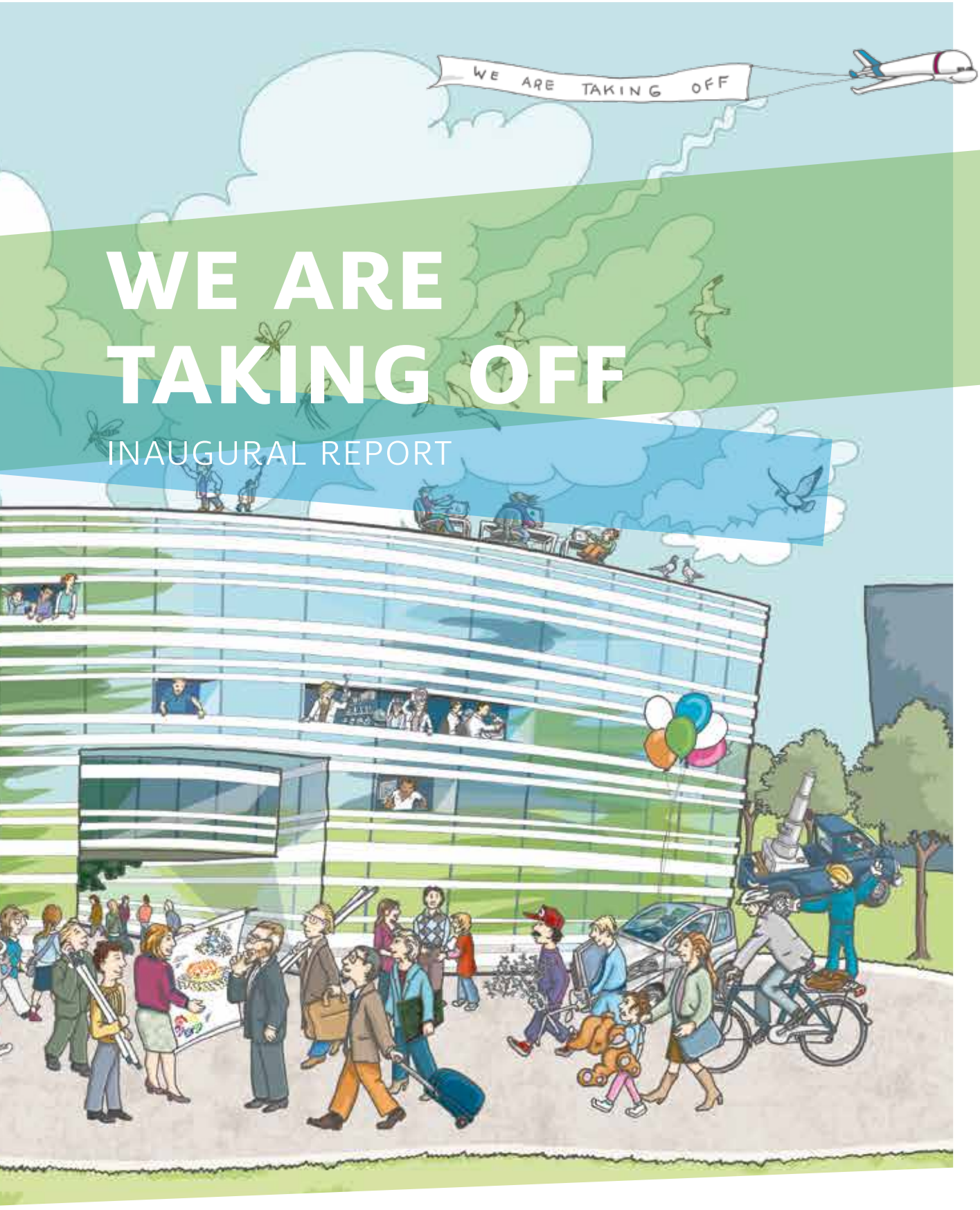
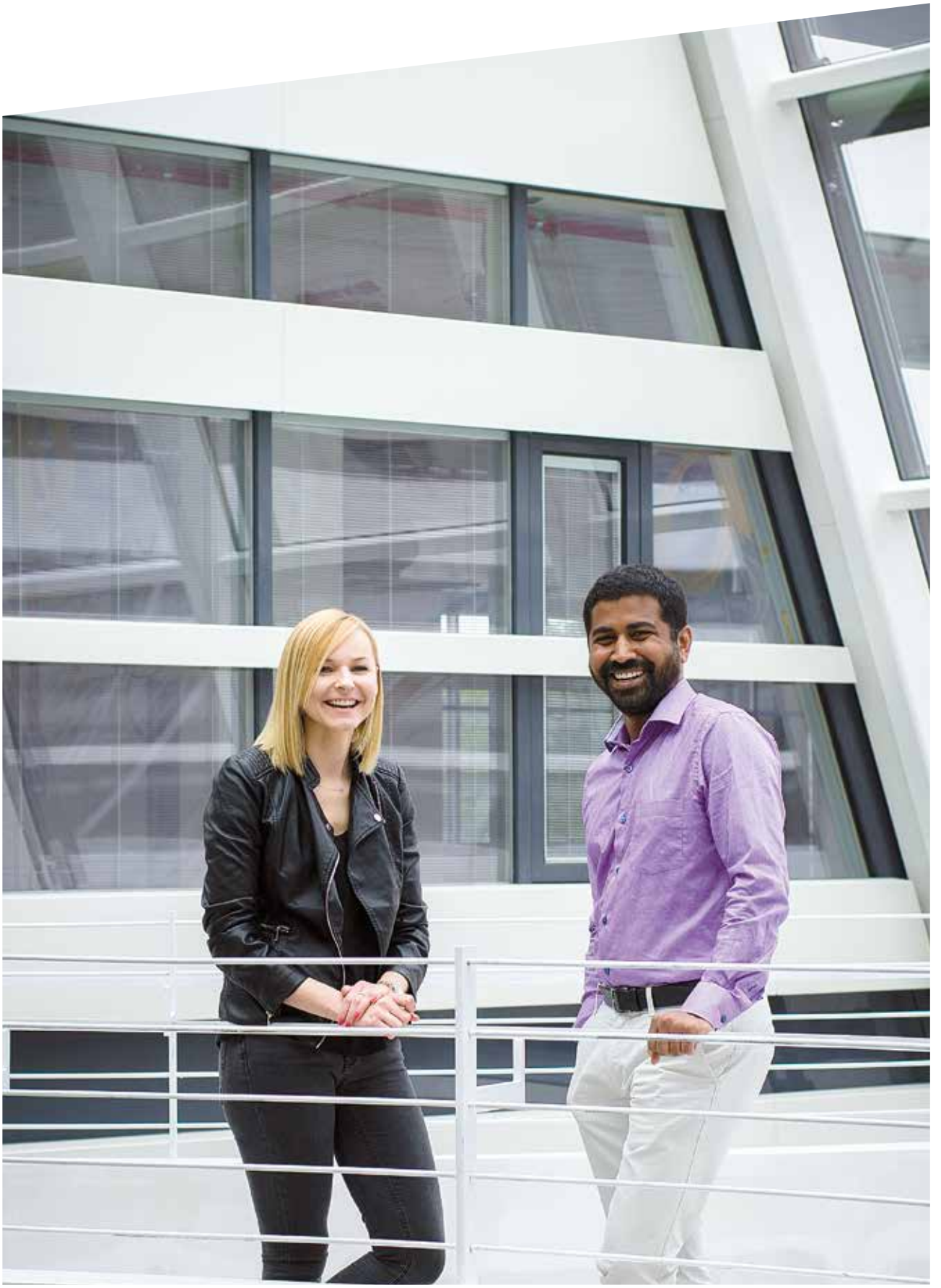
CSSB
Centre for Structural
Systems Biology



WE ARE TAKING OFF

INAUGURAL REPORT



CSSB PARTNERS

BNITM Bernhard Nocht Institute for Tropical Medicine

DESY

EMBL

HELMHOLTZ ZENTRUM FÜR INFEKTIONSFORSCHUNG

HPI

JÜLICH FORSCHUNGSZENTRUM

MHH Medizinische Hochschule Hannover

Research Center Borstel Leibniz-Center for Medicine and Biosciences

UKE HAMBURG

UHH Universität Hamburg DER FORSCHUNG | DER LEHRE | DER BILDUNG

CONTENTS

MISSION, RESEARCH AND MILESTONES

OUR PEOPLE

"There are no signposts in the sky to show a man has passed that way before. There are no channels marked. The flier breaks each second into new uncharted seas." **Anne Morrow Lindbergh, New Jersey, USA**

OUR VISION

Matthias Wilmanns
CSSB Scientific Director

DEAR COLLEAGUES, SUPPORTERS AND FRIENDS OF CSSB

We have all had the experience of sitting in an airplane with our tray tables up and our seat belts fastened, waiting expectantly for take-off. Waiting for the moment when the airplane's wheels leave the runway and we are airborne. Like an airplane, CSSB is poised for take-off.

CSSB is waiting on the runway ready to embark upon an exciting journey of scientific discovery. Our aim is to use the world leading infrastructure here on the DESY campus in combination with cutting-edge, in-house technologies to investigate how pathogens infect humans. Our destination and ultimate goal are to facilitate the development of novel approaches for combatting infectious diseases.

Over the past two years the CSSB partner institutions, scientists and administrative staff have come together to transform an inspirational idea into a reality. The construction of our state-of-the-art research building is almost complete. CSSB scientists, who are international leaders in their fields, have already begun initiating exciting new scientific collaborations. Our partner institutions have created and agreed upon both an operating plan and research concept, thus providing CSSB with a clear strategic path.

CSSB is a cooperation of ten partner institutions. Each institution and individual involved brings their own unique perspectives, strengths and capabilities to this collaborative endeavor. Despite some occasional moments of turbulence, our determination and motivation have propelled us forward to create a unique, new research centre whose combination of methodologies and technologies is truly unprecedented.

Within the pages of our first annual report, we would like to show you how CSSB began and introduce you to the scientists who will carry CSSB forward. I would like to thank all those who have supported CSSB over the past years. Your dedication, vision and hard work has brought us to this point of departure.

CSSB is ready to experience the exhilarating sensation of flight. We are taking off.

Matthias Wilmanns
CSSB Scientific Director

MISSION, RESEARCH AND MILESTONES

OUR MISSION

We use cutting edge technologies and methods to investigate how pathogens infect humans.

1 RESEARCH FOCUS

Together, we research the structure and function of pathogens in order to understand their interaction with the human body. This research facilitates the development of novel methods for combatting infectious diseases.

2 COLLABORATION

We use a collaborative, multidisciplinary and integrative approach to our work in an effort to answer relevant scientific questions.

3 INNOVATION

We use the best research facilities and infrastructures available to investigate the molecular mechanisms associated with infection. For this, we develop new tools and techniques.

4 RESPONSIBILITY

We engage in an active dialogue with the scientific community, society and industry about our research, which serves a civilian and peaceful purpose.

5 EMPOWERMENT

We empower the next generation of scientists to work boldly and collaboratively to tackle the complex problems of tomorrow.

6 RESPECT

We establish respectful working relationships that are founded upon mutual appreciation and regard for intellectual property. We encourage an open exchange of ideas.

7 DIVERSITY

We create a setting in which every colleague can give their best, irrespective of nationality, age or gender. Diversity enhances creativity, drives innovation, provides new perspectives and enriches our working environment.

OUR RESEARCH

Salmonella typhimurium (orange)
adhering to a host cell

A lack of knowledge of the molecular mechanisms underlying most infections presents a roadblock to the development of effective therapies. Molecular, structural and functional analysis, which uncovers and manipulates infection mechanisms, is essential for ensuring a rapid and effective response to current and future epidemics. CSSB is primarily focused on uncovering the molecular properties and ingenuities of infection mechanisms.

CSSB advocates a novel approach which integrates the use of structural biology and imaging techniques with systems biology approaches to attain meaningful insight into the molecular mechanisms of some of the world's most important infections. CSSB will establish a state-of-the-art electron cryo-microscopy research facility as well as four complementary core facilities. These technologies complement the available on-site research infrastructures PETRA III, one of the brightest synchrotron radiation sources, and European XFEL, a free electron laser.

OUR BEGINNINGS

AN INTERVIEW

"We will remain open to new scientific questions and will offer space for excellent collaborative opportunities in biomedical research."

WHAT DO YOU EXPECT FROM CSSB?

PROFESSOR DIRK HEINZ: We are close to the Atlantic coast here, that's why we could think of CSSB functioning like a lighthouse. CSSB should be visible from far away and known for conducting exceptional structural biology research using the world class infrastructure that is currently available here at DESY in Hamburg. I hope that CSSB's focus on infection biology research will further boost the light it casts as this is a highly relevant field for our society. Infectious diseases affect us all in one way or the other. The studies carried out by CSSB scientists will help uncover the molecular principles of infectious diseases at high resolution and bring our overall understanding of these diseases decisive steps further.

PROFESSOR MATTHIAS WILMANNS: The diversity of methods and the exceptional quality of the technology available at CSSB are unique worldwide. Our access to the world-leading infrastructure at DESY and the European XFEL places CSSB in the Champions League of research and, to continue using sports terminology: we are at least in the semi-finals! Our research focus is infection biology but this does not mean that we will concentrate on this exclusively. We will remain open to new scientific questions and will offer space for excellent collaborative opportunities in biomedical research.

Matthias Wilmanns
CSSB Scientific Director

PROFESSOR CHRIS MEIER: I believe a research focus is an essential uniting factor; this does not mean that we must concentrate our efforts solely in this field. My hope is that combining the strengths of the ten different partner institutions, who are constructing and operating this facility together, will result in new synergies in their joint research. This chance to create something truly new is what I find particularly exciting about CSSB. The discussions and debates regarding the developments of CSSB have always been very constructive. My experiences so far lead me to believe that the newly appointed CSSB scientists will conduct research that is both exciting and collaborative. Successful results will then ideally be reflected back onto the CSSB scientists' home institutions, thus resulting in even more opportunities for research collaboration.

YOU HAVE ALWAYS INSISTED UPON THE CREATION OF A RESEARCH BUILDING. WOULD IT BE POSSIBLE TO SUCCESSFULLY CONDUCT THIS RESEARCH VIRTUALLY?

PROFESSOR DIRK HEINZ: The synchrotron light sources are used by several CSSB partner institutions who conduct structure biology research. Our guiding thought was that if we were to construct the research institute directly on the DESY campus, we would have quicker access to these unique light sources than if we were to access them externally. This is an extremely important advantage for the recruitment of excellent scientists to a new research institute. It makes a huge difference if you can say: you can drive 500 km and use this light source three times a year – or if you can offer lab space onsite along with the possibility of developing relationships with and being inspired by other scientists who are also using this infrastructure.

PROFESSOR CHRIS MEIER: I consider it to be very important that high-quality research on complex topics is not only conducted in a virtual setting. We all have virtual, worldwide networks; we cooperate with colleagues in Australia, the US and many other locations. If, however, you meet colleagues from different CSSB partner institutions in the cafeteria, the hallway or over a cup of coffee or tea, then you can spontaneously exchange ideas and gain inspiration from one another. This stimulates research and is unbeatable for scientific cooperation. This is the benefit of having a research centre that physically exists.

PROFESSOR MATTHIAS WILMANNS: Having a joint building is one thing but we have taken it a step further. We deliberately want to go beyond the typical 70s or 80s style research building with long hallways and laboratories and offices with closed doors – this usually prevents communication. We have constructed a research building that supports communication at all levels and facilitates personal interaction as well as spur-of-the-moment exchanges. At CSSB, we want to work together to create a culture of dialogue and trust.

PROFESSOR CHRIS MEIER: This is the reason we gave the architects a very clear directive to create a building in which the architecture actively supports and encourages communication. This is why there are, for example, no individual kitchens in each lab but open kitchens that can be centrally accessed by everyone.

PROFESSOR DIRK HEINZ: And there is a lot of glass and, therefore, lots of transparency so that everyone can be seen and can meet informally.

"I consider it to be very important that high-quality research on complex topics is not only conducted in a virtual setting."

WHERE DO YOU SEE CSSB IN FIVE YEARS?

PROFESSOR DIRK HEINZ: I believe we can consider CSSB a success when it has grown to become its own entity. At the moment, there are ten institutions participating in CSSB. This means ten working groups, who all come from very different environments and cultures, coming together and learning to work together. It would make me happy if in five years the expansive reach of CSSB joint publications and research projects could demonstrate that this concept works: the individuals are working together and the results of their research are greater than the single components provided by each individual partner. And in ten years from now – maybe even a Nobel Prize! We are going to have such excellent technology and methods that structural biology will surely experience another big surge. If this takes place here in Hamburg and one of our scientists also receives such an important prize, this would truly be an achievement for our concept.

PROFESSOR MATTHIAS WILMANNS: I would like to appeal to all those involved in CSSB to promote a "Yes, you can do it!" attitude. My serious hope is that we will have a significant number of European Research Grant (ERC) or Leibniz Prize recipients. This is realistic and will lead to international visibility.

PROFESSOR CHRIS MEIER: Our ambition is to become one of the world's best. In five years, I expect CSSB to be internationally recognised; our mission to be known worldwide; and our research to be renowned for its excellence. I hope that openings in our Research Hotel will be highly sought after by young researchers who see CSSB as a place where they can not only successfully begin their career but can also be empowered to solve the complex problems of tomorrow. I hope that CSSB's reputation will be so well established that we will be involved in both excellence initiatives and specialised research areas. I am, however, slightly sceptical about being awarded the Nobel Prize in ten years.

Chris Meier
CSSB Deputy Director

Dirk Heinz
CSSB Council Chair

WHICH CHALLENGES MUST BE OVERCOME?

PROFESSOR DIRK HEINZ: It is essential that the cooperation is successful. This depends on both the individuals who will be working together and the research institutions involved in CSSB. If the chemistry in this centre thrives the way we hope it will, then the cooperation will be a success. The Council and Directorate must therefore ensure that this cooperation is fortified not only with monetary funds but also with exemplary behaviour and unwavering support. This requires thoughtful leadership, steadfast courage and authentic communication.

PROFESSOR MATTHIAS WILMANNS: There are many challenges. CSSB is a cooperation of so many research partners and this makes it particularly fragile. It requires leadership from all the responsible parties to ensure that this joint vision is brought to life in a way that enables us to work and act as a single unit. To loosely quote Antoine de Saint-Exupery "When you want to build a ship, don't gather everyone together to find wood, assign responsibilities, and distribute work. Instead, teach them to yearn for the vast and endless sea." At CSSB, we are focused on scientific excellence and this goal will guide us.

PROFESSOR CHRIS MEIER: People are the most important factor! If this research centre is going to succeed, we need a high-performing team that works well together and embodies a collaborative spirit. We are under a lot of pressure to succeed and we cannot forget our goal of creating an environment which fosters collaboration. Each partner institution has recruited well-known scientists specifically for CSSB and each will carry a portion of the overall operating costs. The partners institutions who have invested heavily in CSSB will only remain a part of this cooperation, if scientific success is achieved.

PROFESSOR DIRK HEINZ: Compared to other scientific start ups, the advantage the CSSB has is that the scientists have been recruited, the directorate is capable and well-functioning, the operating concept has been approved and everyone was involved in the development of the research strategy and mission statement. The collaborative research can begin now. This is truly unique; CSSB is ready to take off!

IF YOU HAD ONE WISH …

PROFESSOR CHRIS MEIER: I hope that in five years, we are to the point that we can begin construction on the first extension of the CSSB building.

PROFESSOR DIRK HEINZ: It would be nice if CSSB's fundamental research could contribute to the development of a new antibiotic.

PROFESSOR MATTHIAS WILMANNS: I hope that our Research Hotel is so attractive that it will create new research diversity within CSSB.

— **Interview conducted by scientific journalist, Angela Grosse.**

CSSB PARTNERS:

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) | Research Center Borstel (FZB) | Universität Hamburg (UHH) | University Medical Center Hamburg-Eppendorf (UKE)

OUR MILESTONES

FOUNDATION STONE: On 29 September, 2014, together with representatives of CSSB partners, Dr. Dorothee Stapelfeldt, Hamburg's science senator, Andrea Hoops, secretary of state in Lower Saxony's science ministry, and Dr. Matthias Wilmanns, CSSB Scientific Director, laid the cornerstone stone for CSSB's new research building.

1ST CSSB INTERNATIONAL SYMPOSIUM: The 1st CSSB International Symposium, "Systems in Infection Biology – From Molecules to Organisms," took place at the Bernhard Nocht Institute for Tropical Medicine from 9–11 April, 2015. Approximately 130 participants attended the symposium with international speakers from the US, UK, Canada, Spain, Switzerland, Austria and Israel. The symposium was funded by and planned in cooperation with the Joachim Herz Stiftung.

CSSB SPRING SCHOOL: From 13–17 April 2015, seventeen PhD and post-doctoral students attended the first CSSB Spring School "Structural Systems Biology – From Molecules to Organisms" on the DESY Campus. CSSB group leaders taught courses in sample preparation and analysis, advanced microscopy, single particle analysis, structure determination and modelling of structure and dynamics of complexes. The Spring School was also funded by and planned in cooperation with the Joachim Herz Stiftung.

PUBLIC SCIENCE EVENT: On 7 September 2015, CSSB and the Academy of Sciences and Humanities in Hamburg hosted a moderated discussion for the general public with the title "Pathogens Under Super Microscopes: A Journey into Invisible Worlds." The panel discussion featured four renowned experts who not only discussed the threats infectious diseases such as Ebola and HIV pose to our health but also explained how scientists are working together to develop new methods and techniques for combating these infections.

TOPPING OUT CEREMONY: On 9 September 2015, CSSB celebrated its topping out ceremony on the DESY Campus in Hamburg-Bahrenfeld. Katharina Fegebank, Hamburg's Scientific Senator, Dr. Karl Eugen Huthmacher from the German federal government, Kristin Alheit, Schleswig-Holstein's Scientific Minister and Prof. Helmut Dosch, Chairman of the DESY directorate and other representatives of CSSB's ten partners joined Dr. Matthias Wilmanns, CSSB Scientific Director, in celebrating this important milestone.

HAMBURG NIGHT OF SCIENCE: On 7 November 2015, more than 18,000 people visited the DESY campus in Hamburg-Bahrenfeld for the Hamburg Night of Science. Many visitors who came to the CSSB exhibit had in-depth conversations with the CSSB scientists. Children enjoyed creating pathogens out of pipe cleaners. The colorful CSSB balloons and the CSSB logo tattoos were a big hit with visitors of all ages!

CSSB BIOLOGY SCIENCE SLAM: CSSB hosted a Biology Science Slam on the evening of 7 November 2015. Three postdocs from CSSB partner institutions (DESY, FZJ, EMBL) shared their research with the audience in a fun and entertaining way.

FZB JOINS CSSB: On 16 June 2016, Research Center Borstel (FZB) signed a contract for associated partnership with CSSB.

OUR COLLABORATIONS

Collaborative efforts and the integration of methods and insights from different disciplines are essential to gain a holistic understanding of host-pathogen systems. The breadth of available expertise and methodologies at CSSB make it an ideal platform for scientific exchange and a perfect environment for interdisciplinary collaborative work. Below are two examples of collaborative research projects involving CSSB scientists.

MAPPING RED BLOOD CELL INVASION

CSSB scientist, Tim Gilberger, focuses his research efforts on identifying and interfering with the weak spots of the malaria pathogen. Using systems biology approaches in collaboration with the University of Toronto (Andrew Emili) and the Sick Kids Research Institute (John Parkinson), he plans to fine-map the protein interactions taking place in the "invadome", the protein network responsible for red blood cell invasion. Once a map has been generated, cell biology methods will be used to validate this updated network. This approach will be complimented by CSSB scientist, Christian Löw, who will then use x-ray crystallography to obtain high resolution insights into the individual proteins powering and regulating host cell invasion. Determining the structure of specific proteins might enable the identification of proteins or protein sites which could be targeted by small compounds to inhibit this crucial step in the infection cycle.

> "This research project integrates the expertise of four different laboratories. The collaboration will not only enable each laboratory to venture into new research avenues with synergistic effects for this research project, but will also help to implement systems and computational biology approaches within CSSB." **Tim Gilberger, Hamburg**

ACHIEVING ATOMIC ACCURACY

Electron cryo microscopy (cryo EM) is an emerging method used by structural biologists to further our understanding of molecular structures. Although recent technical developments have led to great improvements in cryo EM imaging, the development of methods for building accurate models has lagged behind. To solve this problem, CSSB scientist, Thomas Marlovits, together with international collaborators from the University of Seattle/Howard Hughes Medical Institute (David Baker, Frank DiMaio), the University of California in San Francisco (Yifan Cheng) and the University of Virginia (Edward Egelman) are developing novel refinement protocols as well as analytical tools which enable the generation of atomically accurate molecular models, even if the data itself is only of near-atomic resolution. *Source Article: DiMaio, et al. (2015). Atomic-accuracy models from 4.5-Å cryo-electron microscopy data with density-guided iterative local refinement. Nat Methods. 12(4):361-5*

OUR BUILDING

AN INTERVIEW WITH THE CONSTRUCTION MANAGER

Verena Börschmann
Construction Manager

WHAT EXCITES YOU ABOUT THE MANAGEMENT OF THIS PROJECT?

VERENA BÖRSCHMANN: The biggest fascination for me has been implementing both the sophisticated functional and technical requirements of the CSSB scientists and the demanding design requirements of the building's architect. This has not always been easy. By actively involving the users in the project, we have, however, been able to coordinate any necessary modifications to the design with the architect. In this way, we have optimized the building's utilization while preserving the architect's distinctive signature.

WHAT WERE YOUR BIGGEST CHALLENGES?

VERENA BÖRSCHMANN: Personally, this build has been a challenge for me as I moved from an on-site supervisor role to the project manager position which is more office based but has increased responsibility. I am very happy that as a mother of two children, this role was given to me, even if it is only a short-term project. This "masterpiece" is important as it will enable my further development in the field.

An additional challenge was that this is a laboratory building with diverse technical requirements that must be fulfilled. I first needed to understand how a laboratory functions and what each individual scientist needs to successfully carry out their research.

HOW MANY TRADES, CRAFTSMEN AND OTHER CONSTRUCTION EXPERTS HAVE WORKED ON THIS PROJECT?

VERENA BÖRSCHMANN: Looking back over the entire construction period, there have been between 70 and 75 different trades actively involved on the construction site. For each trade, we employ roughly five workers and one supervisor. This means that approximately 400 people will have worked on the building by the time it is finished in 2017. To ensure that the high demands of our users are met, I have also assembled a team of 25 experts who help make certain that the architect's plans can be realised effectively.

HOW DO YOU COORDINATE THIS WORK?

VERENA BÖRSCHMANN: Everything begins with good planning, the implementation of which is continually controlled. At the very beginning, I created an overall schedule which describes the basic steps for the realisation of the project. Based on this, the architect then created an even more detailed schedule which monitors the implementation of tasks. This schedule includes all the project tasks such as: when the project planner must deliver blue prints; when the administration must issue calls for tender; and when certain craftsmen can begin working. This schedule is tightly monitored to see if we are keeping within the given time and cost framework.

CSSB BUILDING KEY FIGURES

4
floors with a total area of 10,813 square meters

3,038
square meters of safety level 2 (S2) lab space

219
square meters of safety level 3 (S3**) lab space

6
electron cryo microscopes

WHICH CHALLENGES DO YOU FORESEE?

VERENA BÖRSCHMANN: The initial operational phase when all the research groups begin moving into the building will be very exciting. Four months have been scheduled for this. I think we will need this time to make sure that everything works smoothly. The building's climate control alone is highly complicated and the specifications for the safety level S2 labs are just as complex.

WHAT ARE YOU GOING TO DO ONCE THE BUILDING IS IN OPERATION?

VERENA BÖRSCHMANN: I am following the construction activity in and around Hamburg very closely. In the city, there are many exciting projects – and I would like to be involved in one of these in a supervisory position. In the future, when I pay a visit to "my CSSB", I hope to see that research has been brought to life.

— Interview conducted by scientific journalist, Angela Grosse.

182
participant lecture hall

1
"Science bridge"
to PETRA III beamlines

6
junior group leader spaces
in our Research Hotel

4
CSSB core facilities

OUR PEOPLE

INTERNATIONAL PERSPECTIVES

The CSSB Scientific Advisory Board (SAB) has been instrumental in contributing to the scientific direction and development of CSSB. The board's insightful advice and recommendations have helped CSSB position itself meaningfully within the national and international scientific landscape.

"It has been a pleasure and privilege to serve as a member of the CSSB Scientific Advisory Board. This initiative has established a wonderful centre for structural biology research that will make important contributions to German and European science." **Geoffrey Smith, University of Cambridge**

"Bridging systems and structure is an exciting vision, with tremendous opportunities to link the complex behaviours of biological systems in health and disease to key underlying molecular mechanisms. CSSB is poised to make advancements in this important area."

Tanja Kortemme: University of California

"Nowhere else do we see so many opportunities for cutting-edge scattering techniques in one place as on the DESY campus and at CSSB. The natural next step for such a unique infrastructure will be to tackle the biological questions that require structural biology at all levels combined – structural systems biology in a nutshell." **Poul Nissen, Aarhus University**

"CSSB is a unique multidisciplinary research centre, linking the key areas of structural and systems biology to address grand challenges in infection research. It has been exciting to serve on the CSSB SAB and see this farsighted initiative come to fruition." **Simon Phillips, Research Complex at Harwell**

"It has been a remarkable experience to see the vision of integrated scientific research focused on solving the most challenging problems in infection biology go from a concept to a foundational group of talented faculty, staff and students who will be located in a new laboratory building on the DESY campus. The participation of the partner institutions and their willingness to share in a common vision, working with all the stakeholders and considering the advice of the SAB, has been a real highlight in CSSB's start-up phase." **Keith Hodgson, Stanford University, Chair of SAB**

"CSSB provides an address for the greater structural biology community in Northern Germany and generates the critical mass to enable pioneering research in a very important area of biomedicine." **Patrick Cramer, MPI for Biophysical Chemistry**

"CSSB provides unique opportunities for studying infectious diseases by bringing together biologists and state-of-the-art structural biology techniques." **Holger Stark, MPI for Biophysical Chemistry**

"CSSB is set to become a major centre in the increasingly important research area of high-resolution cryo EM during the next few years." **Werner Kühlbrandt, MPI of Biophysics**

OUR SCIENTISTS
TIM GILBERGER

BIOGRAPHY

- Dr. rer. nat. 1999, Universität Hamburg, Hamburg
- Postdoc, Walter and Eliza Hall Institute, Melbourne, Australia (1999–2003)
- Group Leader, Bernhard Nocht Institute For Tropical Medicine, Hamburg (2003–2010)
- Associate Professor, McMaster University, Canada (2010–2014)
- Professor, Universität Hamburg, Hamburg; Department Head, Bernhard Nocht Institute For Tropical Medicine, Hamburg since 2014
- CSSB principle investigator since 2014

FUTURE GOALS

By understanding the molecular mechanism of host cell invasion at the highest possible resolution, we hope to provide a solid basis for the development of novel, anti-malarial strategies that will target this crucial process.

CURRENT RESEARCH

RED BLOOD CELL INVASION OF THE MALARIA PARASITE

One of the most significant steps in the complex life cycle of the malaria parasite is the invasion of human erythrocytes. All clinical symptoms are connected with the modification and destruction of the host cell. The Gilberger group studies the molecular basis for erythrocyte invasion by combining genetic, cellular, biochemical, structural and systems biology based approaches with the aim to deliver a detailed molecular blueprint that will help define novel therapeutic targets.

ADHESIVE AND REGULATORY ELEMENTS

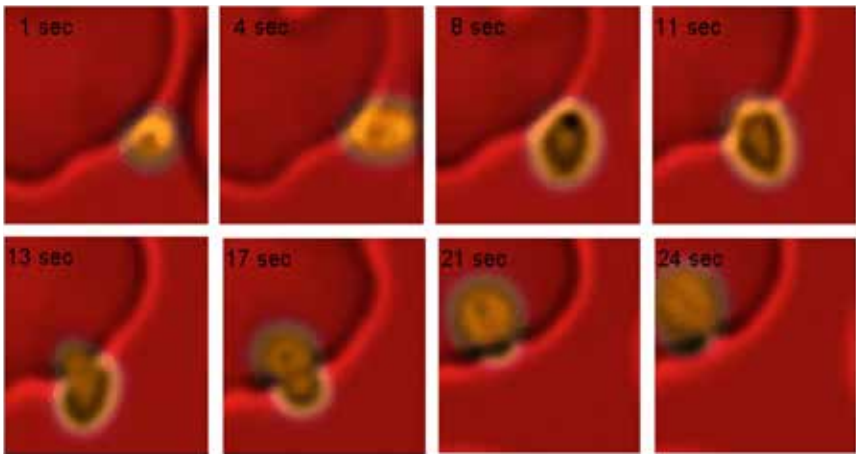
To survive and multiply, the malaria parasite invades red blood cells in less than a minute. The physical link between the parasite and erythrocyte membrane is generated by the interaction of parasite proteins that bind with their adhesive, extracellular domain to specific surface structures of the erythrocyte. This physical bridge between parasite and its host cell is linked to the actin-myosin motor of the parasite powering the invasion.

One cellular control mechanism is the post-translational modification of proteins due to phosphorylation. We are investigating kinase dependent phosphorylation of cytoplasmic domains of type I invasins as a switch mechanism in the molecular cascade triggering and powering the invasion process of human red blood cells. We are interested in the identification of the responsible kinases as well as in the dissection of signaling and effector pathways mediated by the cytoplasmic domain of selected invasins.

STRUCTURAL ELEMENTS

The motor complex is anchored in the membranes of the inner membrane complex (IMC) and is located under the plasma membrane in the malaria parasite. The obvious fundamental role of the IMC stands in contrast to our rudimentary knowledge of its components, dynamics and biogenesis in the malaria parasite. We explored a systems biological approach and subsequent phylogenetic profiling to identify novel IMC proteins. We revealed high levels of diversity in terms of structural organisation and phylogenetic trajectories of Plasmodium IMC proteins, which exemplify the adaptive molecular composition of this structure. Using high resolution and time-lapse microscopy we investigated i) the dynamic of this

CELL BIOLOGY OF HUMAN PARASITES



Time lapse microcopy of the invasion of a human red blood cell by the malaria parasite.
Image: Tim Gilberger

pre-sexual differentiation and iii) its sub-compartmentalisation.

GLOBAL NETWORKS

Erythrocyte invasion is the result of the interplay of a complex protein network. The Bozdech laboratory (NTU, Singapore) constructed a high confidence gene interactome network using a probabilistic Bayesian network approach. Using the assembled interactome network, we identified a sub-network of proteins that are associated with merozoite invasion by retrieving 418 predicted proteins directly linked to previously established invasion associated proteins. Using a GFP-tagging approach, we selected 70 proteins for experimental analysis of their predicted association with invasion. 42 proteins could be localised in the parasite, of which 31 were targeted either to the apical organelles, the parasite surface or the IMC, compartments directly linked to the invasion process. Using reverse genetics, cell biological and biochemical approaches, we are now studying the function of selected individual proteins in the invadome.

SELECTED PUBLICATIONS

Kono, M., et al. (2016) **Pellicle formation in the malaria parasite.** J Cell Sci; 129: 673-80.

Wetzel, J., et al. (2015) **The role of palmitoylation for protein recruitment to the inner membrane complex of the malaria parasite.** J Biol Chem 2015; 290: 1712-28

Kono, M., et al. (2012) **Evolution and architecture of the inner membrane complex in asexual and sexual stages of the malaria parasite.** Mol Biol Evol; 29: 2113-32.

Grüring, C., et al. (2011) **Development and host cell modifications of Plasmodium falciparum blood stages in four dimensions.** Nature Commun; 2: 165.

Hu, G., et al. (2010) **Transcriptional profiling of growth perturbations of the human malaria parasite Plasmodium falciparum.** Nature Biotechnol; 28: 91-8.

OUR SCIENTISTS
KAY GRÜNEWALD

BIOGRAPHY

- Dr. rer. nat. 2000, University Jena
- Postdoc, NIH, Bethesda, USA (2002–2003)
- Group Leader at MPI of Biochemistry, Martinsried (2004–2009)
- Senior Group Leader, University of Oxford, UK Wellcome Trust Senior Research Fellow since 2010
- Professor of Structural Cell Biology, University of Oxford, UK since 2012
- Professor, Universität Hamburg and Designated Head of Department of Structural Virology, Heinrich Pette-Institute, Hamburg
- CSSB principle investigator

FUTURE GOALS

We aim to understand the dynamics of crucial host-pathogen interactions mechanistically using an integrated structural cell biology approach that combines *in vitro* and *in situ* techniques. We will focus on studying the dynamics of macromolecules in their native functional environment and to accomplish this we will push the limits of existing technologies.

CURRENT RESEARCH

Cells constitute the smallest autonomous units of life. Supra-molecular complexes carry out essentially all functions and processes and form the cells structural elements. The tightly regulated structural and functional organization of a cell at this level is currently only rudimentary understood. Viruses and their interactions with host cells provide attractive model systems for studying macromolecular interactions. Unravelling the mechanisms underlying the dynamic interactions of viruses with their host cells at this level is crucial to understand the complexity of a viral infection. The Grünewald group applies electron cryo microscopy in combination with other complementary techniques to approach selected aspects of this highly ordered network by analysing protein complexes *in situ*.

We have pioneered the application of electron cryo tomography (cryo ET) of pleomorphic viruses, to reveal their three-dimensional supramolecular organization. Examples are virion structures of Herpes simplex virus, HIV-1 and various Bunyaviruses. Over the past decade, we have moved towards studying the cell biology of viral infections. Understanding the entirety of a virus' 'life cycle' requires knowledge of its transient structures at the molecular level. We aim to develop a comprehensive picture of the functional interaction between viral protein complexes and cellular structures during the course of the infection. Viruses also serve as dedicated tools for mining the molecular details of cellular tomograms. Examining viruses in their natural states enables us to both follow and probe dynamic cellular processes.

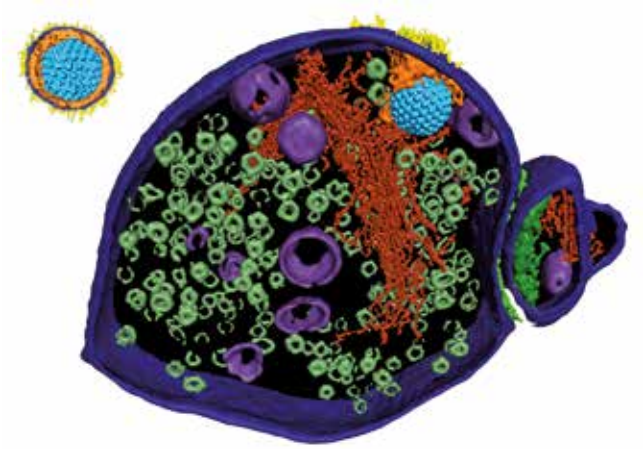
MEMBRANE MODULATIONS

The structural design of viruses provides a remarkable example of simplicity and functionality in biological systems. Viral particles and machineries work as highly effective molecular devices to mediate membrane traversal and transfer viral genomes and accessory proteins into and out of cells and their sub-compartments. We analyse the dynamic interactions of viral and cellular protein complexes leading to perturbations of the curvature of membranes.

INTEGRATIVE STRUCTURAL BIOLOGY

Electron cryo microscopy (cryo EM) provides an excellent platform for combining different approaches, such as biochemical, proteomics and X-ray crystallo-

VIRUS IMAGING



A snapshot of the structural intermediates of herpesviruses entering a synaptosome. Herpes simplex virus 1 entry occurs by fusion of the viral envelope with the plasma membrane of the host cell with several viral glycoproteins and cellular receptors being involved. Image: Maurer et al. PNAS 2008

graphic studies, and then, integrating their results with native sub-cellular structural information. Driven by our biological questions, we are involved in various methods development efforts. This includes the combination of cryo ET with 'single particle' approaches, the use of super-resolution fluorescence cryo microscopy modalities and soft X-ray cryo-microscopy/tomography in a correlative fashion, as well as the micromachining of samples to make them amenable for cryo EM analyses.

IMAGE ANALYSIS AND COMPUTATIONAL BIOLOGY

Image analysis (e.g. sub-volume averaging) is an integral part of cryo EM data processing. We are part of a community effort which, in response to novel hardware developments, seeks to continually advance the processing, visualization and validation tools required for the different levels of resolution and complexity. Integrative structural biology also requires dedicated means to combine data obtained from different modalities such as the fitting of higher resolution into lower resolution structures.

SELECTED PUBLICATIONS

Zeev-Ben-Mordehai, T., et al. (2016) **Two distinct trimeric conformations of natively membrane-anchored full-length herpes simplex virus 1 glycoprotein B.** PNAS 113: 4176-81

Zeev-Ben-Mordehai, T., et al. (2015) **Crystal Structure of the Herpesvirus Nuclear Egress Complex Provides Insights into Inner Nuclear Membrane Remodeling.** Cell Reports 13: 2645-52

Hagen, C., et al. (2015) **Structural Basis of Vesicle Formation at the Inner Nuclear Membrane.** Cell 163: 1692-701

Ibiricu, I., et al. (2011) **Cryo Electron Tomography of Herpes Simplex Virus during Axonal Transport and Secondary Envelopment in Primary Neurons.** PLoS Pathogens 7: e1002406.

Maurer, U.E., Sodeik, B., Grünewald, K. (2008) **Intermediates of membrane fusion in herpesvirus entry captured by cryo electron tomography.** PNAS 105: 10559-10564.

OUR SCIENTISTS
MARTIN HÄLLBERG

BIOGRAPHY

- PhD 2002, Uppsala University, Uppsala, Sweden
- Postdoc, Stockholm University, Stockholm, Sweden (2003–2005)
- Team leader, Structural Genomics Consortium's (SGC's) KI Node (2005–2007)
- Junior Research Fellow, financed by the Swedish Research Council (2007–2010)
- Senior Researcher position and Group Leader, Karolinska Institutet, Stockholm, Sweden (2010–2012)
- CSSB principle investigator since 2012

FUTURE GOALS

We will continue the work in mitochondrial RNA biogenesis focusing on reconstituting larger processing complexes combined with biochemical work to understand the the initial RNA processing order in mammalian mitochondria. Furthermore, we will study what happens with the mitochondrial mRNAs after polyadenylation. This research will not only increase our fundamental understanding of the human cellular energy generation but will also open up new avenues for early diagnosis and intervention.

CURRENT RESEARCH

MITOCHONDRIAL RNA BIOGENESIS

We work with the biochemistry and structural biology of RNA biogenesis. Given that impaired gene expression of mitochondrial DNA can lead to mitochondrial disease and pre-mature aging, the current focus of our group is to study mitochondrial gene expression.

UNDERSTANDING MITOCHONDRIAL RNA PROCESSING

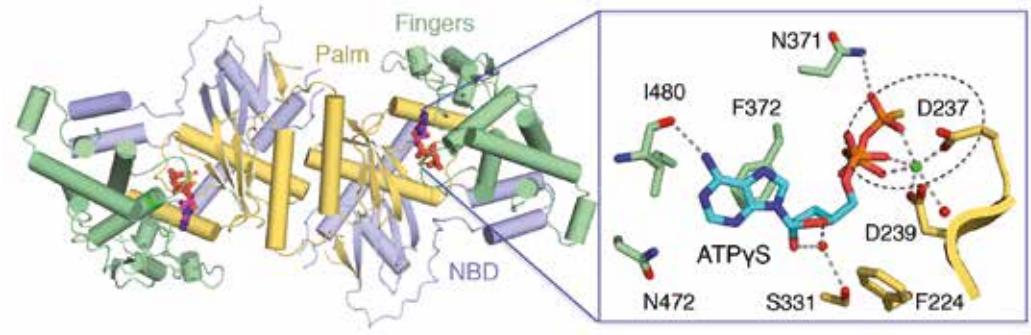
Transcription in human mitochondria gives rise to long polycistronic transcripts that need to be extensively processed in order to obtain mature RNA units that can be used in translation, the synthesis of proteins within the mitochondrion. A multitude of recent work shows that mitochondrial RNase P (mt-RNase P) initiates this processing by cleaving the polycistronic transcripts in the 5'-ends of mitochondrial tRNA genes.

Mammalian mt-RNase P is a tripartite protein complex that consists of MRPP1, MRPP2 and MRPP3. In Reinhard et al. 2015, we determined the structure of the nuclease subunit of the human mitochondrial RNase P (MRPP3) and showed that it has a non-functional active site in isolation. However, the nuclease subunit is capable of reforming a normal active site once its partners MRPP1 and MRPP2 are bound together with tRNA in an induced fit process. This work revealed the molecular basis for regulation of one of the key enzymes of human mitochondrial gene expression.

POLYADENYLATION OF mRNA

Polyadenylation alters the fate of mRNAs in several ways. It can increase mRNA stability, stimulate translation initiation, promote degradation or be required for completing certain stop codons that are not encoded in mtDNA. Polyadenylation is critical for mitochondrial translation and, hence, energy generation by the mitochondrion." In Lapkouski & Hällberg 2015, we determined the first high-resolution structure of a ver-

STRUCTURAL BIOCHEMISTRY OF MITOCHONDRIAL RNA BIOGENESIS



Structure of a vertebrate mitochondrial RNA poly-A polymerase. The enzyme is only functional as a homodimer and a strong dimer interface can be seen in the structure. Right: close-up of the enzyme active site with bound nucleotide.
Image: Mikalai Lapkouski

polyadenines to the 3'-ends of mitochondrial mRNAs.

Through high-resolution co-substrate ternary complexes, we could propose a structure-based hypothesis for why and how a mutation that causes spastic ataxia, a rare neurodegenerative disorder affecting balance, speech, movement of the arms, legs, and tongue, results in strongly reduced mRNA polyadenylation.

MITORIBOSOME BIOGENESIS

After processing, mitochondrial tRNAs and rRNAs need to be chemically modified. In Spåhr et al. 2012, we determined the crystal structure of the MTERF4-NSUN4 complex that methylates the mitochondrial small subunit rRNA and is critical for mammalian mitoribosome biogenesis. Based on the structure, we proposed that MTERF4 acts as a molecular ruler and helps orient NSUN4 for catalysis on the mitoribosome.

SELECTED PUBLICATIONS

Lapkouski, M., Hällberg, B.M. (2015) **Structure of mitochondrial poly(A) RNA polymerase reveals the structural basis for dimerization, ATP selectivity and the SPAX4 disease phenotype.** Nucleic Acids Res. 43, 9065-9075

Reinhard, L., Sridhara, S., Hällberg, B.M. (2015) **Structure of the nuclease subunit of human mitochondrial RNase P.** Nucleic Acids Res. 43, 5664-5672

Posse, V., Shahzad, S., Falkenberg, M., Hällberg, B.M., Gustafsson, C.M. (2015) **TEFM is a potent stimulator of mitochondrial transcription elongation in vitro.** Nucleic Acids Res. 43, 2615-2624

Posse, V., et al. (2014) **The amino terminal extension of mammalian mitochondrial RNA polymerase ensures promoter specific transcription initiation.** Nucleic Acids Res. 42, 3638-3647

Spåhr, H., Habermann, B., Gustafsson, C.M., Larsson, N.G., Hallberg, B.M. (2012) **Structure of the MTERF4-NSUN4 complex that regulates mitochondrial ribosome biogenesis.** PNAS 109, 15253-15258

OUR SCIENTISTS
MICHAEL KOLBE

BIOGRAPHY

- PhD 2002, Max-Planck-Institute for Biochemistry and the Ludwig-Maximilians University, Munich
- Postdoc, Max-Delbrück Centre, Berlin (2001–2004)
- Junior research group leader, Max-Planck-Institute for Infection Biology, Berlin (2004–2015)
- Professor Universität Hamburg, Hamburg; Department Head of Structural Infection Biology, Helmholtz Centre for Infection Research, Braunschweig since 2015
- CSSB principle investigator since 2015

FUTURE GOALS

Our underlying biological goal is to understand in-depth the process by which Type 3 Secretion Systems orchestrate the sequential order of effector translocation and trigger controlled subversion of a target cell. In addition, we aim to explore the potential for therapeutic targeting of the type 3 secretion pathway involved for either bacterial invasion or immune activation depending on the disease state.

CURRENT RESEARCH

BACTERIAL DISEASE AND HOST-PATHOGEN INTERACTIONS

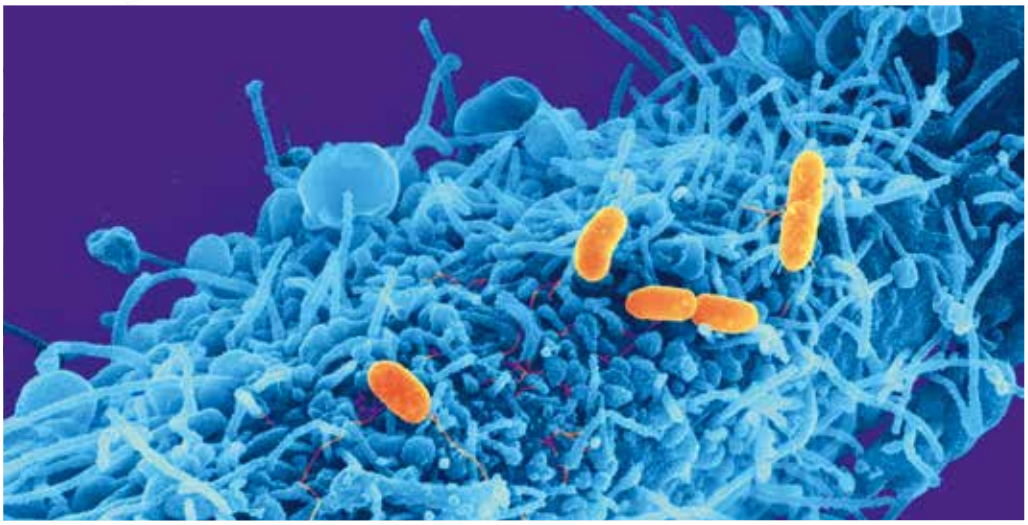
A major interest of the Kolbe group is the study of molecular machines from different pathogens and the host response to them. We focus on understanding how these macromolecular complexes assemble and work to facilitate bacterial infection. Water-borne bacterial pathogens like Salmonella or Shigella utilise a nanosyringe-like structure named Type 3 Secretion System (T3SS) to hijack human cells and prepare them for invasion. We study the structure and function of the T3SS and take a closer look at the atomic architecture of the T3SS throughout the infection process.

We seek to answer questions such as: How do structural switches in the secretion apparatus allow the recognition and secretion of effectors in a hierarchical manner? What kind of protein-protein interactions can drive bacterial invasion? How do bacteria promote the formation of vesicles and adapt to accommodate herein? We integrate high-end technologies like X-ray lasers and electron cryo-microscopes with other biophysical methodologies to examine the dynamic process of a bacterial infection at nanoscale resolution. The study of the structure and the molecular assembly mechanisms of vesicular trafficking induced by pathogenic bacteria in host cells will be a developing interest of the research group.

SUBVERSION OF ANTIBACTERIAL RESPONSE

In addition, we have a strong interest in uncovering the strategies used by Gram-negative organisms to subvert the antibacterial response of human cells. Components of the tip of the T3SS nanomachine interact with host membranes to allow the translocation of bacterial effectors directly to the human cell. How the translocon components assemble and insert into host membranes to form pores is under investigation.

STRUCTURAL BIOLOGY OF BACTERIA



Salmonella typhimurium (orange) adhering to a host cell.
Image: Michael Kolbe and Diane Schad

The group also focuses on the molecular interactions of T3SS effector proteins with host components and their signalling cascade that drives cellular subversion. A developing interest of the lab is to understand, at the atomic level, how innate immune receptors sense the presence of bacterial components, such as T3SS effectors or bacterial pigments, when they gain access to the cell cytosol leading to immune activation. Three-dimensional structural analysis and binding assays of key components of the T3SS pathway and their host interacting partners will allow us to design molecular drugs for the treatment of Gram-negative bacterial infections.

Our overarching goal is to integrate interdisciplinary approaches such as cellular microbiology, computational modelling and structural biology methodologies to advance our understanding of the assembly of macromolecular structures at near atomic resolution and to determine which approaches can also be applied to other biological pathways beyond infectious diseases.

SELECTED PUBLICATIONS

Moura-Alves, P., et al. (2014) **Aryl hydrogen receptor senses bacterial pigmented virulence factors and orchestrates antibacterial defenses.** Nature, 512: 387-392

Dohlich, K., Brotcke Zumsteg, A., Goosmann, C., Kolbe, M., (2014) **A Substrate-Fusion Protein is Trapped inside the Type III Secretion System Channel in Shigella flexneri.** PLoS Pathogens, e003881

Loquet, A., et al. (2012) **Atomic model of the type III secretion system needle.** Nature, 486: 276-279

Poyraz, O., et al. (2010) **Protein refolding is required for assembly of the type three secretion needle.** Nat. Struct. Mol. Biol., 17: 788-792

Lunelli, M., Lokareddy, R.K., Zychlinsky, A., Kolbe, M., (2009) **IpaB-IpgC interaction defines binding motif for type III secretion translocator.** Proc. Natl. Acad. Sci., 106: 9661-9666

OUR SCIENTISTS
JÖRG LABAHN

BIOGRAPHY

- PhD 1994, Freie Universität Berlin, Institute for Cristallography
- Post-Doctoral Research Fellow at Harvard Medical School and Research Center Jülich
- Group Leader at Research Center Jülich (ICS-5)
- Professor for X-ray Crystallography at Heinrich-Heine-University Düsseldorf (2012)
- Outstation of Research Center Jülich (ICS-6) since 2013
- CSSB principal investigator since 2013

FUTURE GOALS

Developing and integrating methods for sample generation, structural analysis of targets and bioinformatics to arrive at a coherent approach to structural systems biology that will allow the modeling of protein networks at the level of atomic and molecular interaction based on structural information. We also plan to conduct crystallographic analysis of uncleaved Presenilin in an effort to unravel the structural basis of autoinhibition and the activation of gamma-Secretase.

CURRENT RESEARCH

METHOD DEVELOPMENT

Progress in the understanding of the molecular base of biological processes is critically dependent on the quality of samples. The properties and interactions of mammalian membrane proteins and natively unfolded proteins are especially difficult to study. The Labahn group develops novel techniques for protein isolation, purification and crystallisation to enable the investigation of these molecules for both research and industrial applications.

Currently, the main focus of method development is on high-throughput approaches for membrane protein crystallisation by lipidic cubic phase crystallization in vapor diffusion experiments and the development of a protein-tag that allows precipitating proteins directly from lysate as well as improved straylight correction for the characterisation of proteins in crystals by spectroscopic means.

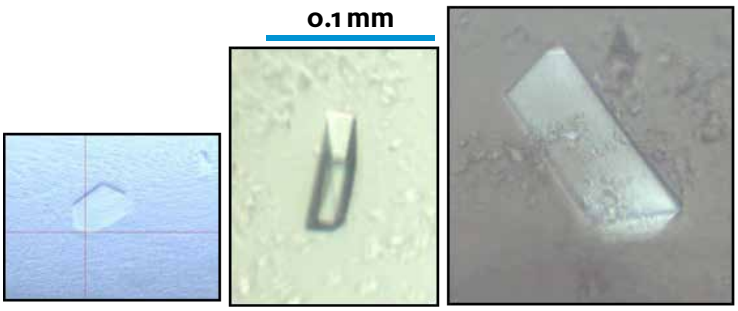
MEMBRANE PROTEINS

Signaling events between cells are facilitated by membrane proteins. Membrane proteins are not only the attack point for many diseases, but can also be targets for curative treatments. By identifying specific types of cells and then changing their behavior in the external stimulation of pathways, new targets can be identified.

Using the crystallographic analysis of different states, we are investigating dynamic processes such as signaling in membrane protein complexes like the sensory rhodopsin/transducer complex. Our current focus rests on the biophysical characterisation of serotonin transporters and gamma-secretase and its constituting protein.

The gamma-secretase complex proteolytically degrades APP to Aß which forms the pathogenic fibrils in Alzheimer disease. Presenilin, the catalytic protein of the gamma-secretase complex, is activated for complex formation by auto-cleavage. But uncleaved Presenilins also play a role in other biological processes like Ca-homeostasis in the endoplasmatic reticulum, the interaction with DRAL, GSK3 and the tau protein, which is also involved in M. Alzheimer.

METHOD DEVELOPMENT FOR MEMBRANE PROTEINS



One of the major obstacles in the structural analysis of human membrane proteins is the optimization from a 1st-hit (left) to diffracting crystals (right). Image: Jörg Labahn

cleavable structure of Presenilin can be found using spectroscopy. Upon cleavage, helical segment of 15 residues in the auto-inhibitory loop allows proteolytic activity of presenilin and gamma-secretase complex formation, whereas for other biological processes independent of gamma-secretase e.g. Ca-homeostasis, the uncleaved helix is essential.

NATIVELY UNFOLDED PROTEINS

In mammalian systems, some functionally important proteins exhibit structural instability to the extent that these proteins have been labeled natively unfolded. We investigate the pro-apoptotic protein par-4 (prostate apoptosis response factor 4, a.k.a. pawr) which is crucial for the sensitisation of cells for pro-apoptotic stimuli and the execution of the apoptotic process by binding the bcl-2 gene. Apoptosis, the programmed cell death, is a major defense of an organism used to terminate aberrant cells. The biological activity of par-4 is stimulated by a diverse set of conditions ranging from cancer to HIV-encephalitis, and is tightly controlled by a network of regulating proteins.

We investigate the 3D-structure of this protein and use the obtained information to model the molecular interactions of par-4 with regulatory proteins thus generating a model of the structural systems biology of par-4 mediated apoptosis. The regulatory domain of par-4 interacts with other proteins by coiled-coil interaction, leucine-zipper formation, or both.

SELECTED PUBLICATIONS

Hendler, R.W., et al. (2015) **Stray light correction in the optical spectroscopy of crystals.** Appl Spectrosc. 69, 1106-11

Kubicek, J., Block, H., Maertens, B., Spriestersbach, A., Labahn, J. (2014) **Expression and purification of membrane proteins.** Methods Enzymol. 541, 117-40

Tiruttani Subhramanyam, U.K., Kubicek, J., Eidhoff, UB., Labahn, J. (2014) **Cloning, expression, purification, crystallization and preliminary crystallographic analysis of the C-terminal domain of Par-4 (PAWR).** Acta Crystallogr F Struct Biol Commun. 70, 1224-7

Kubicek, J., et al. (2012) **Controlled in meso phase crystallization--a method for the structural investigation of membrane proteins.** PLoS One; 7(4):e35458.

Moukhametzianov, R., et al. (2006) **Development of the signal in sensory rhodopsin and its transfer to the cognate transducer.** Nature. Mar 2; 440(7080):115-9.

METHOD PATENTS

PCT/EP2015/070393 (WO2016034741 A1) *"Fusionsprotein und Aufreinigungsverfahren"*
EP20080017180 (EP2168646 A1) *"Method of loading a crystallization device"*

OUR SCIENTISTS
CHRISTIAN LÖW

BIOGRAPHY

- PhD 2008, Martin Luther University Halle-Wittenberg, Halle
- Postdoc, Karolinska Institutet, Stockholm, Sweden (2008–2013)
- Group leader at EMBL Hamburg since 2014
- CSSB principal investigator since 2014

FUTURE GOALS

To obtain comprehensive insights into the transport mechanism of this transporter class, we focus our research on characterising nutrient transporters from bacteria, parasites and humans in various states of the transport cycle using structural methods to decipher the common transport mechanism of major facilitator superfamily (MFS) transporters. We aim to obtain new structural and dynamic insights into the binding mode of transporters to peptides, drugs and inhibitors and to provide molecular insights into structure and function of transport regulators of nutrient transporters.

CURRENT RESEARCH

MEMBRANE TRANSPORT PROTEINS

The Löw group focuses on structural and functional studies of membrane transport proteins. Combining high throughput technologies for structural studies with classical biochemical approaches has enabled us to obtain a first glimpse of the structure and function of nutrient uptake systems. We seek to develop a better understanding and visualisation of the molecular processes of substrate recognition and transport in nutrient uptake systems.

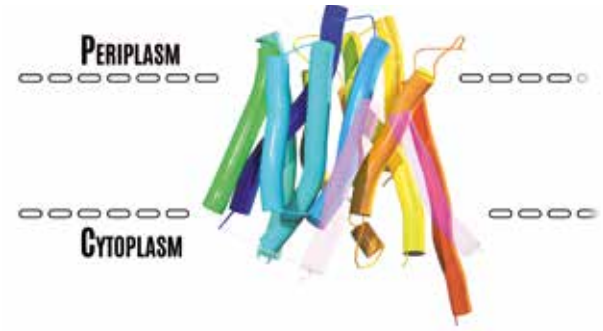
Cell membranes compartmentalise metabolic processes and serve as selective barriers for permeation. Therefore, nutrient transport through the plasma membrane conducted via membrane transport proteins is essential to maintain homeostasis within the cell. Many proton-coupled secondary active transporters of the major facilitator superfamily (MFS) are involved in the accumulation of nutrients above extracellular levels.

Structural and functional analyses of MFS transporters suggest an alternating-access mechanism for the transport of substrates across the membrane. Here, the transporter adopts different conformational states, allowing the substrate binding site to face either side of the membrane. A full transport cycle involves at least three different conformational states – inward open, occluded and outward open –, with each of them in a ligand-bound and ligand-free state. Since MFS transporters are found in all branches of life and often with numerous gene copies, we believe that many if not all of these transporters follow a common transport mechanism.

HUMAN PEPTIDE TRANSPORTERS

We study proton coupled oligopeptide transporters of the PepT family (also known as the POT family) which are responsible for the uptake of a range of different di- and tripeptides, derived from the digestion of dietary proteins, and are highly conserved in all kingdoms of life. The best studied members of this family include the two human peptide transporters, PepT1 and PepT2. These peptide transporters are of great pharmacological

MEMBRANE PROTEIN STRUCTURAL BIOLOGY



Structure of a proton dependent oligopeptide transporter in the inward open conformation (based on 4LEP.pdb). Membrane boarders are indicated.
Image: Christian Löw

of drugs and amino acid-conjugated pro-drugs as substrates. A detailed understanding of the structural basis for substrate recognition can therefore help to convert pharmacologically active compounds into substrates for PepT1 and PepT2 and improve their absorption in the small intestine and subsequent distribution in the body. We, therefore, study the proton-dependent oligopeptide transporter (POT) family using a combination of biochemical and biophysical methods.

TECHNOLOGY DEVELOPMENT

Integral membrane proteins are a challenging class of proteins in terms of their structural and functional characterisation. Over the years, we have developed and established new tools as well as a workflow for protein production and quality control of membrane proteins including functional assays. This allows us to screen multiple samples and parameters in parallel. Furthermore, we are trying to find new ways to stabilise integral membrane proteins in vitro upon extraction from their natural lipid environment.

SELECTED PUBLICATIONS

Frauenfeld, J., et al. (2016) **A novel lipoprotein nanoparticle system for membrane proteins.** Nature Methods 13, 345-51

Quistgaard, E.M., et al. (2016) **Understanding transport by the major facilitator superfamily (MFS): structures pave the way.** Nature Reviews Molecular Cell Biology 17, 123-32

Guettou, F., et al. (2014) **Selectivity mechanism of a bacterial homolog of the human drug-peptide transporters PepT1 and PepT2**. Nat. Struct. Mol. Biol. 21, 728-31

Quistgaard, E.M., et al. (2013) **Structural basis for substrate transport in the GLUT-homology family of monosaccharide transporters.** Nat. Struct. Mol. Biol. 20, 766-8

Guettou, F., et al. (2013) **Structural insights into substrate recognition in proton-dependent oligopeptide transporters.** EMBO Rep. 14, 804-10

OUR SCIENTISTS
THOMAS MARLOVITS

BIOGRAPHY

- PhD 1997, University of Vienna, Vienna, Austria
- Postdoc, University of Vienna, Vienna, Austria, MPI of Biophysics, Frankfurt and Yale University, New Haven, USA
- Group Leader, Research Institute of Molecular Pathology, Vienna, Austria and Institute of Molecular Biotechnology, Vienna, Austria since 2005
- Professor of Structural and Systems Biology, University Medical Center Hamburg-Eppendorf, Hamburg since 2013
- CSSB principle investigator and Deputy Director since 2014

FUTURE GOALS

By understanding the molecular mechanism of TTSS-mediated protein transport at the highest possible resolution, we hope to provide a basis for the development of novel therapeutic strategies that will either inhibit its activity or modify the system for targeted drug delivery.

CURRENT RESEARCH

MOLECULAR MACHINES IN ACTION

A fundamental property of many biological processes is that they are performed by highly organised, multi-component macromolecular assemblies, often referred to as molecular machines. The Marlovits group studies the structural basis for assembly, regulation and function of transmembrane molecular machines. We use a multidisciplinary approach by combining molecular biology, genetic, cellular, biochemical and a wide-range of structural (EM, X-ray, NMR, X-linking/mass spectrometry) tools. We are developing novel imaging and modeling technologies to visualise dynamic molecular processes in unprecedented detail in situ and in action.

MICROBIAL PATHOGENESIS

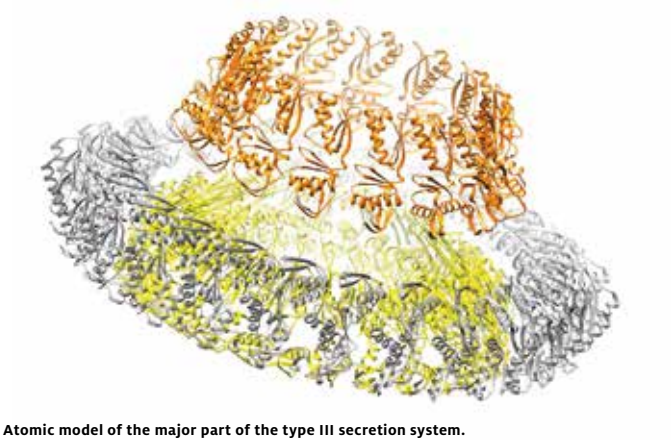
Gram-negative pathogens such as Yersinia, Shigella, Pseudomonas, enteropathogenic/enterohemorrhagic E. coli (EPEC/EHEC) and Salmonella are the causative agents for many diseases known to both animals and humans. These pathogens often originate as food-borne diseases and result in outcomes ranging from a mild stomach ache to death. Central to the pathogenicity are bacterial toxins ('effectors'), which are delivered via the type III secretion system, a large membrane embedded machinery, from the bacterium to its host cell. Once delivered, these translocated effector proteins have the remarkable capacity to modulate various host-cell pathways that induce membrane ruffling and subsequently make the host accessible to bacterial infection.

TYPE III SECRETION SYSTEM: UNFOLDED PROTEIN TRANSPORT ACROSS MEMBRANES

Our recent structural analysis (Schraidt & Marlovits, Science 2010) of the injectisome, the most prominent, cylindrical structure of the type III secretion system, revealed a potential secretion path through the central part of the membrane embedded complex. However, the inner diameter of this path is too small to accommodate a fully folded effector protein, suggesting that either the injectisome must undergo large conformational changes during transport or the effector proteins need to be unfolded.

To investigate the type III secretion of human pathogens, we focused (1) on determining the secretion path of injectisomes, (2) on understanding the mechanism of transport, and (3) on visualising pro-

STRUCTURAL AND SYSTEMS BIOLOGY OF BACTERIA



Atomic model of the major part of the type III secretion system.
Image: Thomas Malovits

tein transport in situ. We discovered that substrates are inserted into the secretion path in a polar fashion – N-terminal regions first – and that they are transported in an unfolded state. To understand, whether such a behavior is in fact observed *in situ*, we analysed protein transport across membranes in a near-native state using cryo electron tomography (Radics et al 2014). For the first time, we were able to visualise pathogenic type III secretion systems in action.

TECHNOLOGY DEVELOPMENT – ATOMIC STRUCTURE DETERMINATION FROM LOWER RESOLUTION CRYO-EM MAPS

Direct electron detectors are key to the recent revolution in structural biology and have made it possible to generate electron density maps at near atomic resolution using electron cryo microscopy from non-crystalline sample material. However, building accurate models into these 3-5Å maps remains a challenge. We recently developed a new modeling approach that integrates Monte Carlo optimisation with local density guided moves, Rosetta all-atom refinement and real space B-factor fitting; thus yielding accurate models from experimental maps for three different systems with resolutions as low as 4.5Å (DiMaio et al Nature Methods 2015). Based on increasing need within the scientific community, we expanded this work by developing easy-to-use modeling tools which build accurate models at the highest possible resolution from single particle electron microscopy maps.

SELECTED PUBLICATIONS

DiMaio, F., et al. **Atomic-accuracy models from 4.5-Å cryo-electron microscopy data with density-guided iterative local refinement.** Nat Methods. 12(4):361-5

Radics, J., Königsmaier, L., Marlovits, TC. (2014). **Structure of a pathogenic type 3 secretion system in action.** Nat Struct Mol Biol. 21(1):82-7

Schraidt, O., Marlovits, TC. (2011). **Three-dimensional model of Salmonella's needle complex at sub-nanometer resolution.** Science. 331(6021):1192-5

Wagner, S., et al. (2010). **Organization and coordinated assembly of the type III secretion export apparatus.** Proc Natl Acad Sci U S A. 107(41):17745-50

Marlovits, T., et., al. (2006). **Assembly of the inner rod determines needle length in the type III secretion injectisome.** Nature. 441(7093):637-40

OUR SCIENTISTS
MATTHIAS PRELLER

BIOGRAPHY

- PhD 2011, Hannover Medical School, Hannover
- Postdoc, Hannover Medical School, Hannover (2012)
- Junior professor of Structural Bioinformatics, Hannover Medical School, Hannover since 2012
- CSSB principle investigator since 2012

FUTURE GOALS

Understanding the molecular mechanisms of mechanotransduction in motor proteins, in particular in the context of the apicomplexan glideosome, is of major interest to our research. Using this information will support the rational development of specific inhibitors of parasitic movement and host cell invasion processes and help us to improve our development strategy.

CURRENT RESEARCH

DE NOVO DEVELOPMENT OF NOVEL INHIBITORS OF THE APICOMPLEXAN PARASITE INVASION

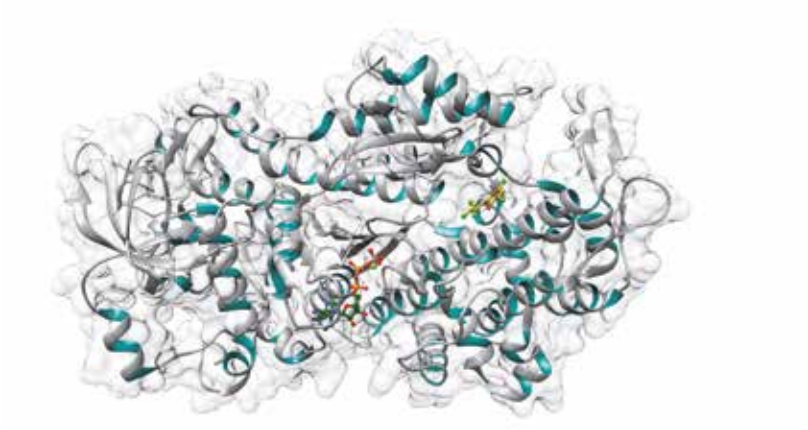
Malaria is one of the most lethal tropical diseases caused by apicomplexan parasites. The Preller group applies a rational, structure-based approach to develop and analyse novel small molecule inhibitors, targeting the motility and invasion machinery of the parasites – the glideosome.

Using a combination of computer-chemical predictions, organic synthesis as well as biophysical and structural analyses, we are designing, stereoselectively and biomimetically producing and mechanistically investigating small molecule glideosome inhibitors which show inhibitory potencies against the blood and liver stages of the parasites. These compounds will be used as tools in biochemical and cell biological studies to understand the host cell invasion process of the parasites.

MOLECULAR MECHANISMS OF CHEMO-MECHANICAL COUPLING IN THE MOTOR-PROTEIN MYOSIN

The acto-myosin system constitutes the functional, contractile unit of the sarcomere in muscle cells. Acto-myosin motor activity is, however, not restricted to muscle cells. Mechanical work and directed movement is generated during the myosin power stroke – the key event of acto-myosin motor activity. However, our knowledge about the molecular events of chemo-mechanical coupling remains incomplete. The lack of structural information of important conformational states, as well as an insufficient description of the allosteric communication pathways within the motor domain have thus far prevented a full description of the mechanotransduction mechanism in molecular detail. We are using a multidisciplinary approach including state-of-the-art biophysical methods, crystallographic analysis and mechanistic simulations to shed light on the allosteric communication pathways within the motor protein that dictate the propagation of chemomechanical coupling signals leading to force production and movement.

STRUCTURAL BIOINFORMATICS OF PARASITIC DISEASE



Crystal structure of a member of the natural product-based inhibitor class of halogenated pseudilin (yellow) bound to the motorprotein myosin . These structures serve as the basis for the rational inhibitor design. Image: Martin et al. 2014; Preller et al. 2011

DYNAMICS AND MECHANISMS OF DISEASE-ASSOCIATED MUTATIONS IN PROTEINS

We are interested in understanding the consequences and molecular mechanisms underlying disturbances of the native function of protein systems by single point mutations.

Recently, we were able to elucidate the mechanisms of two mutations in cytoplasmic β-actin, which are related to severe human developmental malformations and deafness, on the structure, function and dynamics of the molecules (Hundt et al. 2014) as well as the disturbing effect of point mutations in human connexin46 on the proper formation of gap junction channels in the human lens, leading to cataract (Schadzek et al. 2016).

SELECTED PUBLICATIONS

Schadzek, P., et al. (2016) **The cataract related mutation N188T in human connexin46 (hCx46) revealed a critical role for residue N188 in the docking process of gap junction channels.** Biochim. Biophys. Acta 1858, 57-66.

Radke, M.B., et al. (2014) **Small molecule-mediated refolding and activation of myosin motor function.** Elife 3, e01603.

Chaturvedi, A., et al. (2013) **Mutant IDH1 promotes leukemogenesis in vivo and can be specifically targeted in human AML.** Blood 122, 2877-2887.

Preller, M., and Holmes, K.C. (2013) **The myosin start-of-power stroke state and how actin binding drives the power stroke.** Cytoskeleton (Hoboken) 70, 651-660.

Preller, M., Chinthalapudi, K., Martin, R., Knolker, H.J., and Manstein, D.J. (2011) **Inhibition of Myosin ATPase activity by halogenated pseudilins: a structure-activity study.** J. Med. Chem. 54, 3675-3685.

OUR FUTURE

CSSB empowers the next generation of scientists to work boldly and collaboratively to tackle the complex problems of tomorrow. CSSB's collaborative atmosphere and its interdisciplinary research culture provide junior researchers with the essential skills and experience to conduct pioneering and innovative research.

"I am interested in understanding the molecular mechanism of proteins related to human health. The core facilities available at CSSB enable me to easily achieve my research goals." **Udaya Kumar**

"My research focuses on the type three secretion system (T3SS) which is a major determinant of bacterial pathogenicity. The application of recent innovations in the field of systems biology allows decoding the underlying regulatory network of the T3SS in a dynamic fashion. CSSB with its infrastructure and interdisciplinary collaborations provides an innovative framework for my research." **Sebastian Schulz**

"Development of new microscopy methods is happening at the interface of physics and biology. CSSB offers me the ideal environment to establish super-resolution fluorescence cryo-microscopy as a revolutionary tool for structural and cellular biology." **Rainer Kaufmann**

"With the modern technologies and infrastructure at CSSB, I hope to gain new insights into the complex structural and dynamic processes during host cell invasion of the malaria parasites". **Dmitrij Malcev**

"With the move into the new building, all CSSB researchers will bring alive the spirit of CSSB: interdisciplinary communication and collaboration".

Linda Reinhard

"Almost half of all proteins are metalloproteins which require metals to catalyse fundamental metabolic processes. Using the advanced technologies and strong expertise at CSSB, I hope to provide unprecedented insights into the molecular mechanisms of iron metabolism." **Jan Strauss**

CSSB RESEARCH HOTEL:

To help young researchers who are looking to make the transition from postdoc to independent principle investigator, CSSB has reserved space in its new building for a Research Hotel. In the Research Hotel, young principle investigators will have their own lab space and be fully integrated into the CSSB research environment with access to world renowned technologies and infrastructure. Groups in the Research Hotel will benefit from access to all infrastructure, instrumentation, training and funding opportunities open to CSSB scientists.

OUR GOVERNANCE

CSSB DIRECTORATE

TIM GILBERGER, Bernhard Nocht Institute for Tropical Medicine, Universität Hamburg
KAY GRÜNEWALD, Heinrich Pette Institute, Universität Hamburg
MICHAEL KOLBE, Helmholtz Centre for Infection Research
JÖRG LABAHN, Forschungszentrum Jülich
CHRISTIAN LÖW, European Molecular Biology Laboratory
DIETMAR MANSTEIN, Hannover Medical School
THOMAS MARLOVITS, University Medical Center Hamburg-Eppendorf
CHRIS MEIER, Universität Hamburg
MATTHIAS WILMANNS, European Molecular Biology Laboratory
EDGAR WECKERT, Deutsches Elektronen-Synchrotron

JUNIOR GROUP LEADERS

MARTIN HÄLLBERG, Karolinska Institutet, CSSB Research Hotel
RAINER KAUFMANN, Universität Hamburg, CSSB Research Hotel (beginning in 2017)
MATTHIAS PRELLER, Hannover Medical School

CSSB COUNCIL

MARTIN AEPFELBACHER, University Medical Center Hamburg-Eppendorf
CHRISTOPHER BAUM, Hannover Medical School
THOMAS DOBNER, Heinrich Pette Institute
HELMUT DOSCH, Deutsches Elektronen-Synchrotron
DIRK HEINZ, Helmholtz Centre for Infection Research
ROLF HORSTMANN, Bernhard Nocht Institute for Tropical Medicine
CLAUDIA LEOPOLD, Universität Hamburg
IAIN MATTAJ, European Molecular Biology Laboratory
SEBASTIAN SCHMIDT, Forschungszentrum Jülich

CSSB SCIENTIFIC ADVISORY BOARD

PATRICK CRAMER, MPI for Biophysical Chemistry, Göttingen
KEITH HODGSON, Stanford University
TANJA KORTEMME, University of California, San Francisco
WERNER KÜHLBRANDT, MPI of Biophysics, Frankfurt
POUL NISSEN, Aarhus University
SIMON PHILLIPS, Research Complex at Harwell
GEOFFREY SMITH, University of Cambridge
HOLGER STARK, MPI for Biophysical Chemistry, Göttingen

CSSB EXECUTIVE GROUP

THOMAS MARLOVITS, Deputy Director
CHRIS MEIER, Deputy Director
INA PLETTNER, Office Head
MATTHIAS WILMANNS, Scientific Director

CSSB OFFICE

MICHAELA CLAYTON
INA PLETTNER
MELISSA PRASS
JELENA SIEGEL

DESY PROJECT MANAGEMENT FOR CSSB BUILDING

VERENA BÖRSCHMANN
CHRISTIAN HARRINGA
JUDITH KÖSTERS

IMPRINT

PUBLISHER:
Centre for Structural Systems Biology (CSSB)*

REALISATION AND EDITING:
Melissa Prass (Coordination), Michaela Clayton, Angela Grosse, Chris Meier, Matthias Wilmanns

DESIGN:
Holger Ziemann, www.erzaehlzeichen.net

PHOTOS AND ILLUSTRATIONS:
Cover image: Renate Nikolaus
Page 2: Tina Mavric
Page 3: DESY, Tina Mavric
Page 5: Gisela Koehler
Page 9: Michael Kolbe/Diane Schad
Pages 10–13: DESY
Page 14: Lars Berg, Gisela Koehler, DESY (Heiner Mueller-Elsner)
Page 15–16: Gisela Koehler, DESY
Page 18: DESY (Lars Berg)
Page 19: Tina Mavric
Pages 22: Geoffrey Smith, Elisabeth Fall, Lars Kruse, Aarhus University,
Page 23: Simon Phillips, Stanford Chemistry Department, Patrick Cramer/MPI, Holger Stark, Werner Kühlbrandt
Pages 24, 26, 28, 30, 32, 34, 36, 38: DESY
Page 40: Tina Mavric
Page 41: Dmitrij Malcev, Linda Reinhardt, Hugo Neves/EMBL Photolab

PRODUCTION AND IMAGE PROCESSING:
EHS Druck GmbH, Schenefeld

CONTACT:

Postal Address:
DESY
Centre for Structural Systems Biology (CSSB)
Notkestr. 85 (Building 61)
22607 Hamburg/Germany

Head of CSSB Office
Dr. Ina Plettner
E-mail: info@cssb-hamburg.de

Public Relations
Melissa Prass
E-mail: melissa.prass@cssb-hamburg.de

***CSSB IS A COOPERATION WITHOUT ITS OWN LEGAL IDENTITY. ALL PARTNERS ACT EXCLUSIVELY IN THEIR OWN NAME AND ON THEIR OWN ACCOUNT.**

CSSB INVESTORS

Bundesministerium für Bildung und Forschung

Hamburg | Behörde für Wissenschaft, Forschung und Gleichstellung

Niedersächsisches Ministerium für Wissenschaft und Kultur

SH Schleswig-Holstein
Ministerium für Soziales, Gesundheit, Wissenschaft und Gleichstellung

Centre for Structural Systems Biology (CSSB)
Notkestrasse 85 (Building 61)
22607 Hamburg
Germany

www.cssb-hamburg.de
info@cssb-hamburg.de