: It is impressive to see how the topic of infection research has gained importance and become a driver in the Braunschweig–Hannover region. The HZI has doubtlessly played a key role in this. The TU Braunschweig, for example, has established the topic "Infections and Active Compounds" as one of its priorities. The MHH was already very active in this field beforehand, but has recently started many joint initiatives with us. It's remarkable. I don't think there are many research centres that are helping shape and influence their region with their scientific focus to such a degree.

Broer: I think that became very clear at the anniversary event, too: our cooperation partners showed an amazingly strong commitment to the HZI. They appreciate the HZI's contribution in all the different roles we play in the individual cooperations. Sometimes we're the driver and shaper, setting framework conditions, and sometimes we're just a building block, as in the CSSB for example, where we are one of nine partners. But it wouldn't be complete without us. Our partners have seen on many occasions that we work soundly and don't always try to put ourselves in the limelight. I think the HZI has also gained a high degree of acceptance supra-regionally through that.

CONSOLIDATION AND INVESTMENTS

"Sometimes we're the driver and shaper and sometimes we're just a building block."

The HZI will have to cut its expenses in the coming years. How do you intend to ensure that excellent research will still be possible?

Broer: In the past years, the centre has made a lot of investments in infrastructure, facilities and equipment. There were many new – and often well-endowed – appointments. That was sensible, but it brought considerable financial burdens with it. Developments that were out of our hands have further aggravated these burdens. This made the consolidation necessary. We have come to a good consensus with the funding authorities here. They clearly appreciate that something that has been so painstakingly built up should not be endangered for merely short-term savings. So they gave us the option of taking careful and pragmatic measures to reduce costs, spread out over several years. Interim financing prevented adverse impacts for the centre.

Heinz: Aside from the burdens, consolidation also presents opportunities. It also forces a prioritisation: people work more closely together because the resources are more scarce. One deliberates, perhaps, whether to buy expensive equipment together or to use it jointly with partners. This in turn leads to new cooperations that would not have arisen otherwise. If you do it right, you can, in some respects, emerge even fitter after such a phase. Many companies and institutions have gone through these kinds of consolidations unscathed.

Broer: Yes, coupled with the strategic realignment, consolidation makes us even more mobile in some circumstances. Two things are important: one is transparency - for the employees as well – that the facts are on the table and are communicated openly. The second is a clear strategic line. If the strategy is transparent for the funding authorities, then there will be opportunities, even during austerity measures, to make important future investments. Such measures can still become necessary in order to secure the development of the centre and not to lose established potential again. It is a balancing act, and one has to give good reasons for one's decisions. Hopefully, we will reap the benefits in a number of years in the form of top publications and other scientific achievements.

TRANSLATION AND BASIC RESEARCH

The topic of translation is gaining importance. Will basic research continue to be just as important at the HZI?

Heinz: When scientists are busy researching in the laboratory, obviously we mustn't disturb them every five minutes with questions like: And how can we get these results into application now? That won't happen at the HZI. Translation can only work on the basis of excellent, well-funded basic research. Accordingly, basic research is and will always be our core business. At the same time, though, we have a clear mission. The mission of the Helmholtz Association is to contribute towards solving major challenges facing society, science and economy. The funding authorities as well as society as a whole expect our research to reach the patient one day.

What does that mean in practice?

Heinz: It means that we are well advised to keep our sights more keenly trained on possible applications than many other institutions – that is the translation mindset. And I am firmly convinced that through translation, done properly, one can also be more successful in basic science. We are talking here of "back translation". If, for example, you work on a medical topic and interact very closely with clinical partners, basic research will be enriched with questions coming directly from clinical practice.

Broer: It can also be beneficial for researchers if the centre actively supports them in bringing their discoveries closer to application.

Heinz: Yes, some universities say, if you want to patent your discovery, then do it privately, we're not interested. In contrast, here at the HZI we are very interested – here, it's an important part of our mission. I also see this as a unique

selling point. And for a scientist, it is also a great satisfaction when one's own work leads to a new drug, for instance, which is then used in clinical practice. Epothilone is a good example.

OPPORTUNITIES, CHALLENGES AND FUTURE PROJECTS

What are the major opportunities in the next few years – and what are the challenges?

Heinz: The future fields that hold the biggest opportunities and challenges result from our strategy, which we sketched out in the HZI 2025 roadmap: innovative fields of infection research with a clear translational scope. One innovative discipline in particular is RNA-based infection research. To rapidly capitalize on this, we want to establish an institute together with the University of Würzburg, which is an international leader in this field: the Helmholtz Institute for RNA-based Infection Research, HIRI. It would be the first of its kind worldwide.

Broer: The enormous experience we have gained from setting up branches could help here. I have already received many valuable suggestions on this topic from our administration.

Heinz: With the priority topic "therapeutic interventions", we have made it our goal to work on building a discovery and development pipeline, together with partners from industry and other research establishments. We have done a lot in this respect in the field of natural products. And for individualised infection medicine, we are planning a new "Centre for Individualised Infection Medicine", or CIIM, in Hannover. It will be established together with the MHH and will strategically complement the translational research at the TWINCORE in the emerging field of patient specific precision medicine.

Is there a personal message you would like to deliver to the employees of the HZI?

Broer: I would like the colleagues to be aware that, as directors, we often have to move within very narrowly constrained conditions. The general context in which many decisions are made may not always be immediately visible for everyone. But we do in fact very often respond to things that are happening in the outside world and have an impact on the HZI – developments in the research community, in politics, in the Helmholtz Association, and in all the many alliances and networks that are important to us.

Heinz: Indeed, and not just within Germany. We work in an internationally competitive field, and the world has become bigger and more open. Our classic competitors used to be in Europe and the USA, but now countries like China and India are also playing an increasing role. But I want our colleagues to know: we have lived through unsteady times, but we also have used our opportunities and achieved a promising position together – that is recognised from outside as well. Thus, we can rightly assert: we are very well set up for the future!

Interview: Manfred Braun, Birgit Manno and Hansjörg Hauser

MEETINGS, PRIZES, PUBLIC OUTREACH

Highlights of the Years 2014 and 2015

Hands-on research experience: During the European Researchers' Night, the HZI demonstrated practical experiments for all age groups.

SCIENCE FOR THE PUBLIC

European Researchers' Night, an EU-supported annual event taking place at multiple sites across Europe, was held in Braunschweig for the first time in September 2014. The event was coordinated by the Haus der Wissenschaft ("House of Science"). The HZI had a central booth in the forecourt of the Schloss in Braunschweig, where numerous scientists explained their research to the public. The HZI was involved in organising and preparing for the Researchers' Night. The event took place in Braunschweig for the second time in September 2015, again with extensive involvement of the HZI.

In autumn 2014, the HZI informed the public about various infection research topics for the fourth time in the lecture series **"KrankheitsErregend" ("PathoGenic")**. This year's lectures focused on "hospital germs": on three Saturdays in October and November, clinicians and researchers talked about bacteria of the species *Staphylococcus aureus*, *Clostridium difficile* and *Pseudomonas aeruginosa*, and explained to the audience what makes these germs so dangerous and what strategies for combatting them are currently being investigated.

Popular courses: The 20,000th student visiting the school lab BioS belonged to the class of biology teacher Simone Hüger (front row right), who was welcomed by Prof. Robert Hänsch (Technical University Braunschweig) and Franziska Broer (Administrative Director of the HZI).

In April 2015, the Northern German qualifying round for the science communications competition **Fame Lab** was held for the first time at the HZI. Under the heading "Talking Science" eight young scientists from different disciplines presented their works in three-minute talks. The evening's two winners, HZI researchers Marcia Duarte and James Tsatsaronis, qualified for the national finals in Karlsruhe.

Brillant communicators: HZI scientists James Tsatsaronis and Marcia Duarte, winners of the FameLab competition 2015.

The **Biotechnologisches Schülerlabor Braunschweig, BioS (Biotechnical School Lab)** at the HZI has presented a bridge between schools and research since 2002. It provides the opportunity for students in grades 10 to 13 to learn basic bioscientific laboratory methods through their own experimentation. Demand for the diverse courses was considerable from the beginning; the 20,000th participant was welcomed in 2014. By the end of 2015, a total of more than 23,500 school children from 113 schools had already visited the laboratories in the HZI's Gründerzentrum building.

PRIZES AND AWARDS

a) Science

Selected scientific prizes from 2014

Prize winner	**HZI department** (see organisational chart for complete names)	**Award**	**Time**	**Awarding institution**	**Prize money**
Rolf Müller	MINS	Appointment as Honorary Director of *Shandong University – Helmholtz Joint Institute for Biotechnology*	May 2014	Shandong University, China	
Maike Windbergs	DDEL	DPhG Foundation Young Investigator Award	Sep 2014	DPhG-Stiftung (Horst-Böhme-Stiftung), foundation of the Deutsche Pharmazeutische Gesellschaft	€ 5,000
Andreas Müller	INMI	Jürgen Wehland Prize	Oct 2014	HZI	€ 5,000
Johannes Schwerk	RDIF/MSYS	Promotional award (Förderpreis) Arbeitskreis für Zellbiologie und biomedizinische Forschung e.V.	Nov 2014	Arbeitskreis für Zellbiologie und biomedizinische Forschung e.V.	€ 1,000
Sharmila Nair	RDIF/IMMI	Promotional award (Förderpreis) Arbeitskreis für Zellbiologie und biomedizinische Forschung e.V.	Nov 2014	Arbeitskreis für Zellbiologie und biomedizinische Forschung e.V.	€ 1,000
Martin Korte	NIND; TU BS	1st Prize in the competition of the 4th Regional Education Award of the Allianz für die Region GmbH for the project "Learning through teaching: Teach it forward in three ways"	Dec 2014	Allianz für die Region GmbH	€ 5,000 (for TU Braunschweig)

Selected scientific prizes from 2015

Prize winner	HZI department (see organisational chart for complete names)	Award	Time	Awarding institution	Prize money
Maike Kuhn	BIOM	Short-Term Research Fellowship	Dec 2014–Feb 2015	EMBO (ASTF 591-2014)	€ 6,750
Stephanie Pfänder	EVIR	Nutricia Science Award (prize shared with Eike Steinmann)	Jan 2015	Milupa Nutricia GmbH	€ 10,000
Eike Steinmann	VIRT	Nutricia Science Award (prize shared with Stephanie Pfänder)	Jan 2015	Milupa Nutricia GmbH	€ 10,000
Martin Korte	NIND; TU BS	Ars Legendi Faculty Award 2014	Mar 2015	Stifterverband der deutschen Wissenschaft; Verband für Biologie, Biowissenschaften & Biomedizin VBIO	€ 5,000
Cenbin Lu	DDOP	Prize for Most Outstanding Chinese Ph.D. Students Abroad	Mar 2015	Chinese Ministry of Education	
Anggakusuma, Eike Steinmann	VIRT	Bionorica Phytoneering Award	Aug 2015	Gesellschaft für Arzneipflanzen- und Naturstoff-Forschung (GA); Bionorica AG	€ 10,000
Rolf Hartmann	DDOP	Carl Mannich Medal	Sep 2015	Deutsche Pharmazeutische Gesellschaft (DPhG)	
Rolf Hartmann, Anke Steinbach, Christine Maurer, Cenbin Lu, Benjamin Kirsch	DDOP	PHOENIX Pharmaceutical Science Award Category “Pharmaceutical Chemistry”	Oct 2015	PHOENIX Pharmahandel GmbH & Co KG	€ 10,000
Carlos Guzmán, Claus-Michael Lehr	VAC/DDEL	Nanomedicine Award 2015	Dec 2015	European Technology Platform on Nanomedicine (ETPN) + Enabling Nanomedicine Translation (ENATRANS)	

b) Awards for vocational training

Prize winner	HZI department (see organisational chart for complete names)	Award	Time	Awarding institution	Prize money
Franziska Klann	IMCI / PA	First place nationwide in 2014 as apprentice biology laboratory technician	Dec 2014	Deutscher Industrie- und Handelskammertag (Association of German Chambers of Commerce and Industry) DIHK	€ 6,000
Rebecka Wünsche	VIMM / PA	Helmholtz DKB Apprenticeship Award (third place)	Apr 2014	Helmholtz Association and DKB (Deutsche Kreditbank AG)	€ 700
Nina Burgdorf	BIOM / PA	Second place as apprentice biology laboratory technician	Jun 2014	Industrie- und Handelskammer Braunschweig (Braunschweig Chamber of Commerce and Industry)	€ 10,000
Nina Burgdorf	BIOM / PA	Training scholarship	Nov 2014	Stiftung Begabtenförderung berufliche Bildung	€ 6,000

PRIZES FOR EMMANUELLE CHARPENTIER

A particularly large number of prestigious awards in 2014 and 2015 went to Prof. Emmanuelle Charpentier, who headed the HZI department "Regulation in Infection Biology" from 2013 to 2015. Charpentier helped develop the CRISPR-Cas9-based genome editing method, now in widespread use around the world. Since October 2015, she has held the position of a director at the Max Planck Institute for Infection Biology in Berlin. She additionally heads a research group at the University of Umeå, Sweden.

Award ceremony for the Breakthrough Prize in Life Sciences: Dick Costolo (CEO of Twitter) and Prize laureates Emmanuelle Charpentier and Jennifer Doudna with Hollywood star Cameron Diaz. Photograph: Breakthrough Prize in Life Sciences Foundation

The CRISPR-Cas9 system is part of a defence mechanism that protects bacteria against viral infections. During her studies, Emmanuelle Charpentier discovered that the system can serve as a high-precision tool for editing DNA and studying the function of genes. Among other applications, CRISPR-Cas9 can be used for developing new methods for treating severe diseases in humans.

For deciphering the fundamental molecular mechanisms and establishing the basis for a powerful technology, Charpentier has received many prestigious science awards in the past two years (see table). These include the Breakthrough Prize in Life Sciences, the Leibniz Prize, an Alexander von Humboldt Professorship, the Ernst Jung Prize, the Louis-Jeantet Prize for Medicine and the Swedish Göran Gustafsson Prize.

The discovery of the CRISPR-Cas9 system has been named "Breakthrough of the Year" in 2015 by the leading journal *Science*. Thus their discoverer secured a place in *Time Magazine's* 2015 list of the world's most influential people.

Selected Awards for Emmanuelle Charpentier 2014

Award	Time	Awarding institution	Prize money
Göran Gustafsson Prize	Mar 2014	The Royal Swedish Academy of Sciences	SEK 1.5 M + SEK 250,000
Alexander von Humboldt Professorship	May 2014	Alexander von Humboldt Stiftung	€ 5 M
Acceptance as EMBO member	May 2014	EMBO	
Dr. Paul Janssen Award for Biomedical Research	Sep 2014	Johnson & Johnson, USA	$ 50,000
Jacob Heskel Gabbay Award	Oct 2014	Brandeis University, USA	$ 3,000
Grand Prix Jean Pierre Lecocq	Nov 2014	Académie des Sciences, France	€ 30,000
Breakthrough Prize in Life Sciences	Nov 2014	The Breakthrough Prize Foundation	$ 3 M
Listed among “Foreign Policy’s 100 Leading Global Thinkers 2014”	Dec 2014	Foreign Policy Magazine	

Selected Awards for Emmanuelle Charpentier 2015

Award	Time	Awarding institution	Prize money
Louis-Jeantet Prize for Medicine	Jan 2015	Louis-Jeantet-Stiftung, Geneva	SFR 700,000
Hansen Family Award	Apr 2015	Bayer Science & Education Foundation	$ 75,000
Listed in the “Time 100 List”	Apr 2015	Time Magazine	
Ernst Jung Prize	May 2015	Jung-Stiftung für Wissenschaft und Forschung	€ 300,000
Princess of Asturias Award	May 2015	Spanish Royal Family	$ 50,000
Carus Medal	Sep 2015	Nationale Akademie der Wissenschaften Leopoldina	€ 5,000
Gruber Genetics Prize	Oct 2015	Peter and Patricia Gruber Foundation	$ 250,000
Massry Prize	Oct 2015	Meira and Shaul Massry Foundation	$ 40,000
Wissenschaftspreis Niedersachsen	Nov 2015	Ministry of Science of the State of Niedersachsen (Lower Saxony)	€ 25,000
Gottfried Wilhelm Leibniz Prize	Dec 2015 (award announcement for 2016)	Deutsche Forschungsgemeinschaft (DFG)	€ 2.5 M

BUILDINGS

Clinical Research Centre (CRC) opened in Hannover

On 8 September 2014, the Clinical Research Centre (CRC) was inaugurated on the premises of the Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) in Hannover. At this research centre, which is unique nationwide, the HZI, ITEM and Hannover Medical School (MHH) contribute their particular expertise in early clinical trials. Novel drugs can be studied here for safety (Phase I trials) and for effectiveness (Phase IIa trials). The 31 million euro cost of the new building was shared by the State of Lower Saxony and the Fraunhofer-Gesellschaft. In addition, the three founding institutions provided funding for medical equipment.

A topping-out garland for the BRICS. From left to right: Prof. Dieter Jahn, Vice President of the TU Braunschweig, Prof. Jürgen Hesselbach, President of the TU Braunschweig, Renate Müller-Steinweg, supervisor of the Staatliches Baumanagement Braunschweig (Department of building inspection), Peter-Jürgen Schneider, Minister of Finance of the state of Lower Saxony, Ulrich Markurth, Mayor of Braunschweig, Prof. Dirk Heinz, Scientific Director of the HZI. Photograph: TU Braunschweig/Presse und Kommunikation

BRICS soon to be completed

The topping-out ceremony for the Braunschweig Integrated Centre of Systems Biology (BRICS) on the campus of the Technische Universität Braunschweig was celebrated on 9 September 2014. BRICS is a joint establishment of the TU and the HZI. Biologists, physicists, mathematicians, informaticians, chemists and engineers will collaborate here to tackle issues in systems biology, including mathematical and bioinformatic modelling of infections and other biological processes. As of the end of 2015, the new building is almost complete; inauguration and opening are planned for mid-2016. Five institutes of the TU and two departments of the HZI will move into this building.

CSSB: Using high-power light sources against pathogens

Exactly one year later – on 9 September 2015 – another topping-out ceremony was celebrated on the premises of the Deutsches Elektronen-Synchrotron (DESY) in Hamburg. The Centre for Structural Systems Biology (CSSB) is being set up here. Starting in 2017, infection processes will be studied using high-intensity light sources. The HZI is one of nine partners involved in the project. The building will cost 50 million euros and will cover a total area of 13,000 square metres. The laboratories and office building will offer space for 180 researchers on three levels.

HIPS has moved

After around two years of construction, the new building of the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) has been completed and is now occupied. The building, which cost about 26 million euros, was ceremonially inaugurated on 2 October 2015. At this HZI establishment on the campus of the University of Saarland (UdS) in Saarbrücken, 99 employees are researching into new natural compounds, optimising them for application in humans and

A home to pharmaceutical research: The new HIPS building in Saarbrücken was completed in 2015.

developing new methods for delivering drugs at their target sites in the body. HIPS is the first non-university institute for pharmaceutical research in Germany that is publicly funded.

Groundbreaking ceremony for the DRFG

Construction of the Centre for Drug Research and Functional Genomics, DRFG, commenced on 17 November 2015. Numerous guests from industry and politics came to the HZI for the groundbreaking ceremony. The tasks of the DRFG scientists will include investigation of bacterial genes and their functions and of new active compounds against pathogens. The investment costs for construction amount to around 26.9 million euros, 60 percent of which is covered by the Federal Government and 40 percent by the State of Lower Saxony. Alongside HZI researchers, scientists from the Leibniz Institute German Collection of Microorganisms and Cell Cultures (DSMZ) and from TU Braunschweig will also work at the new drug research centre.

SCIENTIFIC EVENTS

“Biological Barriers” conference at HIPS

In 2014, for the tenth time running, experts from around the world gathered in Saarbrücken at the “Biological Barriers” conference. The meeting, which for some time has been jointly organised by HIPS and the University of Saarland (UdS), is held every two years. Alongside lectures, it offers discussion rounds, workshops, poster sessions and laboratory courses. Participants discussed new insights into how drugs reach their target site in the body, how nanotechnology can deliver drug molecules across biological barriers and by what methods these processes can be researched.

NoRDI and Jürgen Wehland Prize

Every year since 2010, experts from all across Europe have shared news about their research at the annual NoRDI Symposium (North Regio Day on Infection) at the HZI. In October 2014, the fifth symposium, NoRDI V, revolved around “Gastrointestinal Infections”. NoRDI VI the following year focused on “Barriers in Infection”. Both of these events hosted the award ceremony for the Jürgen Wehland Prize, with which the HZI has been honouring outstanding young investigators since 2011. The prize went to Andreas Müller of Otto von Guericke University in Magdeburg in 2014, and to Sabrina Schreiner of TU München and the Helmholtz-Zentrum München in 2015. It is awarded in honour of the HZI Scientific Director Jürgen Wehland, who passed away during his term of office in 2010.

Jürgen Wehland Prize 2014: Awardee Prof. Andreas Müller (second from right) with Prof. Burkhard Schraven (University of Magdeburg, left), Brigitte Wehland, widow of Prof. Jürgen Wehland, and Prof. Dirk Heinz, Scientific Director of the HZI.

Jürgen Wehland Prize 2015: Awardee PD Sabrina Schreiner (second from right) with Prof. Thomas Dobner (Heinrich Pette Institute, right), Brigitte Wehland, widow of Prof. Jürgen Wehland, and Prof. Dirk Heinz, Scientific Director of the HZI.

Inhoffen Medal for outstanding pioneer work

The 2014 Inhoffen medal, the most prestigious German award in the field of natural product chemistry, went to Alois Fürstner of the Max-Planck-Institut für Kohlenforschung (carbon research). In 2015, Hiroyuki Osada of the RIKEN Center for Sustainable Resource Science received the prize. Every year since 1994, the HZI and TU Braunschweig have jointly awarded the medal and held the associated Inhoffen lectures in honour of a pioneer in bioscientific research: Prof. Hans-Herloff Inhoffen, who was Director of the TU and later co-founder of the Institute for Molecular Biology, Biochemistry and Biophysics (IMB), from which the HZI subsequently emerged. Inhoffen died in 1992. The Inhoffen Medal, endowed with 5,000 euros, is bestowed by the Friends of the HZI.

VISTRIE Symposia on Viruses and the Immune System

Seven partners from German universities and research establishments and from the Rijeka School of Medicine in Croatia jointly form the virtual institute VISTRIE (Viral Strategies of Immune Evasion). In May 2015 they organised the third VISTRIE symposium, focussed on the interaction between viruses and the immune system. Since its beginning, the symposium has been attended by internationally renowned speakers.

Inhoffen Medal 2014: Laureate Prof. Alois Fürstner with Prof. Dietmar Schomburg (TU Braunschweig and Friends of the HZI) and HZI director Prof. Dirk Heinz.

Prof. Hiroyuki Osada earned the Inhoffen Medal 2015

Focus on natural compounds: The HIPS Symposium
Each year since 2000, HIPS has provided scientists with a forum for exchanging ideas beyond the boundaries of classical disciplines. The HIPS Symposium brings together researchers from the three pharmaceutical fields of natural product research, medicinal chemistry and drug delivery. In particular, it offers young scientists the opportunity to benefit from the expertise of established, internationally renowned colleagues.

Prevention and individualised medicine: The TWINCORE Symposium
The TWINCORE Symposium has established itself as an important platform for topics at the interface between basic and clinical research. In the last two years, TWINCORE invited scientists to Hannover for the sixth and seventh time to share their knowledge. In 2014, the meeting focused on the topic of "Combatting pathogens with preventive and therapeutic strategies"; the 2015 event concentrated on "Individualised infection medicine".

POLITICIANS VISITING THE HZI

In 2015, the HZI welcomed Federal and State politicians as guests on several occasions.

In May 2015, the Federal Government adopted a new strategy to counter antibiotic resistance. As part of this initiative, Federal Minister of Health Hermann Gröhe visited Braunschweig to familiarise himself with the work being done at the HZI and in particular the focal topic of drug research. Also in May, Lower Saxony's Minister President Stephan Weil visited the HZI to gain an impression of the scientific collaboration in the region.

The ceremony for the HZI's 50th anniversary was attended by numerous prominent guests, among them Lower Saxony's Minister of Science and Culture Gabriele Heinen-Kljajić and Parliamentary State Secretary of the Federal Ministry for Education and Research Thomas Rachel.

At the groundbreaking ceremony for the new Centre for Drug Research and Functional Genomics DRFG in November, the HZI welcomed Federal Minister of Research Johanna Wanka as a guest of honour.

Federal Minister of Research Prof. Johanna Wanka

Federal Minister of Health Hermann Gröhe

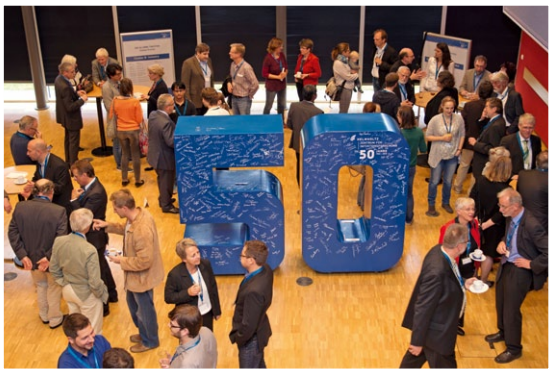
REUNION AT THE FORMER WORK PLACE

First meeting of the HZI Alumni

A campus tour allowed the alumni to discover what had changed since their time. Dr. Michael Böcher (retired, former senior scientist in the department “Genome Analysis” at the GBF/HZI) and Prof. Jürgen Bode (principal investigator at Hannover Medical School and former head of the research group “Epigenetic regulation” at the GBF/HZI, right) visited the fermenter hall.

Fifty Years of cutting edge research – these are also fifty years in which several employees, among them excellent researchers and experts in science or administration, left the centre and became alumni.

The HZI aims to strengthen its alumni network to foster the contact between former employees and staff members working at the HZI now. Particularly current PhD students and postdocs can profit from the experience of alumni working in all kinds of professional fields today. Alumni, on the other hand, gain access to an exclusive talent pool and have the possibility to stay in contact with former colleagues and with the HZI. On the occasion of the 50 year jubilee, the HZI organized the first meeting of the HZI Alumni, which was well received. A varied programme with campus tour, scientific spotlight, career forum and subsequent get-together attracted over 100 participants who took advantage of the opportunity to exchange ideas and network with each other. The HZI plans to further expand these activities with regular meetings and newsletters. All HZI alumni are welcome to join the network at www.helmholtz-hzi.de/hzi-alumni.



The alumni offered their professional expertise to the current PhD-students and postdocs during a career forum.

Around sixty former employees visited today's HZI on the occasion of the first alumni meeting.

Getting together with former colleagues and friends was one aim of the alumni meeting. From left to right: Prof. Rudi Balling (director of the Luxembourg Centre for Systems Biomedicine and former Scientific Director of the HZI/GBF), Prof. Leopold Flohé (retired, former Scientific Director of the GBF) and Prof. Edgar Wingender (director of the Institute of Bioinformatics at the University of Göttingen and former head of the research group "Bioinformatics" at the GBF).

Dr. Stefanos Grammatikos, Vice president Biotech Sciences at UCB Pharma SA and former postdoctoral researcher at the GBF, second from left with PhD-students.

Alumna Dr. Susanne Pistor (Qiagen GmbH and former postdoctoral researcher at the GBF, middle) answered PhD students' questions.

Dr. Harald Dinter (senior vice president Global Biologics at Bayer HealthCare and former postdoctoral researcher at the GBF, middle) joined discussions with young scientists at HZI.

“THE EXPERTS AT THE INTERFACES WILL BECOME INCREASINGLY IMPORTANT”

Prof. Michael Manns, Clinical Director at the HZI, about Translation as a Core Strategic Aim of the HZI

In October 2015, Prof. Michael Manns took up the position of Clinical Director at the HZI. Manns is one of the world’s leading specialists on liver disease and is Head of the Department “Gastroenterology, Hepatology and Endocrinology” at Hannover Medical School (MHH).

Prof. Manns took an active role in the HZI celebration of the 50 years jubilee when contributing to the panel discussion.

Prof. Manns, you are the first Clinical Director of the HZI. This position was newly established in 2015. What is your function?

Translation – meaning the rapid and efficient transferral of results from basic research into application – is a core strategic aim of the HZI. My main responsibilities as Clinical Director include structuring translation into an efficient process and bringing clinical topics into HZI research activities.

What is your approach to achieve this?

Much of my work involves coordinating the HZI’s translational research projects, supporting strategic recruitment to positions related to translational research and advising the scientific management of the HZI. Access to clinical material has to be coordinated and mediated for the HZI workgroups. The aim here is to establish links between clinically relevant HZI research and corresponding projects at MHH. We need to strengthen the Hannover-Braunschweig area within the German Centre for Infection Research, DZIF. We want to make it visible - nationally and internationally - as an outstanding location for infection research with clinical relevance.

Has the HZI, from your point of view, already managed to orientate a part of its research towards translational goals?

The HZI and its partners have indeed already come a long way. Founding TWINCORE was an important structural

measure to bring the centre's researchers closer to MHH as the most important clinical cooperation partner – and thus closer to patients. HZI and MHH have also committed to establishing a Centre for Individualised Infection Medicine, the CIIM. It was founded in December as a virtual centre first, accessible to translational research groups at both institutions, and will serve to network and consolidate workgroups that are firmly anchored in the HZI and established as clinicians at MHH. The CIIM will be home both to internationally outstanding research groups and to medical networks with a focus on infection and with management functions located in Hannover. These include the German Liver Foundation, with its hepatitis network Hep-Net, and the community acquired pneumonia network CAPNETZ. The CIIM needs to be housed in a research building as soon as possible. Although this building has yet to be constructed a building plot next to TWINCORE and MHH already awaits it. Ideally, the building would also house the research and administrative activities of the German Centre for Infection Research, DZIF. This would create a unique focal point for translational research on infection medicine in the Hannover-Braunschweig region.

"We can create a unique focal point in the Hannover-Braunschweig region"

So the partnership between MHH and HZI plays a crucial role in future development?

Yes, this cooperation has enormous potential. Infections are the research focus of the HZI and play a central role at MHH as well. There is thus a high degree of conformity in the scientific alignment. Common use of resources strengthens the competitiveness of both establishments nationally and internationally – also when it comes to acquiring research funds. The DZIF, where MHH and HZI together assume a key position, is an example.

What are the long-term prospects of this partnership, from a scientific point of view?

Translational infection research is inconceivable without access to patients. In this respect, MHH's contribution to research at the HZI is essential in the long term. What distinguishes MHH in particular is its unique patient population. This provides a large reservoir of relevant data and patient material. For instance, MHH is an internationally renowned and extremely prestigious transplant centre; among other things it operates one of the largest and longest-running centres for liver transplants. Also, a considerable number of patients with liver diseases of all origins, some of them severe, including many with the viral hepatitis strains A, B, C, D and E, are treated at MHH. Other core areas of expertise of MHH lie in working with immunosuppressed patients, dealing with multidrug-resistant bacteria and performing clinical trials to approve new treatments. This existing experience and expertise present considerable opportunities for research.

What does that mean for the HZI, exactly?

Given access to a hospital – to patients – basic research comes face-to-face with the most important problems in medical practice. People don't come a long way to MHH to have blood taken for research purposes. They come because they hope to be cured. That is why MHH has such an extensive patient population. The clinical staff is very familiar with everyday care of patients and the problems related to this practice. Basic research can benefit from this in many ways. The close connection with clinical practice will help provide a deeper understanding of the mechanisms of infection and immunity and contribute to the development of novel anti-infective compounds and vaccination strategies.

And how does MHH benefit from this cooperation?

Access to the HZI's excellent technology platforms can bring MHH infection research to a new level. Such a technological and analytical infrastructure simply isn't available at a university hospital, nor is the know-how for its application. What a research centre like the HZI in turn lacks – as I said

”Infection research is inconceivable without access to patients“

– are the patients and the patient material. The partners can complement each other perfectly here.

If the situation now is so favourable, what is there left to do?

There are still many unresolved problems we have to tackle in the long term if we are to put translation on a solid footing. For example: clinical practice and research are generally separate career paths. Those who leave clinical medicine for the pharmaceutical industry or to pursue academic research typically don't come back. And if they do, they have tremendous difficulty in rising to leading positions. But we need capable people who are mobile and move between positions within the triangle consisting of university hospital, industry, and non-university basic research.

What makes this so difficult?

To begin with, working at a hospital takes up a lot of time and is very demanding. The same goes for basic research. Good research projects take time and are labour intensive. One has to cover a lot of ground, as many well know, before one has results that can be published in a high-ranking journal. That's just one more reason why clinical practice and basic research are two career paths that are hard to combine.

Prof. Michael P. Manns is the HZI's first Clinical Director. Photograph: MHH/Kaiser

Is it necessary to combine them? There could be other ways to share the expertise.

Even for that – I am firmly convinced – it isn't enough to meet up occasionally for a symposium in some nice location and talk over a coffee or a beer. You have to really know the daily routine at the hospital and in basic research. Even the approach we spoke of just now – of studying patient material from the hospital with the sophisticated technology of basic research – is not enough on its own. We have to tackle genuinely unsolved problems from clinical practice and, to do that, we have to know and understand them. That is why I maintain: we need to familiarise young clinicians with the possibilities and perspectives of basic research.

What can be done to achieve that?

First and foremost, we can give medical professionals the opportunity to swap for a certain length of time from clinical practice to basic research – be it in Braunschweig or in Hannover. By enabling them to do basic scientific training in HZI workgroups during their postdoctoral phase, for example. Then, when they return to the hospital, they stay in contact with the research at their training laboratory; they are a part of both worlds.

That is the aim of the Clinician Scientist Programme, which the HZI has developed together with MHH and other clinical partners?

Yes. It includes clinical leave stipends as well as clinical colloquia at the HZI. Conversely, basic researchers have to be introduced to specific clinical problems. Lasting, intensive personal contacts can link the two spheres closely and ensure effective translation. In this way, the HZI also gains

“We need to familiarise young clinicians with basic research.”

better access to what is happening at the hospital, to the discussions amongst clinicians – and to a network of research partners within the hospital.

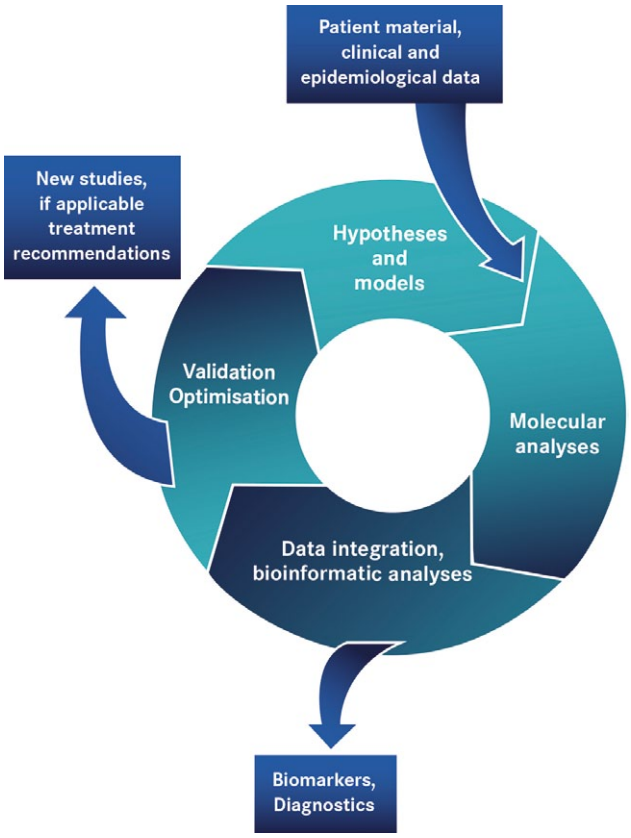
Will the programmes be enough for all that?

They are a crucial step in that direction. But, ultimately, we will have to create opportunities for dual career paths. Paths which combine clinical training with practical experience in basic research, and at the same time offer attractive positions. Of course, enthusiasm for research will always be a big part of the motivation. But we can also foster and stimulate this motivation by giving the young medical professionals the chance to familiarise themselves with research. We will always need pure clinicians and pure basic researchers. But the experts at the interfaces will become increasingly important in years to come.

What do you believe will have to be achieved in the next ten to fifteen years?

I would like to see the young people from our Clinician Scientist programmes assuming leading positions in ten to fifteen years – heading departments at prestigious hospitals. And to see other participants in these programmes working as independent researchers, say at the HZI, and at the same time working with part-time contracts at the hospital and in patient care, or heading outpatient clinical establishments. If we can achieve that, then we will have truly made a lasting contribution towards translation between basic research and clinical practice.

Interview: Manfred Braun



Model for the future: The integration of findings and analysis methods from clinical and basic research will lead to new diagnostics and individualised treatment concepts.

CLINICIAN SCIENTIST PROGRAMMES AT THE HZI

Combining Research Know-how and Medical Expertise

Physician and researcher: Clinician Scientist Dr. Patrick Behrendt, Photographs: MHH/Kaiser

To intensify its clinical focus, the HZI is actively integrating clinicians into its research programme. Medical professionals are given the opportunity to combine their clinical routine – despite its high workload – with scientific training. At the HZI and its partner sites, they can use the sophisticated infrastructure of a laboratory environment equipped to the latest technological standards for their own patient-oriented research projects. The host laboratories in turn benefit directly from first-hand experience with relevant clinical issues. Furthermore, new personal networks established between clinicians and researchers create a kind of "human bridge" between bench and bedside.

Throughout 2014 and 2015 the HZI made significant advances in driving this process forward, employing various models and cooperating with clinical partners such as Hannover Medical School (MHH) and Otto von Guericke University Magdeburg (OvGU). New jobs for clinician scientists were created and several projects with a translational focus and relating to clinical practice were initiated at the HZI and TWINCORE.

These are some of the current projects involving clinician scientists:

The HZI participates in the MHH Young Faculty Program, which funds Clinician Scientists over a total period of three years. Physicians in the Young Faculty Program retain their clinical posts but are freed for 4 months every year to carry out patient-oriented research in their specialised field.

In the second selection round of the programme, which has been running since the summer of 2014, MHH physicians **Dr. Gerrit Ahrenstorf, Dr. Alexandra Jablonka** and **Dr. Bettina Wiegmann** were selected to receive funding for participation in cooperative projects at the HZI and TWINCORE. They will be starting their projects soon.

The physicians selected in the first round, **Dr. Wolfgang Koestner** (*Assessment of autoimmune versus viral myocarditis by optical and MR imaging*), **Dr. Kurt-Wolfram Sühs** (*MicroRNA Profiling within Liquor cerebrospinalis upon reactivation of Varicella zoster*) and **Dr. Martin Wetzke** (*Host and pathogen factors determining disease severity in children with RSV infection*), have reported their first results in conference lectures and are currently preparing manuscripts, demonstrating the success of this programme.

For the second funding period of the Collaborative Research Centre (CRC) programme 854, from 2014 to 2017, the HZI is financing a rotational position (known as a "Gerok position") for a clinical infectiologist from OvGU Magdeburg and has appointed **Dr. Christian Schulz** of the Department of Gastroenterology, Hepatology and Infectiology at OvGU. Schulz took up his position in the HZI research group "Molecular Interactions and Processes" in the autumn of 2014. He is working on a project entitled *The influence of the microbiome of the upper gastrointestinal tract in the pathogenesis of inflammatory and malignant diseases*. The first findings made in this particular cooperation have already been presented at conferences and are currently being prepared for publication.

Existing funding instruments such as DZIF stipends are also being used to integrate researching physicians. **Dr. Benjamin Heidrich** of the Department of Gastroenterology, Hepatology and Endocrinology at MHH was awarded a DZIF Clinical Leave Stipend to work for one year in the HZI research group "Molecular Interactions and Processes" on the project *The influence of the gut microbiome on the development of cirrhosis in patients chronically infected with the hepatitis C virus*. The results, currently in preparation for publication, have secured funding for a successor project.

Dr. André Karch is supported by an MD/PhD Stipend to continue his scientific training with the PhD project *The role of microbiota of the respiratory system for infectious complications and graft survival after lung transplantation - does the donor matter?* within the HZI research group Epidemiological and Statistical Methods.

The success of the DZIF measures is demonstrated not least by the case of **Dr. Patrick Behrendt** who received a DZIF Clinical Leave Stipend in 2012. Meanwhile, his position is being funded equally by the MHH Department of Gastroenterology, Hepatology and Endocrinology and the TWINCORE research group Experimental Virology to ensure that resulting translational projects can continue. At the TWINCORE – in close collaboration with the HZI - he focuses on translational projects, e.g. how hormones from adipose tissue affect hepatitis C virus replication in cell culture.

In the future, the HZI will continue to promote collaboration between basic research and clinical practice by supporting projects involving clinician scientists and planned activities such as those at the Centre for Individualised Infection Medicine (CIIM).

Pioneers of translational research:
The Clinician Scientists at HZI and TWINCORE.

FROM BENCH TO BEDSIDE

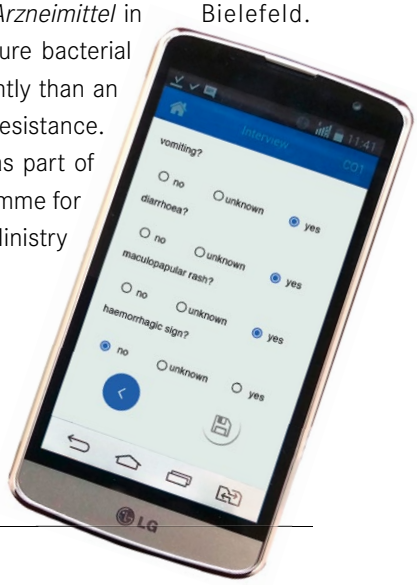
Examples of Innovation and Translation at the HZI

Field testing: HZI epidemiologist Prof. Gérard Krause (first picture, middle) applied the SORMAS technology successfully to monitor disease outbreaks in Africa.

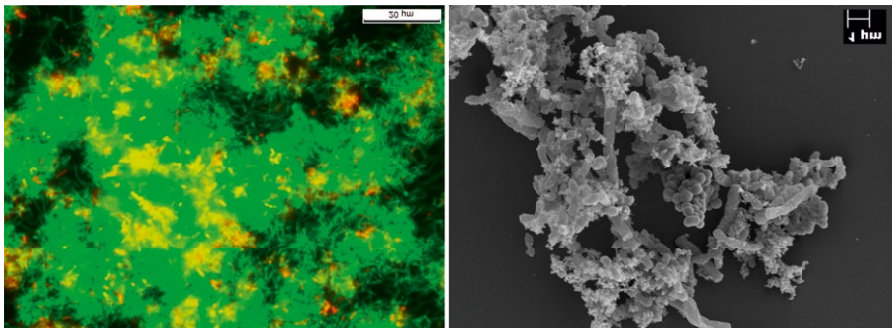
Setting the stage for innovation and promoting the transfer of research results into clinical and pharmaceutical application: the HZI is dedicated to pursuing these key goals in numerous projects, many of which are embedded in close cooperative partnerships and brought to life with third-party funding. In 2014 and 2015, a number of these projects reached critical milestones.

One outstanding example of rapid application of knowledge from basic research for the benefit of patients is SORMAS, funded by the Federal Ministry of Education and Research (BMBF) via DZIF. It is a joint project of the HZI's Epidemiology department with partners such as the Hasso Plattner Institute, the Robert Koch Institute and the Bernhard Nocht Institute, as well as researchers in Nigeria. The partners involved have successfully developed a monitoring and management system to improve the efficiency and timeliness of detecting and containing Ebola outbreaks and epidemics of other pathogens. The "**S**urveillance and **O**utbreak **R**esponse **M**anagement **S**ystem" (SORMAS) is based on decentralized collection of data on new cases and central control of infection prevention measures, and can be employed even in remote regions of Africa as a smartphone app. After nine months of development, the trial in rural areas of Nigeria was very promising. Project coordinator Prof. Gérard Krause, head of the HZI department "Epidemiology", will continue to expand the SORMAS system features for use in West African epidemic regions.

Major advancements have also been made in several other application-oriented projects. The HZI research group "Microbial Communication" led by Prof. Irene Wagner-Döbler is developing a novel medical product in cooperation with the medium-sized pharmaceutical company *Dr. August Wolff GmbH & Co. KG Arzneimittel* in Bielefeld. The product is designed to cure bacterial vaginosis, yet to act more gently than an antibiotic and to produce no resistance. The project is being funded as part of the Central Innovation Programme for SMEs (ZIM) of the Federal Ministry for Economic Affairs.



Smartphone App for disease control: the SORMAS system, co-developed by HZI epidemiologists.



Looking for a cure for Bacterial Vaginosis (BV): HZI scientists have developed a polymicrobial biofilm model for BV to study the interaction of bacteria in the vagina. The model comprises the two pathogens *Gardnerella vaginalis* and *Atopobium vaginae*, as well as *Lactobacillus crispatus* which is an inhabitant of the healthy vaginal flora. Left, fluorescent microscopic picture after staining with the LIVE/DEAD viability stain, and right, scanning electron micrograph. *Lactobacillus crispatus* forms long rods, while the two pathogens cannot be distinguished based on morphology.

The microbial compound carolacton is being further developed in a BMBF joint project involving medical professionals, bioprocess engineers, microbiologists and VOCO GmbH, an internationally leading manufacturer of dental materials. Carolacton acts as a biofilm inhibitor and could be used in practical applications to maintain dental health.

In 2015, the HZI raised more than 2 million Euros in total as funding for cooperative projects with a translational character. For the next few years, the HZI sees particularly great promise in projects involving the development of drug candidates against neglected and poverty-related infectious diseases.

Furthermore, various studies are currently in preparation, for example on optimising dosage intervals of inhaled antibiotic therapies for mucoviscidosis (cystic fibrosis) patients, for controlling zoonotic transmission of campylobacteriosis by targeted use of bacteriophages, and for developing vaccines against respiratory and systemic infections in humans and pigs.

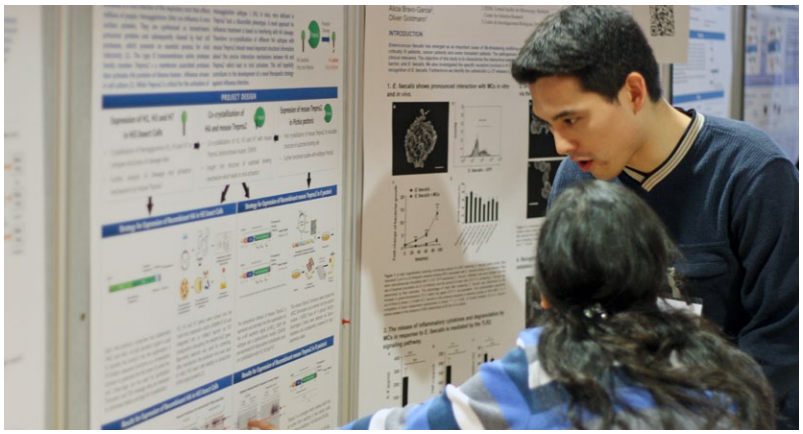
In 2016, the HZI will be working to establish an innovation fund (Pre4D Seed Fund) with the goal of significantly promoting the technological advancement of HZI drug and diagnostic candidates, and to develop basic research results as far as possible along the path to practical application.

The distinctive feature of this measure, which the Helmholtz Association is funding with € 390,000 per year, is its conceptual design. The assessment of early stage projects will be characterized by a high degree of professionalism. An external team of experts from pharmaceutical companies will identify promising developments with a potential for clinical-medical application and evaluate them according to a catalogue of criteria, analysing among other things the property rights situation, the medical need and, if applicable, competing solutions. An external project supervisor will provide competent and professional support on select projects in their early phases.

Dr. Michael Strätz

TRAINING THE NEXT GENERATION OF INFECTION RESEARCH EXPERTS

The HZI's Interdisciplinary Structured PhD Programme



The HZI International Graduate School for Infection Research (GS-FIRE) was initiated in 2009 with the aim of providing structured interdisciplinary training covering all areas of infection research and related fields. The Graduate School facilitates international recruitment, ensures quality in supervision and care of PhD candidates and supports their career development. It complements the interactions and infrastructure in this research field in particular in the Hannover-Braunschweig area and strengthens the region as an integrated and internationally recognised centre of excellence.

EARNING A DOCTORATE IN AN EXCELLENT ENVIRONMENT

Starting in 2009 all new PhD candidates were integrated into the HZI Graduate School. Since the end of 2013 all PhD candidates on campus have taken part in a structured training programme (Figure 1) which can be compiled according to the individual's needs. About 170 young researchers actively participate in the HZI Graduate School. Of these, more than 40% have an international background. In 2015 the Graduate School included people from 27 different countries (Figure 2).

Thorough recruitment of new PhD candidates highly interested in the research areas of the HZI is an essential task for the centre. An elaborate system has been developed that affords stringent selection of candidates. To ensure a choice of excellent applicants an international announcement is launched yearly. The progress and preselection of applications is monitored with an online application tool. Video and on-site interviews complete the process.

High quality standards are in place to ensure optimal training. This includes not only professional recruitment of the PhD candidates but also accreditation of the supervisors, evaluation of the PhD projects on offer and assessment of all available lectures and courses. Supervision is supported and ensured by thesis committees of scientists who meet regularly to track the progress of the PhD project. The training programme complements the individual research activities and aims at optimal preparation of the young researchers for their future careers. They are trained in a broad spectrum of research areas relevant for infection defence and have to master classical and novel techniques. The programme covers basic research and translational aspects, thus qualifying young scientists in the field of infection research.

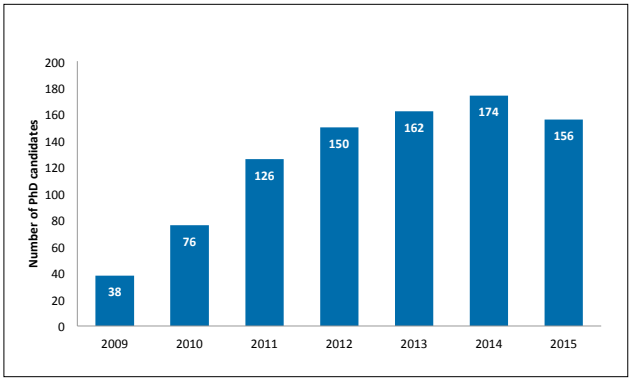


Figure 1: Number of PhD candidates on the HZI campus since the start of the Graduate School in 2009.

CURRICULUM AND SCIENTIFIC MEETINGS

The Graduate School offers project-oriented postgraduate training for PhD candidates in which they learn to carry out thorough and independent scientific work while conducting research on their own three-year scientific project.

The main focus of the curriculum (Figure 3) concerns a series of "topic lectures". Currently eleven topic lecture series are offered, each consisting of eight lectures. These are given by selected researchers from the HZI or partner institutes. Each lecture is supplemented by a seminar in which PhD candidates present relevant original publications. They present and discuss their work annually during a retreat in which all HZI Graduate School participants and supervisors take part. Another occasion involving only young researchers from the Graduate School and external PhD programmes is the "Christmas PhD Symposium". This allows them to present their work as posters and talks, if selected. Awards are given for the best presentations at both retreat and symposium.

The HZI Graduate School organises biennial Summer Schools focusing on state-of-the-art research in infection and immunity. Internationally renowned scientists act as teachers and mentors for these one-week events, allowing intensive scientific discussions of research projects and networking. Fourty PhD candidates participate in these Summer Schools of which six have been organised to date.

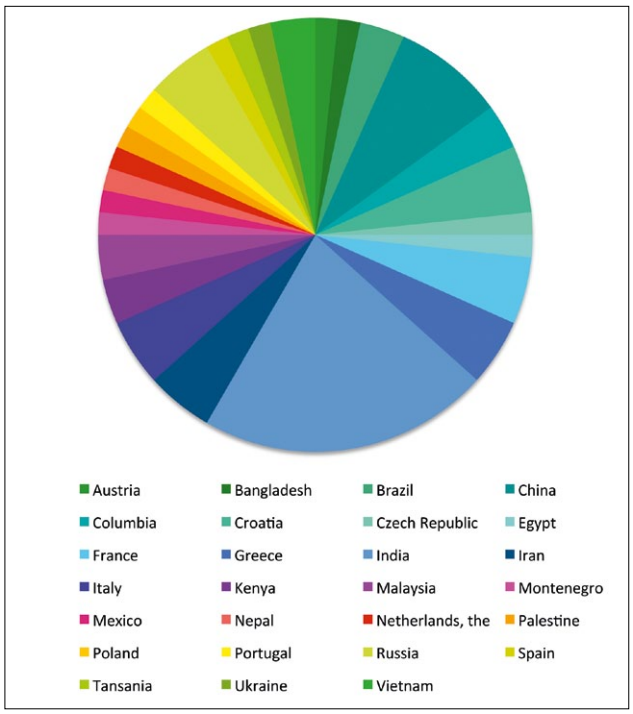


Figure 2: Internationality of the Graduate School: In 2015, the PhD candidates enrolled at the Graduate School originated from 27 different countries (Germany not included).

Mini-symposia entitled "A Day on..." arranged by the HZI Graduate School complement the curriculum. For instance, during the "Day on different aspects of translation" nine speakers from basic research, the pharmaceutical industry and clinical practice presented their approaches to translating research into application.

In addition, practical courses are regularly organised. To see other Helmholtz centres and meet peers there, weekend retreats for newly accepted PhD candidates take place.

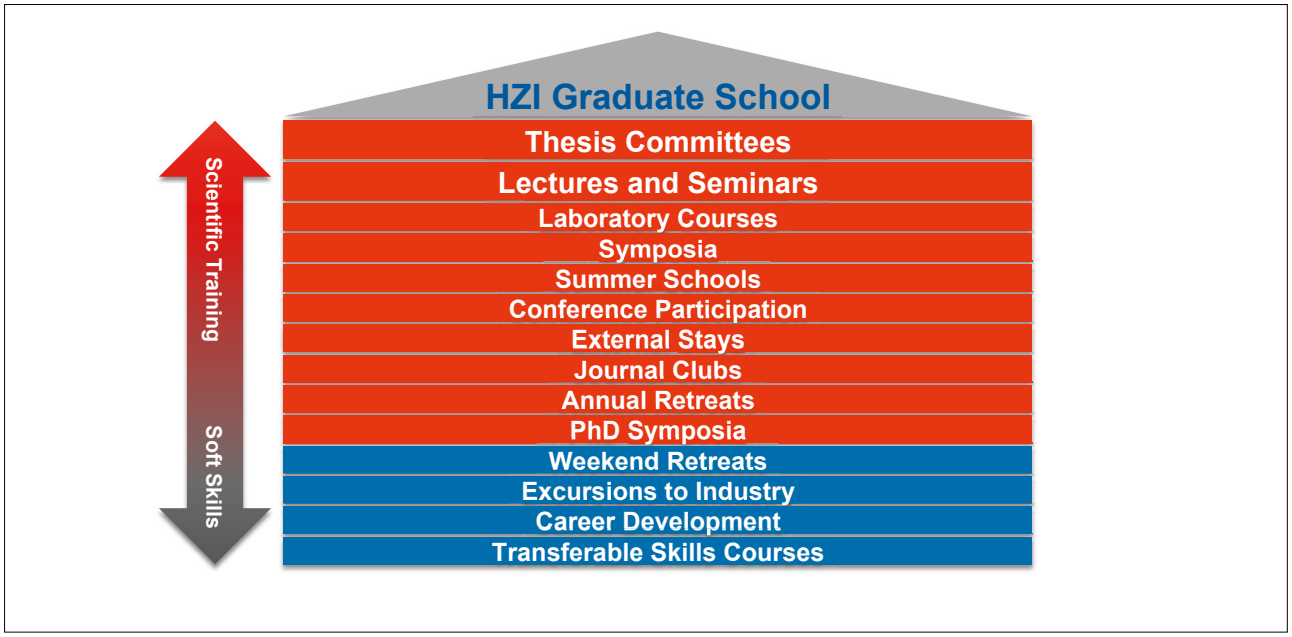


Figure 3: Curriculum of the HZI Graduate School

CAREER SUPPORTING EVENTS

As an integral part of the curriculum, career-supporting events are held. These include monthly workshops with invited speakers from different disciplines. The first Alumni Symposium with former HZI PhD candidates offered the opportunity to discuss scientific questions and personal issues with former HZI members who are now established in their positions in academic research, industry or other professions. Excursions to large pharmaceutical companies are also included in the programme. Transferable courses on career planning are regularly offered. These events alongside the regular curriculum complete the preparation of the next generation of infection researchers.

The programme of the HZI Graduate School has been successively improved and is today recognised as a model for PhD education at Helmholtz centres. During the institutional evaluation of the HZI in 2013 the Graduate School was singled out as an outstanding infrastructure element of the HZI by a panel of international referees.

Dr. Sabine Kirchhoff

SELECTED RESEARCH FIELDS

HEPATITIS C – TIME OF CHANGE

Prof. Thomas Pietschmann, Head of the Division "Experimental Virology", TWINCORE
Co-authors: Dr. Dorothea Bankwitz, Dr. Gisa Gerold, Dr. Sibylle Haid and Dr. Paula Perin

More than 25 years after the discovery of hepatitis C virus (HCV) – the causative agent of hepatitis C, an insidious viral liver disease that leads to liver fibrosis, cirrhosis and hepatocellular carcinoma – efficient and well tolerated treatments are finally available. These new drugs, so called directly acting antivirals (DAAs), can eliminate the virus and cure the infection. Thus, they offer hope and novel life perspectives to millions of chronically infected patients. Control of hepatitis C at the population level seems at close range. In fact, experts contemplate whether regional elimination or even global eradication of HCV is now feasible. Concomitantly, the HCV research field transforms and tackles unsolved challenges.

Hepatitis C virus: A late bloomer

In the late seventies of the past century, when hepatitis A virus (HAV) and hepatitis B virus (HBV) had been discovered and when a prophylactic vaccine for HBV was introduced, nevertheless numerous cases of blood transfusion-associated chronic liver disease (hepatitis) remained. It was at that time when the term "non-A, non-B hepatitis" (NANBH) was coined and when researchers set out to discover the elusive pathogen responsible for this form of transmissible liver disease. Using chimpanzees, the only animal that was found to be susceptible, first clues as to the nature of the pathogen had emerged and pointed towards a viral pathogen with lipid envelope. However, it was not before 1989 when the culprit of NANBH was finally discovered: Michael Houghton from Chiron and his team employed a cDNA-based expression approach. They probed an expression library derived from nucleic acids isolated from the serum of a chimpanzee infected with the NANBH-pathogen with antibodies from human sera of patients suffering from chronic liver disease due to NANBH. By this way they isolated a bacterial expression clone that carried and produced a non-chimpanzee gene sequence that resembled the genome structure of a flavivirus. The novel pathogen was baptized hepatitis C virus (HCV), the search for NANBH was completed and it was the first time a virus was discovered without ever culturing it. Diagnostic screening systems were rapidly implemented to identify HCV in blood and to prevent HCV transmission through transfusion and blood products.

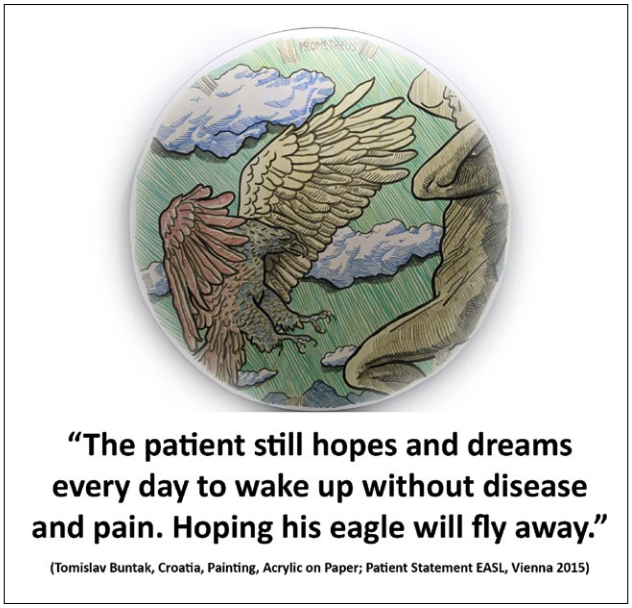


Figure 1: An ancient Greek legend tells the story of Prometheus, who is punished by Zeus in a way that every day, an eagle feasts on his liver. As Prometheus is immortal, his liver regenerates each night. Acute HCV infection is associated with only mild flu-like symptoms. However, in most cases HCV establishes a chronic infection that in the course of decades slowly destroys the liver. As a consequence, chronic HCV infection is a major indicator of severe liver disease in humans. Figure: Patient statement EASL meeting Vienna 2015

Viruses are obligate intracellular parasites which cannot propagate without a suitable host cell. Therefore, to develop drugs that specifically target the virus it is critical to have adequate cell culture models that permit virus replication. However, development of such cell systems turned out to be a major obstacle and road block for many years to come. Human hepatocytes, the primary host cell of HCV, are a highly differentiated and specialized cell type. When taken into cell culture these cells rapidly de-differentiate and die.

Control of Hepatitis C at the population level seems at close range – yet, several problems are still unsolved.

As a consequence HCV, which like all viruses heavily depends on functions of its host cells for propagation, encounters a deteriorating environment and is unable to propagate efficiently. To overcome this, at first, selectable viral minigenomes, so called replicons, were created and introduced into human liver tumour cells. This allowed first insights into the mechanisms of viral RNA replication. Finally, with a delay of 16 years, in 2005, cell culture systems that support complete HCV replication and infection were developed [1]. At last, these models permitted screening for HCV drugs targeting all stages of the viral life cycle by using authentic HCV viruses.

Translational research challenges – what remains to be done?

The fruit of these discoveries, the novel cell culture systems and massive efforts of pharma industry began to mature in May 2011: The FDA approved two drugs that both target the HCV NS3-4A protease – an enzyme the virus needs to digest its proteins into mature building blocks of its replication machinery. These were the first directly acting antivirals (DAAs) to be licensed and they much improved HCV treatment success. They also were the vanguard of a phalanx of second generation DAAs, which address either of three viral targets. Consequently, highly effective combination therapies are now available that are capable of curing chronically infected patients in more than 90% of cases.

However, three challenges remain: First, these drugs are costly which may limit therapy uptake particularly in low-income countries where the disease burden is highest. Second, the majority of infected patients are not diagnosed and thus do not know they are infected. Finally, treatment-induced viral clearance does not protect from re-infection by the virus. This is particularly of relevance in high at-risk groups for virus transmission, e.g. in people who inject drugs. Therefore, global public health efforts are needed to increase diagnosis and treatment uptake and consequently reduce HCV-associated disease burden. Ultimately, a prophylactic vaccine that prevents re-infection of treated patients and protects people at risk of infection could allow global eradication of the virus.

In light of this we investigate key principles for virus replication with an emphasis on the cell entry and virus egress steps. During virus release HCV acquires a coat of host lipoproteins. These host proteins facilitate binding and entry into new host cells and at the same time they protect from circulating antibodies thereby enabling chronic infection (Figure 2). By understanding how HCV engages with lipoproteins we aim to highlight novel strategies to strip HCV of its lipoprotein coat to increase its immunogenicity.

Curing chronic hepatitis C – the arc of a medical triumph.

"It may be possible to imagine the global eradication of HCV infection, but three major challenges remain: infection is often diagnosed at a late stage, the high cost of direct-acting antivirals may lead to selective use, and reinfection remains possible." (Chung R.T. and Baumert T.F. N Engl J Med 2014)

Open Sesame: How HCV sneaks into liver cells

HCV uses a minimal set of four entry factors to infect cells. These include the lipoprotein receptor SR-B1, the tetraspanin CD81 and the tight junction proteins CLDN1 and OCLN. In addition, accessory proteins facilitate virus attachment, trafficking at the cell surface, internalization and membrane fusion. Virus-receptor interactions initiate signalling cascades that prepare the host cell to internalize HCV. Thus, HCV itself sends cues to pave the way for its host cell invasion. To dissect the cell biological processes triggered by HCV receptor engagement we incubated host cells with a high dose of infectious HCV. Subsequently we isolated CD81

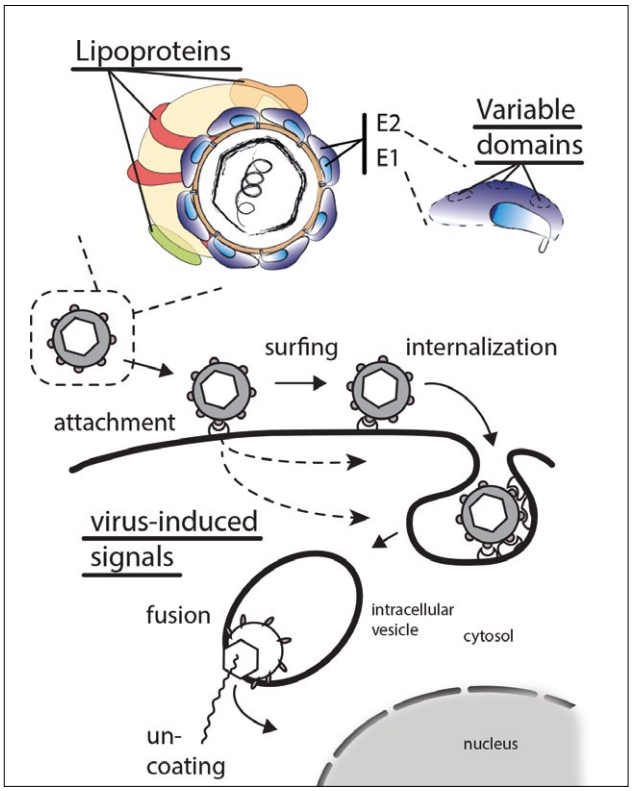


Figure 2: HCV particle composition and cell entry. HCV particles are composed of viral factors (RNA, capsid, envelope proteins E1, E2) and host-derived lipoproteins. The interaction with lipoproteins facilitates virus uptake and evasion from antibodies. The viral E1 and E2 proteins interact with cell surface receptors, thus inducing signalling events that coordinate virus internalization. They also mediate membrane fusion and they are targets for neutralizing antibodies.

and determined associated proteins by quantitative, high resolution mass spectrometry [2]. These studies revealed 26 dynamic CD81 binding partners, which either dissociated upon virus binding or which were recruited to the receptor. Functional follow-up studies showed that at least six of these host proteins facilitate HCV cell entry. This work reveals new avenues of HCV cell invasion and highlights the potential of quantitative proteomics for dissection of dynamic host pathogen interactions.

Catch me if you can: Virus evolution and viral vaccine development

Vaccines have been the most successful and cost effective means of preventing infectious disease burden. In fact, in some cases, rigorous use of vaccines resulted in complete eradication of a pathogen. In case of HCV, development of a prophylactic vaccine could much facilitate disease control and virus elimination. In fact, since there is no animal reservoir for HCV, availability of a vaccine could even allow global eradication of HCV.

HCV is the only RNA virus that readily establishes chronic, life-long infections in the majority of individuals that have been in contact with the virus. It has evolved a number of mechanisms that allow it to evade immune responses. Among these, HCV´s high variability strongly contributes to immune evasion. Due to error-prone replication and its vast replication capacity – HCV produces up to 10^{12} virus variants per day in an infected patient – it is always a step ahead of our immune system and evolves a variant that escapes adaptive immune responses. The structures of the virus that induce strong antibody responses are at the same time highly variable (Figure 2). Thus, the virus tolerates changes where most of the antibodies are attacking. To circumvent this, we and others now turn to identify those rare antibodies that bind highly conserved viral domains. Targeting such epitopes raises the hope that HCV would be unable to evade because functional constraints preclude changes of these epitopes. A few antibodies targeting highly conserved epitopes have been described today. Importantly, these antibodies not only neutralize a single viral strain, they are cross-reactive and inhibit HCV variants from divergent viral genotypes. In the future it will be critical to identify more of these antibodies, to map their target sites and particularly to devise antigens that direct immune responses to these highly vulnerable Achilles heels of the virus. Recently, we could show that deletion of the hypervariable domain 1 of the virus greatly facilitates access of antibodies to conserved viral epitopes [3]. Funded within the international Helmholtz-Alberta Initiative we are now aiming to translate this information into the development of HCV vaccine antigens that expose conserved target sites and thus induce strong neutralizing antibodies.

Over the counter migraine drug is a first in class HCV membrane fusion inhibitor

Together with our partners within HZI, HIPS, MHH and Leibniz Universität Hannover we have been using HCV cell culture models to screen compound libraries for molecules with antiviral activity and to analyse their mode of action. In the course of these studies we investigated molecules from myxobacteria and other natural sources [4]. Moreover, we screened a compound library including a number of licensed drugs [5]. Using this approach, we identified a number of structurally related clinically licensed compounds (phenothiazines and piphenylmethylpiperazines) that inhibit HCV infection in a dose-dependent fashion. Among our primary hits, we focused our attention on flunarizine, a (diphenylmethylpiperazine and) T-type calcium channel inhibitor used

to treat migraine. This drug specifically inhibits HCV cell entry at the membrane fusion step (Figure 3) and also reduces HCV infection in a mouse model for HCV cell entry. Of note, we were able to select for flunarizine resistance in cell culture. However, resistance mutations map to highly conserved viral domains. Moreover, these mutations rendered the virus more susceptible to neutralizing antibodies. Therefore, antibodies and functional constrains may limit viral drug resistance *in vivo*. Interestingly, these mutations map to a subdomain in the viral E1 envelope protein which has previously been implicated as viral fusion peptide. These findings should also help to further dissect the molecular principles of HCV membrane fusion.

Embedded into research consortia at the HZI and its partners and through collaborations within the focus topic "Chronic viral infections" we aim to unravel key principles of HCV replication and pathogenesis. Moreover, we aim to find new approaches and a model for HCV vaccine development and cost effective management of HCV patients.

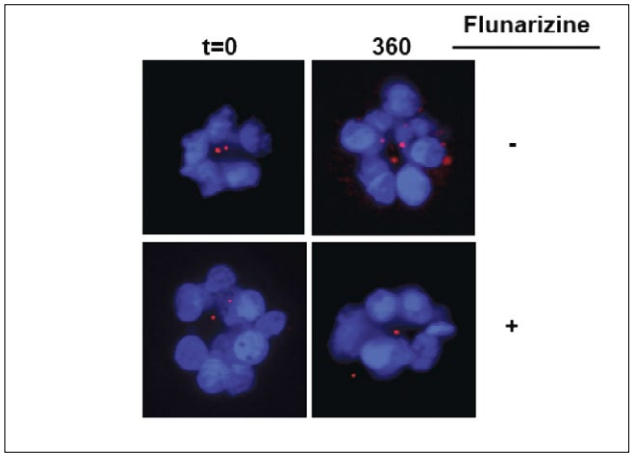


Figure 3: Single particle tracking of labelled HCV virions into liver organoid cultures. Flunarizine specifically inhibits membrane fusion of HCV particles (red puncta). Nuclei are stained in blue. Courtesy of Glenn Randall, University of Chicago, USA. Reprinted from Perin PM et al., Flunarizine prevents hepatitis C virus membrane fusion in a genotype-dependent manner by targeting the potential fusion peptide within E1, Hepatology (2015), with permission from John Wiley and Sons.

THOMAS PIETSCHMANN

born in **1971** in Würzburg, studied biology with emphasis on biochemistry, animal physiology, virology and immunobiology at the University of Würzburg and the Duke University (Durham, NC, USA). After completing his studies in **1996**, he received his Ph.D. degree in biology at the Institute for Virology of the University of Würzburg and subsequently worked as postdoc at the Institute for Virology in Mainz and in the Department for Molecular Virology in the University Clinic of Heidelberg. There, Thomas Pietschmann established an independent research group that investigated the mechanisms of morphogenesis and cell entry of the hepatitis C virus. Since **2006** his group was supported by an Emmy Noether fellowship from the German Research Community. In **2007** he was appointed with his work group to TWINCORE. He now leads the Institute for Experimental Virology there and holds a professorship at the MHH.

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THE BACTERIAL CRISPR-CAS9 SYSTEM

Prof. Emmanuelle Charpentier, Head of the Department "Regulation in Infection Biology"
Co-Author: Dr. Jennifer Debarry

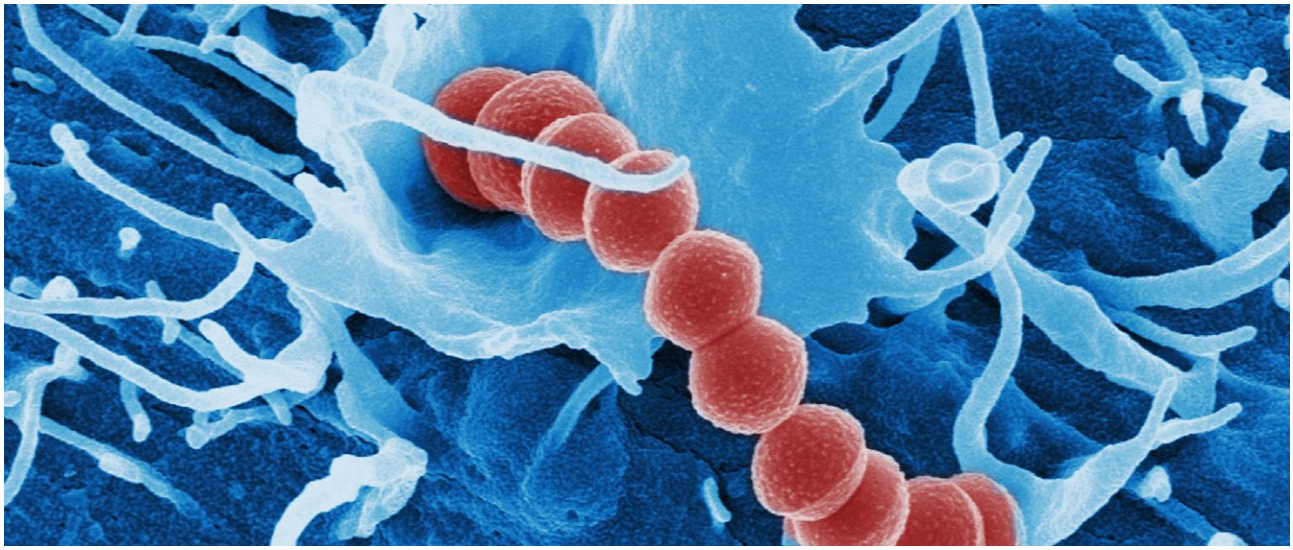

Figure 1: Electron microscopic image of *Streptococcus pyogenes* on human epithelial cells. Study of RNA regulation processes in this pathogen led to an in depth understanding of the bacterial CRISPR-Cas9 defence system and its subsequent exploitation as genome editing tool (Image: HZI/Manfred Rohde).

With groundbreaking findings in the field of RNA-mediated regulation based on the CRISPR-Cas9 system in the human pathogen *Streptococcus pyogenes*, the foundation for the development of a novel, highly versatile and specific genome editing tool has been laid. The mechanism of dual-RNA programmable CRISPR-Cas9 endonuclease in bacteria has been harnessed into a novel broad, versatile and efficient tool for genome engineering in cells and organisms of the three kingdoms of life. It could be demonstrated that dual-RNAs or dual-RNAs engineered as single transcripts can be programmed to target any DNA sequence of interest. Recently, a novel enzyme from a new type of CRISPR-Cas systems has also been studied in our laboratory.

An ancient bacterial defence system

CRISPR-Cas9 genome engineering stems from the initial discovery of trans-activating CRISPR RNA (tracrRNA), a small RNA molecule in bacteria. Originally, our research was aiming at understanding RNA-mediated regulation in the human pathogen *Streptococcus pyogenes*. By conducting a computational search for novel RNAs with possible regulatory functions in bacterial virulence, tracrRNA was highlighted as a small RNA encoded in the vicinity of a type II CRISPR-Cas locus.

CRISPR-Cas is an RNA-mediated adaptive immune system that protects bacteria and archaea from invasion of mobile genetic elements such as phages and plasmids. The system is composed of an operon of *cas* genes encoding the CRISPR-associated (Cas) proteins and a CRISPR array of short identical repeats interspaced with short invader-targeting unique spacers (the abbreviation CRISPR stands for "Clustered regularly interspaced short palindromic repeats") encoding the CRISPR RNA (crRNA) components. The CRISPR-Cas systems are highly diverse in sequence and *cas*

gene organization, and have evolved in six main types with multiple sub-types.

The CRISPR-Cas dogma proposed in the years 2005-2006 and demonstrated in subtypes I and III in the years 2007-2008 was as follows. The CRISPR array is transcribed into a precursor crRNA molecule that undergoes processing by a Cas endoribonuclease to generate the individual mature crRNAs. These crRNAs serve then as guides to target a complex of Cas proteins to cleave the cognate target nucleic acid invader.

Although this mechanism was initially thought to function for all CRISPR-Cas subtypes, we set to investigate whether the tracrRNA in *S. pyogenes* would not have a regulatory role in the expression, activation and function of the type II immunity elements. A detailed genetic and biochemical analysis of these elements resulted in the discovery of a novel mechanism for the maturation of the short guide crRNAs that is unique to type II CRISPR-Cas [1].

tracrRNA contains an anti-repeat sequence that allows it to base-pair with each of the repeats of the precursor crRNA. The duplex RNAs stabilized by the CRISPR-associated protein Cas9 are cleaved by the bacterial enzyme RNase III, leading to mature tracrRNA bound to intermediate forms of crRNAs, which undergo a second maturation event to produce the dual-tracrRNA:crRNAs. With the involvement of RNase III, the pathway was found to be reminiscent of mechanisms of RNA interference described in higher organisms, however unique in all kingdoms of life [1].

A novel tool for biotechnology

Further characterization of the mechanism responsible for invader nucleic acid targeting aimed at demonstrating the potential of the minimal type II CRISPR-Cas9 system as a genetic tool. All components of the maturation process, tracrRNA, pre-crRNA, RNase III and Cas9 were shown to be essential in the defence against temperate phages [1]. A biochemical study followed that resulted in the discovery of a novel nucleic acid interference mechanism specific for type II CRISPR-Cas systems and distinct from type I and type III, which use a single crRNA to guide a complex of Cas proteins to the invader.

We showed that in type II, dual-tracrRNA:crRNA characterized by the typical bipartite anti-repeat–repeat structure guides the single enzyme Cas9 to cleave site-specifically cognate target DNA [2]. It was demonstrated that the system could easily be simplified by combining tracrRNA and crRNA into a single guide RNA (sgRNA) molecule that would still retain the dual-RNA structure. Further achievements resulted in the demonstration that, as predicted, such devised system could have high potential to revolutionize methods for site-specific genome editing in the three kingdoms of life [2].

In addition, we showed that the endonuclease Cas9 naturally uses two distinct nuclease domains to cleave each strand of the target DNA, allowing easy conversion of the enzyme into a site-specific RNA-guided nuclease, nickase or DNA-binding enzyme [2]. This unique RNA-programmable genome editing technology has already proven to provide substantial advantages over previously established gene manipulation strategies (e.g. zinc-finger or TALEN nucleases) that require significant protein engineering and cannot easily be manipulated to promote functions other than site-specific DNA cleavage. With the CRISPR-Cas9 tool, only the RNA component of the system has to be engineered newly by adjusting the guide sequence of the sgRNA according to the DNA target to be silenced or regulated (e.g. double-stranded DNA cleavage using Cas9 as a nuclease, nicking of the DNA using Cas9 as a nickase, regulation of transcription using Cas9 as a DNA-binding protein fused or not to other regulatory components) or modified (e.g. using Cas9 as a DNA-binding protein fused to a DNA modification enzyme).

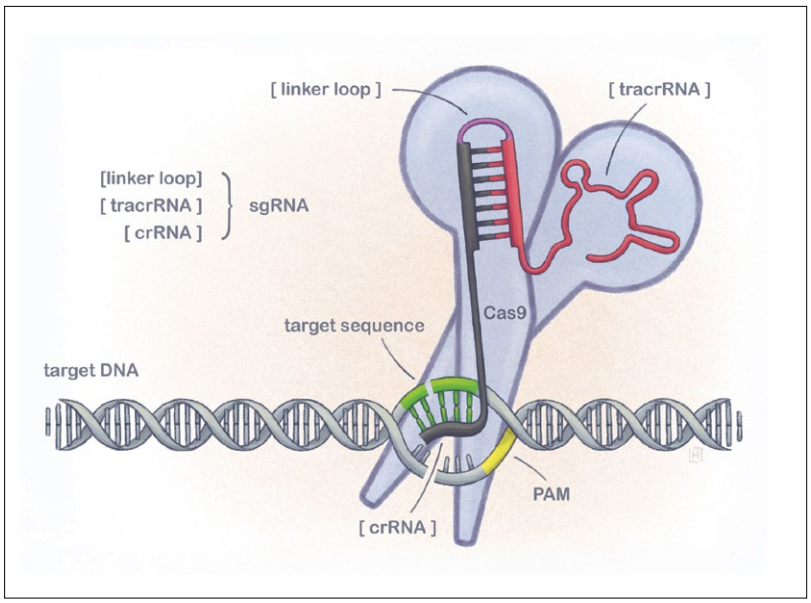

Figure 2: CRISPR-Cas9, the RNA-programmable "DNA scissors": The enzyme Cas9 (blue, represented as scissors) is guided by a single guide RNA (dual-tracrRNA(red)-crRNA(black), linked together with a linker (purple)) programmed to cleave the target DNA in a sequence specific manner. A PAM sequence (yellow; NGG for the Cas9 of *Streptococcus pyogenes*) located downstream of the targeted DNA sequence on the non-target sequence is required. *Deltcheva et al., 2011 (Nature); Jinek, Chylinski et al., 2012 (Science); Drawing by Linnea Holmström Ljung.*

The CRISPR-Cas9 genome engineering device that is now widely used by the scientific community originates from the natural tracrRNA:crRNA-Cas9 system of the bacterial pathogen *S. pyogenes*. Orthologous systems to the *S. pyogenes* system [3, 4], which we identified and for which we demonstrated a functional orthogonality expand the possibilities for multiplex genome editing and could provide means to improve the specificity and versatility of the RNA-programmable Cas9 tool.

Recent findings

CRISPR–Cas systems that provide defence against mobile genetic elements in bacteria and archaea have evolved a variety of mechanisms to target and cleave RNA or DNA. The well-studied types I, II and III utilize – as described above – a set of distinct Cas proteins for production of mature crRNAs and interference with invading nucleic acids.

Recently, we discovered a novel feature in CRISPR–Cas immunity: the CRISPR-associated protein Cpf1 exhibits dual, RNA and DNA, cleavage activity. In contrast to CRISPR-Cas9, Cpf1 is able to process the pre-crRNA on its own and then use the processed RNA to specifically target and cut DNA [5].

More specifically, we have shown that type V-A Cpf1 from *Francisella novicida* is a dual-nuclease that is specific to crRNA biogenesis and target DNA interference. Cpf1 cleaves pre-crRNA upstream of a hairpin structure formed within the CRISPR repeats and thereby generates intermediate crRNAs that are processed further, leading to mature crRNAs. After recognition of a specific protospacer adjacent motif on the non-target DNA strand and subsequent probing for a seed sequence, Cpf1 introduces double-stranded breaks in the target DNA. We showed that the RNase and DNase activities of Cpf1 require sequence- and structure-specific binding to the hairpin of crRNA repeats and that Cpf1 uses distinct active domains for both nuclease reactions.

This study uncovers a new family of enzymes with specific dual endoribonuclease and endonuclease activities. Not requiring a host derived RNase and the tracrRNA makes

it the most minimalistic CRISPR-Cas system described so far allowing for possible new avenues of sequence specific genome engineering [5].

Outlook

The described mechanisms of interference with nucleic acids by the CRISPR-Cas9 system have received considerable attention from the scientific community and are meanwhile in common use in laboratories around the world. Within a very short time, the efficacy of the device to manipulate genes and their expression in human cells and numerous other cells and organisms (e.g., plants, mice, drosophila, monkeys) has been demonstrated.

CRISPR-Cas research has rapidly developed into one of the most dynamic and fastest-moving fields in life sciences and holds great promise for future biotechnical and biomedical applications. In particular, it is expected that the system could be a very valuable tool for the repair of selected genes in human cells, and thus could be exploited for human therapeutics. This includes gene therapy for human genetic disorders and potential treatment of chronic diseases.

EMMANUELLE CHARPENTIER

Emmanuelle Marie Charpentier was Head of Department at the HZI and Professor at Hannover Medical School from **2013 to 2015**. She is currently a Scientific Member of the Max Planck Society and Director at the Max Planck Institute for Infection Biology in Berlin. Charpentier is also Alexander von Humboldt Professor and Visiting Professor at the Laboratory for Molecular Infection Medicine Sweden at Umeå University. Prior to her appointments in Germany and Sweden, she was Assistant and Associate Professor at the Max F. Perutz Laboratories at the University of Vienna. She has also held research associate positions at the Rockefeller University, New York University Langone Medical Center, the Skirball Institute of Biomolecular Medicine and the St. Jude Children's Research Hospital. Charpentier's research unveiled the key mechanisms of the CRISPR-Cas9 technology, laying the foundation for CRISPR-Cas9 as a broadly developed genome engineering technology. She has received a number of national and international awards and distinctions for this work.

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Chylinski K, Makarova KS, Charpentier E, Koonin EV. *Classification and evolution of type II CRISPR-Cas systems.* Nucleic Acids Res. **42**(10):6091-6105. (2014)

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IMPLEMENTATION OF MYCOLOGICAL DIVERSITY IN DRUG RESEARCH

Prof. Marc Stadler, Head of the Department "Microbial Drugs"
Co-authors: Dr. Eric Kuhnert, Dr. Frank Surup

Figure 1: Potential drug sources from nature: Earth stars in the jungle of Northern Thailand (Christian Richter)

The HZI has a strong tradition with regard to the discovery of natural products with great potential in pharmaceutical and agrochemical applications. The advent of multi-resistant pathogenic microbes has recently led to a renaissance of this research, since most marketed antibiotics are derived from natural sources. The research in this field at the HZI was hitherto based on gliding bacteria, from which numerous novel anti-infectives have recently been discovered. Since 2012, fungi and their secondary metabolites have been introduced as a complementary source of chemical innovation, to be added to the existing screening libraries. Here, we summarise our strategy and highlight the most important accomplishments to date.

Focussing on unexplored fungi

Fungi have been important sources of novel antibiotics and other drugs ever since the advent of drug research. Examples like the beta-lactam antibiotics (penicillins, cephalosporins), immunomodulators like cyclosporin and scyphostatin, the antimycotic caspofungins, and above all the statins, the most successful class of therapeutic drugs ever discovered, amply illustrate the importance of fungi in drug research. The organisms that produce the aforementioned drugs are mainly soil-inhabiting fungi that belong to a rather restricted number of genera and families. Like the streptomycetes among the bacteria, they can easily be cultured and have now been explored for over 70 years.

Nevertheless, modern phylogenomics and molecular ecology research has meanwhile revealed that the traditional drug producers are heavily outnumbered by other fungal species that inhabit ecological niches such as plants and insects, and that even the soil contains numerous species that have apparently never been cultured and studied. The number of existing fungal species is now estimated to exceed 5 million, while only ca.100,000 species are known to science as yet, most of which have never been explored systematically for antibiotics production.

Fungi have been important as sources for novel antibiotics and other drugs ever since the advent of drug research.

Figure 2: Impressions from the Thai jungle (Robert Lücking)

Our rationale is that we must focus on these unexplored organisms, using sophisticated isolation strategies and classical taxonomic as well as microbiological know-how, in order to increase the probability of finding new lead structures. We are aiming at establishing a screenable library of fungal compounds as soon as possible to integrate into the general screening activities at the HZI. For this purpose, an international collaboration network was established that involves mycologists and natural product researchers from around the world. Some case studies and projects show how this network works and what has been accomplished so far.

International collaboration networks

In collaboration with scientists in Germany, Belgium, Kenya, South Africa and Thailand, we are systematically exploiting the tropical basidiomycetes for novel antibiotics and other useful secondary metabolites.

A collaboration funded by DAAD (Deutscher Akademischer Austauschdienst) and the Thailand Research Fund (TNF) has been ongoing since summer 2012. The project started with joint field work and involved several academic exchanges of PhD students and postdoctoral researchers between the partner institute at Mae Fah Luang University (MFU, Chiang Rai) and the HZI. Several new species were discovered in the rainforests of Northern Thailand. Over 150 mycelial cultures were made on-site, purified at MFU and then transferred to Germany where Thai and German PhD students were involved in their screening. Even though it took some time to produce the extracts of these slow-growing untapped organisms, and the scale-up of production is sometimes tedious, the first antibiotics from these organisms have already been published [1].

A similar collaboration with Egerton University (Kenya), University of KwaZulu-Natal (South Africa), the culture collection BCCM/MUCL (Louvain, Belgium) and chemists from the Technische Universität Berlin has been funded by the EU in the ERAFRICA program. Several students from Asia and Africa have meanwhile visited the HZI for internships or are even about to get their PhD in Germany. Another pro-

Figure3: D. Anuparma Daganarama (left) and Benjarong Thongbai (middle), Ph. D. students of Mae Fah Luang University who are being co-supervised by HZI researchers on a foray at the Mushroom Research Centre, Chiang Mai Province

Figure 4: Thai and HZI PhD students Benjarong Thongbai (right), Christian Richter and Eric Kuhnert (standing) at work – isolation of cultures from freshly collected specimens with M. Stadler (Robert Lücking)

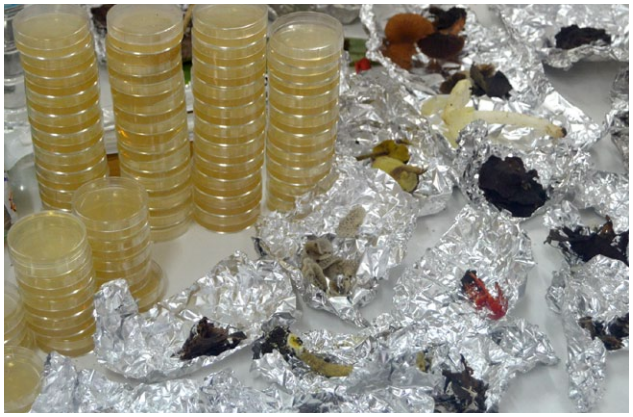

Figure 5: Agar plates and herbarium specimens (Robert Lücking)

Figure 6: Cascade near Chiang Mai (Robert Lücking)

ject on international academic and staff exchange, involving the research institute BIOTEC, Thailand, and the CBS-KNAW Biodiversity Centre (Utrecht, the Netherlands) has recently received substantial funding from the EU in the course of the Horizon 2020 programme. Several other collaborations with scientists in e.g., Algeria, Argentina, Japan, China, USA and all over Europe have also been started. Numerous novel anti-infectives can result from these projects in the near future, but most importantly, these activities will help to create an international network of young scientists. They will be able to combine traditional knowledge and modern technology in natural product-based drug research from first-hand experience in a multicultural environment.

Correlations between biodiversity and chemical diversity in the "endophyte model family" Xylariaceae

The rationale for pre-selection of interesting organisms for screening can best be demonstrated by our ongoing work on the ascomycete family Xylariaceae. These fungi are ubiquitously present as wood degraders, but are also often encountered as endophytes of living plants, and some genera and species are constantly associated with insects. While the species of the temperate zone have already been studied extensively, the tropical Xylariaceae are still widely unknown and appear to be an excellent source for new bioactive metabolites.

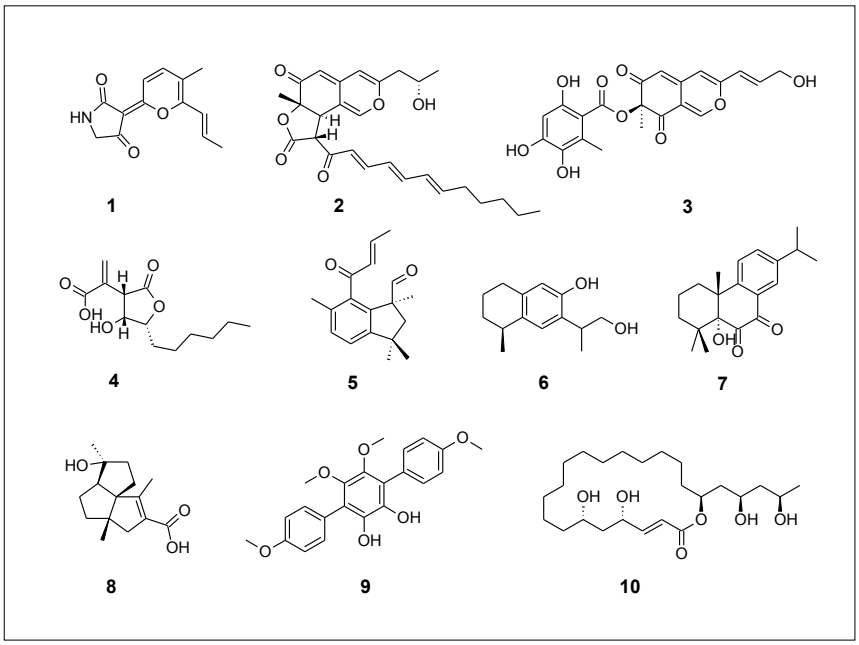

Figure 7: Chemical structures of bioactive compounds from *Hypoxylon* species discovered at the HZI. **1** Hypoxyvermelhotin A (tetramic acid derivative, see Kuhnert et al., Fungal Biol. 2014, 118: 242-252); **2** Lenormandin B (azaphilone polyketide, see Kuhnert et al., Fungal Divers. 2015, 71: 165-184); **3** (+)-6"-hydroxymitorubrinol (azaphilone polyketide, see Sir et al., Mycol. Progress 2015, 14: 28; **4** Sporothric acid (polyketide; see Surup et al., Mycol Int J Fungal Biol 2014, 5: 110-119); **5** (Novel botryane sesquiterpenoid); **6** Hypoxylane A (14-noreudesmane sesquiterpenoid); **7** rickitin A (abietane diterpenoid, for **5-7**: see Kuhnert et al., Phytochemistry 2015, 117: 116-122); **8** rickinic acid (silphiperfolene sesquiterpenoid, see Surup et al., Nat Prod Bioprospect 2015, 5: 167-173); **9** rickenyl A (novel terphenyl, see Kuhnert et al., Phytochemistry 2015, 118:68-73; **10** rickiol A (unpublished polyketide with novel carbon skeleton). Compounds **5-10** and over 20 further metabolites were isolated from the same strain of *Hypoxylon rickii*, and all depicted compounds **1-10** were obtained in the course of a single PhD thesis (Kuhnert et al., Dissertation TU Braunschweig 2015).

Our monographic studies [2, 3] involving a combination of chemotaxonomic, molecular phylogenetic and morphological techniques have been ongoing since 2001, and data on almost 10,000 specimens are now available. This facilitates the recognition of rare, unexplored or even novel species, from which often unprecedented metabolites can be expected [4]. The fruiting bodies and cultures often contain entirely different metabolites [5], and upon optimisation of culture media and fermentation conditions as well as subsequent scale-up to stirring fermentors, it is often possible to obtain more than 50 compounds from a single strain. Recent studies on the genome sequences of the ascomycete order Xylariales have revealed that some strains actually contain almost 100 genes and gene clusters encoding for secondary metabolites. From a single fermentation of *Hypoxylon rickii*, we were able to isolate more than 20 novel metabolites that belong to various biosynthetic classes, including five different scaffolds of terpenoids, terphenyls and polyketides (see Eric Kuhnert, PhD thesis, Technische Universität Braunschweig, 2015).

Fungal metabolites in drug research at the HZI

The fungal culture collection at the HZI has grown rapidly and currently already comprises over 1,000 strains. Many other strains are accessible via collaborations with our global network of leading biodiversity researchers. The challenge for the future will be to prepare extracts for screening and provide those to the partners at the HZI and associated collaboration partners, e.g. in the DZIF.

It will take time and additional labour to set up a comprehensive fungal screening library and maintain the follow-up work for hit-to-lead characterisation. Nevertheless, over 100 novel antibiotics and other bioactive compounds have already been obtained by students and guest researchers in the mycochemical lab, and many of them are already part of the HZI screening library.

Moreover, the first collaborations on the biosynthesis and total synthesis of our fungal lead compounds have already commenced, and several highly promising projects with partners from German universities as well as other Helmholtz centres are currently under way. Therefore, we can hope that fungal metabolites will soon play a pivotal role in the drug research scenario at HZI.

MARC STADLER

has been head of the department “Microbial Drugs” at the HZI **since 2012**. He also is teaching at Technische Universität Braunschweig. He received his PhD in Biology/Biotechnology in **1993** at University of Kaiserslautern. After a postdoctoral research stay at Lund University, Sweden (Org. Chemistry, **1994/95**), he joined Bayer Pharma division in **1995** and gained in total 16 years of industrial experience. He has in parallel received the venia legendi for Mycology at Bayreuth University in **2009** and was appointed as Visiting Professor at the Institute of Microbiology, Chinese Academy of Science, Beijing, in **2013**. He is Acting Vice President of the International Mycological Association (IMA) and member of the Editorial Board of several leading mycological scientfic journals.

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THE LONG TERM VISION: INFECTION RESEARCH WITHIN THE GERMAN NATIONAL COHORT

Prof. Gérard Krause, Head of the Department "Epidemiology"
Co-Author: Dr. Yvonne Kemmling

Figure 1: Taking blood samples is part of the examination procedure at the study centre.

Figure 2: The medical history is taken routinely for all participants.

It is well known that chronic infections contribute to non-communicable conditions such as cardiovascular, metabolic, malignant or neurodegenerative disease. However, increasing evidence suggests that this holds true for acute infections as well. One important challenge in establishing such associations is that they cannot be investigated experimentally in humans. Examining this hypothesis in patients who have already developed such a non-communicable disease carries the problem that the alleged contributing infections are likely to have occurred a long time before and it is no longer possible to detect them. In order to overcome such challenges the German National Cohort (GNC) study, Germany's largest epidemiological project, will examine the health status of 200,000 adults from the general population prospectively over several decades. Those individuals who later develop cardiovascular illness, for instance, will then be assessed with respect to past infections and other risk factors, and will be compared to those individuals who remain healthy.

Linking infections to non-communicable diseases

Establishing causal associations between infections and subsequent non-communicable diseases has immense consequences because it will inevitably open the way for new diagnostic, preventive and therapeutic measures. A good example for this is cervical cancer, of which some forms can now be prevented by vaccination against human papilloma virus. For past acute infections such associations are much more difficult to detect than for persistent chronic infections because the pathogens, or even their immunological traces, may no longer be detectable by the time the non-communicable diseases become evident. There is therefore an urgent need for prospective cohort studies to validate and quantify these potential associations.

In this cohort study, 200,000 people aged between 20 and 69 from across Germany will be medically examined and questioned on their living habits

The aim of this long-term population survey is to explain the causes of widespread diseases such as cardiovascular disorders, cancer, diabetes, dementia and infections, and to identify risk factors, highlight effective forms of prevention and identify options for their early detection. In this cohort study, 200,000 people aged between 20 and 69 from across Germany will be medically examined and questioned on their living habits, e.g. physical activity, smoking, diet, occupation etc. In addition, all participants will supply blood samples which will be stored in a central biobank for later research projects. After four to five years, they will be invited to complete a second questionnaire and undergo another examination at their local study centre. They will continue to be observed over the following 5 - 15 years, during which time some are bound to develop diseases. We will then be able to look back at the data collected earlier from these individuals. Compared to other large scale cohort studies worldwide one unique characteristic of the GNC project is that infections are part of the research portfolio. The Department "Epidemiology" at the HZI coordinates infection-related research activities within the GNC project.

The German National Cohort ("Nationale Kohorte") is unique in its dimensions and level of detail

The Hannover study centre

The Department "Epidemiology" has established the Hannover GNC study centre and is responsible for vaccination assessment at all 18 GNC sites. The facilities of the Hannover GNC study centre are located in the newly established Clinical Research Center (CRC) in Hannover. Three strong partners - HZI, Fraunhofer ITEM and MHH - founded the CRC jointly. The study centre staff of the HZI comprises healthcare professionals such as physicians, nurses, a receptionist and technical assistants, all trained and certified according to the standards of the GNC. It is equipped with examination rooms, interview rooms and touch-screen work stations. Medical equipment includes: Philips iE33 Echocardiography System, Sana Bike 350 F ergometer, Easy on-PC Spirometry, NIOX VERO® for assessing airway inflammation, VASCULAR EXPLORER for automatic detection of arteriosclerotic vascular lesions, equipment for skin autofluorescence measurement of advanced glycation end products and a digital non-mydriatic fundus camera.

Since the start of the main study over 1,900 participants have been recruited at the Hannover site. Between now and 2018, 10,000 participants from the greater metropolitan area of Hannover will have to be examined and interviewed.

Basic GNC measurements include anthropometric indices, blood pressure, vascular stiffness, spirometry, physical activity and a tooth count. The medical history taken during the GNC study centre visit consists of a computer-assisted personal face-to-face interview (CAPI), and a touch-screen questionnaire containing up to 183 questions on a large variety of diseases, demographic information and exposure (e.g. dietary habits, tobacco use, occupational exposure). In addition, biomaterials including blood, urine, saliva, nasal swabs and stool are collected from all participants.

Figure 3: Measurements at the study centre include a tooth count.

For near-instantaneous sample preparation, we have access to a biosafety level 2 laboratory equipped with a HAMILTON automated pipetting system.

The second level of the GNC study, which involves 20% of the main cohort, consists of an intensified protocol involving an oral glucose tolerance test, electrocardiography, measurement of airway inflammation, vaccination data collection and assessment of oral health (see also: http://nako.de/wp-content/uploads/2015/07/Wissenschaftliches-Konzept-der-NAKO2.pdf).

In addition, add-on Level 3 studies may be conducted at some or all of the study centres. Level 3 studies allow more detailed phenotyping and depend on external funding. We have developed a plan for a set of such surveys on infectious diseases to be carried out in Hannover and at other collaborating study centres. These include novel methodological approaches to capture acute infections prospectively as they occur during the five year intervals between examinations. Preparatory investigations and pilot studies have been successfully completed [1]. Implementation of these Level 3 studies would make it possible to investigate the association between frequent acute infections and predisposing risk factors, and also between acute infection types and subsequent non-communicable diseases.

GÉRARD KRAUSE

studied medicine at the University of Mainz and completed his research doctorate in tropical hygiene with the University of Heidelberg. From **1993 to 1998** he worked as clinician and research associate at different hospitals in Germany before he went on to work as epidemic intelligence service officer at the Centers for Disease Control and Prevention, Atlanta, USA. In **2000** he movedto the Robert Koch Institute (RKI) in Berlin, first as head of the surveillance unit, from **2005** to **2013** as director of the department for infectious disease epidemiology. In **2005** he received the venia legendi in Epidemiology and Hygiene at the Charité University Medicine in Berlin. In **2011** Krause accepted the position as full professor for infectious disease epidemiology at Hannover Medical School (MHH) and became head of the Department "Epidemiology" at the HZI. In **2013** he founded the PhD Programme Epidemiology at MHH.

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PERSPECTIVES OF NON-INVASIVE VACCINE DELIVERY

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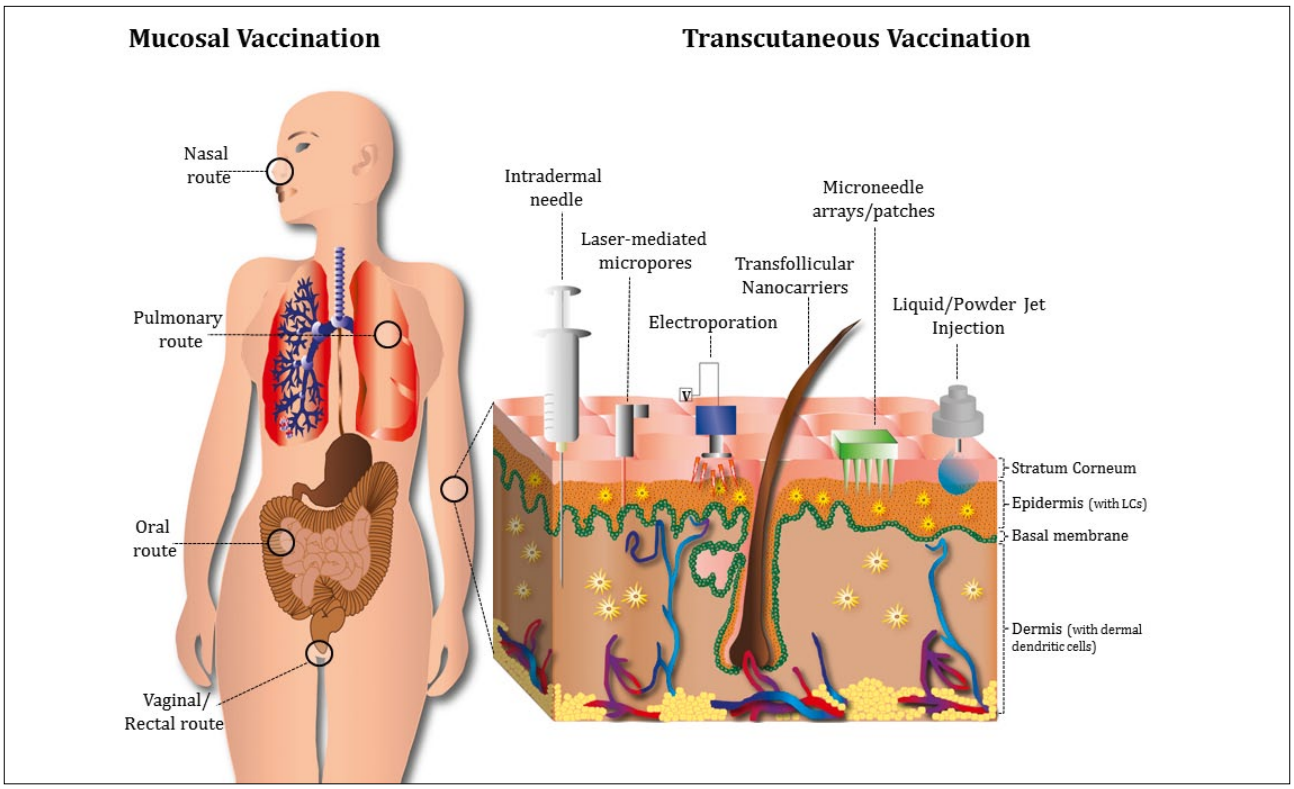

Figure 1: Different approaches for mucosal and transcutaneous vaccination (TCV). For TCV the goal is to reach the epidermal and dermal Langerhans cells (LCs) and dendritic cells with minimal impairment of the protective barrier function, mainly represented by the uppermost layer (stratum corneum). Image: HZI/Hanzey Yasar

For over 150 years, vaccines have been commonly administered via needle and syringe. Significant efforts in the field of vaccine research currently focus on new technologies and strategies which offer minimally- or non-invasive alternatives to injectable vaccination. In this respect mucosal administration (e.g. oral, intranasal, sublingual), but also needle-free (NF) administration via the skin, have recently shown considerable promise. Recent progress in non-invasive vaccine delivery research has been made at the HZI/HIPS, as emerging from collaboration between the Department "Vaccinology and Applied Microbiology" (VAC) and the Department "Drug Delivery" (DDEL).

Mucosal and transcutaneous vaccination

Vaccination is widely regarded as one of the foremost contributions of medical research to society, lowering both morbidity and mortality associated with infectious diseases. The therapeutic use of vaccines against both communicable and non-communicable diseases is also raising considerable interest. The routine administration of vaccines using needle and syringe is however associated with numerous disadvantages, e.g. lack of patient comfort and compliance, requirement of trained personnel for injection administration, and potential risks to safety associated with needle-stick accidents or needle reuse.

New technologies and strategies offer "needle-free" alternatives to injectable vaccination.

To overcome these limitations a variety of NF methods for vaccine administration have been developed, which are continually being further improved and tested. Two general application pathways for NF vaccination are used in this respect: mucosal vaccination approaches comprise vaccine delivery to the mucosal membranes such as those of the eye, nose, lung, vagina, gut and rectum, whereas transcutaneous vaccination (TCV) involves vaccine application either into or onto the skin (Figure 1). One major advantage of mucosal application with respect to needle-based parenteral vaccination is the stimulation of both systemic and mucosal immune responses. The mucosa is the major port of entry for infectious agents. Thus, the stimulation of an efficient local immune response at mucosal sites would not only lead to protection against disease (i.e. symptoms), but would also promote effective protection against infection (i.e. colonization). This will in turn reduce the potential risk of horizontal transfer to susceptible contacts. On the other hand, the skin also represents a particularly attractive target for non-invasive vaccination, since it constitutes an easily accessible administration site.

Skin Vaccination Methods

The skin is also equipped with a rich pool of immune cells known as antigen presenting cells (APCs), which are capable of detecting, taking up, processing and presenting antigens to naïve T cells. Interaction with a particular subset of APCs known as Langerhans cells (LCs) located in the epidermal skin layer is both a possibility and a distinct advantage of TCV, as this interaction offers the potential for induction of strong immune responses. Thus, a broad spectrum of novel TCV methods designed to facilitate entry of vaccines into or through the skin in a minimally-invasive manner are currently under investigation. These include vaccine administration by means of liquid or powder jet injection, application of micron-sized needles (microneedles) to create microscopic skin pores, electrical pulsing of the skin (electroporation), use of agents to increase skin permeability, and laser-mediated generation of micropores (Figure 1).

While such techniques represent a significant step forward from the use of needles and syringes, they continue to rely on disruption of the outermost stratum corneum (SC) skin layer in order to facilitate vaccine permeation. Reduction of this protective barrier may lead to an increased risk of infection - an unfavourable situation in mass vaccination campaigns where maintenance of a high standard of hygiene is difficult, or during vaccination of immune compromised individuals. Therefore, the establishment of NF strategies, which do not involve reduction of the SC barrier, would be definitely advantageous.

Transfollicular delivery of nanoparticles

Nanoparticles have previously demonstrated their considerable promise as delivery systems for TCV. Incorporation of vaccine components into nanoparticles protects them from biochemical degradation (e.g. enzymatic degradation, pH) and allows for mimicking the dimensions and appearance of infectious organisms – the natural target of the immune system. Such properties afford nanocarriers the ability to improve and modulate immune responses to associated vaccine components. While permeation of nanocarriers into the skin has traditionally required disruption of the SC by using skin pre-treatment methods such as plucking, waxing or stripping, it has been shown that nanoparticles are in fact able to penetrate into the skin structure via natural interruptions in the SC barrier - the hair follicles - in the absence of any skin pre-treatment (Figure 1).

Early studies revealed that nanoparticles migrate into and accumulate within hair follicles in a size-dependent manner [1], where they are then able to freely interact with localized LCs. This transport of substances to the deeper skin layers by way of the hair follicles is called transfollicular delivery. Such studies have formed the basis of work conducted to date within the VAC/DDEL collaboration, which aims to further investigate the promise of transfollicular nanoparticle delivery as a non-invasive vaccination strategy. In a first study [2,3], we employed a double emul-

sion method to formulate biocompatible and biodegradable polymeric nanoparticles consisting of poly(lactic-co-glycolic acid) (PLGA), or PLGA with an outer coating of chitosan (CS) (Figure 2A). Ovalbumin (OVA) was incorporated into both nanoparticle formulations as a model antigen.

Application of such nanoparticle systems to excised pig ear skin [3] resulted in the accumulation of higher amounts of OVA within the hair follicles, as compared to application of OVA in solution (Figure 2B). *In vitro* studies also demonstrated the potential of both PLGA and CS-PLGA nanoparticles loaded with OVA to induce activation and maturation of APCs, and illustrated the further ability of such activated APCs to interact with and stimulate proliferation of antigen-specific T-cells [2]. CS-PLGA nanoparticles with incorporated OVA, as a particularly promising candidate, were then tested for their ability to act as a transfollicular vaccine delivery system in an *in vivo* mouse model. Co-administration of OVA-loaded nanoparticles with the immune-stimulating adjuvant bis-(3',5')-cyclic dimeric adenosine monophosphate (c-di-AMP) onto intact mouse skin was seen to promote the highest production of OVA-specific antibody across all tested formulations, as well as in comparison to formulations administered to skin pre-treated by stripping (Figure 2C). Application of OVA-loaded nanoparticles together with c-di-AMP also resulted in the strongest CD8 T-cell responses of all tested formulations, as well as the highest level of cytokine production [4].

Perspectives of transfollicular applications

In summary, a variety of novel approaches are currently under development to allow for minimally-invasive, NF vaccination against infectious diseases. Our studies have demonstrated the potential of nanoparticle delivery via the transfollicular route as a strategy for TCV without compromising the SC barrier. In particular, we have shown that CS-PLGA nanoparticles incorporating the model vaccine antigen OVA co-administered with the adjuvant c-di-AMP were able to

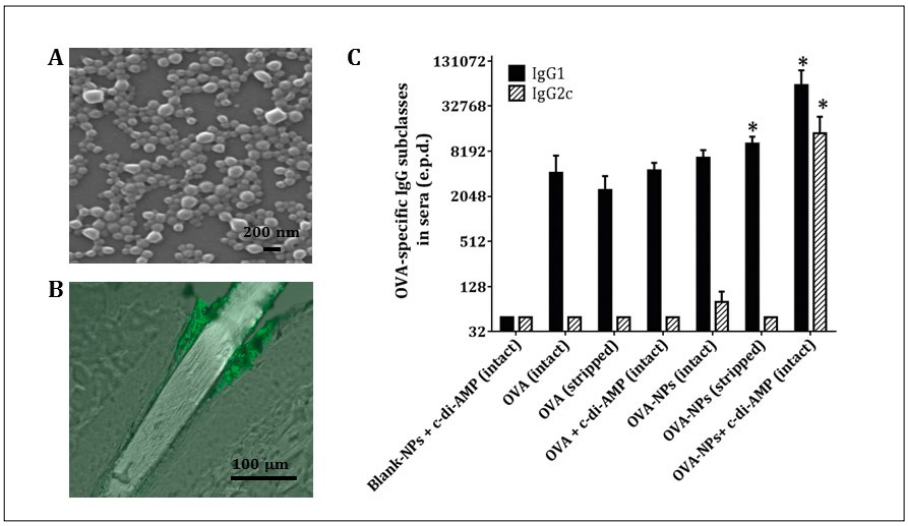

Figure 2: (A) Scanning electron micrograph of the *in vivo*-tested vaccine formulation (OVA-loaded CS-PLGA nanoparticles [NPs]). (B) Accumulation of fluorescently-labelled nanoparticles in the hair follicle of pig ear skin. (C) Antibody response after application of various vaccine formulations to the skin of BALB/c mice. The best balanced humoral/cellular (IgG1/IgG2c) immune response was achieved as a result of OVA-nanoparticle administration in combination with a suitable adjuvant (c-di-AMP), even without stripping the stratum corneum [2-4]. Figures reprinted from [2] and [4] with kind permission from Elsevier.

penetrate into hair follicles and effectively interact with perifollicular APCs, resulting in significant OVA-specific immune responses. Besides exploring new carrier technologies, such as e.g. Inverse Micellar Sugar-Glass (IMSG) nanoparticles ([5], see also the highlight article "Nanoparticle-mediated Vaccination" in this research report), our next objective is to substitute the model antigen OVA with hemagglutinin, a vaccine-relevant antigenic fragment of the influenza virus. This will allow us to investigate the ability of our nanoparticle system to stimulate protective immune responses in a clinically relevant setting.

CARLOS GUZMÁN

studied medicine and graduated as physician at the National University of Rosario, Argentina. He received his MD in **1988** as well as his PhD in Microbiological Sciences in **1993** from the University of Genoa, Italy. He then worked as a research fellow at the University of Genoa and the German Research Centre for Biotechnology (GBF) in Braunschweig. In **2000** Guzmán qualified as a professor at Hannover Medical School. He is an honorary professor of Medical Microbiology at the Hannover Medical School. He was head of the department "Vaccinology" at the GBF/HZI from **2005 to 2008**, when he became head of the department "Vaccinology and Applied Microbiology" at the HZI. His main research interests are vaccination strategies and the understanding of the molecular basis of host response to infection and vaccination.

CLAUS-MICHAEL LEHR

After studying pharmacy at the Universities of Mainz and Hamburg, Claus-Michael Lehr received his PhD from Leiden University, The Netherlands, in **1991**. He then worked as a postdoc at the University of Southern California, USA, and as a research associate at the Leiden/Amsterdam Center for Drug Research. Subsequently, Lehr was an associate professor at the Philipps University in Marburg and head of the department "Biopharmaceutics and Pharmaceutical Technology" at Saarland University Saarbrücken. Since **2010** he leads the department "Drug Delivery" at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) in Saarbrücken. He focuses on biological barriers, in vitro models and nanocarriers.

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FROM QUANTIFICATION OF T CELL RESPONSES TO PREDICTIVE MODELS

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Prof. Michael Meyer-Hermann, Head of the Department "Systems Immunology"
Co-Author: Philippe A. Robert

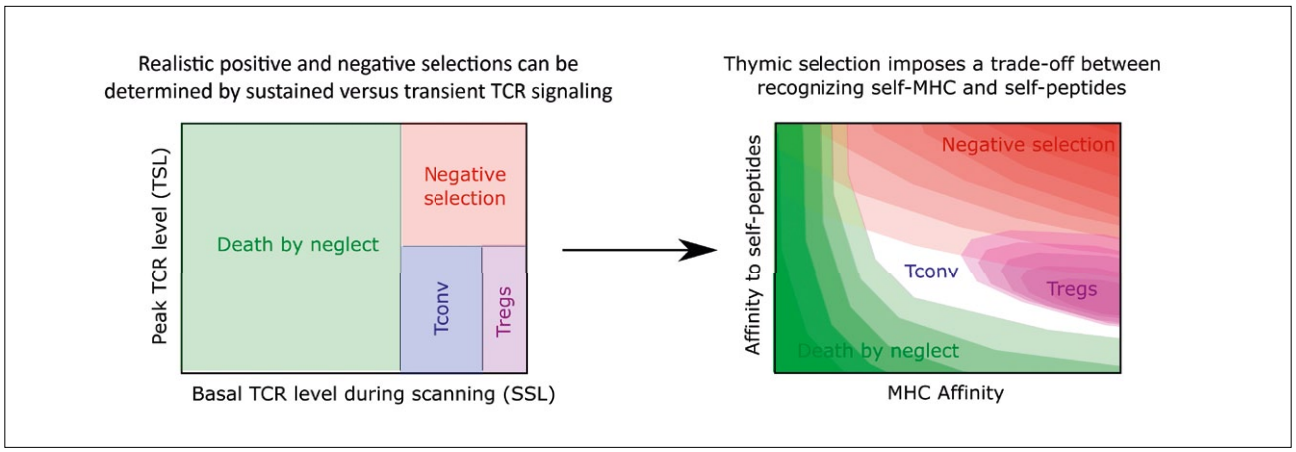


Figure 1: How developing T cells decide their fates based on the signal they receive (left) and the consequence in terms of MHC or self-peptide recognition (right) (Figure: Philippe Robert).

T cells are lymphocytes that play an important role in cell-mediated immunity. A subset of them, the CD4⁺ T helper cells, are critical regulators of immune responses. In the thymus, virgin CD4⁺ T cells make a first decision between becoming a regulatory T cell (Treg) harbouring immunosuppressive properties or a conventional CD4⁺ T cell (Tconv). In the periphery, these Tconv can further differentiate into various subtypes having specialized functions to combat different pathogens. Since dysregulation of the Treg and Tconv compartments can cause autoimmunity, allergy or chronicity of infection, the HZI departments "Experimental Immunology" and "Systems Immunology" have joined forces to generate quantitative data and build predictive computational models precisely describing development, homeostasis and differentiation of CD4⁺ T helper cells in order to develop novel therapeutic concepts.

Early steps of thymic selection shape the T cell receptor repertoire

Each T cell carries a unique T cell receptor (TCR), by which it can sense protein fragments (peptides) at the surface of other cells, when presented on a carrier molecule called MHC. In the thymus, all developing T cells express their unique TCR through a random gene recombination process, and are subsequently positively selected depending on the capacity of their TCR to bind the carrier molecule MHC. However, if the affinity to MHC loaded with self-peptide is too high, T cells might get activated by self-molecules and induce autoimmunity. For that reason, all positively selected T cells with a too high affinity have to be eliminated in a process called negative selection to avoid the exit of potentially dangerous, self-reactive T cells from the thymus. A part of the high-affinity T cells can escape negative selection and can develop into Tregs.

We wondered how T cell progenitor cells make a decision about whether to die, survive, or become a Treg. The main signal a developing T cell receives comes from its TCR, through repeated interaction with self-peptides presented on MHCs of thymic antigen-presenting cells (APCs). This interaction comprises two components: Binding to MHC and binding to the presented self-peptide. We developed the first agent-based model to simulate the integrated signal a thymocyte receives over time in the course of scanning APCs that carry random self-peptides. In the model, it is assumed that the

T cell progenitor cells make a decision about whether to die, survive, or become a Treg.

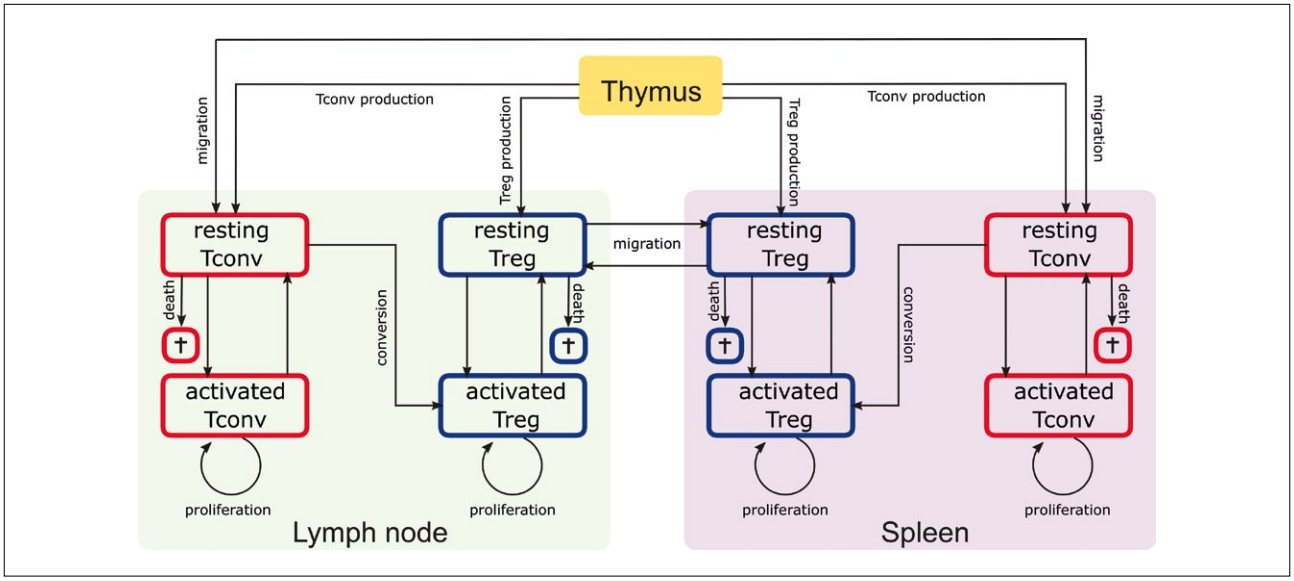


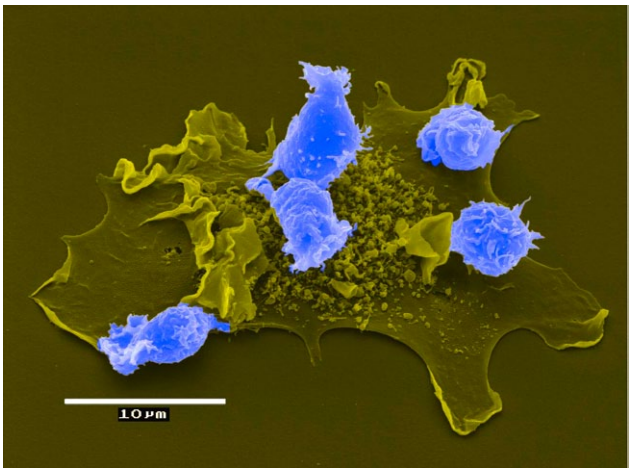
Figure 2: Mechanisms underlying Treg homeostasis (adapted from Milanez-Almeida et al. Eur. J. Immunol. 2015)

signal inside a given T cell is increased depending on the affinity of the TCR to the presented self-peptides, and decays over time. These simple assumptions allowed us to reveal the main properties of the signal sensed by the developing T cells: Since the MHC molecule at each interaction is always very similar, the basal value of the signal was correlated to the affinity of the TCR to the MHC molecule. Similarly, the peaks of the signal were due to encounter with high affine peptides, meaning the developing T cell was self-reactive. By defining "death based on the peak signal" and "survival based on a sufficiently enough basal level", we could explain both positive selection of T cells recognizing MHC molecules and negative selection of self-reactive T cells (Figure 1). We further deduced that Tregs have higher affinity to the MHC molecules, and therefore are much easier to activate. The model provides a credible explanation of how developing T cells can decide their fate thanks to the integrated TCR signalling over time [1].

In future projects, the validated model will be applied to mimic pathogen access to the thymus, leading to the presentation of their peptides. We would like to predict how this situation impacts on the repertoire of TCRs and on the risk of developing self-reactive T cells capable of causing autoimmune diseases. A related question is under which conditions peptides like tumour antigens that are similar to self-peptides would be tolerated, even if not explicitly presented in the thymus. Finally, in the case of autoimmune diseases like multiple sclerosis, it would be of interest to know if and how providing of new brain-specific self-antigens to the thymus would help to restore immune balance. The developed computational model can provide relevant hints for these and similar questions.

A close liaison – the homeostasis of Tregs and Tconv is tightly controlled

Multiple mechanisms can account for the homeostasis of the Treg population: output of freshly produced Tregs from the thymus, homeostatic proliferation in the periphery, cell death, and conversion from Tconv into Tregs (Figure 2). Quantitative information about the relevance of these different mechanisms was lacking and is now provided for the



Electron microscopic image of T cells (shown in blue) interacting with dendritic cells.

first time through the development of a computational model of peripheral Treg homeostasis in a joint experimental and theoretical approach by our departments. Using transgenic mice in which Tregs can be experimentally killed in a highly specific manner, we induced a deletion of the Treg population and followed the kinetics of their rebound. Various parameters were determined and used to design an Ordinary Differential Equations model to simulate the amount of Tregs and Tconv in different lymphoid organs over time. Using this model, we could not only predict the previously unanticipated possibility that Tregs regulate migration of Tconv between spleen and lymph nodes, but also could for the first time precisely quantify the conversion rate from Tconv to Tregs in these lymphoid organs. This newly developed computational model will be basis for additional modeling approaches describing T cell-mediated immune responses during acute and chronic infections [2, 3].

To be or not to be: decision-making during CD4⁺ T helper cell differentiation

During their differentiation, CD4⁺ T helper cells integrate numerous environmental cues, ranging from costimulatory signals and cytokines to hormones and local metabolites. Despite intense research the precise order and combination of signals received by CD4⁺ T cells during their differentiation process is only incompletely understood, and the highly diverse CD4⁺ T helper subsets (e.g. Th1, Th2, Th17, Th22 and TFH) have been reported to be induced by many different ways. In order to better understand how the intracellular decision is made during CD4⁺ T helper cell differentiation, we performed prototypical in vitro differentiation cultures and followed the expression kinetics of major transcription factors and cytokines. This extensive data set is now being used to determine parameters in a computational model of CD4⁺ T helper cell differentiation based on the underlying gene regulatory network. The aim is to identify the importance and kinetics of

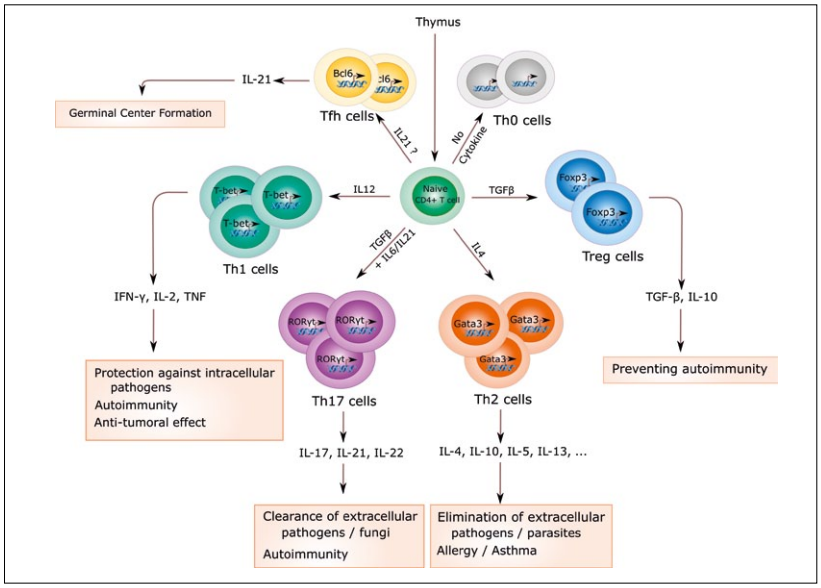


Figure 3: Major CD4+ T helper subsets, their inducing signals and biological functions (Figure: Philippe Robert).

main pathways and critical mechanisms, and to predict *in silico* the effect of different combinations of extracellular signals. The model will considerably help to understand under which conditions the differentiation programmes are robust or plastic. There is an increasing demand for the generation of stable T cell subsets for various therapeutic purposes, like immunotherapy, and we hope that our model will help to optimize or describe the limits of such protocols.

JOCHEN HUEHN

studied biochemistry and molecular biology at the University of Hamburg and worked on his PhD project at the Bernhard-Nocht-Institute in Hamburg. After a postdoc time at the Charité University Medicine in Berlin, he became a Junior Professor for Immune Regulation with a focus on rheumatology and clinical immunology. **Since 2008**, he heads the Department of Experimental Immunology at the HZI Braunschweig and holds a professorship at the Hannover Medical School (MHH). **Since 2014** he is an elected Advisory Board Member of the German Society of Immunology and **since 2015** he is coordinating the EU-funded Innovative Training Network ENLIGHT-TEN. The Department "Experimental Immunology" is studying various aspects of immune regulation with a particular focus on regulatory T cells.

MICHAEL MEYER-HERMANN

studied Physics, Mathematics, and Philosophy in Frankfurt/Main and Paris and accomplished his PhD in Theoretical Elementary Particle Physics. He initiated new research groups for Systems Immunology in Dresden (Germany), Oxford (UK), and at the Frankfurt Institute for Advanced Studies (FIAS, Germany). **Since 2010**, he heads the Department "Systems Immunology" at the HZI and holds a professorship at the Technische Universität Braunschweig. Further, he is in the board of directors of the newly founded Braunschweig Integrated Centre of Systems Biology (BRICS). He develops mathematical models for the dynamics of host-pathogen interactions and uses these for scientific predictions and more efficient disease treatment. Since **2015** he is coordinator of the intercontinental Human Frontiers Science Program (HFSP) project on information processing in humoral immune responses.

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Scientists at the HZI published about 1000 articles in the years 2014/2015. A large proportion of these papers appeared in top-tier journals. The HZI regularly acknowledges exciting publications by awarding a prize for the so-called "HZI Paper of the Month". The winners are selected by an in-house jury and the prize is financially supported by the "Friends of the HZI". In this section, a selection of these excellent publications is presented by the authors.

HIGHLIGHT PUBLICATIONS

MODULATION OF TLR SIGNALLING BY HERPESVIRUSES

Prof. Melanie Brinkmann, Head of the Junior Research Group "Viral Immune Modulation"

Toll-like receptors (TLR) play a crucial role in the detection of invading pathogens. Some viruses efficiently block TLR signaling, among them the human gammaherpesvirus Kaposi's sarcoma-associated herpesvirus (KSHV) and the murine gammaherpesvirus 68 (MHV68). One KSHV protein, the replication and transcription activator (RTA), has found an elegant way to block TLR signalling: it directly targets the receptor and thus inhibits the entire downstream signalling cascade. As a key regulator of the viral life cycle and a viral protein that targets multiple innate immune signalling proteins, RTA makes an extremely attractive target for antiviral drug development.

The interplay between the innate immune system and invading pathogens is crucial for the outcome of infection. As the innate immune system attempts to inhibit amplification of the invading microorganisms, pathogens have developed sophisticated mechanisms to modulate the immune system to ensure the progression of the infection. An early host defence mechanism involves recognition of pathogen-associated molecular patterns by pattern recognition receptors (PRR). The Toll-like receptors (TLR) are type I transmembrane proteins and were the first PRR to be characterized. TLR localize to either the plasma membrane or intracellularly in endolysosomal compartments. TLR recognize a variety of

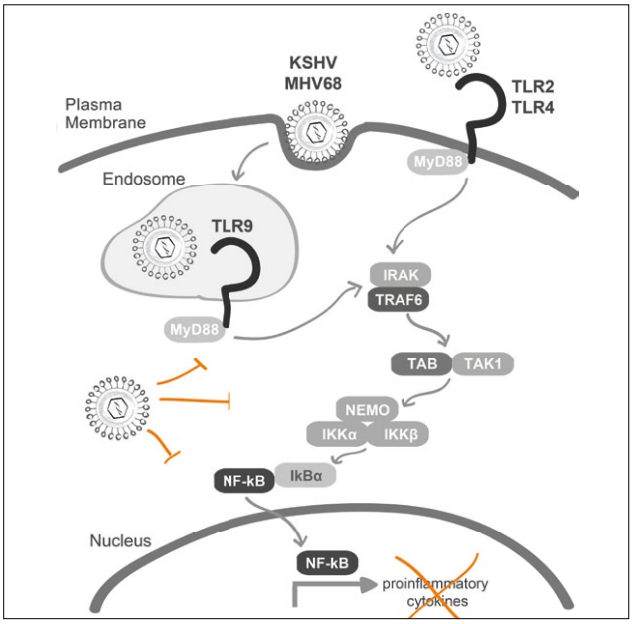

Figure 1: Infection with MHV68 and KSHV inhibits TLR-dependent induction of proinflammatory cytokines. Toll-like receptors are known to detect the gammaherpesviruses KSHV and MHV68 and mount an antiviral response. Herpesviruses establish lifelong infections in their hosts and have evolved elegant mechanisms to counteract TLR signalling and thereby endure the successful progression of infection.

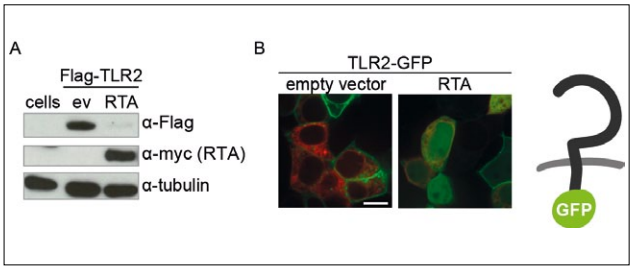

Figure 2: (A) TLR2 expression is reduced in the presence of RTA. 293T cells were transfected with Flag-tagged TLR2 and either empty vector or myc-tagged RTA. Protein levels in whole-cell lysates were analysed by immunoblotting against Flag (TLR2), myc (RTA), and tubulin was utilized as a loading control. (B) GFP-tagged TLR2 does not localize to the plasma membrane in the presence of RTA. TLR2-GFP normally localizes to the plasma membrane (green, left image). In the presence of RTA, the GFP signal is diffuse and cytoplasmic (green, right image). The endoplasmic reticulum is shown in red.

"non-self" patterns: cell surface TLR detect microbial surface molecules, while endosomal TLR recognize nucleic acid moieties. Upon binding their specific ligands, TLR induce signalling cascades leading to proinflammatory cytokine and type I interferon production.

Herpesviruses are large, double-stranded DNA viruses, which establish lifelong infections in the host. Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic virus and the etiological agent of Kaposi's sarcoma and B-cell proliferative disorders including primary effusion lymphoma. Because of herpesviral host species specificity, murine gammaherpesvirus 68 (MHV68) has emerged as an animal model for the human gammaherpesviruses Epstein-Barr virus and KSHV. Multiple TLR have been previously identified to be important for detection of MHV68 and KSHV; additional studies have shown that both gammaherpesviruses modulate multiple aspects of TLR signalling.

We infected macrophages with KSHV and MHV68 and found that both viruses induce a negligible proinflammatory cytokine response. Furthermore, we found that TLR signalling is inhibited in macrophages that were previously infected with KSHV or MHV68. The lack of response of infected cells to TLR stimulation suggests that both KSHV and MHV68 specifically inhibit TLR signalling pathways (Figure1).

To identify viral proteins involved in inhibition of TLR signalling, we examined the ability of 85 KSHV-encoded proteins to inhibit the TLR2-dependent proinflammatory cytokine response. Among others, the KSHV lytic switch protein "replication and transcription activator" (RTA) strongly inhibited both TLR2 and TLR4 signalling. Interestingly, both TLR2 and TLR4 protein levels were reduced in the presence of RTA (Figure 2A), and RTA expression resulted in disruption of the normal plasma membrane localization of both TLR2 (Figure 2B) and TLR4 (not shown).

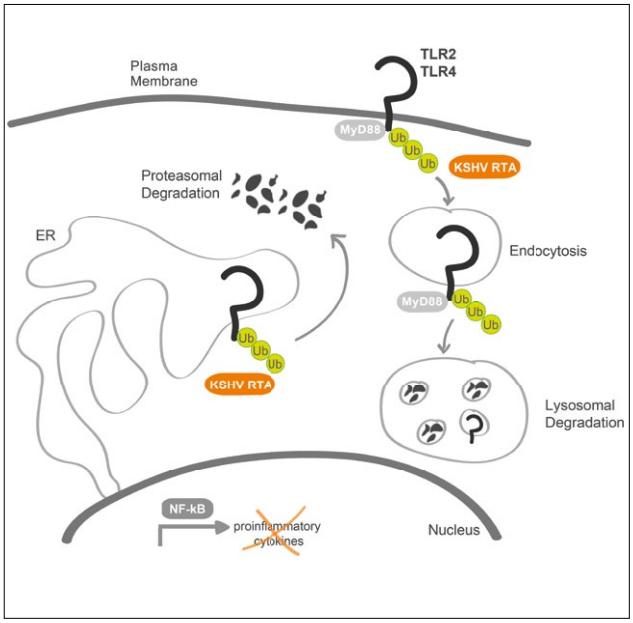

Figure 3: KSHV RTA is a key viral factor involved in inhibition of TLR signalling. Expression of RTA reduces levels of TLR2 and TLR4, possibly by targeting TLR for proteasomal or lysosomal degradation.

The KSHV RTA protein is a multifunctional protein. In addition to its essential role as a transcriptional activator of the KSHV lytic gene programme, it is known to be an E3 ubiquitin ligase. Previous studies have found that RTA targets multiple cellular and viral proteins, and we have now shown that RTA mediates downregulation of TLR. RTA may downregulate TLR and other proteins by targeting them for proteasomal or lysosomal degradation (Figure 3); understanding the molecular mechanisms involved is an essential part of future studies.

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TYPE-III PROTEIN SECRETION IN *SALMONELLA ENTERICA*

Dr. Marc Erhardt, Head of the Junior Research Group "Infection Biology of *Salmonella*"

The incidence of foodborne outbreaks caused by Enterobacteriaceae like *Salmonella* remains substantial and constitutes a significant socioeconomic burden also in Europe. Of special importance is the increasing emergence of multidrug-resistant Enterobacteriaceae. The targeting of their virulence-relevant factors is a promising approach for the development of novel anti-infectives. The aim of this project was thus to decipher the molecular mechanisms of important virulence traits of *Salmonella* with a focus on the molecular mechanism of type-III protein secretion.

Gastrointestinal infections by gram-negative, pathogenic Enterobacteriaceae like *Salmonella enterica* represent a severe health problem worldwide with high economic implications. Two important virulence factors of the bacterium - motility organelles (flagella) and needle-like injectisome systems - rely on type-III protein secretion for assembly and export of substrate proteins.

The type-III protein secretion apparatus is a complex nanomachine responsible for secretion of building blocks and substrate proteins of the flagellum and the virulence-associated injectisome needle complex. Although the key proteins have been identified, the mechanisms of substrate recognition, energy usage and substrate export remain poorly understood. Type-III secretion systems utilize energy of the proton motive force and ATP hydrolysis of an associated, cytoplasmic ATPase complex to drive substrate export (Figure 1). The cytoplasmic components of the secretion system share strong homology to the F_OF_1-ATP synthase of the respiratory chain and it was thought that the flagellum was derived from a proto F_OF_1-ATP synthase where ATP hydrolysis energized the export process.

Here, we studied the energy requirement of the type-III secretion apparatus using a combination of sophisticated bacterial genetics, protein biochemistry and immunofluo-

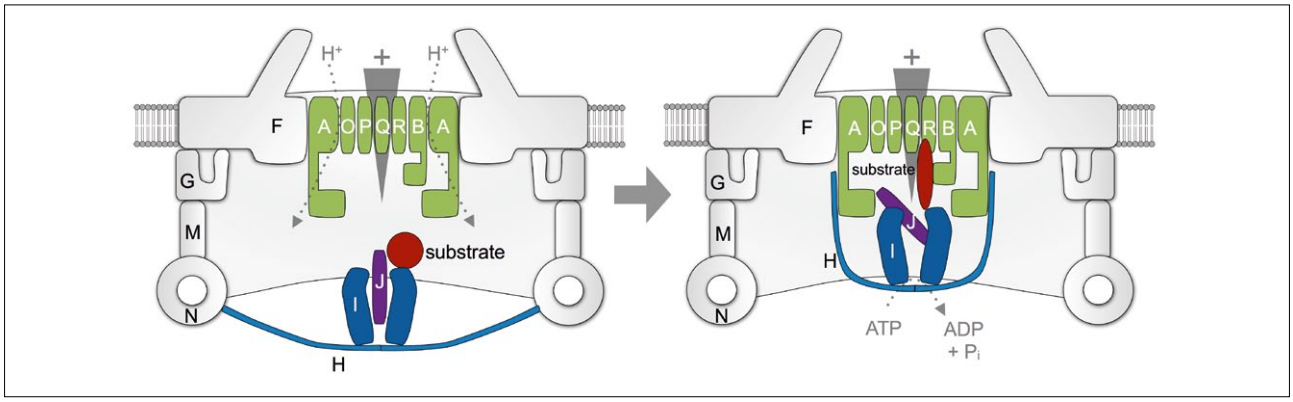


Figure 1: Model of the flagellar type-III protein export process.

rescence techniques. We found that the activity of the associated ATPase complex is dispensable for the function of type-III protein secretion systems in *Salmonella*. We demonstrated that formation of functional flagella is possible in the absence of the type-III secretion system associated ATPase by mutations that increased the proton motive force and flagellar substrate levels (Figure 2). We also showed that increased proton motive force bypassed the requirement of the virulence-associated type-III secretion system ATPase for secretion of substrate proteins via the injectisome complex.

Our findings have important implications for the evolution of the bacterial flagellum and type-III secretion systems. They suggest that a proto ATPase was added at a later time during evolution to a primordial proton-powered type-III export system to facilitate substrate recognition and unfolding, which resulted in optimally efficient protein export of contemporary type-III secretion systems.

In conclusion, our results demonstrate that the type-III protein secretion apparatus is intrinsically a proton motive force-driven proton-protein antiporter and the associated ATPase functions in a supporting role to enhance protein export efficiency. A better understanding of these mechanisms may provide new ways to counteract *Salmonella* virulence.

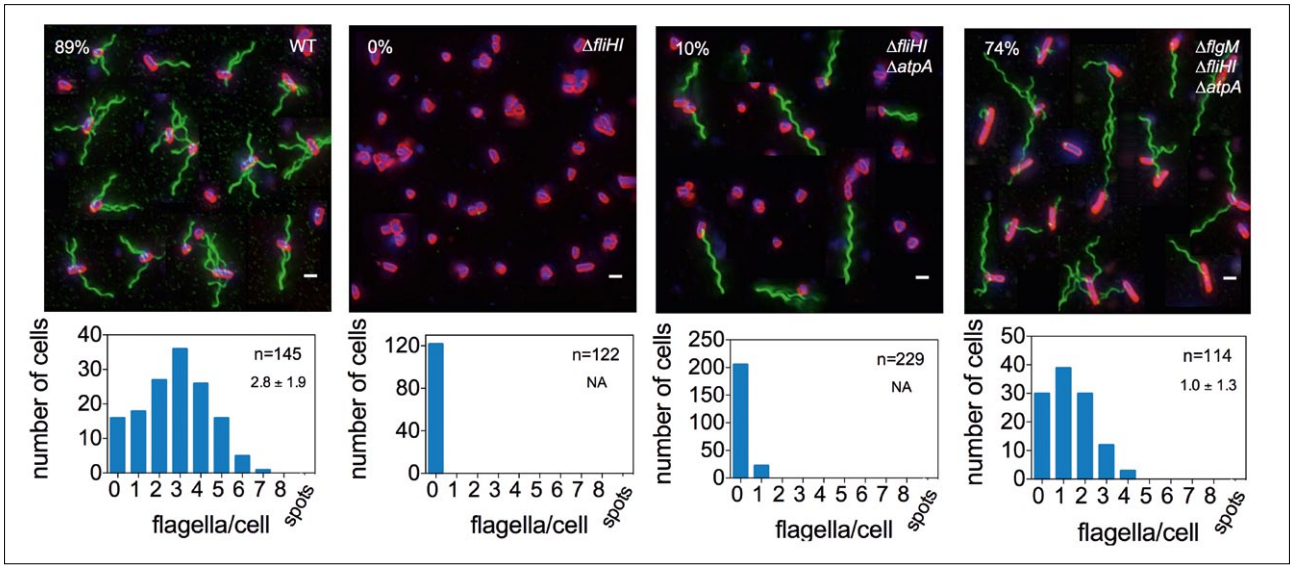


Figure 2: Flagellation of *Salmonella* can be restored in an ATPase deletion strain by providing excess secretion substrate and proton motive force. Top: exemplary immunofluorescence images of flagellated or non-flagellated Salmonella mutants. Bottom: quantification of flagella numbers per cell used to measure the activity of the flagellar type-III secretion system. Figure: Adapted from Erhardt et al., *PLoS Genet* (2014)

Erhardt, M., Mertens, M. E., Fabiani F. D., Hughes, K. T. **ATPase-Independent Type-III Protein Secretion in *Salmonella enterica*.** *PLoS Genet* **10 (11)**, e1004800 (2014)

NANOPARTICLE-MEDIATED VACCINATION

Prof. Carlos A. Guzmán, Head of the Department "Vaccinology and Applied Microbiology"
Prof. Claus-Michael Lehr, Head of the Department "Drug Delivery"

Today, most of the vaccines approved for human use are adminstered by needle injection. However, we were able to demonstrate that vaccines can be delivered across an intact skin barrier using nanoparticle-based adjuvanted formulations.

Vaccination constitutes the most efficient approach to protect against many infectious diseases. Conversely, people are often frightened by potential side effects and injection-associated pain. Thus, there is an increased vaccine fatigue resulting in the re-emergence of already controlled diseases (e.g. measles). In order to counteract this trend we focused on the development of novel non-invasive vaccination strategies, which should result in higher acceptance and compliance by the public. Non-invasive vaccination strategies, such as mucosal or transdermal application approaches, are painless and offer the possibility of self-administration. This is a major asset when mass vaccination campaigns for populations living in countries with limited access to medical care are considered.

Typically, the skin barrier protects the body against penetration of foreign and potentially dangerous entities, such as allergens (e.g. pollen) or infectious agents. However, by penetrating into hair follicles particles may get in contact with dermal antigen-presenting cells (APC), such as Langerhans cells or dendritic cells (Figure **1**). In a recent series of papers (see article "Perspectives of Non-Invasive Vaccine Delivery") we could demonstrate that nanotechnology in combination with proper adjuvantation may allow to efficiently deliver the model antigen Ovalbumin (OVA) via this pathway. This in turn leads to the stimulation of efficient humoral and cellular immune responses without any untargeted disruption or modulation of the natural skin barrier function provided by the stratum corneum.

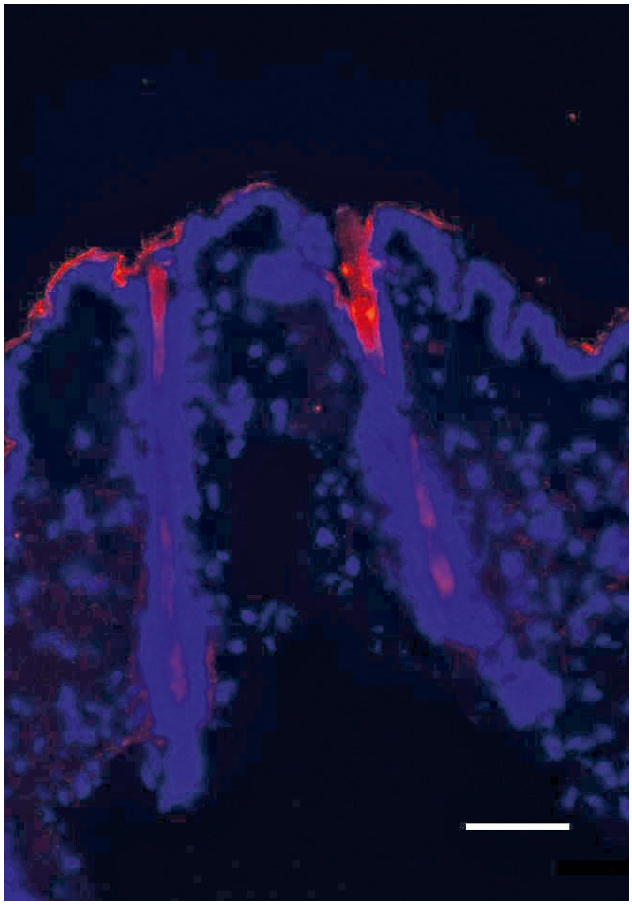


Figure 1: Localization of Dialkylcarbocyanine (DiD, lipophilic fluorescent dye) loaded nanoparticles four hours after topical application on the skin of the flank of mice. The analysis of cryosections (6 µm) showed nanoparticles, which are visible on the skin surface and penetrate inside the hair follicles. Scale bar is 50 µm.

The aim of the present study was to evaluate the potential of a new nanocarrier technology based on inverse micellar sugar glass nanoparticles (IMSG NPs) for such purposes. In addition, we performed a comparative evaluation of the strength and type of immune response elicited after IMSG NPs administration by three different routes, namely intranasal, transfollicular and intradermal. To this end, the model antigen OVA and the adjuvant bis-(3',5')-cyclic dimeric adenosine monophosphate (c-di-AMP) were co-encapsulated in the same carrier.

While the immune response stimulated after intranasal administration was negligible, significant humoral and cellular responses were observed after intradermal and, most importantly, transfollicular vaccination. More specifically, robust antigen-specific antibodies, as well as $CD4^+$ and $CD8^+$ T cell responses (including multifunctional T-lymphocytes) were stimulated in the immunized mice (Figure 2). The results of this study underscore the potential of transfollicular vaccination, but also the need for optimizing both nanocarriers and adjuvants.

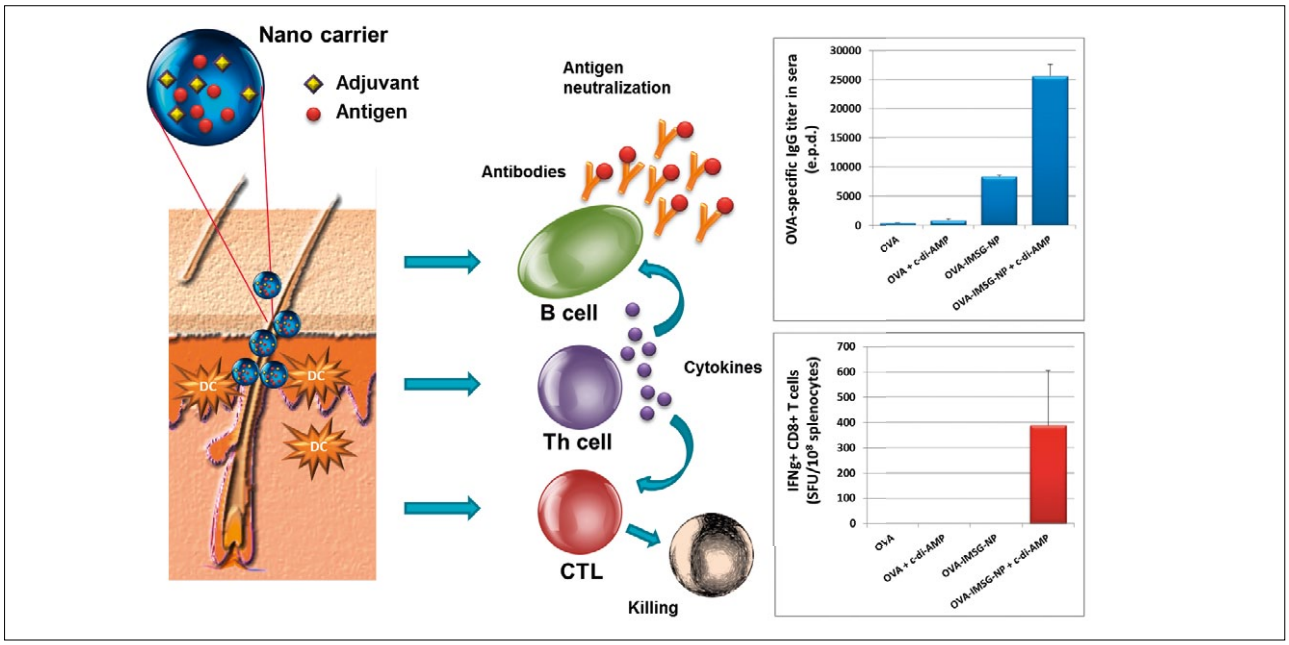


Figure 2: Non-invasive needle-free vaccination strategies are needed to meet the current challenges of vaccine development. Transfollicular vaccination using an adjuvanted nanoparticulate system to deliver antigens to the abundant peri-follicular APCs without compromising the stratum corneum barrier function stimulates efficient humoral and cellular immune responses.

Mittal, A., Schulze, K., Ebensen, T., Weißmann, S., Hansen, S., Guzmán, C. A. & Lehr, C.-M. **Inverse micellar sugar glass (IMSG) nanoparticles for transfollicular vaccination.** *J Control Release* **206** 140-157 (2015)

VIRULENCE OF *PSEUDOMONAS AERUGINOSA*

Prof. Rolf Hartmann, Head of the Department "Drug Design and Optimization"

Antibiotic-resistant bacterial infections result in a high demand for novel anti-infectives that are less prone to tolerance. A promising strategy is to selectively target non-vital functions associated with the pathogenicity of bacteria such as the production of virulence factors and biofilm formation. The human pathogen *Pseudomonas (P.) aeruginosa* causes acute and chronic persistent infections and progressive deterioration of the lung e.g. in cystic fibrosis patients. It controls the expression of virulence factors via a cell density-dependent extraordinary cell-to-cell communication system (known as *quorum sensing*, QS) by using signal molecules. PqsR is the key receptor of a *P. aeruginosa*-specific QS system that regulates multiple virulence factors such as pyocyanin at the transcriptional level. Targeting the receptor PqsR by antagonists that do not affect the bacterial viability is considered to be a novel promising approach towards the development of anti-virulence therapeutics as this strategy is expected to abate resistance development.

Based on the scaffold of the natural ligand of PqsR, HHQ (2-Heptyl-4-quinolone), we developed a compound (compound 1, see Figure 2) that is potent in *Escherichia coli* reporter gene assay, but only moderately reduced pyocyanin in *P. aeruginosa*. Therefore, we characterized the compound in a reporter gene assay in *P. aeruginosa* and discovered a dose-dependent agonistic activity, which gave us a hint at a possible biotransformation of the antagonist within *P. aeruginosa* cells. We turned our attention on the enzyme PqsH that is involved in the QS signaling pathway and converts HHQ into the more potent PqsR ligand PQS (*Pseudomonas* quinolone signal). Mass spectrometry experiments in wild-type

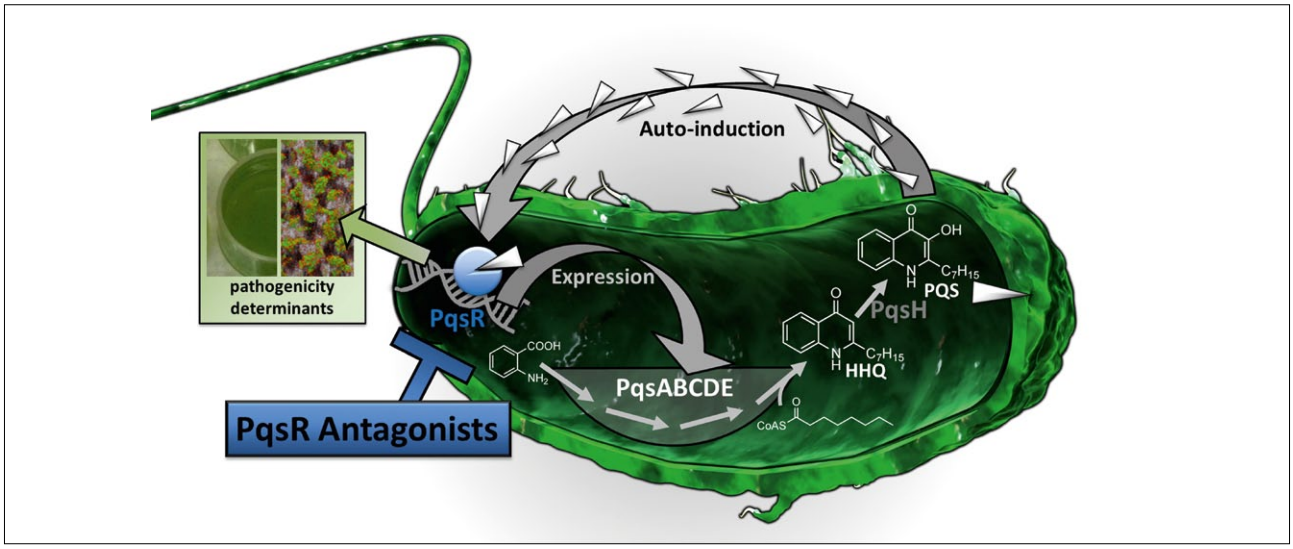


Figure 1: PqsR has key functions in virulence and pathogenesis and is activated by the signal molecules HHQ and PQS. Blockade of PqsR by antagonists is a novel approach towards the development of antivirulence therapeutics.

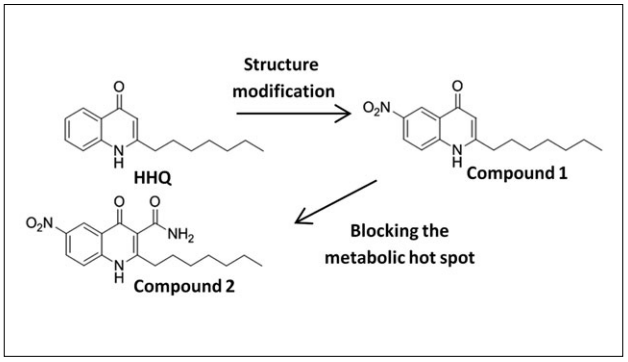


Figure 2: Based on the scaffold of HHQ, a first PqsR antagonist (compound 1) exhibiting low efficacy in *P. aeruginosa* has been developed. Blockade of the metabolic hotspot resulted in a highly potent and stable antagonist (compound 2).

and *pqsH* knock-out mutant *P. aeruginosa* cells revealed that the initially antagonistic compound is converted into a potent agonist by the synthase PqsH resulting in a low *in vivo* efficacy of the compound. Pursuing a rational development of a potent and stable PqsR antagonist, the susceptible position was chemically blocked by the substitution of a hydrogen by a carboxamide. This novel compound (compound 2, see Figure 2) turned out to exhibit highly potent pure antagonistic properties without displaying any agonistic activity in the *P. aeruginosa* reporter gene assay. Importantly, this antagonist demonstrated promising efficacy in cell-based assays and strongly reduced the virulence factor pyocyanin. Applying the improved compound in two animal models, the nematode *Caenorhabditis elegans* and the larvae *Galleria mellonella*, we could show that the mortality rate caused by *P. aeruginosa* is significantly reduced and, thus, we provided the first proof-of-concept for a PqsR antagonist as an anti-virulence agent.

These findings provide an exciting example that a compound suffering from ineffectiveness under *in vivo* conditions can be rescued by rational consideration of other potential factors than just penetration or efflux problems applying state-of-the-art medicinal chemistry strategies. They may open new avenues for the development of novel species-specific anti-infectives, which might help to minimize adverse effects observed with broad-spectrum antibiotics.

Lu, C., Maurer, C. K., Kirsch, B., Steinbach, A. & Hartmann, R. W. **Overcoming the Unexpected Functional Inversion of a PqsR Antagonist in *Pseudomonas aeruginosa*: An In Vivo Potent Antivirulence Agent Targeting *pqs* Quorum Sensing.** *Angew. Chem. Int. Ed.* **53** 1109–1112 (2014)

STRUCTURE ELUCIDATION AND TOTAL SYNTHESIS OF THE BACTERIAL GROWTH INHIBITOR β-LIPOMYCIN

Prof. Markus Kalesse, Head of the Department "Medicinal Chemistry"

The structure elucidation of natural products is one of the pivotal tasks before chemists can pave the way towards making these compounds accessible by synthesis. The most valuable tool for structure elucidation is NMR spectroscopy, which allows determination not only of the constitution (connection of atoms) of natural products but also of their configuration, which is the three-dimensional arrangement of atoms. Recently, researchers have provided an analysis to predict the structure of a class of secondary metabolites called polyketides to a large extent. However, a complete and detailed structure analysis was not possible with this method. We proposed a statistical method, the so-called hidden Markov Model (HMM), which allows prediction of the configuration at small residues of the molecule, the methyl groups, that were previously not accessible to this kind of analysis. In order to confirm the validity of our analysis and to use its value for synthetic chemistry we analysed the configuration of β-lipomycin, which was first isolated in 1972 from a strain of *Streptomyces aureofaciens* and inhibits the growth of several Gram-positive bacteria. Our synthesis provides support for the correct structural assignment and facilitates the production of large amounts of this natural product.

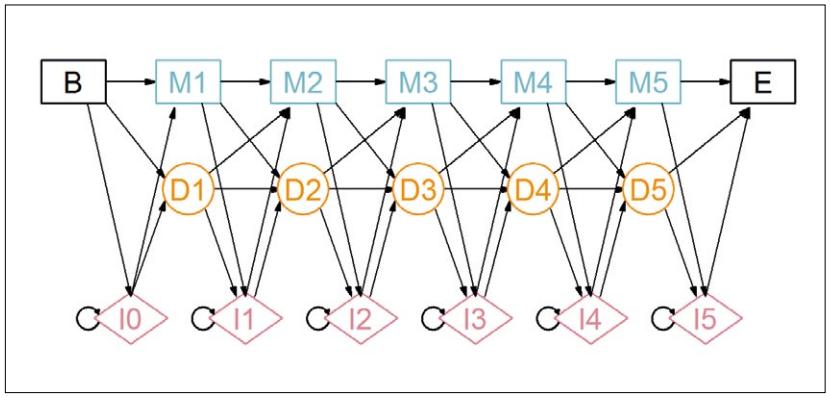


Figure 1: Architecture of the profile HMM with five match states. Blue squares indicate match states; pink diamonds insert states and orange circles delete states. B and E refer to the begin and end of an amino acid sequence. Figure: Reprinted from Hartmann, O. et al., The Structure Elucidation and Total Synthesis of β-Lipomycin, with permission from John Wiley and Sons.

Lipomycins are natural products that received their name due to the fact that their antibiotic activity was antagonized by naturally occurring lipids. Like other polyketides, lipomycins are biologically highly active. Today, researchers are able to predict the configuration of secondary alcohols in type I polyketides based on the amino acid sequence of keto reductases. Configurations at methyl branches were not accessible by such analyses though. The advantage of using the hidden Markov model for predicting the configuration of modular polyketides is the fact that this model uses all amino acids (M) for the configurational prediction and not only selected residues. Additionally, it accounts for insert (I) and delete (D) states, which correspond to additional or missing amino acids. That leads to higher reliability of the prediction and makes a previous sequence alignment dispensable. The term "profile" refers to different emission and transition probabilities of individual positions. Moreover, the absolute value of the *ScoreDiff* value derived from the HMM provides a measure of reliability of the predicted configuration.

Using our profile HMM approach all amino acids of the specific subset of each ketoreductase were taken into account to allocate them to one or another family that generates one or another stereochemistry. Additionally, we used Viterbi scores to quantify the reliability of our predictions. In the case of the C13 alcohol of the lipomycins we found a negative *ScoreDiff* value (-53.96) that is consistent with the L configuration and therefore supports a previous configurational assignment made by others. For the C12 methyl group we found a ScoreDiff of -39.37 and by that identified the stereocenter to be L configured as well. This led to the structure of β-lipomycin (1) as depicted in Figure 2.

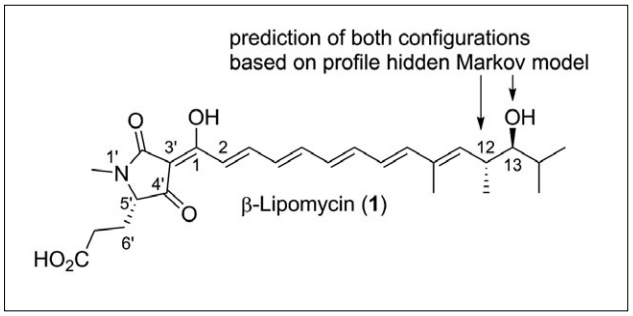


Figure 2: The proposed absolute configuration of β-lipomycin (**1**).
Figure: Reprinted from Hartmann, O. et al., The Structure Elucidation and Total Synthesis of β-Lipomycin, with permission from John Wiley and Sons.

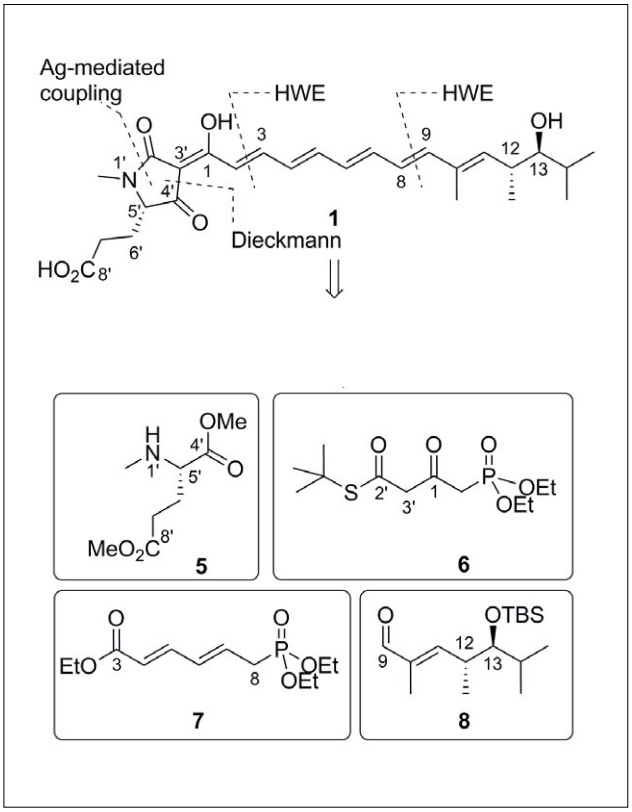


Figure 3: Retrosynthetic analysis of β-lipomycin (**1**).
Figure: Reprinted from Hartmann, O. et al., The Structure Elucidation and Total Synthesis of β-Lipomycin, with permission from John Wiley and Sons.

In retrosynthetic direction β-lipomycin (**1**) can be divided into four segments **5, 6, 7** and **8** (see Figure **3**).

With these fragments in hand we were able to complete the synthesis of β-lipomycin (**1**) in a longest linear sequence of 12 steps starting from commercially available substances with a yield of 17.0%. This synthesis and its preceding structural assignment solely rely on the statistical analysis of the pivotal ketoreductase and confirm the validity and practicability of this analysis.

Hartmann, O. & Kalesse, M. **The Structure Elucidation and Total Synthesis of β-Lipomycin**. *Angew. Chem. Int. Ed.* 53, 7335–7338 (2014)

PREVALENCE OF CHRONIC HEPATITIS B VIRUS INFECTION

Prof. Gérard Krause, Head of the Department "Epidemiology"

Hepatitis B virus (HBV) infection is a health concern worldwide since it can lead to severe liver diseases. HBV is also a major human carcinogen causing primary liver cancer. Information on the prevalence of chronic HBV infection (sero-prevalence of the hepatitis B surface antigen, HBsAg) and the number of individuals living with HBV in each country has been lacking but is important to identify high endemicity areas and to investigate the impact of vaccination and health programs.

In the study, pooled global and country-specific estimates of chronic HBV infection were generated and the number of persons living with this infection was calculated. The input data included around 10,000 data points derived from over 1,900 research reports that met the inclusion criteria and covering more than 100 million study subjects. According to the results, about 3.6% of the world`s population is chronically infected with HBV and potentially at risk of developing related diseases. In addition, infected individuals provide a reservoir for further virus transmission. The wide variation between countries is remarkable: In some African countries like Liberia, Swaziland and South Sudan as well as in the West Pacific countries Kiribati and the Solomon Islands, around 20% of the general population is estimated to be chronically infected with the HBV. Compared to that, chronic HBV infection is rather uncommon in North America and Western Europe. In Europe, the prevalence increases eastwards from 0.01% in the UK general population to above 10% in Kyrgyzstan. A similar tendency is seen for the Americas where a north to south gradient is obvious with a share of over 13% of the population being chronically infected in a Caribbean state. Some of the most populous countries on the globe have intermediate and high HBV endemicity. In China, for example, over 5% of the general population carries HBV; in Nigeria, almost 10% of the population is chronically infected. This has an impact on the absolute number of people living with chronic HBV infection thus that an estimated 74 million people are chronically infected with HBV in China.

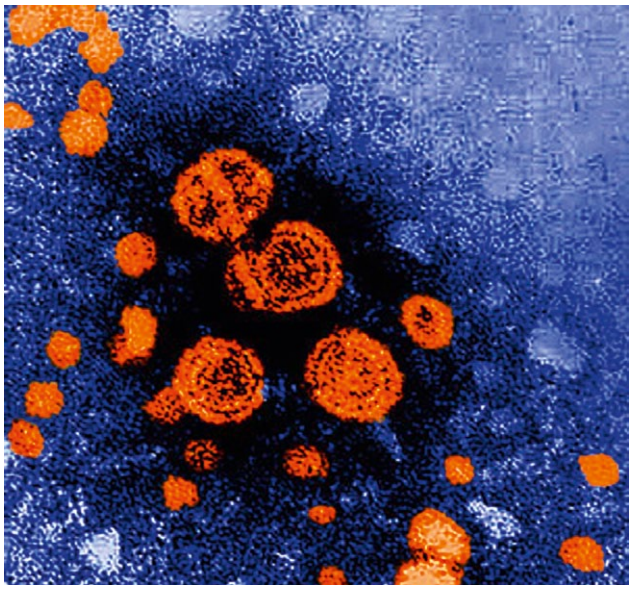


Figure 1: Infections with Hepatitis B Virus are among the most common infectiouos diseases worldwide. Picture: Erskine Palmer, CDC

Looking at overall changes in HBV prevalence, globally and in many countries, chronic HBV infection has decreased, namely in Eastern Mediterranean countries but also in South East Asia. Nevertheless, the vast majority of countries in Africa are at high HBV endemicity and, given the sparse study data from this continent, more detailed investigations are needed, e.g. regarding the most vulnerable age-groups and the rural-urban endemicity patterns. Changes in HBV prevalence are likely to be associated with preventive activities like HBV vaccine implementation but particular reasons for

changes as well as for stable or increasing patterns of prevalence in some countries require further analyses. In the light of population developments and movements, regular nationally representative surveys are needed to both monitor prevalence profiles and to adapt target groups for vaccination and treatment allocation. The results highlight the regionally high prevalence of a chronic infectious disease like HBV infection and its heterogeneity across countries, and thereby provide the basis for assessing potential impacts on population health in a highly mobile society.

Corresponding author: Dr. Jördis J. Ott

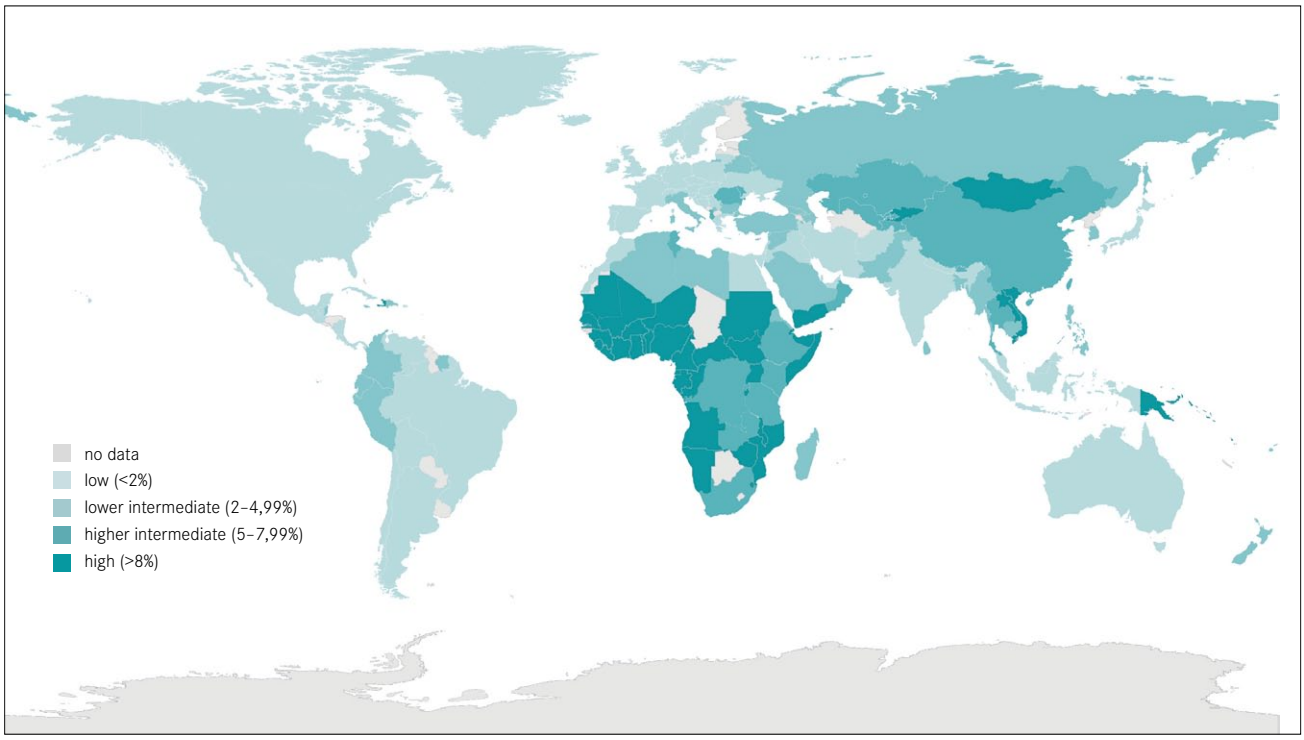


Figure 2: Prevalence of chronic HBV infection, 1957–2013. Figure: Reprinted from The Lancet, 386, Schweitzer, A. et al., Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013, 1546 – 1555, Copyright (2015), with permission from Elsevier.

Schweitzer, A., Horn, J., Mikolajczyk, R. T., Krause, G. & Ott, J. J. **Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013.** *The Lancet* **386**, 1546 – 1555 (2015)

INTERFERON REGULATORY FACTOR-1 PROTECTS FROM FATAL NEUROPATHIC VIRUS INFECTION

Dr. Andrea Kröger, Head of the Research Group "Innate Immunity and Infection"

Invasion of an organism by a pathogenic virus is followed by a viral disease. Infectious virus particles attach to and enter susceptible cells. If we are attacked, the virus is recognized by highly specialized receptors, initiates the release of messengers and activates the immune system. Some of these messengers are type I interferons (IFNs). IFNs form the first line of defence against viral infections. They protect cells against invading viral pathogens and inhibit the replication and spreading of these pathogens. However, many viruses have developed strategies to circumvent the type I IFN system to ensure their replication in the host. We discovered that in addition to the type I IFN system the interferon regulatory factor (IRF)-1 mediates an alternative intrinsic antiviral response. Although both type I IFN and IRF-1 mediate their antiviral action by inducing overlapping subsets of IFN stimulated genes, the functional role of this alternative antiviral action of IRF-1 in the context of viral infections remains unknown.

IRF-1 is essential to counteract the infection with the neurotropic vesicular stomatitis virus (VSV), and mice with a loss of function of IRF-1 succumbed to infection. Although IRF-1 was initially identified to regulate type I IFN response, using sensitive reporter mice we were able to show that type I IFN response was normal in the absence of IRF-1. We found that type I IFN and IRF-1-dependent antiviral responses act sequentially to create a layered antiviral protection program against VSV infection. Whereas early during infection, type I IFN controls viral replication, in a later phase, when IFN is downregulated to prevent inflammatory responses, IRF-1 takes over to control viral replication and spreading. In the absence of IRF-1 the virus re-emerges in the brain and cannot be controlled.

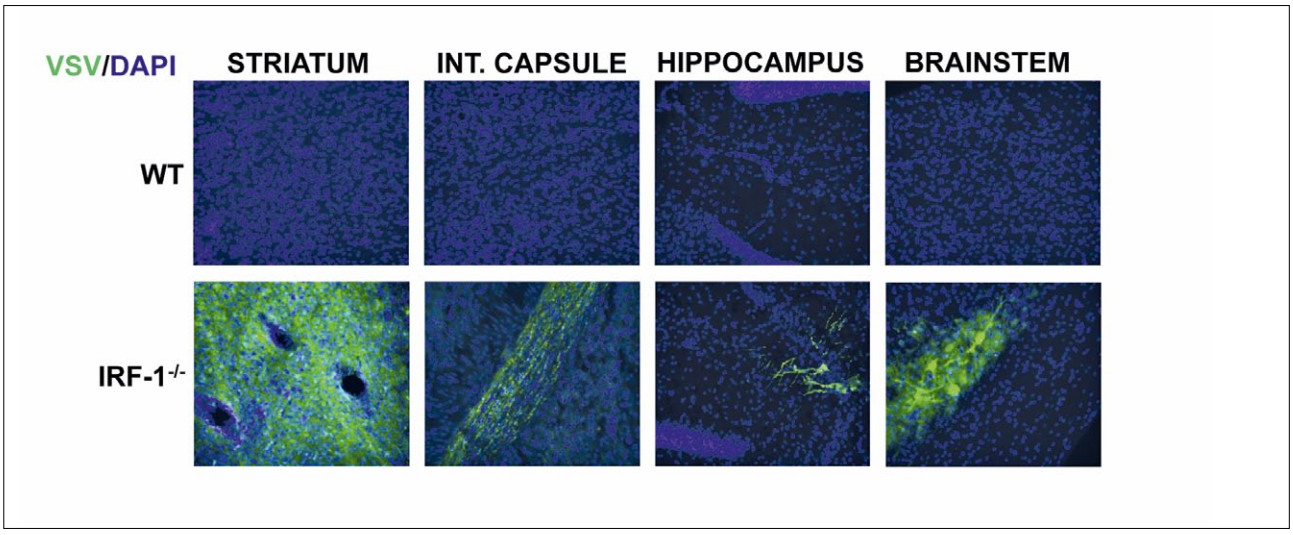


Figure 1: IRF-1 mediated antiviral effect is critical for controlling viral replication in the brain at later stages of virus infection. WT and IRF-1$^{-/-}$ mice infected with VSV-eGFP. Representative pictures of immunohistological analysis of VSV-eGFP protein in different brain parts. VSV-eGFP (green), DAPI (blue). Figure: Nair S. et al., *PLoS Pathog.* (2014)

Because the function of IRF-1 is not essential for cells mediating the adaptive immune response and as the cellular tropism of the virus is not changed, we conclude that IRF-1 inhibits viral replication by an intrinsic antiviral response. The antiviral function of IRF-1 is effective directly in the infected neurons.

These data suggest a temporal, non-redundant antiviral function of type I IFN and IRF-1, the latter playing a crucial role at late time points of VSV infection in the brain. Although type I IFN action is a prerequisite for survival from the infection, IRF-1 becomes increasingly crucial in neuronal tissue at a time point where clearance of the virus has not been achieved. The data highlight the importance of IRF-1 as an antiviral agent that acts in combination with the IFN system.

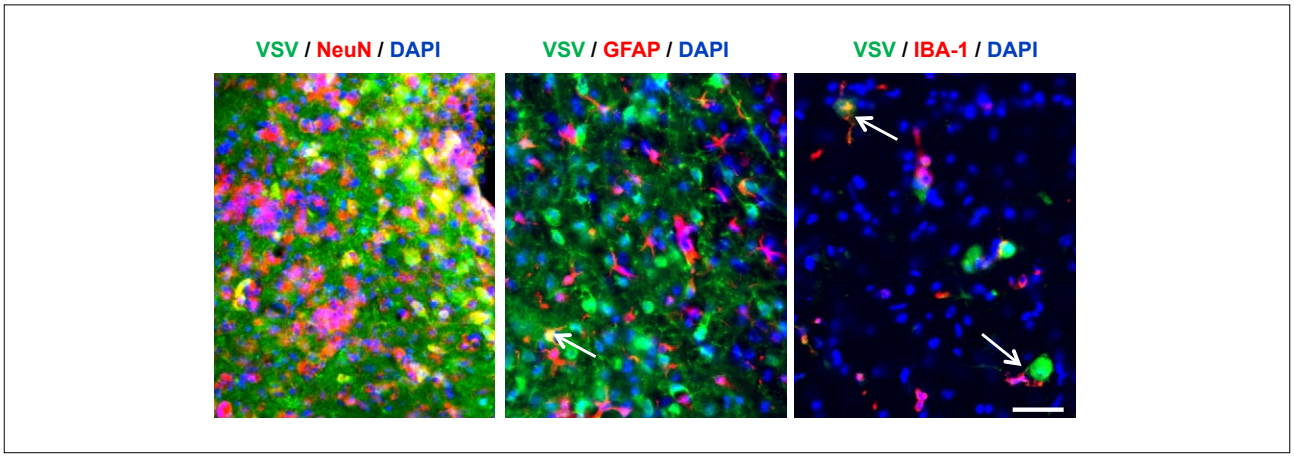


Figure 2: IRF-1 inhibits viral replication in neurons. Immunohistological analysis of infected brain cells. Detection of VSV (green) in neurons (NeuN: red), astrocytes (GFAP: red) and microglia (IBA: red) 6 days post infection. Scale bar 50µm. Figure: Nair S. et al., *PLoS Pathog.* (2014)
- arrows indicating co-localization of microglia with infected neurons

Nair S., Michaelsen-Preusse K., Finsterbusch K., Stegemann-Koniszewski S., Bruder D., Grashoff M., Korte M., Köster M., Kalinke U., Hauser H., Kröger A. **Interferon regulatory factor-1 protects from fatal neurotropic infection with vesicular stomatitis virus by specific inhibition of viral replication in neurons.** *PLoS Pathog.* **10(3)** e1003999 (2014)

ANTITUBERCULOSIS COMPOUNDS WITH A NOVEL MODE OF ACTION

Prof. Rolf Müller, Head of the Department "Microbial Natural Products"

Tuberculosis (TB) remains a major global health problem that caused an estimated 1.5 million deaths in 2013. The spread of drug-resistant TB additionally prioritizes the need for new drugs. In collaboration with Sanofi we showed that griselimycins are *Streptomyces*-derived lead compounds that exert potent activity against *Mycobacterium tuberculosis*, both *in vitro* and *in vivo*, by targeting the β-subunit of the DNA polymerase (also called sliding clamp or DnaN). We discovered that resistance to griselimycins is associated with amplification of the molecular target DnaN and connected to severe fitness costs. Our results demonstrate that griselimycins have high translational potential for TB therapy, validate DnaN as an antimicrobial target and capture the process of antibiotic pressure-induced gene amplification.

TB was declared a global public health emergency by the WHO in 1993 and still remains the second most common reason of death caused by an infectious disease (after HIV). Control of TB is further challenged by the emergence of drug-resistant *M. tuberculosis.* New drugs with novel modes of action and new therapy approaches with shortened treatment periods are needed.

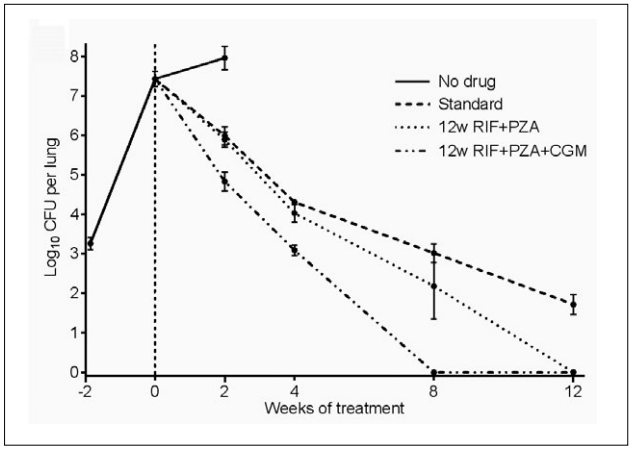


Figure 1: Co-administration of CGM with first-line anti-TB drugs in mouse models of TB. The data represent mean and standard deviation of the detectable, viable bacilli (colony-forming units, cfu) isolated from TB-infected mice during the intensive phase of treatment. Mice were either treated with RIF+INH+PZA followed by RIF+INH (standard regimen) or with RIF+PZA+CGM or with RIF+PZA for comparison. Co-administration with CGM resulted in no detectable viable bacilli after two months of treatment compared to the standard treatment, where viable bacilli were observed after three months of treatment. Drug doses: Rif, 10mg/kg/day, INH, 10 mg/kg/day, PZA, 150 mg/kg/day, CGM, 100 mg/kg/day. Figure: from Kling, A. et al., Science 348:1106 (2015). Reprinted with permission from AAAS.

Griselimycin (GM) and methylgriselimycin (MGM) are derived from *Streptomyces* strains and are highly active against *M. tuberculosis*, both *in vitro* and *in vivo*. For further *in vivo* studies the GM analog cyclohexylgriselimycin (CGM) was chosen from a library of synthetic GMs, based on its improved pharmacokinetic properties and its prolonged half-life in human plasma. The standard TB regimen consists of an intensive phase of daily rifampin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB) for two months, followed by a continuation phase of daily RIF and INH for four months. Compared to the standard TB regimen, an improved *in vivo* activity in mouse models of TB was observed when CGM was co-administered with first-line anti-TB drugs.

The observation that the GM biosynthetic gene cluster in *Streptomyces sp.* contains an additional DnaN homolog (GriR) and that overexpression of GriR results in the loss of sensitivity in other *Streptomyces*, led to the conclusion that GriR mediates self-resistance in *Streptomyces*. In mycobacteria GM resistance was found to be mediated likewise by target amplification. Genomic analyses of *Mycobacterium smegmatis* mutants selected *in vitro* by exposure to increasing concentrations of GM revealed an amplification of a chromosomal segment containing the *dnaN* gene. A similar situation was observed in CGM-resistant *M. tuberculosis* selected *in vivo*. Frequency of resistance was found to be very low (10^{-10}) and connected to severe fitness loss for the resistant pathogen.

GMs specifically exert bactericidal activity against *M. tuberculosis* and other organisms of the *Corynebacterineae* suborder. Binding analysis using surface plasmon resonance (SPR) confirmed that GMs bind to the mycobacterial sliding clamp with binding constants in the picomolar range. No interaction of GMs with the human sliding clamp analog was observed. Crystal structures of mycobacterial DnaNs with bound GMs revealed that GMs bind to the peptide binding site of DnaN, which is also responsible for the binding of DNA polymerases and other DNA-modifying enzymes. Further studies suggested that GM indeed blocks binding of the DNA polymerase to DnaN and leads to impaired DNA replication and to DNA damage caused by loss of DNA repair. Taken together these results demonstrated a clear potential for TB therapy by targeting the DNA polymerase sliding clamp and thus validated DnaN as a novel target in antimicrobial therapy.

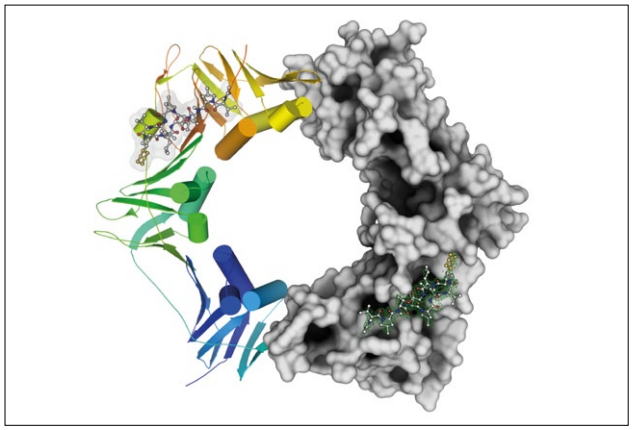


Figure 2: Co-crystal structure of the *M. tuberculosis* DnaN homodimer with CGM at a resolution of 1.9 Å (PDB: 5AGV). The left half of the dimer is depicted as a cartoon (coloured from the N-terminus (blue) to the C-terminus (red) of the peptide chain). The right half of the dimer is depicted as the molecular surface. CGM is depicted in white as ball and stick model with the cyclohexane moiety coloured in orange. The green mesh around the right CGM molecule represents the positive mF_o-F_c difference electron density (contoured at +2 σ) present in the binding-site after crystallographic refinement with the ligand omitted.

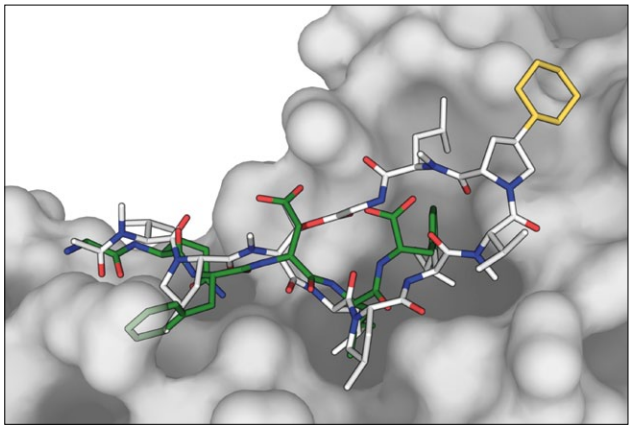


Figure 3: CGM blocks binding of the DNA polymerase (DnaE1) to DnaN. Superpositions of *M. tuberculosis* DnaN (shown as surface) in complex with CGM (white/orange) with the internal DnaN binding site of mycobacterial DnaE1 (*GQFDLF*, green). The DnaE1 derived peptide has been modelled based on the crystal structure of a C-terminal peptide (*GQLGLF*) from *E. coli* DNA polymerase II in complex with the *E. coli* β-clamp (PDB: 3D1E). The two *in silico* substitutions are modeled as the most probable rotamers.This internal peptide of DnaE1 comprises the residues 945-950, which have been shown to contain the main sequence (QFDLF) responsible for interaction of mycobacterial DnaE1 with DnaN.

Kling, A., Lukat, P., Almeida, D.V., Bauer, A., Fontaine, E., Sordello, S., Zaburannyi, N., Herrmann, J., Wenzel, S.C., König, C., Ammerman, N.C., Barrio, M.B., Borchers, K., Bordon-Pallier, F., Brönstrup, M., Courtemanche, G., Gerlitz, M., Geslin, M., Hammann, P., Heinz, D.H., Hoffmann, H., Klieber, S., Kohlmann, M., Kurz, M., Lair, C., Matter, H., Nuermberger, E., Tyagi, S., Fraisse, L., Grosset, J.H., Lagrange, S. & Müller, R. **Targeting DnaN for tuberculosis therapy using novel griselimycins.** *Science* **348**, 1106-1112 (2015)

FATTY ACID METABOLISM AND Th17 CELLS

Prof. Tim Sparwasser, Head of the Institute for Infection Immunology, TWINCORE

Soraphen A, a specific Myxobacteria-derived natural compound, targets the cellular fatty acid metabolism and shifts mouse and human T cell development towards an anti-inflammatory phenotype. The value of this approach was confirmed in a preclinical animal model in which mice were protected against chronic inflammatory disease when treated with a pharmaceutically-optimized Soraphen A. The results of this work not only mark a significant conceptual advance in the field of immunology, but may also have an immediate therapeutic perspective for the treatment of various inflammatory diseases

Regulatory T cells (Treg) play a crucial role for maintaining immunological tolerance. Due to their strong capacity to suppress the development and function of inflammatory effector T cell subsets, Treg cells have an essential impact on autoimmunity, anti-tumour responses and the immune reactions against infectious pathogens. In contrast to Treg cells, Th17 cells represent an effector T helper cell lineage with profound pro-inflammatory capacity, contributing critically to inflammation and pathology in autoimmune diseases. Regarding the essential role of T cells for both the induction and the control of immune responses, it is evident that new approaches with the potential to modulate the formation of inflammatory Th17 versus anti-inflammatory Treg cells will have a major impact not only on the development of novel treatments for autoimmune diseases, but also in the field of tumour immunology and infection research.

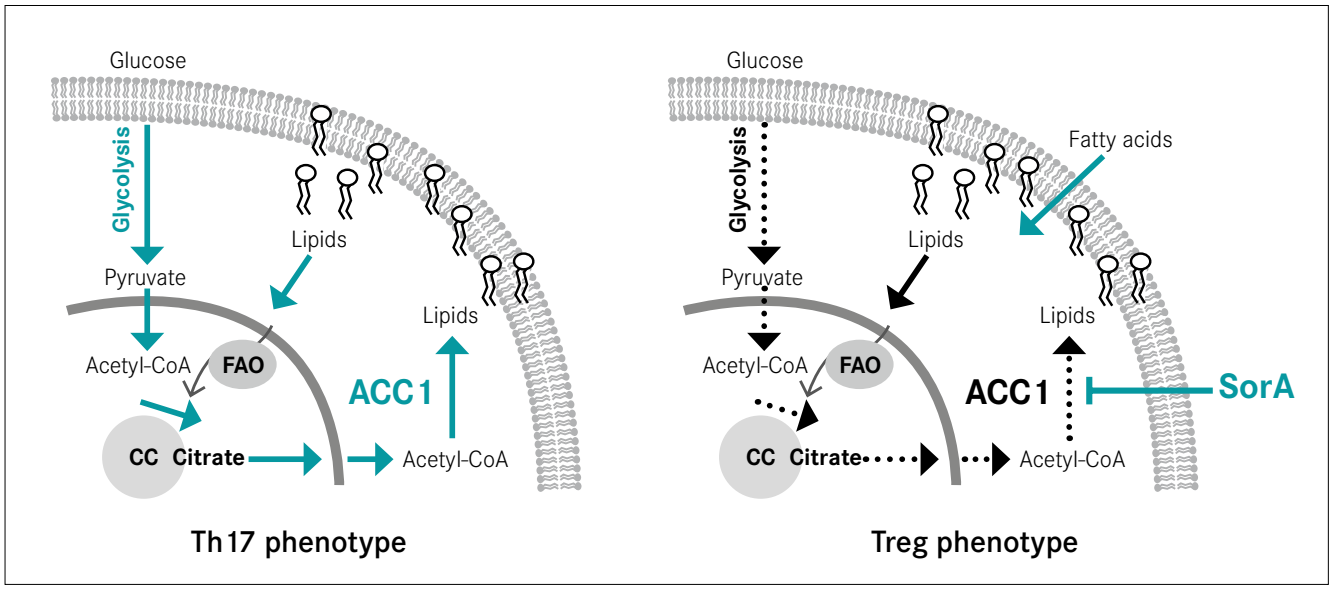


Figure 1: Induction of a Treg phenotype by inhibition of ACC1. Th17 development depends on the activation of the "glycolytic-lipogenic pathway" (left, green arrows). Glucose is taken up by developing Th17 cells and metabolized via glycolysis to pyruvate and acetyl-CoA, which is shuttled via the citrate-cycle (CC) towards de novo synthesis of fatty acids. Blocking this pathway by Soraphen A (SorA) inhibits Th17 development and leads to the induction of Treg cells, which can take up fatty acids from the environment (right, green arrow) and rely on fatty acid oxidation (FAO).

A collaboration of scientists from HZI, TWINCORE, HIPS and MHH led to the identification of a Myxobacteria-derived compound with the capacity to selectively restrain the development of pro-inflammatory T cells such as Th17, while at the same time favouring the induction of Treg cells. Our results demonstrate that the compound Soraphen A binds to and inhibits the function of the enzyme Acetyl-CoA-Carboxylase 1 (ACC1) in T cells. ACC1 plays an essential role in the cellular fatty acid metabolism by catalysing the rate-limiting step in the *de novo* synthesis of long chain fatty acids which have important functions for the biology of the cell, e.g. as crucial components of cellular membranes. Using a range of different metabolic assays including ^{13}C tracing as well as real-time extracellular metabolic-flux analyses we could show that Th17, but not Treg cell development depends on the carbon transfer from glucose via glycolysis and the mitochondrial citrate-cycle towards *de novo* fatty acid synthesis. Thus, interfering with this "glycolytic-lipogenic pathway" by ACC1 inhibition shifts T cell development from Th17 towards Treg cells (Figure **1**). Using Soraphen A treatment or mice carrying a T cell-specific deletion of ACC1, we could furthermore show that inhibition of ACC1 has a strong effect on the development of Th17-associated inflammatory reactions *in vivo*. Both Th17 induction and disease-associated pathology was significantly reduced in experimental autoimmune encephalomyelitis, the mouse model for multiple sclerosis. Finally, the translational potential of this approach was established in a series of additional *in vitro* experiments which showed that Soraphen A has the same immune modulatory effect also in human T cells.

Together, these results demonstrate that manipulation of specific processes of the intracellular T cell metabolism such as *de novo* fatty acid synthesis can have profound effects on the development of inflammatory versus regulatory T cell subsets. Targeting ACC1 may therefore represent a fruitful strategy for controlling inflammatory and autoimmune diseases.

Berod, L., Friedrich, C., Nandan, A., Freitag, J., Hagemann, S., Harmrolfs, K., Sandouk, A., Hesse, C., Castro, C. N., Bähre, H., Tschirner, S. K., Gorinski, N., Gohmert, M., Mayer, C. T., Huehn, J., Ponimaskin, E., Abraham, W.-R., Muller, R., Lochner, M. & Sparwasser, T. **De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells.** *Nat Med* **20** 1327-33 (2014)

HOST-DERIVED INHIBITION OF HEPATITIS C VIRUS REPLICATION

PD Eike Steinmann, Head of the Research Group "Virus Transmission", TWINCORE

Infections with hepatitis C virus (HCV) are responsible for a major health burden worldwide. In this study, we found that HCV infection causes the upregulation of the interferon-stimulated gene encoding human cholesterol-25-hydroxylase (hCH25H) in primary human hepatocytes and chronic hepatitis C virus patients. 25-hydroxycholesterol (25HC), the product of this enzyme, profoundly blocks HCV RNA replication by affecting the biogenesis of the membranous viral replication factory. Our results contribute to a better understanding of *in vivo* interferon-driven antiviral responses as well as of the effect of interferon treatment.

Hepatitis C virus, a member of the *Flaviviridae*, is a positive-strand RNA virus that primarily infects human hepatocytes. Worldwide, an estimated 140 million people are chronically infected with HCV and are at high risk for developing severe liver damages, including hepatic steatosis, fibrosis, cirrhosis and hepatocellular carcinoma. For the past 25 years, therapy consisted of treatment with interferon (IFN)-alpha and the nucleoside analogue ribavirin. Recently, the licensing of directly acting antivirals (DAAs) targeting the HCV non-structural proteins improved cure rates profoundly, now reaching levels of over 90%. However, many infected individuals have not been diagnosed and a prophylactic vaccine is not available, which likely is required for global control and even eradication of HCV.

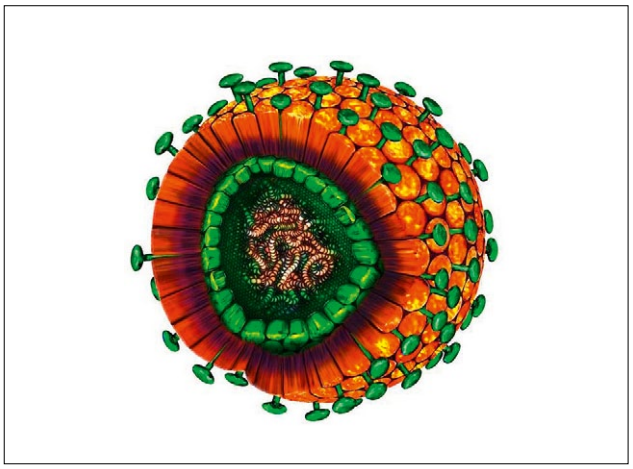


Figure 1: Model of hepatitis C virus.

The first line of immune defence against HCV is based on cell-intrinsic innate immunity in the liver cells that leads to the induction of the type-I and -III IFN system. These cytokines induce a plethora of genes exerting a strong antiviral effect. Nevertheless, the modes of action of only a few interferon-stimulated genes (ISGs) have been well elucidated. Recent studies have reported that cholesterol-25-hydroxylase (CH25H) is expressed as an ISG and mediates antiviral activities against different enveloped viruses through the production of 25HC. However, the intrinsic regulation of human CH25H (hCH25H) expression within the liver as well as its mechanistic effects on HCV infectivity remain elusive.

In this study, we characterized the expression of hCH25H using liver biopsies and primary human hepatocytes (PHH). In addition, the antiviral properties of this protein and its enzymatic product, 25HC, were further characterized against HCV in tissue culture. Interestingly, here we found that hCH25H mRNA levels were significantly upregulated both in HCV-positive liver biopsies and HCV-infected PHH. Apparently, the expression of hCH25H in PHH was primarily and transiently induced by type-I IFN. Next, we performed transient expression of hCH25H in human hepatoma cells followed by HCV challenge. Here, we observed that transient expression of hCH25H resulted in strong restriction on HCV infection in a genotype-independent manner. This antiviral effect required the enzymatic activity of the hydroxylase as the main antiviral effect was mediated through the presence

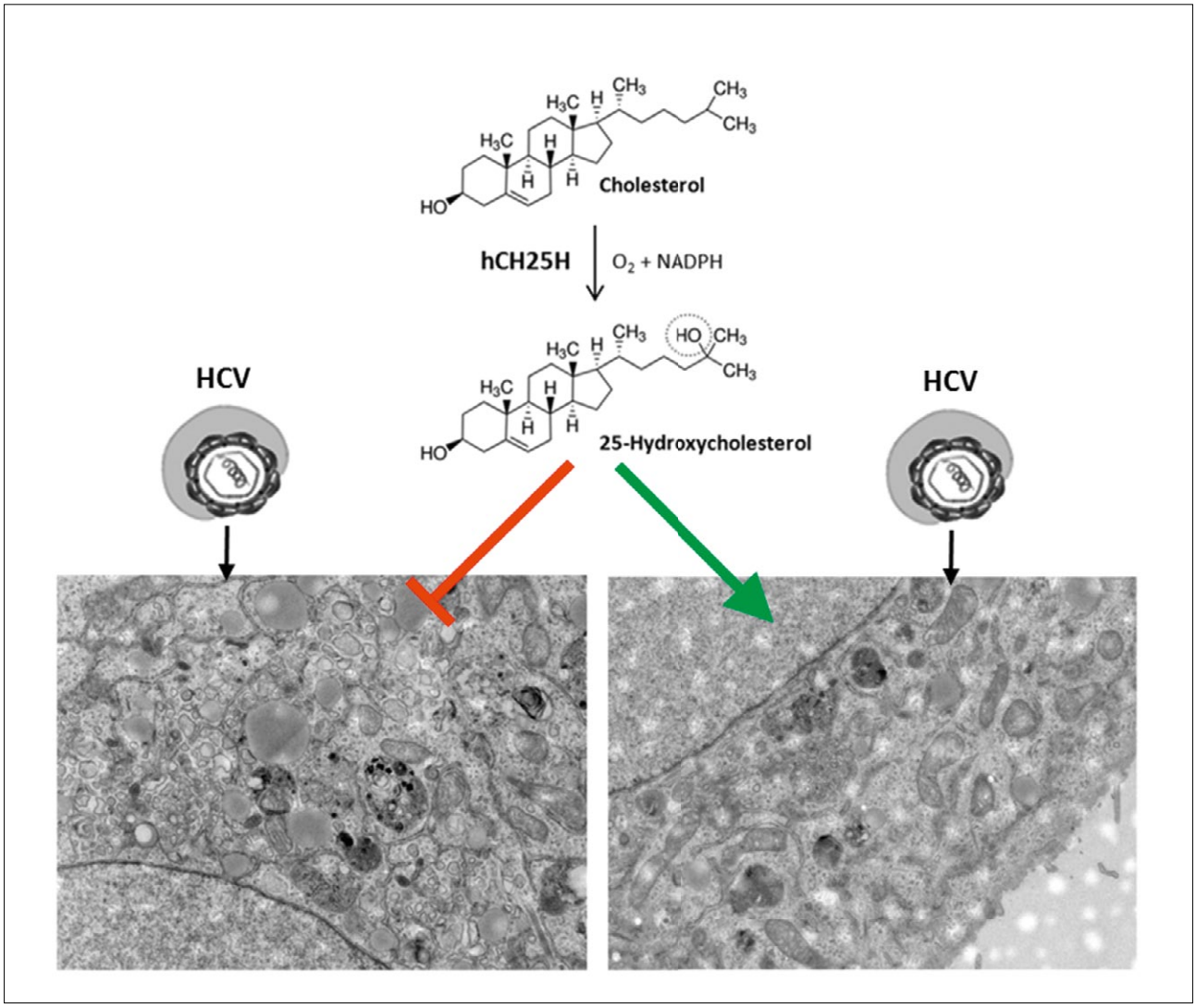


Figure 2: Depicted in the upper part is the synthesis of 25HC by CH25H catalysis, molecular oxygen and cholesterol as substrates and NADPH as a cofactor. The lower part demonstrates the scheme to analyze the effect of 25HC on the formation of double membrane vesicles, the main constituent of the membranous web induced by HCV. The inhibitory action of 25HC on membranous web formation after HCV infection is shown on the right compared to a mock-treated liver cell on the left, as assessed using transmission electron microscopy. Figure: HZI/Steinmann with kind permission from Inés-Romero-Brey, Heidelberg University Hospital

of 25HC. In an attempt to find the mode of action, we observed an inhibition of viral membrane fusion during the entry process by 25HC, which was not due to a virucidal effect. Yet the primary effect by 25HC on HCV was at the level of RNA replication, which was observed by using subgenomic replicons of two different genotypes. Further analysis using electron microscopy revealed that 25HC inhibited formation of the membranous web, the HCV replication factory, independent of RNA replication. In conclusion, HCV infection causes the upregulation of interferon-inducible CH25H in vivo. Its product 25HC restricts HCV primarily at the level of RNA replication by preventing the formation of the viral replication factory.

Anggakusuma, Romero-Brey, I., Berger, C., Colpitts, C. C., Boldanova, T., Engelmann, M., Todt, D., Perin, P. M., Behrendt, P., Vondran, F. W., Xu, S., Goffinet, C., Schang, L. M., Heim, M. H., Bartenschlager, R., Pietschmann, T. & Steinmann, E. **Interferon-inducible cholesterol-25-hydroxylase restricts hepatitis C virus replication through blockage of membranous web formation.** *Hepatology* **62** 702–714 (2015)

QUORUM SENSING IN *STREPTOCOCCUS MUTANS* CONTROLS ANTIBIOTIC SYNTHESIS AND COMPETENCE

Prof. Irene Wagner-Döbler, Head of the Research Group "Microbial Communication"

***Streptococcus mutans (S. mutans)* is a bacterium of human dental plaque that contributes to caries development by forming thick biofilms and excreting organic acids. It uses two small signaling peptides for its cell-density dependent communication – a process that is called quorum sensing (QS). They control (1) the synthesis of peptide antibiotics that kill certain other bacteria of the dental plaque microbiome, and (2) the synthesis of a membrane apparatus for genetic competence, i.e. the ability to take up external DNA and integrate it into its own genome. It was unknown how the two QS systems are connected, and why under certain conditions genetically identical *S. mutans* cells can split into a competent and a non-competent subpopulation.**

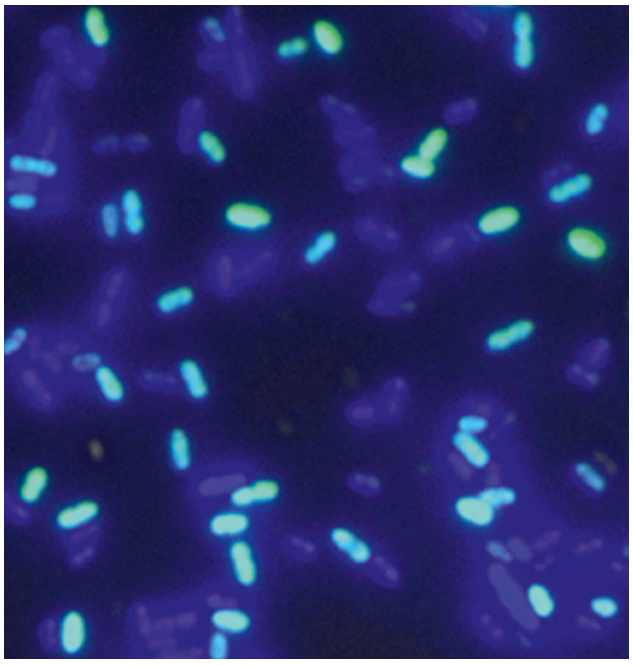


Figure 1: Bacteriocin synthesis is expressed unimodally, while competence is expressed in a subpopulation of *Streptococcus mutans* only. The promoter of the gene for competence *(comS)* is tagged by a green fluorescent protein, while the gene for mutacin synthesis *(cipB)* is tagged by a blue fluorescent protein. Cells were cultivated in complex media and stimulated by competence stimulating peptide (formerly called CSP, now called MIP (mutacin-inducing peptide).)

In order to clarify the genetic hard-wiring of the components of the QS signaling cascade in *S. mutans* we developed a toolbox of single and dual fluorescent reporter strains for the key genes of both signaling cascades and followed signal propagation on the single cell level. We found that the QS signal historically discovered first, the competence stimulating peptide (CSP), does not control competence, but exclusively induces the synthesis of peptide antibiotics. These are called mutacins in *S. mutans*, thus we suggested renaming CSP as MIP (mutacin-inducing peptide).

The second signal, SigX-inducing peptide (XIP), not only controls competence development, but also mutacin synthesis through the alternative sigma factor SigX, which therefore is the master regulator of QS in *S. mutans*. The simultaneous expression of bacteriocins and competence has been observed previously, but no genetic connection between the two traits had been found. We demonstrated that ComE, the response regulator for bacteriocin synthesis, is regulated by SigX because it carries the so-called cin-box in its promoter region, a conserved nucleotide sequence, which is required for transcription by SigX-activated RNA polymerase. This genetic integration of both signaling systems through the alternative sigma factor SigX suggests that *S. mutans* increases its genetic repertoire via QS-controlled predation on neighboring species in its natural habitat.

We investigated modality, i.e. whether the population is homogenous and uniformly expresses a certain factor or whether it is heterogeneous and shows a certain phenotype only in a subpopulation. In complex media, mutacins are produced by all cells of *S. mutans*, while only a subpopula-

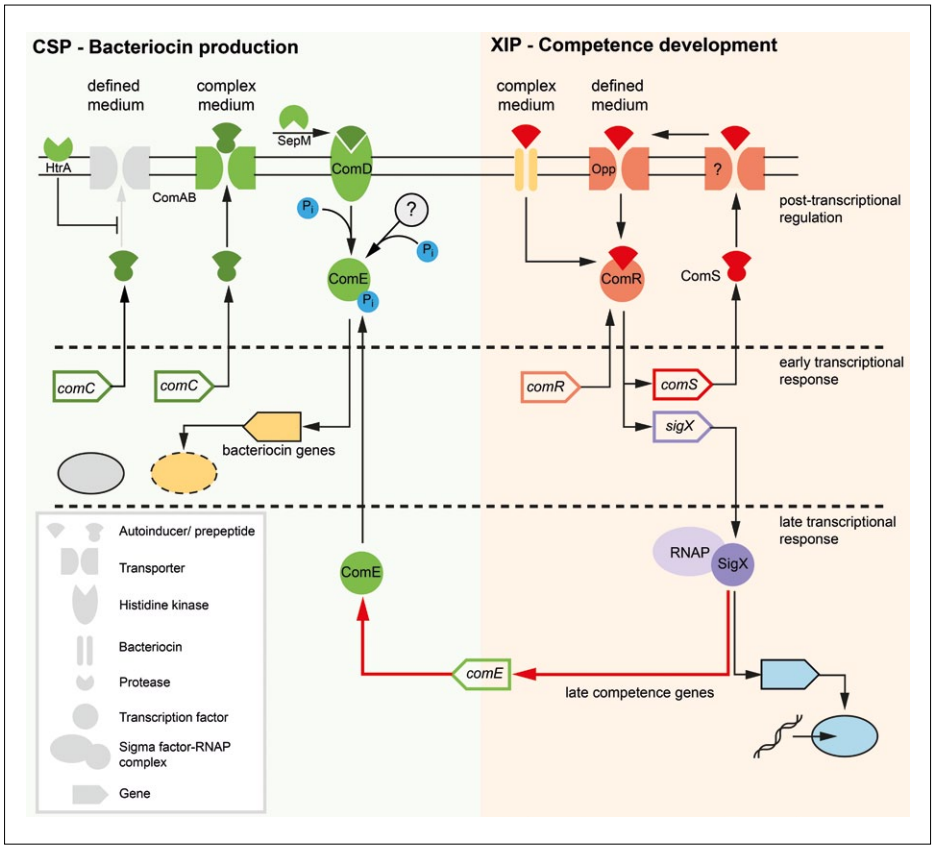


Figure 2: Model of competence development in *S. mutans*. Two quorum sensing pathways are operating in *S. mutans*. Bacteriocin expression is regulated via CSP signaling and the ComDE two-component system (green box). CSP should be renamed MIP (mutacin-inducing peptide). Competence development is regulated via XIP signaling and the ComR regulator (red box). Both systems are connected via the alternative sigma-factor SigX, which controls *comE* transcription (red arrow). Competence regulation proceeds in three successive steps: (1) Instantaneous post-transcriptional activation of the regulator ComR. (2) Early transcriptional response: Transcription of *comS*, resulting in a positive feedback loop for signal synthesis, and of sigX. (3) Late transcriptional response: Transcription of the *SigX* regulon, including the competence genes and the *comE* response regulator. Upon competence development the medium determines via which mechanism XIP is imported into the cell. Figure adapted from Reck et al., PLoS Genet (2015)

tion becomes competent (Figure 1). The reason for this puzzling observation was found using mutants of the response regulator ComE that mimic the phosphorylation status of the protein. These mutants demonstrated that mutacin production is regulated posttranslationally through phosphorylation of ComE, which instantaneously triggers mutacin synthesis in the entire population. By contrast, the modality of competence is transcriptionally regulated through a two-stage signalling cascade.

The origin of bimodality where the population splits into two subpopulations differing in competence was discovered using over-expression of key genes. The *comRS* module is responsible for bimodality. It consists of the gene *comS*, which encodes the intracellular pre-peptide for the XIP signal, and the gene for the response regulator ComR. Both are connected through a positive feedback-loop, i.e. ComR induces expression of *comS*. Cell sorting of fluorescent reporter strains showed that the modality of *comRS* determines the modality of the downstream regulators, including the alternative sigma factor SigX and the response regulator ComE.

The system appears to be efficiently tailored towards the complex microbiome of the oral cavity by ensuring not only death of competitors, but the simultaneous use of their DNA for genetic adaptation.

Reck M., Tomasch J., Wagner-Döbler I. **The Alternative Sigma Factor SigX Controls Bacteriocin Synthesis and Competence, the Two Quorum Sensing Regulated Traits in *Streptococcus mutans*.** *PLoS Genet* **11(7)**, e1005353 (2015)

UNRAVELING A NOVEL MECHANISM OF VIRAL ANTAGONISM

Prof. Dagmar Wirth, Head of the Research Group "Model Systems for Infection and Immunity"

Viral pathogens use a variety of different strategies to counteract the immune response. Frequently, they target the type I interferon system, which is a potent cellular defence program rapidly induced upon infection. Viruses counteract this host response by expressing virus-encoded antagonistic proteins. This sets the stage for a race between the infected cells, which immediately start their antiviral program, and the viruses, which initiate expression of encoded antagonistic proteins.

To elucidate the dynamics of this process we designed a cellular system that allows these counteracting activities to be uncoupled.

While the key factors that control interferon production have been identified, the dynamics of interferon induction and the counteracting viral protein(s) have been largely unknown. This limits the rational development of antiviral therapies that target these antagonistic proteins. To get insights into the dynamics of virus/host interaction we established a synthetic biology-based approach. Rather than relying on natural infection processes in which induction of interferon and production of antagonistic proteins are fixed and cannot be modulated, we genetically modified cells to independently control these two counteracting processes. To this end, we employed synthetic doxycycline–dependent expression cassettes to control the production of fluorescently tagged antagonistic proteins, in particular NS3/4a

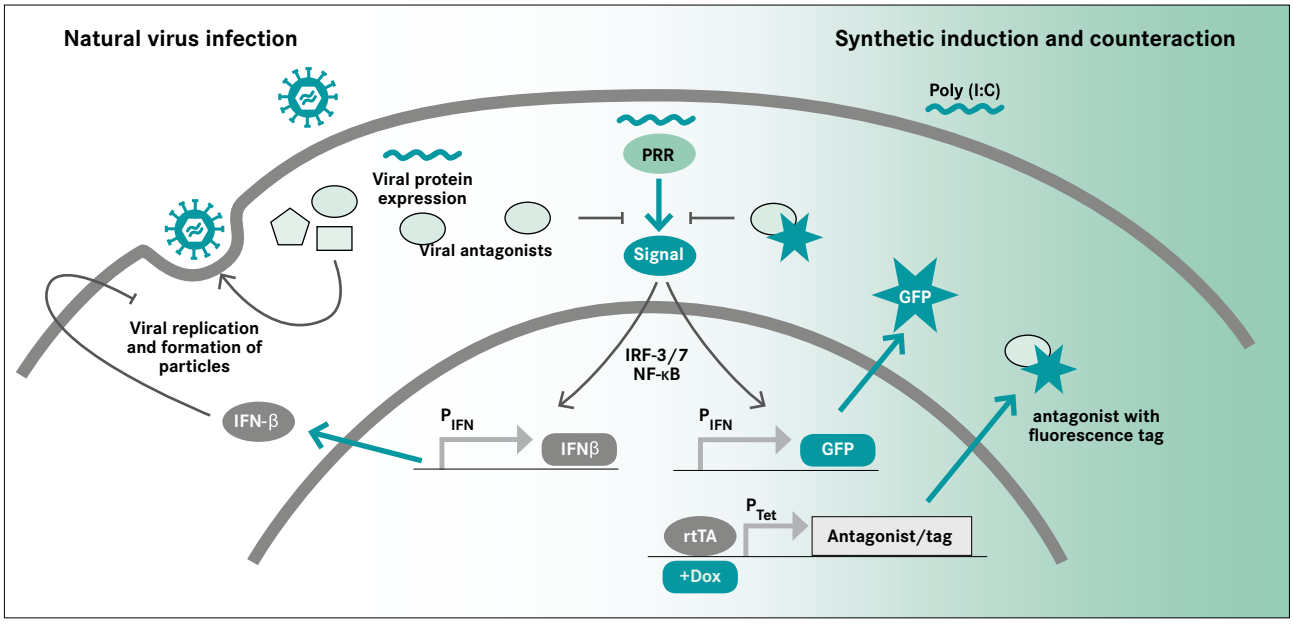


Figure 1: Synthetic intervention in the interferon system. Natural viral infection (left side): Virus infection leads to induction of IFN-ß, which blocks viral replication. Viral antagonists in turn have an inhibiting effect on the IFN-β cascade.
Synthetic setting (right side): Synthetic dsRNA is sensed by intracellular receptors (PRR) and leads to the induction of the IFN-ß promoter driving the green fluorescent protein (GFP). A synthetic doxycycline-dependent expression cassette is used to control expression of viral antagonistic proteins and can be followed by red fluorescence.

protease from hepatitis C virus and NS1 protein from influenza virus. These synthetic modules were integrated in IFNß-GFP reporter cells that authentically monitor the activity of the interferon ß promoter by inducing GFP. Upon stimulation of the cells with synthetic triggers we induced antagonist expression and followed the course of the counteracting reactions over time. To overcome cell-to-cell heterogeneity in the onset of expression, we employed time-lapse microscopy of a large number of individual cells.

We determined the specific starting points of interferon expression and of antagonistic protein production in individual cells. Thereby, we could confirm that an early onset of antagonist expression can completely block induction of the interferon promoter. An unexpected observation was made when we monitored cells in which the interferon expression started first and was followed by subsequent induction of the antagonist: in this setting we saw that interferon expression was halted. This suggests that the interferon cascade – when previously initiated – can be blocked even by late onset of viral antagonist expression, i.e. in retrospect. Statistical analysis revealed a correlation between onset of antagonistic protein expression and halt of interferon induction. This proves that the interferon cascade does not follow a "hit-and-run" principle, a mechanism that would be expected to provide optimal protection. Rather, the cascade requires ongoing stimulation for maximal expression. Accordingly, viral antagonist expression can limit interferon production even when it comes late.

Together, the investigation of the dynamics of virus/host interaction in a controlled setting revealed a new mechanism of viral counteraction. In future, this cell system might also serve for validating compounds that selectively restore the immune response to viral infection.

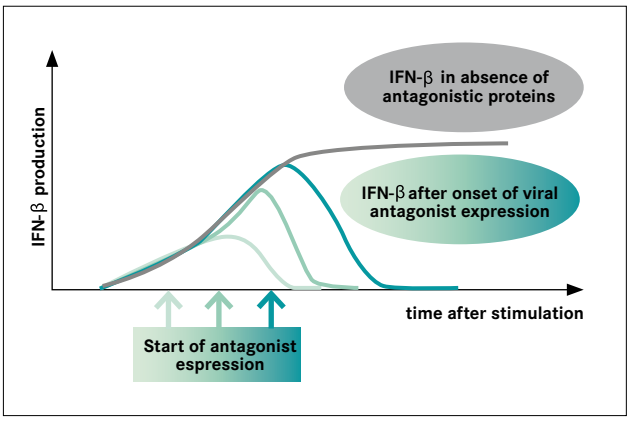


Figure 2: Schematic representation of IFN-β expression after induction by dsRNA (grey line) and upon subsequent doxycycline-induced expression of an antagonist (turquoise) in the same cell by means of time-lapse microscopy (live-cell imaging).

Rand, U., Hillebrand, U., Sievers, S., Willenberg, S., Köster, M., Hauser, H. & Wirth, D. **Uncoupling of the dynamics of host-pathogen interaction uncovers new mechanisms of viral interferon antagonism at the single-cell level.** *Nucleic Acids Res* **42** e109 (2014)

Myxobacterial swarming cells on an agar surface.

PARTNERS AND NETWORKS

THE LINK BETWEEN BASIC RESEARCH AND CLINICAL MEDICINE

Prof. Ulrich Kalinke, Excecutive Director of TWINCORE

THE TWINCORE CENTRE FOR EXPERIMENTAL AND CLINICAL INFECTION RESEARCH

Founded in 2008 as a joint venture between HZI and MHH, the TWINCORE Centre for Experimental and Clinical Infection Research aims to enhance the interaction between basic researchers and clinician scientists. At TWINCORE, multidisciplinary teams strive to channel new knowledge into clinical practice and to translate clinical observations into research projects that will improve our understanding of disease mechanisms.

Research institutes at TWINCORE

Currently four W3 professorships are established at TWINCORE. The positions are held by the heads of the main divisions of TWINCORE: Prof. Ulrich Kalinke, Executive Director of TWINCORE and Director of the Institute for Experimental Infection Research, Prof. Thomas Pietschmann, Director of the Institute for Experimental Virology, Prof. Tim Sparwasser, Director of the Institute for Infection Immunology, and Prof. Susanne Häußler, Director of the Institute for Molecular Bacteriology.

The research group “Gene and Cell Therapy” at TWINCORE, headed by Prof. Michael Ott, has been delegated to TWINCORE by the Department of Gastroenterology, Hepatology and Endocrinology at MHH (headed by Prof. Michael P. Manns).

TWINCORE
Zentrum für Experimentelle
und Klinische Infektionsforschung

Translational infection research at TWINCORE

Research at TWINCORE focuses on the analysis of pathogen-host interactions. During long periods of co-evolution, pathogens and their hosts have developed complex strategies enabling both host populations and pathogen populations to survive. Researchers at TWINCORE are studying the mechanisms that maintain this balance. To induce protective immunity in the host organism, recognition of the properties ”foreign“ plus ”danger” is needed. Current work is therefore examining how “danger” signals are sensed and how regulatory cells affect pathogen-specific immunity.

New mechanisms of pathogen inhibition are being investigated at TWINCORE. To this end, biological compound libraries are being examined to find antiviral and antibacterial substances. There are still numerous infectious diseases for which no vaccine is available. Novel vaccination strategies are therefore a main research focus at TWINCORE. New therapeutic or prophylactic approaches discovered in basic research need to be extensively analysed in preclinical assays prior to first-in-human testing. At TWINCORE, new preclinical models are being developed with the objective of better predicting reactions in humans.

Clinical cooperations

To reinforce collaboration with clinical partners, research at TWINCORE focuses on (i) chronic viral infections, (ii) gastrointestinal infections, (iii) pulmonary infections, and (iv) infections of the immunocompromised host. Twinning projects between clinician scientists from MHH and basic researchers from TWINCORE have turned out to be particularly effective. The Young Academy founded by the MHH and supported by the HZI trains clinician scientists over a period of three years, enabling them to devote four months each year to research while keeping their clinical posts. Additionally, the TRAIN Academy “Translational Research and Medicine: From Idea to Product” offers 2-year in-service training courses for basic researchers as well as physicians and clinician scientists.

Research at TWINCORE also helps to pave the way towards the establishment of individualized infection medicine.

Research results

In 2014 and 2015, remarkable progress was made in several TWINCORE research projects with high clinical relevance. TWINCORE researchers elucidated cellular mechanisms of inhibiting the replication of the hepatitis C virus, thus opening up new perspectives for potential therapeutic approaches against this widespread pathogen. They also identified the antiviral effect of compounds like flunarizin as inhibitors of the cell entry of hepatitis C viruses.

Other TWINCORE scientists resolved key processes in the interplay between fatty acid metabolism in T cells and the balance of inflammatory versus regulatory T cell subsets (see also section “Highlight Publications”). These insights are of particularly high relevance with respect to the role of inflammatory processes in the onset of various diseases. Virologists at TWINCORE decoded a hitherto unknown camouflage mechanism of cytomegalovirus (CMV), bacteriologists uncovered the genetic basis for the particularly high resistance shown by a variant of biofilm-forming *Pseudomonas aeruginosa.*

In the upcoming years TWINCORE will further enhance collaboration with clinician scientists. In this context exploration of the potential of molecular imaging in infection research and medicine will be one new focus.

TOP-LEVEL PHARMACEUTICAL SCIENCE

Prof. Rolf Müller, Managing Director of the HIPS

THE HELMHOLTZ INSTITUTE FOR PHARMACEUTICAL RESEARCH (HIPS)

The Helmholtz Institute for Pharmaceutical Research (HIPS) was founded jointly by HZI and Saarland University (UdS) in 2009 with the aim of combining the outstanding expertise of both institutions in natural compound research, medicinal chemistry and drug delivery. The new institute developed vigorously and now employs more than 100 people in its three departments and three junior research groups. In 2015, the main construction work for the new HIPS research building was finished. Two years after the groundbreaking ceremony and six years after the foundation of HIPS, the inauguration of the new building took place on 2 October, 2015. In the past two years, the HIPS departments have made promising advances in their respective research fields.

The Department **Microbial Natural Products** (MINS, head: Prof. Rolf Müller) focuses on the identification and development of microbial compounds, primarily from myxobacteria and actinomycetes. The main accomplishments of MINS in 2014/2015 include the discovery of disciformycins (active against multiresistant staphylococci) and the discovery and mode of action studies of cystobactamides, representing novel and highly potent broad-spectrum antibiotics. This work led to a research project funded through the German Center for Infection Research (DZIF) and carried out jointly by HIPS, HZI and Leibniz University Hannover (LUH) to address total synthesis, production and strain improvement and to optimise cystobactamides using medicinal chemistry techniques. Furthermore, studies on novel griselimycins - depsipeptides showing superb *in vivo* antituberculosis activity caused by targeting the DNA polymerase sliding clamp - were the outcome of a successful collaboration between HIPS/HZI and Sanofi. Two junior research groups are affiliated to MINS: “Metabolic Engineering of Actinomycetes” and “Structural Biology of Biosynthetic Enzymes”.

The Department **Drug Design and Optimization** (DDOP) headed by Prof. Rolf Hartmann specialises in pharmaceutical and medicinal chemistry. One main focus of DDOP is the rational design of compounds abolishing bacterial pathogenicity without affecting cell viability, so-called pathoblockers. In a ligand-based approach, the DDOP team has developed a quorum sensing inhibitor (QSI) with promising *in vivo* potency against *Pseudomonas aeruginosa* infections based on the fact that it inhibits formation of biofilms by the bacteria. While this QSI might be suitable for pulmonary delivery, current efforts employing a fragment-based approach are concentrating on the development of compounds for systemic applications. In further projects, researchers at DDOP are addressing bacterial metalloproteases such as collagenases from *Clostridium* and *P. aeruginosa* LasB as promising anti-virulence targets. The junior research group "Chemical Biology of Carbohydrates" has developed novel derivatives of D-mannose which show high affinity towards *P. aeruginosa* virulence factor LecB rendering them promising candidates for application in an anti-adhesive strategy.

Helmholtz-Institute for Pharmaceutical Research Saarland

HELMHOLTZ ZENTRUM FÜR INFEKTIONSFORSCHUNG

UNIVERSITÄT DES SAARLANDES

The department **Drug Delivery** (DDEL, head: Prof. Claus-Michael Lehr) is examining the tailored transport of drugs to the disease's site of origin. With the aim of accelerating the translation of anti-infectives and vaccines for clinical use, DDEL has designed biodegradable nanoparticles for transcutaneous vaccination. These nanoparticles elicit a significant immune response after needle-free delivery of the antigen and innovative adjuvants to the immune cells of the skin. Further, by encapsulating a novel quorum sensing compound in solid lipid nanocarriers, they were found to improve delivery approximately sevenfold in a disease relevant reporter assay. In addition, DDEL researchers have successfully developed a model of the inner membrane of gram-negative bacteria and are currently investigating the predictive value of this model with selected anti-infective compounds.

Regular internal events such as monthly HIPS-talks and HIPS-papers sessions encourage the close contact between the departments and junior research groups. The *HIPS Symposium* on pharmaceutical sciences devoted to infection research takes place once per year and attracts approximately 200 scientific participants from industry and academia to HIPS and the UdS Campus.

The inauguration of the new HIPS building took place in October 2015.

SYNCHROTRON LIGHT SOURCES IN INFECTION RESEARCH

Prof. Michael Kolbe, Head of the department “Structural Infection Biology”

Image: hammeskrause architekten

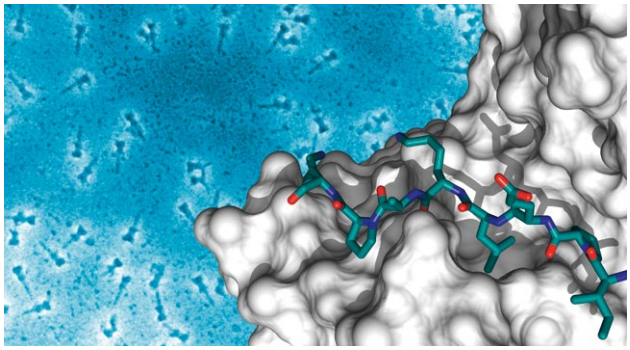
THE CENTRE FOR STRUCTURAL SYSTEMS BIOLOGY (CSSB)

To study biological processes using high-intensity light sources: that is the mission of the Centre for Structural Systems Biology (CSSB), founded jointly by nine partner institutions in North Germany. Since the beginning of 2015, the HZI has been represented at the CSSB by the department “Structural Infection Biology” (STIB). A new CSSB building is currently being constructed on the DESY (Deutsches Elektronen-Synchrotron) campus in Hamburg, in the direct vicinity of the particle accelerator PETRA III. Completion of construction and commencement of operation are scheduled for 2017.

With powerful synchrotron radiation and novel X-ray laser sources at DESY, the structure and dynamics of biomolecules can be studied at unprecedented spatial and temporal resolution. HZI researchers want to use these radiation sources above all to elucidate the function of the type III secretion system (T3SS) of gram-negative bacteria, which plays a key role in the delivery of pathogenicity factors into host cells. Their interest is focussed specifically on the function of the needle complex comprising twenty to thirty different proteins.

CSSB
Centre for Structural
Systems Biology

Using DESY radiation sources, HZI researchers obtain data by crystallography and other spectroscopic analytical methods as well as electron microscopy and NMR-based techniques. They intend to apply such methods to study the components of the T3SS, in particular the interactions between these components and with host proteins, at atomic resolution. The overarching goal of their studies is to explain the transport mechanisms of this secretion system, which is common to so many bacterial pathogens, in order to be able to switch it off using targeted agents and thus deprive the bacteria of an important virulence factor.



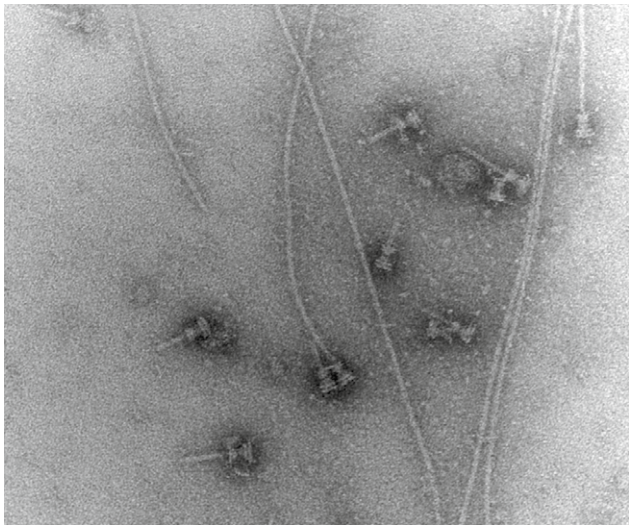
Structure of the IpaB/IpgC virulence factor complex from *Shigella flexneri*. In the background: electron microscopic image of isolated T3SS needle complexes (also from *S. flexneri*). Image: HZI/Kolbe

Organisationally, the departments and research groups at the CSSB formally belong to their native institutes and pursue their own respective projects. Beyond this, however, the concept of the centre is strongly oriented towards collaboration and fruitful interaction. The fact that all research groups at the centre work on infection-related topics from a structural biological perspective should be conducive to this. All laboratories at the CSSB correspond to biological safety standards S2 or even S3, allowing high-level infectiological work with medically relevant human pathogens.

New experimental approaches and ideas are expected to flow in with the guest scientists staying at the "Research Hotel". This can accommodate up to seven groups with a total of 30 to 35 employees, who can conduct research for a limited time using the infrastructure of the CSSB and the DESY radiation sources. This is expected to promote junior groups and strengthen scientific cooperation. The constant turnover of research groups at the CSSB gives the centre continuous access to current developments in research and technology.

There is no risk of the CSSB running out of space too quickly, even if there is booming growth ahead: the building is designed to be expandable by twice to three times its size with further extensions if necessary.

Prof. Michael Kolbe has headed the HZI's STIB department since 2015. He was appointed jointly by the HZI and University of Hamburg and represents the HZI on the CSSB's Board of Directors.



Electron microscopic image of isolated needle complexes of the bacterial type III secretion system (T3SS) from *Shigella flexneri* which is in the research focus for the HZI researchers at the CSSB. The long needle-shaped structures were formed in vitro by mixing the needle complex with the purified needle subunit. Image: HZI/Kolbe

The nine partners involved

- Bernhard Nocht Institute for Tropical Medicine (BNITM)
- Deutsches Elektronen-Synchrotron (DESY)
- European Molecular Biology Laboratory (EMBL)
- Forschungszentrum Jülich (FZJ)
- Hannover Medical School (MHH)
- Heinrich Pette Institute (HPI)
- Helmholtz Centre for Infection Research (HZI)
- Universität Hamburg (UHH)
- University Medical Center Hamburg-Eppendorf

Bodies of the CSSB:

CSSB Council (Kuratorium)
All CSSB partner research organisations are represented with voting rights on the CSSB council. The council meets at least once a year. The present chair of the CSSB council is Prof. Dirk Heinz (HZI).

CSSB Directorate (Direktorium)
The CSSB directorate is composed of the principal investigators appointed by the CSSB partners and is chaired by the scientific director. The present research director is Prof. Matthias Wilmanns (EMBL).

CSSB Scientific Advisory Board
The CSSB scientific advisory board advises the CSSB directorate and council on all relevant activities, particularly on its research portfolio and future plans. It meets at least once a year. The present chair of the CSSB scientific advisory board is Prof. Keith O. Hodgson, Stanford University and Stanford Synchrotron Radiation Lightsource (SSRL).

CSSB Office (Geschäftsstelle)
The CSSB office is in charge of all organizational and administrative matters related to CSSB. The present head of the CSSB office is Dr. Ina Plettner.

ALIGNING TRANSLATIONAL INFECTION RESEARCH

Dr. Timo Jäger, Managing Director of the DZIF

In 2014, the annual meeting of the DZIF took place at the HZI in Braunschweig.

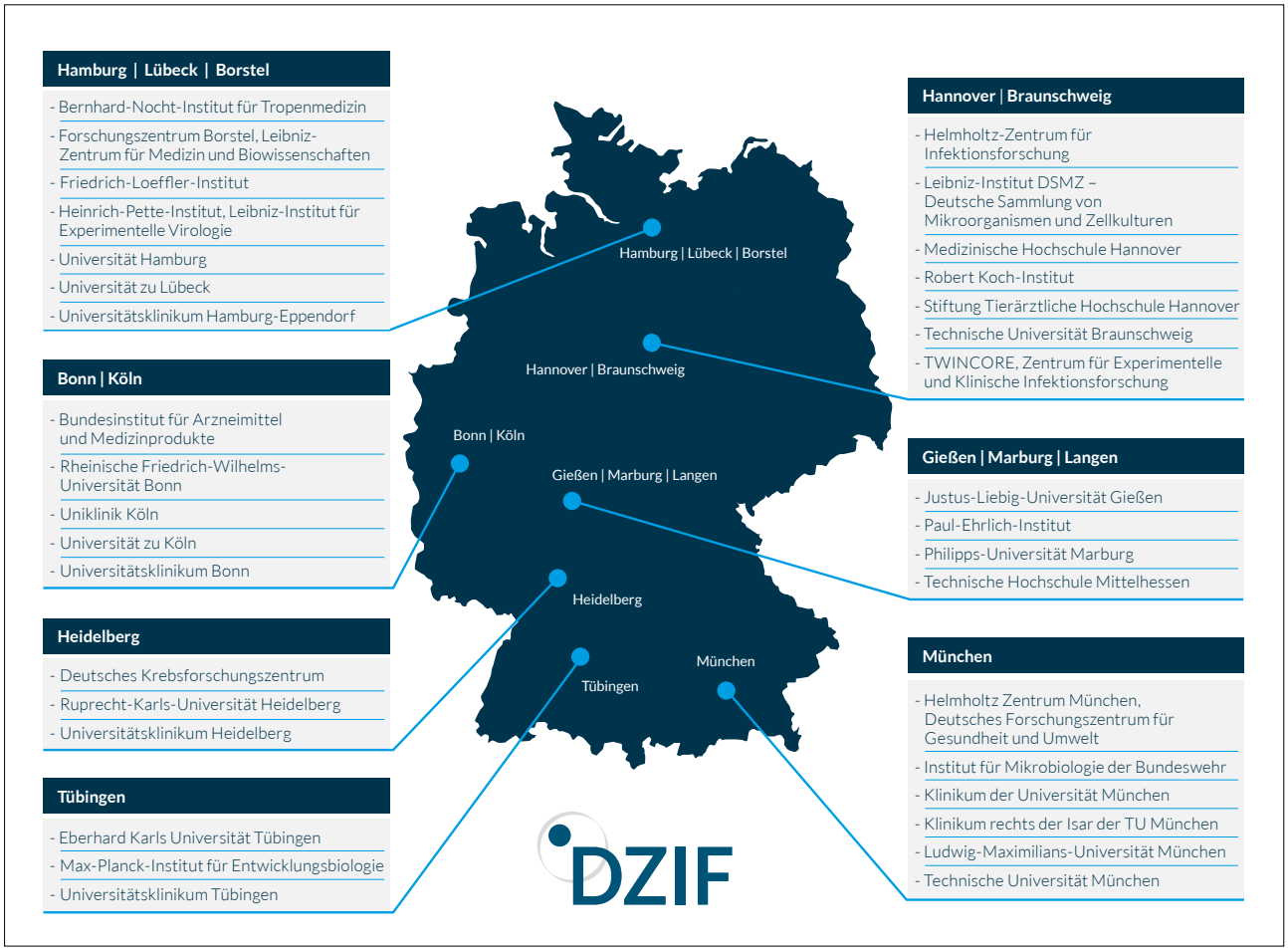
THE GERMAN CENTER FOR INFECTION RESEARCH (DZIF)

The German Center for Infection Research (DZIF) was established in 2012 as an extensive collaborative network of researchers and clinicians. Its aim is to tackle the most urgent challenges in infection research. From the beginning, the HZI has played a key role in the foundation and development of the DZIF, especially at the partner site Hannover-Braunschweig. Today, more than 350 scientists and clinicians from 35 member institutions at seven partner sites and 12 associated partner institutions work together in an integrative approach comprising nine Thematic Translational Units (TTUs) and six Translational Infrastructures (TIs) (see text box).

The DZIF aims to close existing gaps between basic research and clinical applications; its research targets the practical need of the patient. Numerous collaborations with external scientific institutions and the industry strengthen the DZIF´s position. In addition, strong interactions with partner sites in Africa and Eastern Europe have been established. In this way, infectious diseases that are rare in Germany such as malaria, Ebola and tuberculosis, can be investigated in endemic areas.

Tackling infectious diseases and rapidly responding to outbreaks

In 2013, members of the TTU Malaria began to test the effectiveness and safety of a new approach to malaria vaccination. The researchers injected volunteers with viable malaria pathogens while simultaneously administering an anti-malarial that weakened the plasmodia causing the disease. The results indicated a moderate vaccine protection of 30 to 50 percent. In response, in 2014, a phase I clinical trial was initiated at a DZIF partner institution, the Centre de Recherches Médicales de Lambaréné (CERMEL) in Gabon.



DZIF is an affiliation of 35 research institutes, located at seven sites distributed throughout Germany. Twelve additional sites are associated partners: Max-Planck Institute for Informatics, University of Münster, University of Freiburg, Medical Center – University of Freiburg, Charité -Universitätsmedizin Berlin, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Universitätsklinikum Schleswig-Holstein, German Liver Foundation, Goethe University Frankfurt, University of Würzburg, Leibniz Universität Hannover, RWTH Aachen.

Moreover, the dramatic outbreak of Ebola in West Africa in 2014 requested rapid response strategies among the entire scientific community. Thus, DZIF has initiated a consortium to strengthen Ebola research and to close the knowledge gaps as quickly as possible in the fight against the epidemic. This network, named “EBOKON”, was financially supported by the BMBF until the end of 2015. Existing DZIF structures and facilities, as well as the expertise brought in by the participating Ebola scientists, ensure the rapid implementation of measures. Furthermore, a phase I clinical trial for a potential vaccine against the Ebola virus has been supported by DZIF. In November 2014, 30 healthy adults were administered the vaccine candidate at the University Medical Center Hamburg-Eppendorf under the supervision of DZIF-Professor Marylyn Addo.

Novel antiinfectives

The development of novel antiinfectives is another important research field addressed by the DZIF. Scientists from the universities of Tübingen, Münster and Munich together with the company Hyglos join forces to prepare clinical studies on an active substance against the hospital pathogen *Staphylococcus aureus*. A highly effective protein from bacteriophages shall rapidly kill the bacteria and prevent especially the spread of methicillin-resistant *Staphylococcus aureus* (MRSA), which is unsusceptible to many of the commonly used antibiotics. In fact, DZIF also supports the optimization of the recently discovered cystobactamides, a novel class of broad-spectrum antibiotics from myxobacteria. Experiments at the DZIF member institutions HZI and HIPS showed that these substances possess an effect even against gram-negative bacteria such as *Escherichia coli* and

"Translation City" symbolizes DZIF´s network character

Acinetobacter baumannii. The planned chemical modifications will hopefully enhance and broaden the effect against the dreaded hospital pathogens.

DZIF groups its research activities into Thematic Translational Units (TTUs) and Translational Infrastructures (TIs):

Thematic Translational Units:
- Emerging Infections
- Tuberculosis
- Malaria
- HIV
- Hepatitis
- Gastrointestinal Infections
- Infections of the Immunocompromised Host
- Healthcare-associated and Antibiotic-resistant Bacterial Infections
- Novel Antiinfectives[1]

Translational Infrastructures:
- Product Development Unit [2]
- Clinical Trial Unit
- African Partner Institutions
- Natural Compound Library[3]
- Biobanking
- Bioinfomatics[4]
- DZIF Academy

1 Co-coordinator located at the HZI
2 Translational Project Management Office at the HZI
3 Coordinated by the HZI
4 Coordinated by the HZI

The best investment into infectious diseases research is probably fostering talents of the next generation. The DZIF Academy offers a comprehensive and structured training and education programme for young MDs and PhDs at all levels of their academic careers and particularly attracts medical trainees into infection research. In 2014 alone the Academy supported 52 junior talents.

Translation City – the ideal place for translating basic research into application

Short routes between the institutes and researchers are pivotal for targeted and rapid action in medical research. This has been realized in the German Center for Infection Research. In 2015 the DZIF published its new image brochure "Translation City". This virtual city allegorizes one of the centre´s missions: to establish a network that facilitates communication and that offers the infrastructure and know-how to enable research for the patient. Under the motto "United against infections", DZIF researchers will continue to close the translational gap in infection research and to coordinate and strategically align translational infection research with the aim of developing new diagnostic, preventive and therapeutic methods for treating infectious diseases.

ON TRACK FOR APPLICATION

Prof. Ulrich Kalinke, Coordinator of TRAIN

A flagship project of the TRAIN alliance:
The Clinical Research Centre (CRC) in Hannover. Foto: Ralf Mohr,
©Fraunhofer ITEM.

TRANSLATIONAL ALLIANCE IN LOWER SAXONY (TRAIN)

The biomedical Translational Alliance in Lower Saxony (TRAIN) was founded in December 2008 with one primary aim: the cooperative development of new approaches to the diagnosis, therapy and prevention of diseases. From the outset, the intention of the partners involved has been to cover the entire value chain from idea to application in clinical practice and pharmacy. The Alliance has made good progress along this path, combining the many competences of various university and non-university research establishments in Lower Saxony.

Bringing together expertise in infection research

The initiative was started when it was recognised that the Hannover-Braunschweig region harbours outstanding expertise in the investigation and combatting of infectious diseases – albeit scattered across different locations. TRAIN was formed for the special purpose of consolidating these competences.

In the initial phase, TRAIN assisted in the construction of new research buildings and the creation of infrastructure to provide a basis for new translational research and development projects.

September 2014, for example, saw the launch of the Clinical Research Centre (CRC). Jointly created by MHH, HZI and ITEM and located on the ITEM campus close to MHH, the CRC is to perform early clinical trials. Also residing at the CRC are the Hannover Unified Biobank (HUB), in which biological samples from study participants and patients are stored according to the latest quality standards, and the National Cohort.

Another inauguration in September 2014 was that of the Centre of Biomolecular Drug Research (BMWZ) on the LUH campus. At the same time, the Research Centre for Emerging Infections and Zoonoses (RIZ) was established on the TiHo campus, dedicated to researching pathogens that can infect both humans and animals. The Braunschweig Integrated Centre for Systems Biology (BRICS) on the TU-Bs campus (see interview with Prof. Dieter Jahn) is expected to commence operations in 2016. Also set to start in 2016 is construction of the Drug Research and Functional Genomics Centre (DRFG) at the HZI. Together with the BMWZ, the Center of Pharmaceutical Engineering (PVZ) of TU-Bs and other HZI and TU establishments, it will form the central infrastructure of the Lower Saxony Centre of Drug Research (NWZ), which is dedicated to the optimisation and utilisation of natural products. Alongside the HZI, the Leibniz Institute DSMZ-German Collection of Microorganisms and

Cell Cultures will also have laboratories and workgroups at the DRFG.

The TRAIN mission

After the successful establishment of new research buildings and admission of new partners, the TRAIN partners have agreed upon a catalogue of measures for improving communication and coordination of translation activities within the Hannover-Braunschweig location. Currently, the activities of TRAIN include the following tasks:

- Coordination of new translational infrastructure projects and location development concepts
- Consolidation of expertise with joint implementation and use of platform technologies
- Establishment of new teaching formats
- Identification (and where necessary funding) of TRAIN projects developing new drugs or medical products

Insights into translation: The TRAIN Academy

In 2015 the TRAIN Academy came into being with the development of the curriculum for its first project, a part-time course for professional workers in this field entitled "Translational Research & Medicine: From Idea to Product". The course was launched on 15 October. The programme is organised into modules on preclinical and clinical development, quality control and approval of drugs. They cover "classical" (i.e. small molecule based) substances and also biological and innovative drugs and medical products. The course will give scientists and medical professionals special insights into the field of translation. The Academy will also act as a catalyst in the networking of experts which, in turn, will further promote cooperation in the region.

Founding members:

Helmholtz Centre for Infection Research (HZI), Hannover Medical School (MHH), Leibniz Universität Hannover (LUH), Technische Universität Braunschweig (TU-Bs), University of Veterinary Medicine Hannover, Foundation (TiHo) and Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM).

New members of the TRAIN Alliance since 2008:

Vakzine Projekt Management GmbH (VPM), TWINCORE – Centre for Experimental and Clinical Infection Research, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE)

QUANTITATIVE BIOLOGY: AN INTERDISCIPLINARY APPROACH

Prof. Dieter Jahn, Vice President Research of the Technische Universität Braunschweig, about the Braunschweig Integrated Centre of Systems Biology (BRICS)

The new BRICS building: On four floors, laboratories, offices and meeting rooms are planned for joint experimental and theoretical work. The building also provides a training lab, a computer pool and large meeting rooms for student education. Image: Claudia Hurtig

On the campus of Technische Universität (TU) Braunschweig, HZI and TU have jointly set up the "Braunschweig Integrated Centre for Systems Biology" (BRICS). At BRICS, scientists from both establishments will collaborate on the mathematical and bioinformatic modelling of infections and other biological processes. Prof. Dieter Jahn, Vice President of the TU Braunschweig and speaker for BRICS, explains the aims and approaches of the centre, which is set to commence work soon.

Prof. Jahn, how would you explain, in layman's terms, what will be researched at BRICS?

I would start with a brief revisit to the past: Last century, certain physical and chemical processes were made predictable through mathematics. The derived rules in turn became the basis on which modern engineering was developed. Through computer science, we are now able to process large volumes of data as well. We can simulate even highly complex processes on the computer, like the flight of a new plane, for example. An entire Airbus A380 is planned this way, its behaviour is mathematically modelled, and then it is built – and it flies. We now want to harness these possibilities for biology as well. Many engineers ask us why we didn't do that a long time ago.

What is your answer to that?

Biological processes are extremely complex; there are a huge number of parameters influencing them – some we don't even know about yet. Until a few years ago, we hardly had any effective methods or tools for modelling complex biological processes as a whole.

And that has now changed?

Yes, in particular through the "omics" technologies. They allow us to collect a wealth of data relating to processes such as an infection, and then integrate these data using bioinformatics. Genomics tells us what genes exist in the pathogen and the host. Transcriptomics studies how and when these genes become active, proteomics looks at what proteins are

"There is no systems biology without lab work."

formed, and metabolomics shows us what metabolic products exist inside a cell at a given time. With these methods – and with powerful bioinformatics – we can now collect data on the most important processes of a cell holistically, and can introduce those into models and ultimately simulations.

Why is there a need for a dedicated systems biology centre?

Spatial proximity and constant contact tremendously facilitate any close collaboration. Systems biology is highly interdisciplinary – it requires input from mathematicians, physicists, chemists, computer scientists, engineers and biological scientists. Each has to learn from the other first, to understand each other's jargon and way of thinking, before they can tackle joint scientific projects. If everyone is scattered across various institutes, then close collaboration is difficult. Yes, people can meet up and talk from time to time, but the work becomes much more efficient through close daily contact. And efficiency is important to us because systems biological research involves not only theory but also a practical component, which is expensive and demands sophisticated technology. It would hardly make sense to set up that sort of thing over five different locations.

Why is Braunschweig the right place to run such a centre?

The partners involved are already set up to complement each other in those disciplines that are critical for systems biology, and have practiced interdisciplinary collaboration over many years. Scientists and engineers have been collaborating in systems biotechnology for ten years in a biotech-oriented collaborative research centre that is unique in Germany. And we have evolved together technologically as well. In proteomics, for example, the HZI works intensively on the host side while the TU concentrates on the proteins of bacterial pathogens. The Leibniz Institute German Collection of Microorganisms and Cell Cultures, DSMZ, is another

Prof. Dieter Jahn, BRICS Speaker.

partner involved in the research at BRICS – it has the necessary competence in biodiversity in the form of its extensive culture collection together with the latest corresponding genome research. The HZI is well equipped and has excellent know-how for genomics and transcriptomics. We have also systematically expanded our expertise in bioinformatics together at the TU and HZI over the years. And, last but not least, we are well integrated into the network of DZIF, the German Centre for Infection Research. We want to set up BRICS as the competence centre for bioinformatics and systems biology within this network.

What issues will be pursued with priority at BRICS?

We will tackle relatively large joint projects by preference, where the specialised infrastructure of BRICS can be effectively put to best use. The molecular interactions during an infection will be an important focus, though doubtlessly not the only one – BRICS can also address questions from ecology and biotechnology. One of the first big projects of infection biology is research on *Clostridium difficile*, or *C. diff*. This pathogen is appearing increasingly often in hospitals at the moment and is hard to treat with conventional antibiotics. We expect that *C. diff* will soon overtake the well-known and feared MRSA strains as the most important problem germs in hospitals. There is not much research on this bacterium in Germany yet.

How can systems biology help to bring such pathogens under control?

C. diff is being researched by a consortium involving TU Braunschweig and HZI as well as Hannover Medical School MHH, TWINCORE, DSMZ and the Universities of Göttingen and Greifswald. At BRICS, the main objective will be to understand and model the metabolism and corresponding genetic-regulatory and enzymatic networks of the bacterium. Because its metabolism has many peculiarities, it can offer targets for new therapies or preventative approaches.
Other models of biological systems will be developed and investigated by HZI departments like those of Alice McHardy and Michael Meyer-Hermann.

BRICS research revolves around mathematical and bioinformatical models. What place is left for "classical" biology in the laboratory?

There is no systems biology without lab work! An excellent example for that is a joint research project of the TU and HZI on the temperature dependence of infectiousness of the pathogenic bacterium *Yersinia*. The HZI department of Prof. Petra Dersch has researched deeply into this topic. Put simply, at a specific environmental temperature, the pathogen switches on a set of genes it needs to penetrate into the host organism. The phenomenon was first studied down to the last detail in genetic, cell biological and biotechnological experiments in the lab. Working from a multitude of available data, a model was developed for this process using systems biological methods. To our surprise, the model exactly predicted the system's behaviour for hitherto unstudied areas. Experiments have since confirmed these predictions. In brief: before modelling, while recording the initial data, and later during the verification, lab work is indispensable.

Is that why there will always be laboratories where experiments are done, even at BRICS?

Yes. Although, systems biology will allow us to ask much more targeted questions. Accordingly, fewer experiments will be required in the long term. So, even in biology and in particular in infection research, we will arrive at new results more efficiently. To compare once again with the aviation example: better simulations make it possible not to have a hundred planes crash during test flights anymore before ultimately an Airbus can take off.

Interview: Manfred Braun

TRACKING DOWN INDIVIDUAL DISEASE RISKS

Prof. Susanne Häußler, Head of the Department "Molecular Bacteriology" (HZI/TWINCORE);
PD Frank Peßler, Head of the Research Group "Biomarkers in Infectious Diseases" (TWINCORE)

THE HELMHOLTZ CROSS-PROGRAMME INITIATIVE PERSONALISED MEDICINE (IMED)

Personalised approaches are gaining importance in many fields of medicine. In 2013, the Helmholtz Association called the Cross-Programme Initiative "Personalised Medicine" (iMed) into being as a measure to combine its research activities in this sector. Involved are the HZI, HMGU, DKFZ, MDC, DZNE and UFZ, and their clinical partner institutions. The HZI's role within the iMed Initiative is to coordinate the field "Infectious Diseases".

Personalised medicine is the approach of employing systematic diagnostics, targeted prevention and tailored treatments directed at the specific needs of individual patients or patient groups in order to raise the quality and effectiveness of therapy to a new level. A core part of this involves the molecular analysis of patient samples using methods known collectively as "omics" technologies.

One of the first actions the iMed Initiative undertook was to create common platforms and infrastructures for high throughput and information technologies. The Initiative primarily focuses on molecular diagnostics, risk stratification, personalised therapies and prevention. Diseases addressed include cancer, metabolic and cardiovascular diseases, diseases of the nervous system, infections and lung diseases.

To support the respective medical research fields of the centres involved, funding authorities are providing the Initiative with a total of 25 million euros until the end of 2018. The budget for the HZI and TWINCORE, which represent the field of "Infectious Diseases", amounts to 1.16 million euros per year. Since the kick-off meeting in Heidelberg in 2014, a Scientific Advisory Board has been appointed, with Prof. Jordi Vila (Barcelona) and Prof. Oliver Cornely (Cologne) as two internationally renowned experts representing the field of infectious diseases.

Every centre involved in iMed has committed itself to set up a new research group whose activities coincide with the aims of iMed. At the HZI, this is the group "Biomarkers for Infectious Diseases", the head of which is appointed jointly by HZI and MHH as a W2 professor. Each centre furthermore allocates ten percent of its iMed budget to platform costs of projects involving at least one other Helmholtz centre, either directly or through a corresponding translation centre. In this regard, the newly established Microbiomics Platform is the flagship of the HZI.

Numerous projects involving HZI researchers are already under way as part of the iMed Initiative, including

- Establishing a molecular diagnostic platform for rapid pathotyping and resistance typing of *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

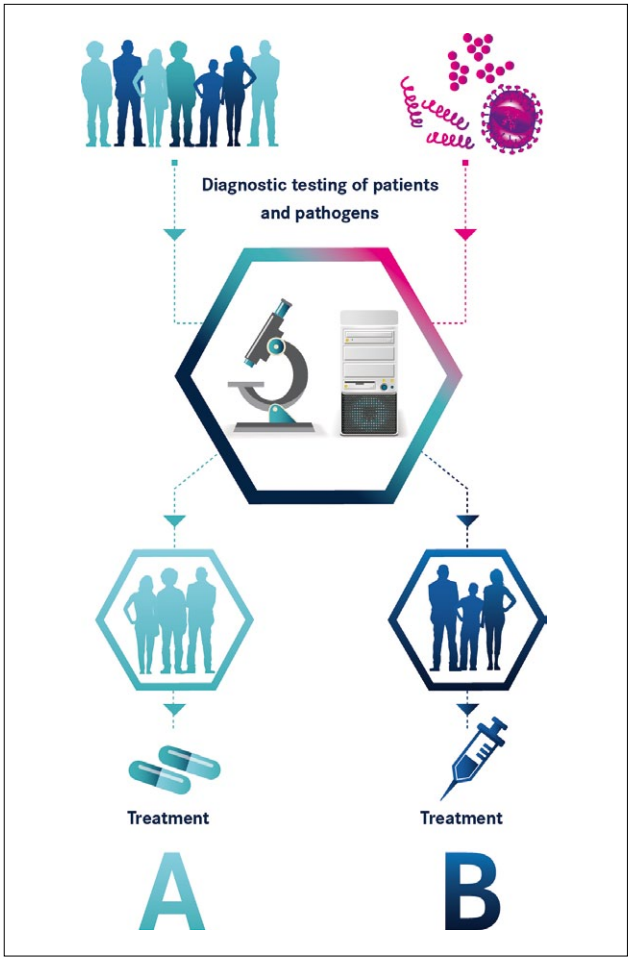
- Identifying genetic markers for severe RSV infections in children, in particular for giving suitable early treatment to children at high risk
- Determining individual immune signatures for the rapid verification of the success of vaccinations - and, where applicable, for estimating the chances for such a success already during the preliminary stage
- Researching the influence of infections on the individual risk of metabolic diseases
- Investigating molecular risk factors for asthma.

The platforms in which the HZI is intensively involved encompass, among others, infrastructures for microbiomics, genomics, proteomics, immunomonitoring and systems medicine.

The Microbiomics Platform will steadily gain importance over the coming years – beyond the projects described – in that two major cooperative projects are starting with the plan to analyse several thousand stool microbiomes (*Microflora of the gut as a factor in the onset of colon carcinoma* together with the DKFZ; *Microflora of the gut as a factor for neurocognitive decline* together with the DZNE Bonn).

Given the opportunity to support a new, highly networked project in the scope of the second funding phase of iMed, Vaccinology has been another high priority research field since the summer of 2015. Focus is primarily on studies of the individual response to vaccines, for example against hepatitis B or influenza. Partners of the HZI in this project are the HMGU, DKFZ, DZNE and MDC.

Frank Peßler and Susanne Häußler represent the HZI in the iMed initiative



The most suitable therapy for each patient: Individualised medicine aims to categorise patients and define groups, based on the most promising treatment for the respective individual (“stratification”).

ORGANISATION CHART

WK
Scientific Advisory Committee
Prof. Dr. Wolf-Dietrich Hardt, Chair

AR – Supervisory Board
MinDir'in Bärbel Brumme-Bothe (BMBF), Chair
MinDirig Rüdiger Eichel (MWK Niedersachsen), Vice Chair

KD
Clinical Director
Prof. Dr. med. Michael P. Manns

LA
Steering Committee
Prof. Dr. Dirk Heinz, Chair

WISKO
Council of Scientists
Prof. Dr. Susanne Häußler, Chair

GFW - Scientific Management — *Prof. Dr. Dirk Heinz*
GFA - Administrative Management — *Franziska Broer*

Topic 1: Bacterial and Viral Pathogens

Dep. **BIFO**
Computational Biology of Infection Research
Prof. Dr. Alice McHardy

Dep. **EPID**
Epidemiology
Prof. Dr. Gérard Krause

RG **ESME**
Epidemiological and Statistical Methods
Prof. Dr. Rafael Mikolajczyk

Dep. **EVIR** — TC
Experimental Virology
Prof. Dr. Thomas Pietschmann

RG **AIVE** — TC
Innate Immunity and Viral Evasion
Prof. Dr. Christine Goffinet

RG **IMMI**
Innate Immunity and Infection
Dr. Andrea Kröger

RG **VIRT** — TC
Virus Transmission
PD Dr. Eike Steinmann

Dep. **INFG**
Infection Genetics
Prof. Dr. Klaus Schughart

RG **TEE**
Experimental Animal Unit
Dr. Bastian Pasche

Dep. **MIBI**
Molecular Infection Biology
Prof. Dr. Petra Dersch

JRG **IBIS**
Infection Biology of Salmonella
Dr. Marc Erhardt

RG **MINP**
Microbial Interactions and Processes
Prof. Dr. Dietmar Pieper

RG **KOM**
Microbial Communication
Prof. Dr. Irene Wagner-Döbler

RG **ZEIM**
Central Facility for Microscopy
Prof. Dr. Manfred Rohde

Dep. **MOBA**
Molecular Biology
Prof. Dr. Susanne Häußler

RG **GMAK**
Genome Analytics
Dr. Robert Geffers

RG **MOLI**
Molecular Immunology
Dr. Siegfried Weiß

Dep. **REGI**
Regulation in Infection Biology
Prof. Dr. Emmanuelle Charpentier

RG **INI**
Infection Immunology
PD Dr. Eva Medina

Dep. **SFPR**
Structure and Function of Proteins
Prof. Dr. Wulf Blankenfeldt

RG **CPRO**
Cellular Proteome Research
Prof. Dr. Lothar Jänsch

RG **MPRO**
Microbial Proteomics
Prof. Dr. Susanne Engelmann

RG **NBSC**
NMR-based Structural Chemistry
Dr. Teresa Carlomagno

RG **RPEX**
Recombinant Protein Expression
Dr. Joop van den Heuvel

JRG **SBAU**
Structural Biology of Autophagy
Dr. Andrea Scrima

Dep. **STIB**
Structural Infection Biology
Prof. Dr. Michael Kolbe

Dep. **ZBIO**
Cell Biology
Prof. Dr. Theresia Stradal

RG **MZBI**
Molecular Cell Biology
Prof. Dr. Klemens Rottner

Topic 2: Immune Response and Interventions

Dep. **EXIM**
Experimental Immunology
Prof. Dr. Jochen Hühn

JRG **VIMM**
Viral Immune Modulation
Prof. Dr. Melanie Brinkmann

Dep. **EXPI** — TC
Experimental Infection Research
Prof. Dr. Ulrich Kalinke

RG **BIOM** — TC
Biomarkers in Infection and Immunity
PD Dr. Frank Pessler

Dep. **IMMK**
Immune Control
Prof. Dr. Burkhart Schraven

RG **INMI**
Intravital Microscopy in Infection and Immunity
Prof. Dr. Andreas J. Müller

RG **IREG**
Immune Regulation
Prof. Dr. Dunja Bruder

RG **SIME**
System-oriented Immunology and Inflammation Research
Prof. Dr. Ingo Schmitz

RG **NIND**
Neuroinflammation and Neurodegeneration
Prof. Dr. Martin Korte

Dep. **SIMM**
Systems Immunology
Prof. Dr. Michael Meyer-Hermann

RG **SMID**
Systems Medicine of Infectious Diseases
Dr. Esteban Vargas

Dep. **VAC**
Vaccionology and Applied Microbiology
Prof. Dr. Carlos Alberto Guzmán (Deputy)

RG **CMIK**
Chemical Microbiology
Dr. Wolf-Rainer Abraham

JRG **IMCI**
Immune Aging and Chronic Infections
Prof. Dr. Luka Cicin-Sain

RG **MIDI**
Microbial Diagnostics
Prof. Dr. Manfred Höfle

JRG **MIKI**
Mircrobial Immune Regulation
Dr. Till Strowig

RG **MSYS**
Model Systems for Infection and Immunity
Prof. Dr. Dagmar Wirth

Other Locations:

Helmholtz Centre for Infection Research GmbH
Inhoffenstraße 7
38124 Braunschweig

HIPS Helmholtz Institute for Pharmaceutical Research Saarland, Universitätscampus E8.1, 66123 Saarbrücken

TC TWINCORE, Centre for Experimental and Clinical Infection Research GmbH, Feodor-Lynen-Str. 7, 300625 Hannover

HELMHOLTZ CENTRE FOR INFECTION RESEARCH

50 YEARS 1965 – 2015

BR - Staff Council
John Aubert, Chair

VPS - Represent. Body for Disabled Employees
Carolin Schaper

BEM – Occupational Re-entry Management
Angela Walter

GB - Equal Opportunity Commissioner
Evelyn Rohn-Stenzel

Topic 3: Anti-Infectives

Dep. **CBIO**
Chemical Biology
Prof. Dr. Mark Brönstrup

RG **BISA**
Biological Systems Analysis
Prof. Dr. Ursula Bilitewski

Dep. **DDEL** HIPS
Drug Delivery
Prof. Dr. Claus-Michael Lehr

Dep. **DDOP** HIPS
Drug Design and Optimization
Prof. Dr. Rolf Hartmann

JRG **CBCH** HIPS
Chemical Biology of Carbohydrates
Dr. Alexander Titz

Dep. **MCH**
Medical Chemistry
Prof. Dr. Markus Kalesse

Dep. **MINS** HIPS
Microbial Natural Products
Prof. Dr. Rolf Müller

JRG **AMEG** HIPS
Actinobacteria Metabolic Engineering Group
Dr. Andriy. Luzhetskyy

RG **MISG**
Microbial Strain Collection
Dr. Joachim Wink

JRG **SBBE** HIPS
Structural Biology of Biosynthetic Enzymes
Dr. Jesko Köhnke

Dep. **MWIS**
Microbial Drugs
Prof. Dr. Marc Stadler

Administrative Management

Departments

EM - Purchasing Department
Olaf Reusner

FA - Finance Department
Dirk-Michael Reinhardt

JUR - Legal Affairs and Licences
Dr. Christiane Kügler-Walkemeyer
(Technology Transfer Commissioner)

ORG - Organisation and Administration-IT
Harald Ohrdorf (Data Prot. Manager)

PA - Human Resources
Jörg Schinkel

PS - Patents
Dagmar Meseke

RZ – Computer Centre
Dr. Joachim Metge

SU - Safety and Environmental Affairs
Dr. Erwin Grund

TB – Technical Services
Olaf Rabe

Staff Units

BIB - Library
Axel Plähn

CO - Controlling
BCO *Elisabeth Gerndt*
DMC *Dr. Michael Strätz* (Deputy)
PWC *Dr. Rolf-Joachim Müller*

FASI - Occupational Safety Specialist
Carsten Strömpl

GS – HZI International Graduate School for Infection Research
Dr. Sabine Kirchhoff

IR - Internal Auditing
Richard Lomberg
(Anti-Corruption Commissioner)

PuK - Public Relations
Susanne Thiele

SKO – Strategic Communication
Manfred Braun

WST - Scientific Strategy
Dr. Hansjörg Hauser

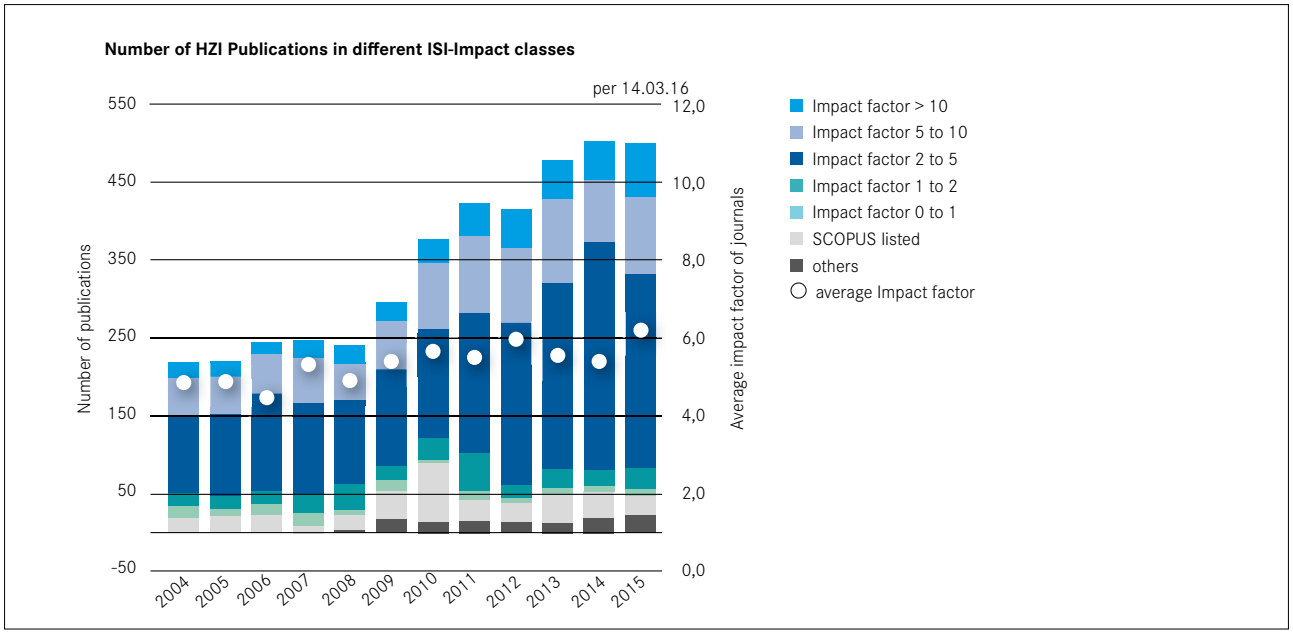
Version: 1. November 2015

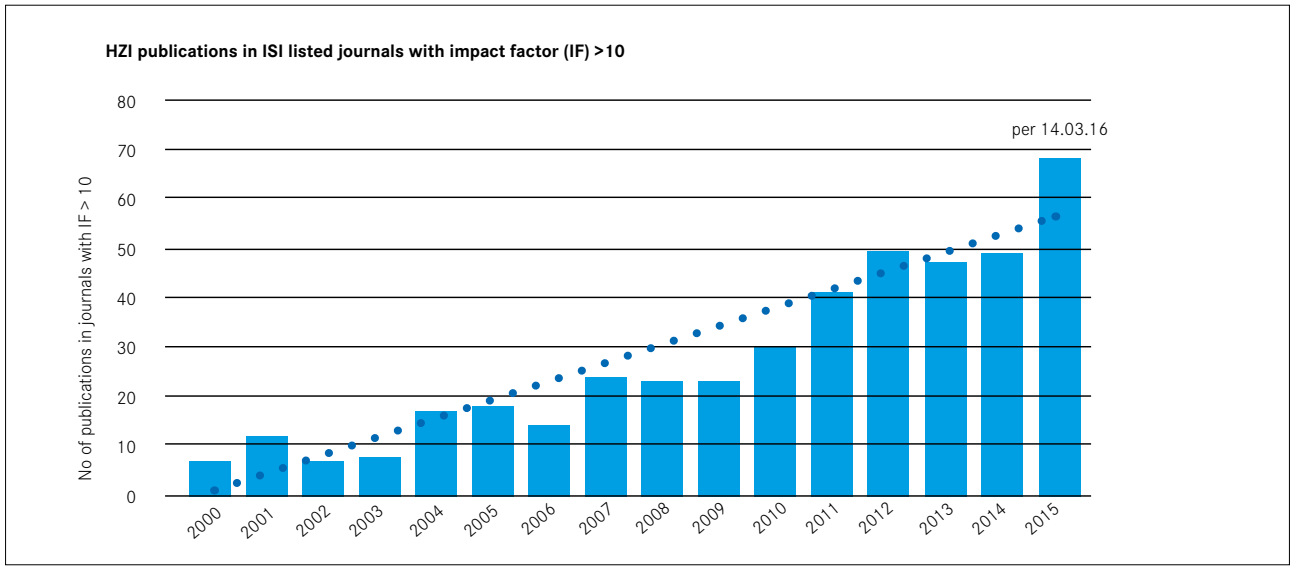
Dep.: Department
RG: Research Group
JRG: Junior Research Group

BCO: Financial Controlling
DMC: External Funding Controlling
PWC: Planning Programme and Scientific Controlling

PUBLICATIONS

In 2014 and 2015, more than 1000 scientific articles were published by HZI scientists. In recent years, the number of publications in general as well as the number of articles in high impact journals have been increasing.





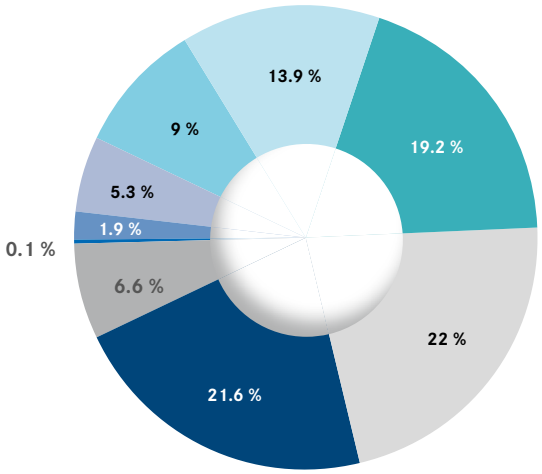
FINANCING

In 2015, the complete budget was 68 million € including 17 million € of external funding. (Within the latter amount, investments in the construction of the new HIPS building and for the National Cohort are not included).

More than 50 % of the external funding came from national research programmes. About 28 % came from EU programmes and industry.

External funding of Research in 2015 (in 1000 €)

Source	Full cost
BMBF	2,342
DFG	3,239
EU	3,701
Helmholtz Association	3,637
Industry	1,122
DAAD	23
State of Lower Saxony	328
State of Saarland	893
Others	1,530
Total Sum	**16,815**



PARTICIPATION IN RELEVANT RESEARCH NETWORKS

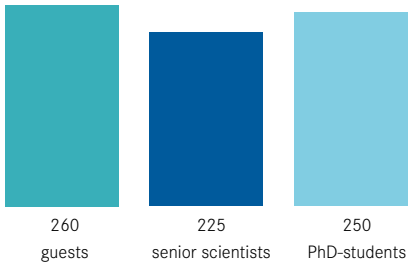
In 2015, the HZI participated in 17 DFG programmes and projects (including DFG Priority Programmes, Collaborative Research Centres, Clusters of Excellence, European Research Training Group), 4 ERC Starting Grants, 26 EU projects (incl. ERC, Marie Skłodowska-Curie actions, IMI) and 30 BMBF / BMWI projects.

Patents, property rights and licenses

	2014	2015
Priority based applications	7	4
Granted patents	26	15
Total number of held property rights	527	502
Licence agreements	40	40
Licence proceeds (thousand €)	2,062	1,833

PERSONNEL

At the end of 2015 the HZI staff comprised 920 full time and part time employees. Additionally, 260 guests worked in various projects, receiving their payment from third parties. Along with 225 senior scientists, around 250 PhD-students were working at the HZI.



BOARDS AND COMMITTEES OF THE HZI

Members of the Supervisory Board (SB) and the Scientific Advisory Committee (SC), including the Clinical Board (CB) as a subcommittee (Status: January 2016)

Function	Name, Titel	Organisation	Ort
Chairman SB	Brumme-Bothe, MinDir'in Bärbel	BMBF	Berlin
Vice-Chairman SB	Eichel, MinDirig Rüdiger	NMWK	Hannover
SB	Mees, Christian	Staatskanzlei, Saarland	Saarbrücken
SC / CB	Addo, Prof. Dr. Marylyn Martina	University Clinic Eppendorf	Hamburg
SC	Autenrieth, Prof. Dr. Ingo	University	Tübingen
SC	Brakhage, Prof. Dr. Axel	HKI	Jena
SB	Baum, Prof. Dr. Christopher	MHH	Hannover
SB	Buer, Prof. Dr. Jan	University Clinic	Essen
SC	Förster, Prof. Dr. Irmgard	University	Bonn
SB	Gastmeier, Prof. Dr. Petra	Charité	Berlin
SB + SC, Chairman SC	Hardt, Prof. Dr. Wolf-Dietrich	ETH	Zürich
SC	Hesselbach, Prof. Dr. Jürgen	Technical University	Braunschweig
SB + SC, Vice-Chairman SC	Kisker, Prof. Dr. Caroline	University	Würzburg
SB	Lang, Prof. Dr. Christine	Organobalance GmbH	Berlin
SB	Medina, Prof. Dr. Eva	HZI	Braunschweig
SC	von Mering, Prof. Dr. Christian	University	Zürich
SC	Ott, Prof. Dr. Melanie	University of California	San Francisco
SB	Rohde, Prof. Dr. Manfred	HZI	Braunschweig
SC	Sauer, Prof. Dr. Markus	University	Würzburg
SC / CB	Suttorp, Prof. Dr. Norbert	Charité	Berlin
SC / CB	Tacconelli, Prof. Dr. Evelina	University Clinic	Tübingen
SC	Vollmar, Prof. Dr. Angelika	University	München
SC	Waldmann, Prof. Dr. Herbert	MPI Molecular Physiology	Dortmund

Photographs

Breakthrough Prize Foundation – pages: 28
Christian Richter – pages: 56
DZIF collection – pages: 104, 105, 106
Fotostudio Franz Fender – pages: 41
hammeskrause Architekten – pages: inside cover "The HZI at a Glance", 102
HIPS collection – pages: inside cover "The HZI at a Glance", 31, 96 (Ronald García), 100
HZI collection – pages:

- 17, 24, 25, 32, 33, 41, 42, 43, 44, 45, 46, 48, 50, 54, 63, 72, 73, 74, 76, 77, 78, 79, 87, 88, 94, 95, 112;
- inside cover "The HZI at a Glance" (Sondermann)
- 6, 15, 21, 23 (Grabowski)
- 7, 10, 31, 55, 59, 62, 66, 70, 72, 74, 76, 80, 82, 84, 92, 94, 112 (Hallbauer & Fioretti)
- 8, 9 (Steuer)
- 9, 18 (Waldthausen&Kreibig)
- 14, 15, 32, 33, 34, 35, 36 (Verena Meier)
- 16 (Marcia Duarte)
- 16, 25 (Christian Stern)
- 17 (Birgit Manno)
- 20/21 (Klaus Schughart)
- 51, 90 Heinz Gramann
- 52, 69 (Manfred Rohde)
- 60, 61 (Misiak)
- 90 (Britta Mießen)
- 92 (Michael Reck)

Jenko Sternberg Design – pages: inside cover "The HZI at a Glance", 10, 11, 39, 113
MHH collection – pages: 38, 40, 41 (Kaiser); 98, 107
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UdS/Jörg Pütz – pages: 66, 76, 78, 86, 100, 101

For all other photographs, the authors and/or institutions are mentioned below the legends.

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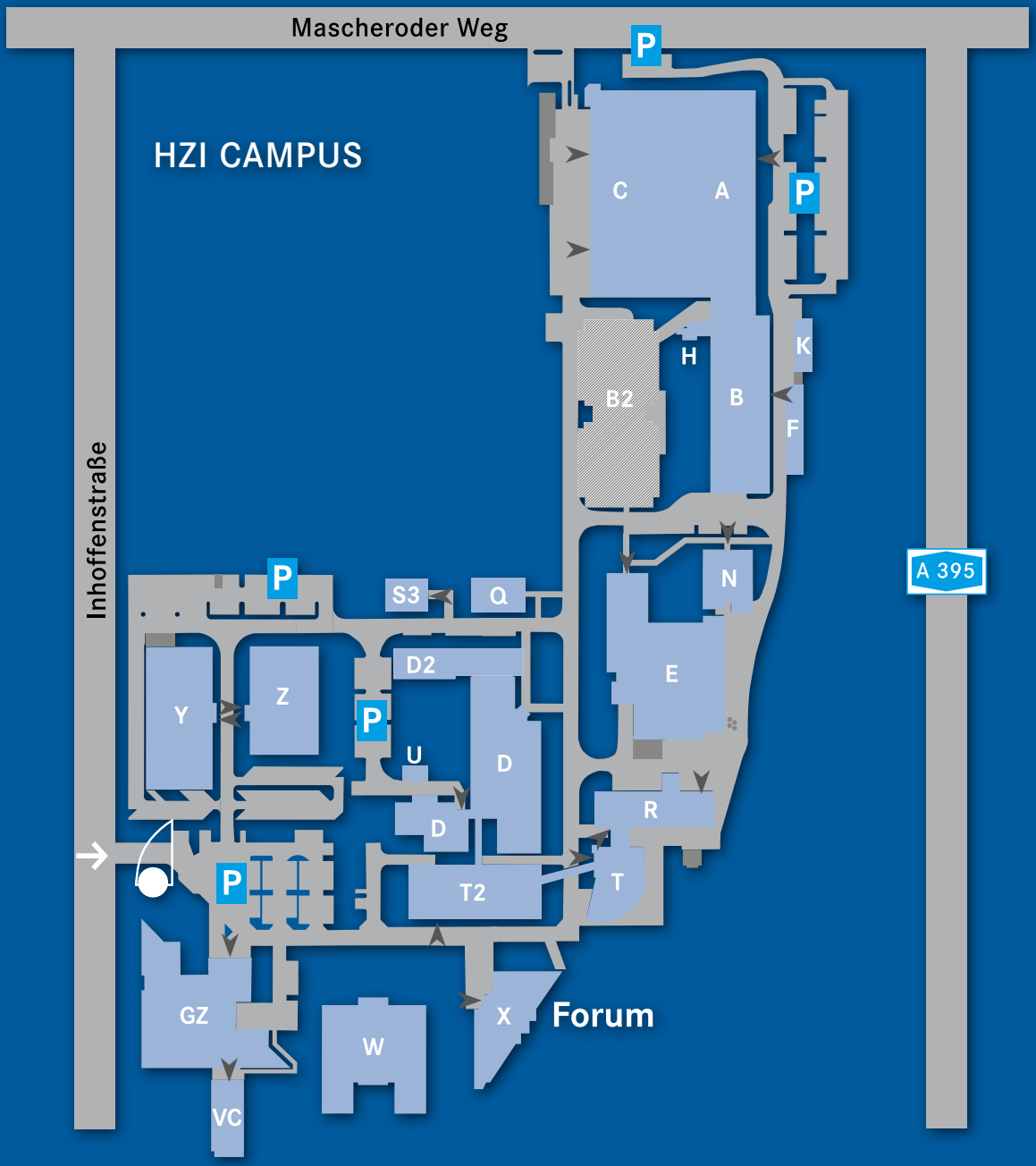

The HZI Campus

A, B, C, D, GZ	Research Labs
B2	Drug research & Functional genomics (planned)
D2	Offices
E, F, H, K, Q, R, U	Infrastructure
S3	S3-Facility
T, T2	Animal Facility
N	Infrastructure & Administration
VC	Administration
W	Administration, Library, Canteen
X	„Forum“ – Event & Seminar Building
Y	Research Labs, Fraunhofer ITEM
Z	Leibniz Institute DSMZ

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INFECTION RESEARCH

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