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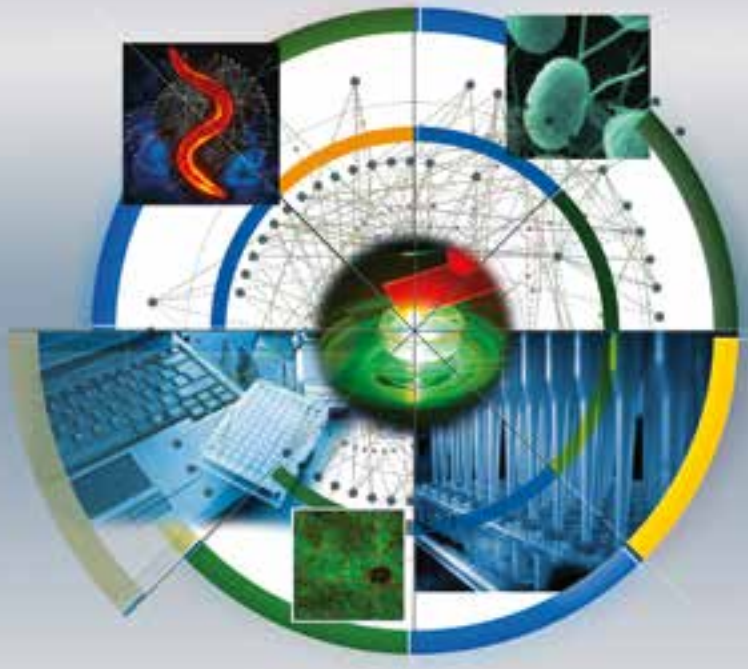

Photo: Derichs Kommunikation GmbH, Jülich, using pictures from Prof. Dr. Ralf Baumeister

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Systems biology is a young and dynamic discipline that sees the whole picture. As part of the life sciences it builds a bridge between sophisticated laboratory experiments and mathematical modelling, between high-tech data measurements and computer-aided data evaluation. Its research subjects are the network-like entangled activities of signal transduction and metabolism in cells, tissues, organs and organisms. Systems biology research deals with this complexity by organising itself into interdisciplinary networks. Experience this fascinating, upcoming branch of science and what answers it provides to previously unresolved questions about human life.

Cover photo: Andrey Kuzmin – Fotolia.com

welcome note

Dear Reader,

What micro-organisms live in the sea? How do corals develop? Systems biology is seeking answers to these questions through an interdisciplinary approach. Although the seas and oceans cover around 70 percent of the Earth's surface and are our planet's largest habitat, they present us with many riddles which we are still unable to solve. Not only do they play a key role in the Earth's climate system, they are also the source of valuable raw materials. At the same time, the oceans provide many people with nourishment and jobs.

The Federal Ministry of Education and Research wants to heighten people's awareness of the fascination and importance of the seas. We have therefore devoted Science Year 2016*17 to the seas and oceans under the motto "Discover. Use. Protect". The more we find out about this sensitive ecosystem, the more we can do to protect it and its resources sustainably and ecologically. To do so we need committed researchers who are interested in making new findings, including researchers in the field of systems biology.

Marine research is just one of many fields where systems biology is helping to fathom the secrets of life. The systems biology approach is a combination of practical experiments and mathematical modelling. It is eminently suitable for elucidating complex life processes and tackling issues of societal relevance – from health research to energy supplies to plant breeding. We have already contributed around 660 million euros to over 20 successful national and international funding measures in this field.

The diverse projects featured in the current issue of systembiologie.de highlight the wide potential of the systems biology approach. I hope that you will enjoy reading about them.

Prof. Dr. Johanna Wanka
Federal Minister of Education and Research

Photo: Bundesregierung / Steffen Kugler

greetings

Dear Reader,

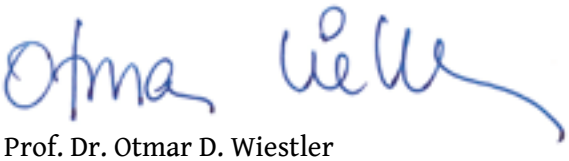
Systems biology attempts to understand biological processes in their entirety. Thus, a holistic image of these processes is developed – from the genome to the proteome, from organelles to the entire organism. To achieve this goal, systems biology deploys a combination of mathematical models and suitable experiments. Within the Helmholtz Association, systems biology has an increasingly important role within different research areas, such as medicine, environmental and marine research. In all these areas, we contribute to solving the urgent issues our society faces.

For example, researchers at the Alfred-Wegener-Institute (AWI), the polar and marine research center within the Helmholtz Association, are attempting to model the food web of the Wadden Sea in order to assess this ecosystem's ability to withstand stress. One of the main research priorities are the wide-ranging external influences, such as invasive species or climate change and their impact on the shallow sea just off Germany's northwest coast (p. 48).

Systems biology also makes a significant contribution to medicine, as it is crucial for our understanding of how internal processes unfold in the body. For instance, metabolic changes play a key role in the formation of brain tumors. Junior group leader Christiane Opitz at the German Cancer Research Center (DKFZ) is studying alterations in brain tumors' metabolic processes and signaling pathways. At the same time she is working as a resident at Heidelberg's university clinic (p. 74). Traditional biological approaches are often no longer sufficient if we want to gain a better understanding of tumor formation. There are simply too many different processes in play, and many of them seem interconnected in a myriad of ways. Systems medicine researchers are therefore combining experimental methods with computer modelling. This approach gives them a better grasp of which signaling pathways play a role in the formation of a tumor. In the long term, this more accurate understanding should help us better combat illnesses such as cancer.

Marine research and medicine are just two of the many fields where systems biology, still a relatively young discipline, may be able to generate invaluable advances. "Seas and Oceans" is the theme of Germany's Science Year 2016*17.

Taking this as our inspiration, the latest edition of *systembiologie.de* explores what systems biology can offer to marine research. I hope you enjoy reading these articles.



Prof. Dr. Otmar D. Wiestler
President of the Helmholtz Association

Photo: Steffen Jänicke / Helmholtz Association

foreword

Dear Reader,

"Systems biology has now turned into mainstream biology," stated Dr. Hiroaki Kitano, one of the pioneers in the field of systems biology, in 2014 in an interview with the magazine *npj Systems Biology and Applications*. In other words, systems biology has arrived. Within two decades of research activity, it has gone all the way from an emerging new discipline to an established field of science.

Its interdisciplinary approach combining techniques drawn from mathematics, physics and IT has become an established tool within many different fields, leading to lasting changes. To pick an example, systems medicine has a wide range of applications when it comes to medical research focusing on issues such as cancer, ageing, haematology and mental illnesses (p. 52). It does not stop there, as systems biology is of high importance to research in the fields of botany, biotechnology and ecology as well. At present, the marine ecology is the focus of particular attention due to Germany's Science Year 2016*17 – Seas and Oceans.

Marine expert Jacques-Yves Cousteau once said, "The prerequisite for knowledge is curiosity". Especially the biggest ecosystems of our planet, the oceans and seas, still contain countless secrets that remain largely unstudied. By uncovering new facts about their sensitive balance, we will be in a better position to protect them, an essential undertaking given the tremendous importance they have for the environment, our climate and – ultimately – for us humans.

Researchers at James Cook University in Australia have recently discovered that climate change is driving the most severe coral bleaching event ever recorded at the Great Barrier Reef, which could possibly cause the collapse of the reef itself. Working together with Dr. Roberto Iglesias-Prieto and Dr. Mónica Medina, Dr. Kitano has also dedicated himself to researching coral bleaching. They analyzed the acclimatizing and adaptation capacities of corals by using genome-based, physiological and systems biology approaches in order to counteract a future coral bleaching event (p. 20). Climate change is also having an impact on our coasts in Germany. The Alfred-Wegener-Institute is studying the ecological condition of the Wadden Sea and using modelling techniques to simulate the effects of climate change on communities of organisms in the Wadden Sea (p. 48).

On shore, the use of mathematical models can improve plant breeding. Dr. Amine Abbadi and Dr. Gunhild Leckband describe how, for example, the effects of changes to genetic and environmental factors can improve the predictions of crop plant yields (p. 24). When it comes to basic research, Dr. Lars Kuepfer and his team believe that systems biology will reduce the need for animal testing, as equivalent models based on human cells and computer-supported approaches will be able to replace these experiments (p. 28). Dr. Sylvia Escher and Dr. Jeannette Koschmann show that the transfer of findings from essential experiments on animals can then be improved by pharmacokinetic computer models (p. 32). Gene technology expert Prof. Bärbel Friedrich also believes that "it is not possible to use animal models for every type of research". She is in favour of new methods such as the molecular gene scissors CRISPR-Cas9, which can be deployed to change genetic material in a simple and targeted manner, such as deactivating genes that cause cancer (p. 36). This key technological tool will also be invaluable for strategies designed to help animals, plants and ecosystems cope with environmental changes.

I hope you enjoy reading this edition of the magazine, linked with fruitful insights into the different aspects of systems biology, be they underwater or on land.

Yours, Roland Eils

Editor in Chief

Photo: Tobias Schwerdt / DKFZ

index

findings from a sea of data

How systems biology contributes to our understanding of marine ecosystems

by Renzo Kottmann and Frank Oliver Glöckner

Mankind has a close and very special relationship with the planet's seas and oceans: not only do they provide food for millions of people, but they have been essential trade routes for millennia. More than half of the world's population live on or near a coastline. As an important economic factor, tourism has also increasingly shaped coastal regions in the past decades. All of these human activities influence and change, directly or indirectly, marine life forms' complex interactions. In view of this, a fundamental and holistic understanding of the marine ecosystem is of crucial importance, particularly in an era of global change, if we want to be capable of predicting the impact of mankind's actions.

The importance of microorganisms in the oceans, the planet's largest ecosystem

The oceans and seas form the largest ecosystem on our planet and cover 70 per cent of its surface. They are the home of countless species of organisms, many of which have not yet been studied. A single drop of seawater teems with more than one million microorganisms. They produce 50 per cent of our planet's oxygen and absorb the same amount of greenhouse gas CO_2. In doing so, they have a direct impact on life both on land and in the sea (Karl, 2007). Nevertheless, we still know virtually nothing about these sea-dwellers, invisible to the naked eye.

Over the past few years, "omics" technologies such as metagenomics, metatranscriptomics and metaproteomics have enabled us to study the genetic material of all microorganisms present in an environmental sample. In particular, the revolutionary advances in the field of *next generation sequencing* (NGS) give marine scientists the tools to analyze microbial DNA quickly and in high resolution. This has enabled marine research institutions to gather huge volumes of data within just a few years.

This omics data has grown in quality and applicability, which now puts us in a position to expand the systems biology approach, i. e. the holistic understanding of all processes in one cell or organism, to ecosystems biology. As a result, we are now able to study the molecular basis underlying the dynamics and interactions of entire microbial populations in marine ecosystems. This transformation provides us with the scientific framework for the systemic integration of molecular and oceanographic data, thereby giving us the key to a better understanding of the ocean's ecosystem. In addition, this approach encourages the discovery and investigation of new molecular processes for economic uses.

Standards

Standards are essential for ecosystems biology, as they make the automated exchange of information possible and so permit the wholesale integration of data. To promote the use of standards in molecular biology, Renzo Kottmann, Frank Oliver Glöckner, and led by Dawn Field, established the *Genomic Standards Consortium* (GSC) together with a number of other research scientists in 2005. This group currently has more than 100 members, and their objective is to enhance the identification, comparability and integration of data by setting out minimum requirements regarding the publication of genomes by the scientific community. We recently expanded our standards to include the description of metagenomes and marker genes, with the option of further additions (Yilmaz *et al.*, 2011). This led to the development of the "Minimum Information about a Biosynthetic Gene cluster (MIBiG)" standard, which is used to describe biosynthetic gene clusters (Medema *et al.*, 2015). It is now possible to link biotechnology even more closely with issues relating to ecosystems biology.

Figure 1: Research scientists at Roscoff Marine Station (France) measure environmental parameters for OSD (Photo: Roscoff Marine Station).

The GSC standards are especially important to our ecosystems biology research because they define how environmental parameters are described in connection with the habitat in question. They require the inclusion of the geographic origin of the original environmental sample with every molecular sequence, something that is, in turn, gaining in importance for biotechnology as part of efforts to comply with the Nagoya protocol. Ratified by the EU in October 2014, this agreement governs international access to genetic resources in addition to the balanced and fair sharing of the benefits arising from their use.

Big data infrastructures

When dealing with "big data", i. e. very large volumes of information, ecosystems biology research needs long-term and efficient data storage, management and analysis solutions. High-performance big data infrastructures therefore play a central role in making ecosystems biology a success in Germany, and these are currently being established on a national and European basis in the form of GFBio, de.NBI, Micro B3-IS and ELIXIR. As described below, we play an active role in shaping these systems.

German Federation for Biological Data (GFBio)

Since the end of 2013, Jacobs University has been working with eleven other universities, the archives of seven museums and collections in Germany as well as selected molecular biology archives and services to form the German Federation for Biological Data (www.gfbio.org), which is funded by the German Research Foundation (DFG). The long-term, service-focused, national and collaborative data infrastructure established by GFBio will be capable of archiving and integrating heterogeneous data from all manner of disciplines, thereby enabling the efficient generation and use of extensive and complex data products for innovative approaches. It will also be possible to make later, efficient use of large volumes of data for biodiversity research.

German Network for Bioinformatics Infrastructure (de.NBI)

Funded by the German Federal Ministry of Education and Research (BMBF), the de.NBI infrastructure initiative (www.denbi.de) was established in March 2015 to deliver sustained solutions concerning the availability of computer and storage capacities, in

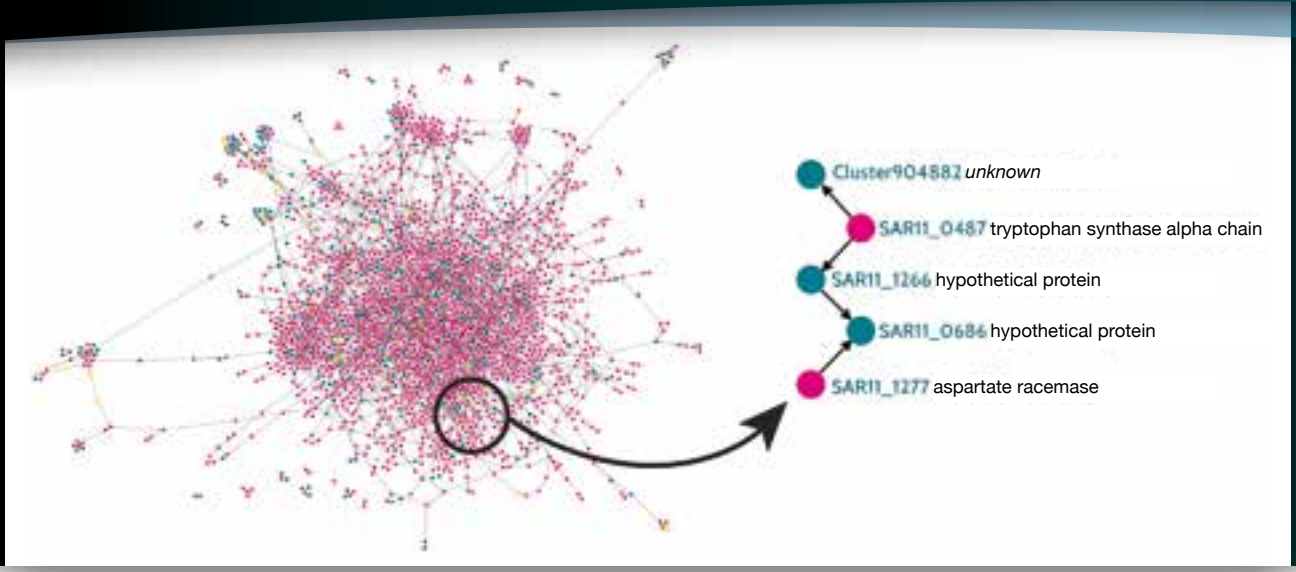


Figure 2: A network of identified and unidentified genes makes it possible to guess the roles of the latter group by illustrating connections to genes whose functions we already know.
This diagram is an excerpt of a network based on Global Ocean Survey (GOS) data (Source: Antonio Fernandez-Guerra).

addition to improving data resources and bioinformatics tools in the natural sciences. The initial eight de.NBI centers with a total of 22 national project partners are structured according to subject matter and possess specialist bioinformatics expertise and resources that they make available as a service offering.

ELIXIR

The ELIXIR European infrastructure project (www.elixir-europe.org) promotes access to the results of state-funded research and bioinformatics resources on a European level. By pooling capacities from across the continent, it delivers unfettered access to data and information in the life sciences, making it one of the key players in the data integration process in systems and ecosystems biology. Represented by de.NBI, Germany has been an ELIXIR member since August 2016.

The Micro B3 information system: marine microbial biodiversity, bioinformatics and biotechnology

Several years ago, we started working on a central, geo-based information system for marine microbial ecosystems biology (Kottmann *et al.*, 2010). We recently launched the Micro B3 information system (http://mb3is.megx.net), which pools information from environmental databases such as PANGAEA, SeaDataNet and EurOBIS, plus sequence data from the *European Nucleotide Archive* (EMBL-EBI/ENA). Micro B3-IS thereby facilitates the in-depth integration of environmental data and molecular sequence data, making it possible to produce flexible analyses and visualizations of large, multidimensional data sets from ongoing biodiversity studies and long-term monitoring.

Ocean Sampling Day – microbial ecosystems biology in coastal regions

Despite the important role that coastal regions play as the immediate interface between people and the seas, there are, at present, only a few global studies about the importance of microorganisms in these regions. We organized Ocean Sampling Day (OSD) as an event to counteract this shortfall in information. Taking place on the summer solstice, June 21, the event was initiated in 2014 as an occasion for research scientists around the world to analyze water samples and to compare microbial diversity and activity using the standardized protocols that we have developed (see Figure 1). More than 200 marine research stations from a huge range of locations across Europe, North America, Africa, Australia and Asia take part in OSD every year.

Since 2015, we have also been encouraging members of the public to get involved via the MyOSD "citizen science" project. As part of the Science Year 2016*17 campaign focusing on marine research and funded by the BMBF, we distributed 1,000 kits for taking samples from the North Sea and Baltic Sea, along with the rivers discharging into them. Work is currently under way on analyzing the findings of the campaign. This undertaking will produce a comprehensive systems biology snapshot of the impact that nutrients and pollutants transported by rivers have on microbial communities along Germany's coasts, revealing important findings on how organisms adapt and what their importance is regarding the protection and use of our coastlines.

Figure 3: The relative proportion of PETase genes in assembled metagenomic samples taken on OSD 2014.
On display here is the relative frequency of the PETase gene at each location on the map in a range of one hit in 1,000 (1e-04) to one hit in 10,000 (1e-05) (Source: Chiara Vanni).

New and unexpected insights

Today, international campaigns such as OSD/MyOSD and high-performance infrastructures make it possible to obtain in-depth information about the marine ecosystem. We are currently plotting, in the form of networks (see Figure 2), the relationships between all known and unidentified genes from ocean and sea samples that have so far been sequenced. These networks will enable us to make more accurate predictions about the potential role of genes that have so far been totally unknown to us, resulting in new insights into ecosystems biology and new targets for biotechnology. For example, researchers recently published an article on a bacterium that can break down polyethylene terephthalate, or PET (Yoshida *et al.*, 2016). PET is one of the main components in the seven million tons of plastic rubbish that make their way into our planet's seas and oceans every year. Using the PETase gene sequence now available to us, we were able to search the OSD data sets to see where PETase-like sequences occur in the oceans and in which variations. Initial findings show that PET-decomposing bacteria are present in marine ecosystems from north of the equator as far as Greenland (see Figure 3). In other words, the information provided by OSD suggests that the genetic ability to break down PET is considerably more widespread than originally assumed.

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Photo Frank Oliver Glöckner: MPI-Bremen; Photo Renzo Kottmann: MPI-Bremen

phytoplankton, an essential component of life on earth

An ideal model system for expanding molecular systems biology to the ecosystem level

by Agostino Merico

The complexity of biological systems has led over time to the development of a range of sophisticated computational and mathematical tools for conducting systems-level studies at organizational scales ranging from molecules to ecosystems. Biological and ecological data collected at different spatial and temporal scales can be integrated today with contextual physical observations thus providing us with the opportunity to gain a holistic understanding about life and its role within the Earth system.

Phytoplankton and the Earth system

Phytoplankton communities constitute one of such complex biological systems. Phytoplankton (Figure 1) are **photosynthesizing** microscopic organisms freely drifting with the currents in the sunlit surface layer of marine and fresh waters. The community structure and ecological functions of marine ecosystems depend critically on these unicellular organisms. The oxygen in our atmosphere has been formed about two billion years ago as a result of oxygenic photosynthesis by primitive phytoplankton cells. This dramatic event poisoned most of the Earth's anaerobic organisms of the Precambrian and provided a basis for the development of the oxygen-dependent metabolism. Phytoplankton produce biomass from inorganic compounds and for this reason they are called primary producers. Today, phytoplankton constitute only 1 % of the Earth's photosynthetic biomass, but they account for almost half of our planet's annual net primary production. Phytoplankton are highly sensitive to changes in climate while they also contribute to those changes because they remove carbon from the atmosphere and move it down into the ocean interior when they die. Bacteria recycle part of this dead material back into inorganic compounds, which are made available again to phytoplankton by mixing processes. A small fraction of the dead material sinking down into the ocean, however, is permanently buried in marine sediments. Over millions of years, heat and pressure in the sediments gradually turn this carbon-rich material into crude oil and gas, which we extract to fuel our modern societies. Changes in phytoplankton community structure and in the spatial and temporal distribution of phytoplankton blooms have therefore tremendous implications for the Earth's climate.

Traditional ecology has made a great progress in understanding various kinds of interactions, for example between preys and predators, but it has been less successful at elucidating the multiple connections between populations, communities, and ecosystems and at predicting the patterns and dynamics generated by these interactions. An accurate understanding of life and its role in the Earth system requires a holistic systems biology approach that couples different aggregation levels while considering the individual components that affect these biological systems from cells to communities and ecosystems.

The trait-based perspective to the ecology of phytoplankton

In recent years, there has been a renewed interest in the study of ecological systems from a trait-based perspective. Traits are observable properties of organisms, usually measured at the individual level, that have an impact on the fitness of the organisms. Examples of traits are basal metabolic rate, body size, and photosynthetic rate. If adopted comparatively across species, trait-based approaches offer the possibility to explore questions at different levels of biological organization

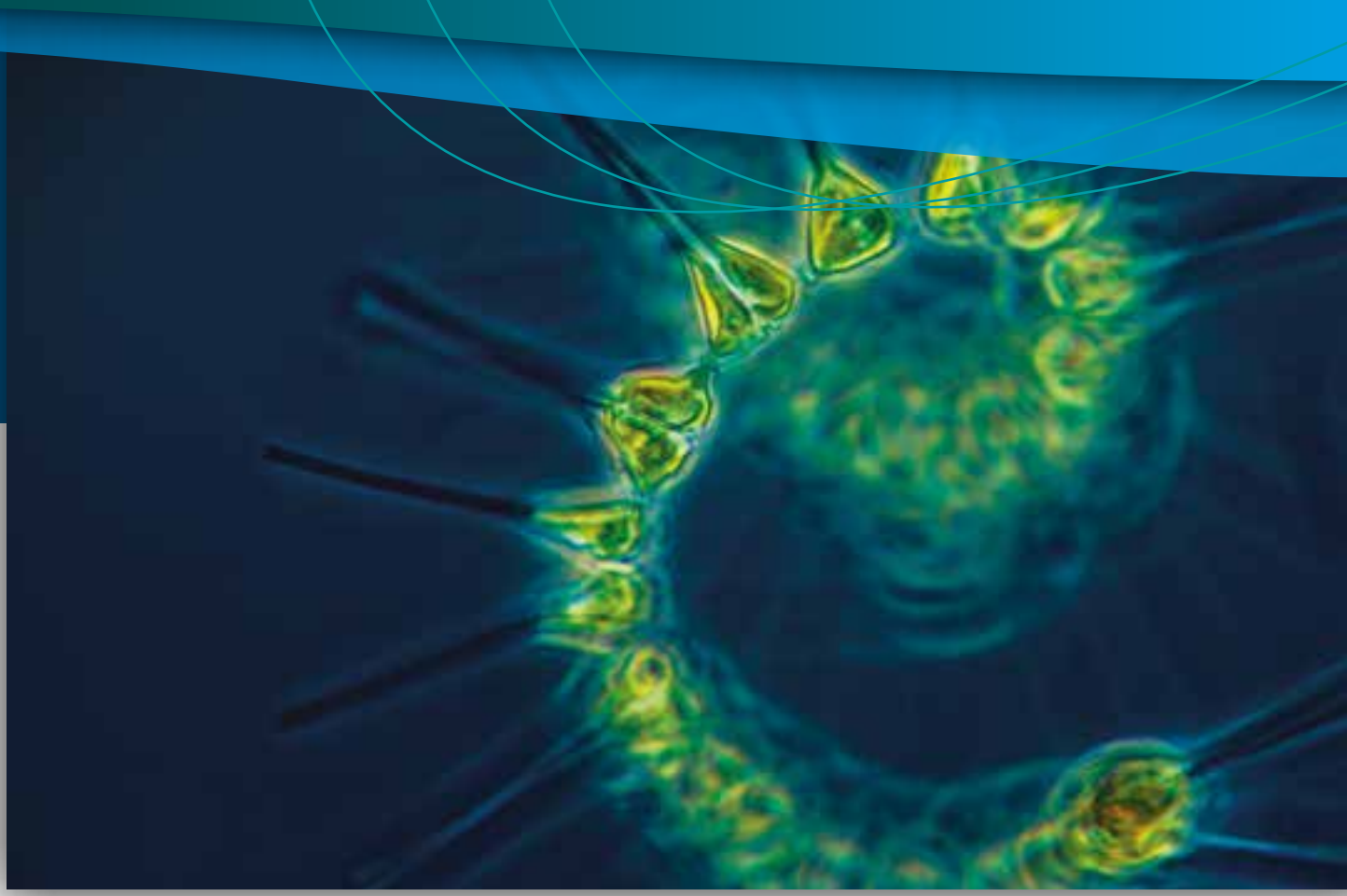

Figure 1: Phytoplankton, microscopic unicellular plants at the base of aquatic foodwebs (Source: National Oceanic and Atmospheric Administration, MESA Project).

and at the intersection between ecology, biogeography, and evolution. Trait-based approaches constitute a stimulating conceptual framework also for predicting species distributions, assessing community composition, and determining biodiversity-ecosystem function relationships.

For example, it is well known that different regions of the oceans are characterized by contrasting phytoplankton community compositions in terms of cell size, a key trait that affects virtually every aspect of phytoplankton biology. Differences in phytoplankton size structures are associated with differences in the efficiency of carbon burial into ocean sediments, because communities dominated by large cells lead to a more effective sedimentation of organic matter than communities dominated by small cells. It is also understood that biodiversity increases from the poles to the tropics, a phenomenon often referred to as "latitudinal diversity gradient". Explaining the latitudinal diversity gradient has been and still is one of the great challenges of ecology. This increasing diversity when moving towards the tropics has been proposed also for phytoplankton. However, much to our surprise, we recently found that phytoplankton size diversity (which is only one component of biodiversity, albeit an important one) peaks in temperate regions and not in the tropics.

Insights from mathematical modeling

By adopting a mathematical model that describes the phytoplankton community by means of a relevant trait (in our case cell size) and that allows for the integration of environmental and biological data, we could predict changes in phytoplankton community properties, namely total biomass, mean trait, and trait variance (representing the trait diversity of the community), along broad environmental gradients and over a centennial time scale (Acevedo-Trejos *et al.*, 2015).

In contrast to the typical "latitudinal diversity gradient" paradigm, our model results suggested that phytoplankton communities of the tropics are on average less size diverse than those of temperate regions (Figure 2). This implies that the adaptive capacity of phytoplankton, i.e. the capacity to reorganize under

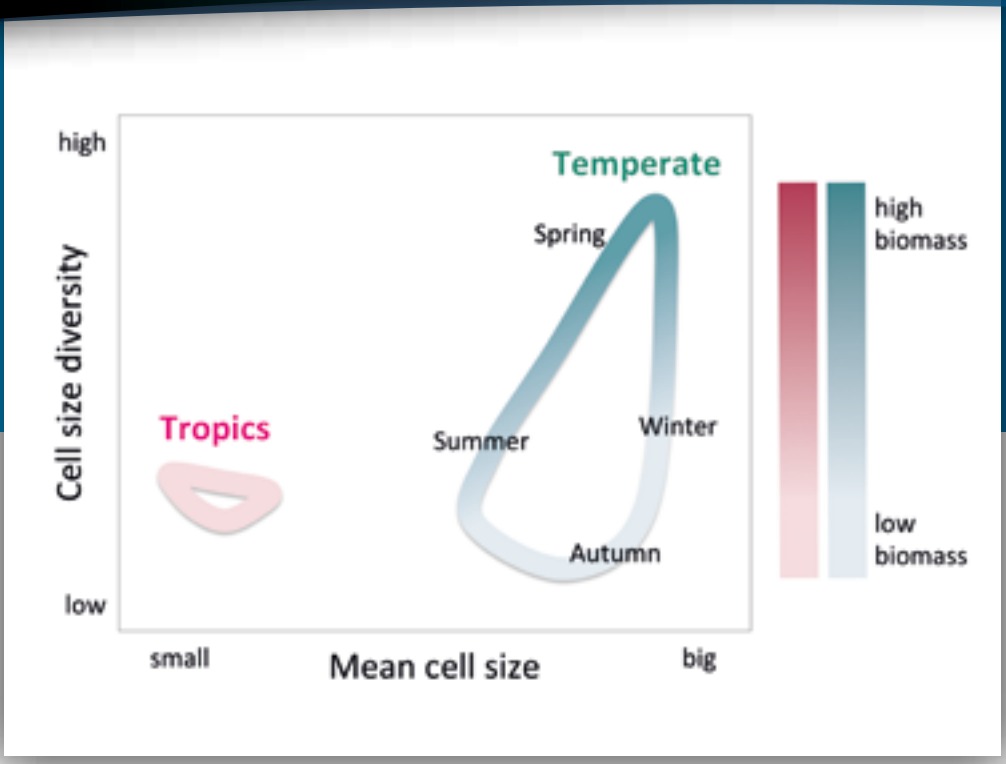

Figure 2: Interrelationships among phytoplankton community properties (total biomass, mean cell size, and size diversity) in two environmentally contrasting regions of the Atlantic Ocean. Results are from model simulations (Source: Acevedo-Trejos *et al.*, 2015).

changing environmental conditions via shifts in cell size composition, is smaller in the tropics than at higher latitudes. It turns out that the biogeography of phytoplankton size structure can be explained by biophysical principles of nutrient diffusion through boundary layers. Small cells, thus, have a competitive advantage with respect to large cells in tropical waters where nutrients are scarce because the effects of nutrient diffusion limitations are reduced in small cells.

In addition, our model predicted that phytoplankton communities of the future will be on average less productive, smaller in mean size, and less size diverse than modern ones, especially in regions characterized by a pronounced seasonality (Acevedo-Trejos *et al.*, 2014). This is a consequence of global warming, which is expected to change the stratification of the oceans and hence the amount of inorganic nutrients that mixing processes will make available to phytoplankton (Figure 3). However, in polar waters (which are characterized by strongest seasonality) ocean warming and freshening (as a consequence of ice melting) is likely to stimulate productivity by decreasing light limitation, thus causing opposite changes, i. e. increase in cell size and perhaps also increase in size diversity.

Upscaling molecular systems biology to the ecosystem level

Decades of research have revealed the important role of these tiny fascinating organisms in the Earth system. Yet, many questions remain unanswered. There is a pressing need, for example, to understand the physiological and metabolic processes that underpin phytoplankton responses to environmental change. How are the genes involved in specific metabolic processes regulated? How will they respond to environmental change? What will be the effects on the whole community?

Recent advances in molecular systems biology are creating the conceptual and technical ground for answering these fundamental questions. The advent of various high-throughput techniques (metagenomics, metatranscriptomics and meta-metabolomics) provides molecular systems biology with tools for understanding and describing the complex set of molecular processes and interactions that contribute to the functioning of ecosystems (Raes and Bork, 2008). Phytoplankton constitute an ideal model system, and an important one given its relevance to the Earth system, for engaging with these challenges.

Acknowledgments

I am grateful to Emilio Marañon, whose insightful comments and suggestions on an early version of this manuscript helped improve the clarity of the text.

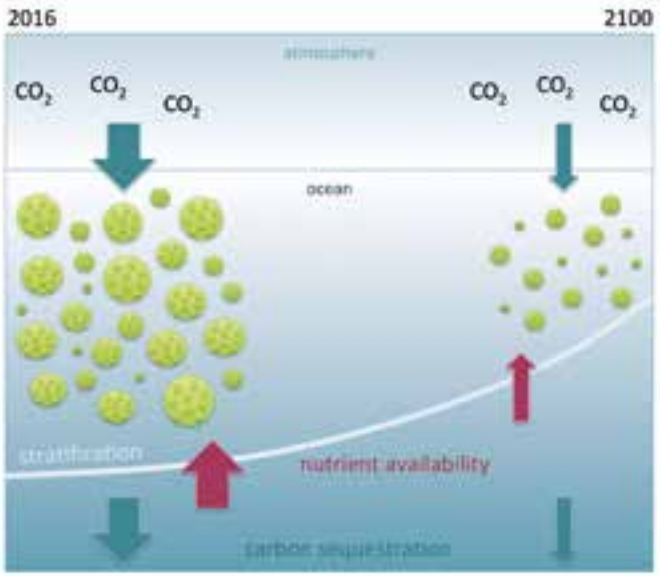

Figure 3: A glimpse into the future composition of phytoplankton communities. In the future, lower nutrient availability in the surface of the oceans (as a result of global warming and enhanced stratification) will lead to phytoplankton communities characterized by less biomass, smaller mean size, and lower size diversity, causing less carbon being permanently sequestered into the ocean sediments. Results are from model simulations (Acevedo-Trejos *et al*., 2014, Source: Agostino Merico/ZMT Bremen).

Research project profile:

The mathematical modeling results presented here are part of a research project entitled "The adaptive capacity of multitrophic plankton communities in a changing ocean" and financed by DFG via the Priority Program "Flexibility matters: Interplay between trait diversity and ecological dynamics using aquatic communities as model systems (DynaTrait)". This research has been conducted at the Leibniz Center for Tropical Marine Ecology. Principal Investigators are Prof. Dr. Agostino Merico and Dr. Esteban Acevedo-Trejos.

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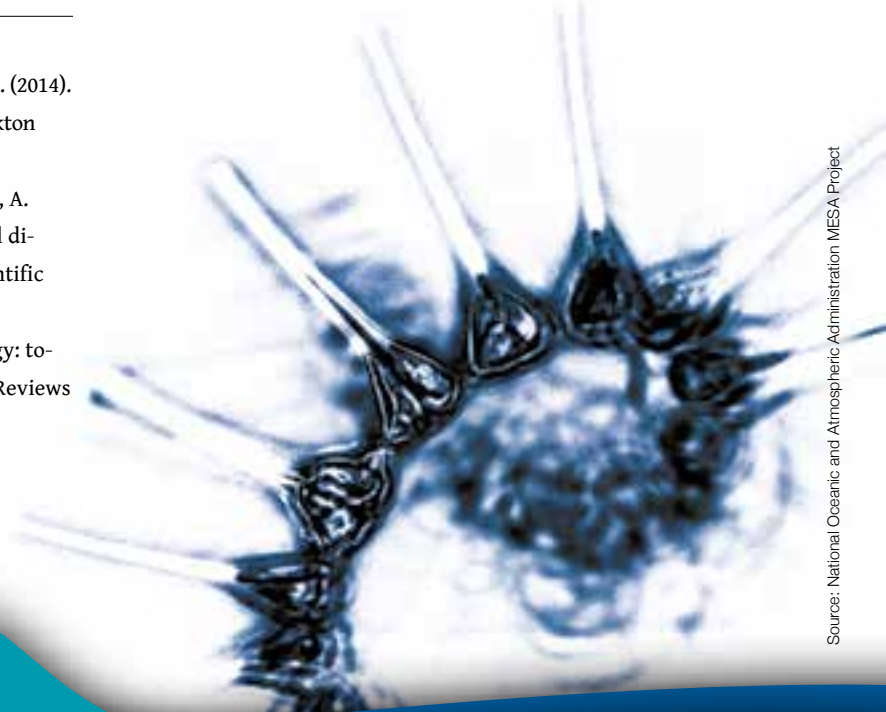
Photo: Prof. Dr. Agostino Merico



Source: National Oceanic and Atmospheric Administration MESA Project

systems biology of the marine *roseobacter* group

The emergence of systems biology in marine research

by Ralf Rabus, Susanne Engelmann, Dieter Jahn, Jörn Petersen, Dietmar Schomburg, Stefan Schulz and Irene Wagner-Döbler

The seas and oceans cover 70 % of our planet's surface and, with approx. 10^{30} of cells, they contain about as many microorganisms as are present in the soil of all of the Earth's landmasses combined. In the light-filled upper layers of the ocean's waters, cyanobacteria and algae are responsible for a significant proportion of the planet's primary production processes. In global terms, the oceans produce 70 % of O_2 and absorb 50 % of CO_2. Conversely, the microbial mineralisation of waterborne biomass to form CO_2 plays a vital role in maintaining global materials cycles, in particular with regard to carbon. This key task falls to bacterioplankton, such as organisms from the *Roseobacter* group.

Members of this genus are present around the world and can account for up to 25 % of all marine bacteria in key ecological regions, such as the Antarctic Ocean. They display an exceptionally wide range of metabolic processes, from aerobic anoxygenic photosynthesis to the synthesis of bioactive secondary metabolites. They are also at home in practically every marine habitat, from the water's surface to deep-sea sediments, from the tropics to the Arctic, living free as bacterioplankton or in close relationships with algae, corals, sponges, crustaceans and fish larvae. Funded by the German Research Foundation (DFG), the TRR 51 cross-regional collaborative research centre studies the ecology, physiology and molecular biology of the *Roseobacter* clade, in order to develop a systems biology understanding of this globally important clade of marine bacteria.

Two members of the clade were selected for systems biology research: the photoheterotropic, facultatively anaerobic and algae-associated *Dinoroseobacter shibae* DSM 16493_T, and the versatile heterotrophic, biofilm-forming *Phaeobacter inhibens* DSM 17395 (Figure 2), which produces secondary metabolites. Their genomes have been fully sequenced, they grow in a reliable and reproducible manner, are genetically accessible and serve as ideal examples of the physiological variety present in the group. The objective of the systems biology approach is to use both of these model organisms to obtain molecular-mechanistic insights into the remarkable ecological success of *Roseobacter*. Reproducing the diversity, dynamic nature and complexity of marine habitats in the lab requires not just state-of-the-art systems biology but also a broad spectrum of specific experimental approaches and methods.

Skilled in the art of surviving

Aerobic anoxygenic photosynthesis is widespread in the *Roseobacter* group. Light is used as an additional source of energy, something that gives *D. shibae* an advantage over purely heterotrophic marine bacteria when it comes to survival. This is especially true when nutrition is scarce, which is a typical situation among bacterioplankton. Studies using continuous cultures subjected to a daily change from light to dark conditions displayed a pronounced temporal dynamic in the cell response to light at the transcriptome level. The energy obtained during photosynthesis must come at the price of higher stress from singlet oxygen, which requires a finely tuned regulation of expression for a host of different cellular models [1]. Computer-based metabolic models document the complex response of *D. shibae* to the availability of light.

Cell-to-cell communication via autoinducers (quorum sensing, or QS) is an important mechanism that *Roseobacter* members use to adapt to their given surroundings. *D. shibae* possesses a particularly complex QS system composed of three synthases that generate a large number of autoinducers (some of which are being studied for the first time), along with five response regulators. The gene-regulating QS network is hierarchically organised and defines the phenotypic heterogeneity of the population.

Figure 1: On our way to developing systems biology for the world's oceans with the new research ship, RV Sonne (Source: Dr. Thomas Badewien, ICBM, University of Oldenburg).

If it is deactivated, the expression of flagella genes is reduced, for example, and the variety of the population's cell division mechanisms is lost. Interestingly, *D. shibae* not only reacts to its own signals, but also to a wide range of QS signals, with a broad signal spectrum being typical of the organisms in the *Roseobacter* group [2].

The interaction between *D. shibae* and the dinoflagellate *Prorocentrum minimum* transitions from a symbiotic phase, when the bacteria provide the algae with vitamins, to a pathogenic phase that sees the bacteria kill the algae. The 191 kb plasmid of *D. shibae* is responsible for this [2], as strains without this "killer" plasmid are unable to kill the dinoflagellate.

Being capable of facultatively anaerobic growth can also be beneficial to marine bacteria in a range of habitats, such as on suspended particles, what is also known as "marine snow", on the surface of microalgae at night, in O_2-free sediment layers and in the low-O_2 zones of the open ocean (pelagic) that are expanding dramatically due to the mounting oversupply of nutrients (eutrophication) in the seas. The systems biology approach to the genome-wide transposon mutagenesis (formation of mutants due to gene relocation) showed that, along with the known denitrification genes, there is a whole series of other genes that are essential for the growth of *D. shibae* in nitrate-reducing conditions. The transition from oxic to anoxic conditions therefore requires comprehensive regulatory and metabolic adaptations [3]. The adaptation to oxidative stress is particularly important in consideration of "poisoning" by bacteriochlorophyll in light (Engelmann and colleagues, unpublished). *D. shibae* protects itself from stress due to high salt levels (5 %) by forming a range of compatible solutes, such as α-glucosylglycerol, for example, which has been proven to exist in alphaproteobacteria [4].

The all-rounder

A key characteristic of *P. inhibens* is its high substrate versatility. This property is relevant for converting organic material, which can be present in seawater in greater quantities on a regular basis during the course of the year, such as following the collapse of algal blooms. As a result, one initial focus of research into this model organism was to study its metabolic network and how this is adapted to a range of nutrient conditions by applying a combination of physiology, proteomics and metabolomics. During growth with complex media, *P. inhibens* was shown to simultaneously absorb and convert a wide variety of nutrients, such as phospholipids, amino acids and carbohydrates. The metabolic network for breaking down amino acids consists of a series of reaction stages that do not correspond to those of standard bacteria and that required extensive reannotation. Overall, this decomposition network was revealed as archetypical of the entire *Roseobacter* group [5]. Like most members of the *Roseobacter* group, *P. inhibens* uses the Entner-Doudoroff pathway to process carbohydrates.

The cell envelope and the proteins anchored in it play a major role in the organism's interaction with its environment. By studying the subproteomes in the different cell envelope fractions, the research scientists were able to reconstruct the various compartments in detail, particularly with regard to the secretion and sorting of proteins, the direct export of effector molecules (e.g. proteinogenic exotoxins and antibiotic secondary metabolites) and cell envelope biogenesis [6]. As with metabolic reconstruction, differential proteinogenomics have revealed or redefined the functions of a large number of genes, which means that it will be necessary to reannotate the *P. inhibens* genome (along with the majority of the *Roseobacter* group's genomes).

Nitrogen, particularly in the form of ammonium, and phosphate are the most important macroelements for bacterial (as with algae) activity and growth in the oceans. Recent studies have shown that *P. inhibens* deploys a new and remarkable strategy to obtain and defend external ammonium for it and its progeny. In addition, the elementary stoichiometry of *P. inhibens* cells diverges significantly (N:P = »16) from the canonical Redfield ratio, which illustrates the carbon, nitrogen and phosphorus (C:N:P = 106:16:1) composition of living and dead matter in the world's oceans (Trautwein and Rabus, unpublished).

Plasmids and secondary metabolites as common characteristics

The presence of plasmids is typical of the *Roseobacter* group, and up to 11 extrachromosomal elements (ECRs) have been discovered in certain members (Figure 2). Along with its own chromosome, *P. inhibens* possesses further three ECRs. The 65 kb plasmid is necessary for biofilm formation [7], the catalase coded in the 78 kb plasmid provides protection from oxidative stress (Michael and Petersen, unpublished), and the 262 kb plasmid features material such as the gene for the biosynthesis pathway of antibiotic tropodithietic acid. The presence of these three plasmids goes hand in hand with high energy requirements and consequently reduces the growth of *P. inhibens* [8]. Studying the molecular basis for this interesting interplay between the genomic structure and growth physiology is the subject of current systems biology research.

D. shibae possesses five plasmids in addition to its chromosome. One of these is particularly important for the organism's survival when nutrients are scarce [9]. The successful transfer of both syntenic sister plasmids from *D. shibae* to *P. inhibens* by means of conjugation demonstrates for the first time inter-genus horizontal gene transfer within the *Roseobacter* group and illustrates an evolutionary mechanism for its ecological adaptability [2].

Another competitive advantage of algae-associated *Roseobacter* members comes from the formation of a large number of secreted metabolites that have antibiotic, probiotic or communicative functions and effects. Some of these offer potential for the biotechnology field (e.g. [10]).

Conceptual development and outlook

Initially, the focus of systems biology research into the *Roseobacter* group concentrated on establishing the basics, i.e. reconstructing metabolic and regulatory networks. In the second stage, attention has been turned to mechanisms connected to genome organisation, cell-to-cell communication, phenotypic heterogeneity, interaction with eukaryotic algae and adaptations to changing environmental conditions (oxygen limits, nutrients, light, hunger, oxidative stress). In the years ahead, systems biology research is to focus on the interaction between different organisms, i.e. symbiosis, competition and pathogenicity. This work will see us concentrate on bacterioplankton, the biofilm on the surface of macroalgae and on microalgae's immediate surroundings (phycosphere, the marine equivalent of the rhizosphere), controlled solely by diffusion. This reductionalistically generated molecular-causal understanding will ultimately help us to better interpret metaOMICs field data in terms of function, and it will be particularly important for data gathered during the summer 2016 Pacific expedition on board the new RV SONNE research ship stationed in Wilhelmshaven (Figure 1). A systems biology understanding of the oceans is the ultimate objective. It is an ambitious project, but one that is urgently needed given the threats facing the planet's seas and climate.

Research project profile:

Systems biology research into the *Roseobacter* group takes place within the framework of the TRR 51 cross-regional collaborative research centre ("Ecology, Physiology and Molecular Biology of the *Roseobacter* clade: Towards a Systems Biology Understanding of a Globally Important Clade of Marine Bacteria"), funded by the DFG. The spokespersons for TRR 51 are Prof. Dr. Meinhard Simon (University of Oldenburg) and Prof. Dr. Dieter Jahn (Braunschweig University of Technology). TRR 51 integrates the three fields of ecology and evolution, genetics and physiology, and systems biology in order to develop a comprehensive understanding of the evolutionary and adaptive success of the *Roseobacter* group in the marine ecosystem. The project leaders of the systems biology division are drawn from Braunschweig University of Technology (Prof. Dr. Susanne Engelmann, Prof. Dr. Dieter Jahn, Prof. Dr. Dietmar Schomburg, Prof. Dr. Stefan Schulz), the Helmholtz Centre for Infection Research in Braunschweig (Prof. Dr. Irene Wagner-Döbler), the Leibniz Institute (DSMZ) in Braunschweig (PD Dr. Jörn Petersen) and the University of Oldenburg (Prof. Dr. Ralf Rabus).

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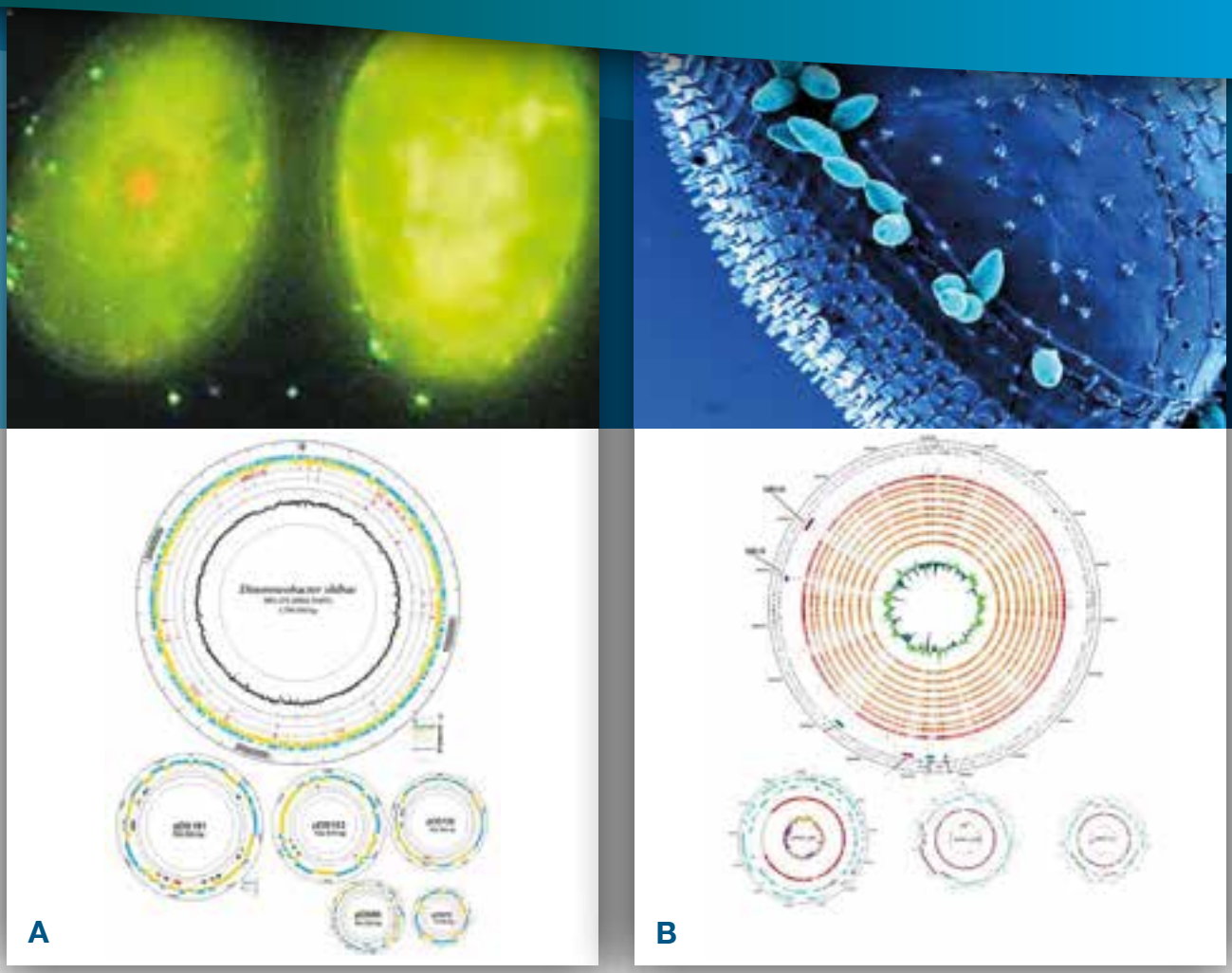

Figure 2: Microscope images and genome (chromosome plus several plasmids) of the two model organisms *Dinoroseobacter shibae* DSM 16493$_T$ (A, in Wagner-Döbler *et al.*, 2010 ISME J 4:61-77) **and *Phaeobacter inhibens* DSM 17395 (B**, in Thole *et al.*, 2012 ISME J 6:2229-2244; image: Manfred Rohde)**, both in association with the dinoflagellate *Prorocentrum minimum*.**

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the future of coral reefs under a global changing environment

Robustness trade-offs in symbiotic organisms

by Roberto Iglesias-Prieto, Mónica Medina and Hiroaki Kitano

Coral reefs are one of the most productive and diverse ecosystems on the planet. These ecosystems provide goods and services to the local populations ranging from food security to coastal zone protection under extreme meteorological events. Moreover, coral reef-derived natural services are critical for the well-being of the human population in excess of 500 million, localized primarily in the developing world.

Paradoxically, these ecosystems have been flourishing in nutrient-deprived shallow waters of tropical oceans for the last 200 million years. The paradoxical nature of coral reefs has been partially resolved after the realization that reef-building corals maintain ecologically obligated endosymbiosis with photosynthetic microalgae. These microalgae, commonly known as zooxanthellae, are dinoflagellates belonging to the genus *Symbiodinium*. Since corals, *Symbiodinium* and the associated microbial community behave as an ecological unit the term holobiont was coined. The translocation of reduced carbon in the form of glycerol or glucose from the algae to the host represents, in some cases, more than 80 % of the total algal production, rendering the intact association (holobiont) autotrophic in terms of carbon. Conversely, the algal partners benefit by using byproducts of the animal metabolism such as ammonia. The nutritional advantages derived from the symbiotic nature of reef-building corals allows them to deposit calcium carbonate at rates that exceed those of erosion, therefore being responsible for the formation and maintenance of the reef structure.

Unfortunately coral reefs are threatened locally from overfishing, pollution and sedimentation, and globally by ocean warming and acidification (Hoegh-Guldberg *et al.*, 2007). Thus there is an urgent need to understand how corals acclimate or adapt to changing environments. The objective of our collaboration is to identify the biological properties that allow reef corals to be robust on geological time scales, enduring 200 million years of environmental variations, while being extremely fragile to the environmental perturbations associated with global change.

Robustness trade-offs

Robustness is a property that allows a system to maintain its functions despite external and internal perturbations (Kitano, 2004). In general, allocating more resources to maintain functionality attains robustness. An interesting feature ubiquitously observed in biological systems is the emergence of intrinsic trade-offs associated with robustness. The most obvious example derived from the increased allocation of resources is the trade-off between robustness and efficiency (Figure 1A). Organisms can evolve to be robust against a range of perturbations, but almost always bring upon themselves extreme fragility against unexpected perturbations (Kitano, 2004). It has also been observed that a general increase in robustness is often achieved at the cost of the system's performance (Kitano, 2004) (Figure 1B).

Harvesting solar radiation needed to energize photosynthesis is one of the most nitrogen demanding processes in all primary producers. The synthesis and maintenance of the protein scaffolding required to properly accommodate the photosynthetic pigments requires a significant allocation of amino acids. As previously mentioned, coral reefs exist in nutrient-poor environments. Under those conditions a key adaptation has been to increase the efficiency with which they collect solar radiation.

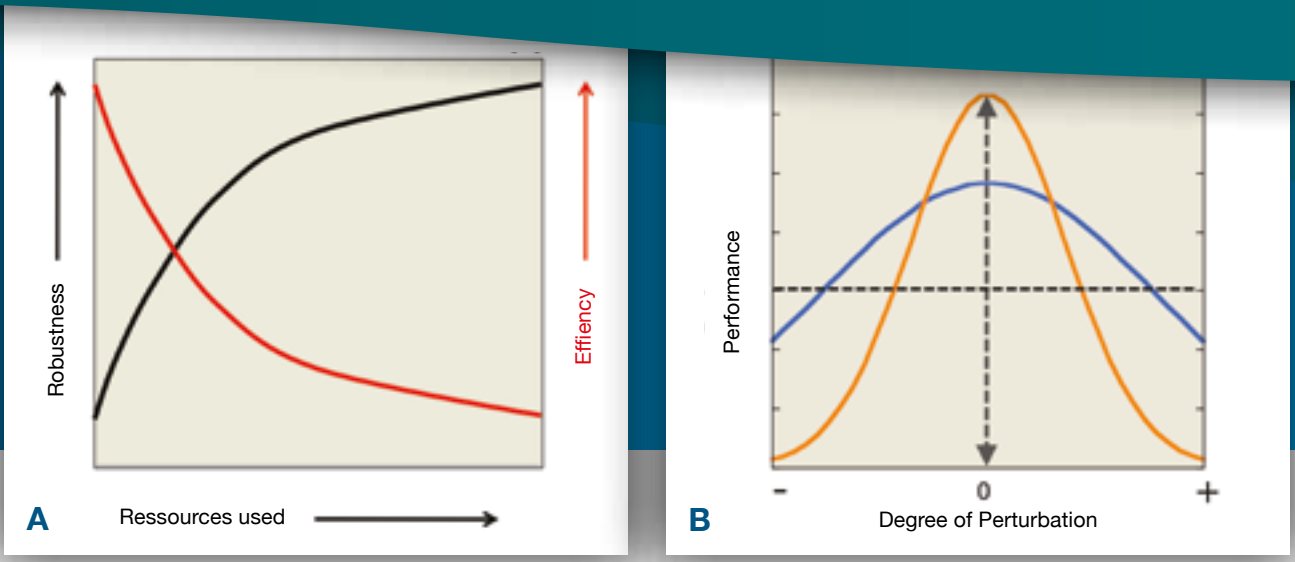

Figure 1: Robustness trade-offs

A Trade-off between robustness and efficiency as a function of the amount of resources allocated to maintain a function.

B Robust systems tend to be able to retain functionality under a wide range of perturbations (blue line; low-performance high-robustness case), while efficient systems exhibit higher performance although they are more fragile (yellow line; high-performance low-robustness case). The broken horizontal line represents the limit of functionality for the system (Source: modified from Kitano, 2007).

Symbiotic corals are the most efficient light harvesters per unit area and chlorophyll α in nature. They are capable of collecting the same amount of solar radiation as their competitors with approximately an order of magnitude less photosynthetic pigments, as well as nitrogen investment. The high efficiency for solar radiation collection achieved by *Symbiodinium* in corals is the result of the multiple scattering of light on the highly reflective calcareous skeleton of the coral (Figure 2A). The scattered radiation increases the probability of absorption of a photon by a photosynthetic pigment. This high efficiency implies an inherent internal fragility when the system is exposed to an environmental perturbation (Figure 2B). Corals experience seasonal variations in their pigment content. Those seasonal responses are controlled by changes in seawater temperature and solar radiation, and the response is correlated to changes in both symbiont cellular pigment concentration and in symbiont densities. In summer, corals achieve their maximal performance by reducing their chlorophyll density, while maximizing their light harvesting efficiency. Under those conditions, a mild increase of 1.5°C above the long-term average sea surface temperature may trigger further reductions in chlorophyll density overwhelming the algal photo-protective capacity. This cascade can ultimately lead to coral bleaching and, if severe enough, a potential collapse of the reef ecosystem (Enríquez *et al.*, 2005) (Figure 3). As a consequence of the seasonal variations in pigment concentration corals are more robust to thermal perturbations in winter than they are in summer months. This inherent fragility must be considered in any future effort to artificially increase the robustness of the coral holobiont to thermal stress by manipulating the algal partner.

There is a large geographical variation in the temperature threshold to trigger coral bleaching. That observation suggests on the one hand a high degree of local adaptation in coral holobionts, while on the other it indicates that current seawater temperature anomalies derived from climate change are outside the range that corals have been exposed to during the process of local adaptation. In this context, we believe that the combined use of genomic, physiologic and systems biology approaches would result in the identification of key traits responsible for local adaptation in coral holobionts and the assessment of their capacity to acclimate or adapt to different future scenarios of climate change.

Possible mechanisms to increase robustness

Biological systems tend to incorporate foreign biologic entities, which allows the organism, in this case the coral, to acquire novel functions as well as robustness by either flexibly reshaping the composition of symbionts or by merging functions through their mutualistic relationship (Kitano and Oda, 2006). Coral larvae can harbor different symbionts, which can provide a broad physiological repertoire to cope with a fluctuating environment. Adult corals, however, harbor less diversity indicating more specificity for certain symbionts. For example, *Orbicella faveolata*, one of the most important reef-building corals in the Caribbean, harbors a limited set of genetically and physiologically unique *Symbiodinium* species. We have developed a suite of genomic and physiological tools that allowed us to examine the response of the coral-*Symbiodinium* partnership at the transcriptome level. Several unexpected patterns of the association have emerged through these studies. Namely, in *O. faveolata* larvae, we found

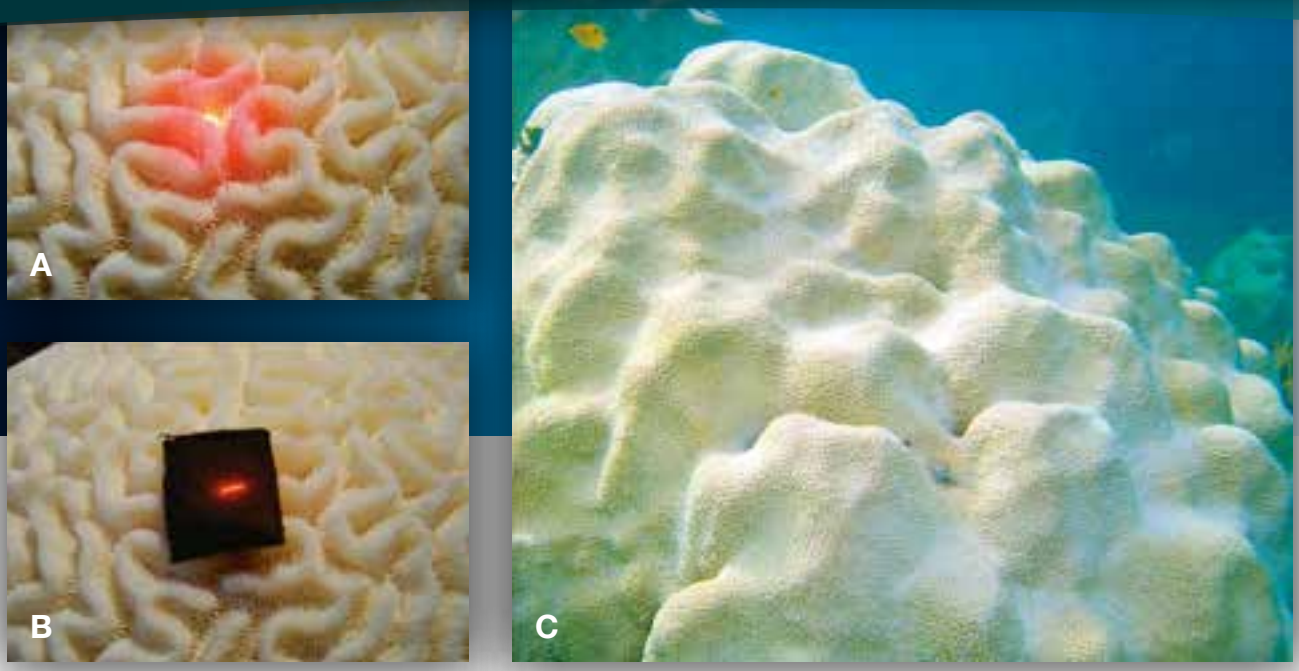

Figure 2: A + B Multiple scattering by a coral skeleton. Comparison of light scattering patterns from a laser pointer directed in **A** at a coral skeleton, with the same laser beam in **B** reaching an opaque plastic target. **C** Naturally bleached *Orbicella faveolata* colony in the Caribbean (Photos: R. Iglesias-Prieto).

that if successfully infected, the early cell recognition signaling induced in the coral host by contact with *Symbiodinium* is similar regardless of the algal genetic identity. Differential gene expression is detected, however, in coral larvae that reject certain algal strains. Thus, our data suggest that successful coral–algal symbioses depend mainly on the symbionts' ability to enter the host in a stealth manner, rather than a more active response from the coral host (Voolstra *et al.*, 2009). In adult corals, when examining the breakdown of symbiosis during coral bleaching we found that the *O. faveolata* transcriptome appears to be more susceptible to algal symbiont type than to thermal stress (DeSalvo *et al.*, 2010).

Increased robustness exemplarily shown with *Symbiodinium* species

Theoretical models predict that for a system to improve its robustness against different stressors, it must **1)** embrace mechanisms to dynamically switch responses to these stressors, **2)** sacrifice performance (e. g., growth rate), or **3)** use both of the above strategies. One of the typical means to dynamically switch a response is to harbor diverse symbionts so that symbiont composition can dynamically change to cope with a given environmental stress (Kitano and Oda, 2006). Recently, we partially tested these models by comparing the performance, determined as their calcification rates, of *O. faveolata* specimens harboring four different *Symbiodinium* species. Interestingly, the most significant result indicates that specimens harboring the thermally tolerant invasive *Symbiodinium trenchii* experienced a 50 % reduction in their calcification rates relative to specimens containing only the three different homologous *Symbiodinium* species (Pettay *et al.*, 2015). This finding confirms that an increase in robustness of the holobiont against thermal stress derived from harboring thermally tolerant *Symbiodinium* results in a reduction of the physiological performance of the holobiont. The employment of similar systems biology approximations will allow us to address several critical questions regarding the future of coral reefs under changing environments. We would like to explore the ecological consequences of changes in the relative dominance of robust low-performance *Symbiodinium* types for individual coral species. We aim to generate explicit models able to predict if changes in the relative abundance of low performance temperature-tolerant holobionts would be able to maintain reef functionality and therefore ecosystem services. The time is also ripe to incorporate the coral microbiome in an effort to determine the potential role of the microbial community for the robustness of the holobiont. This type of research could provide invaluable information regarding the selection of robust phenotypes to increase the success of future coral reef restoration projects.

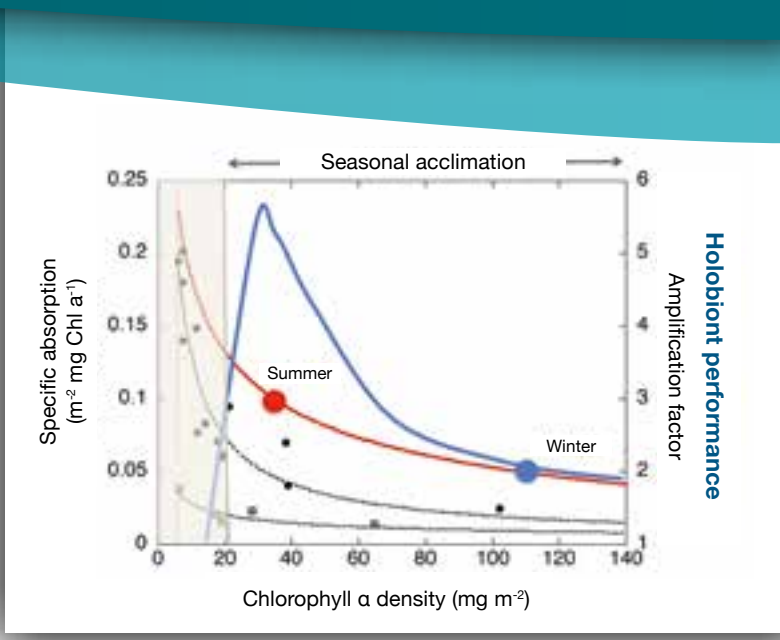

Figure 3: Holobiont efficiency
Seasonal changes in pigment concentration in corals. Black circles represent the specific absorption coefficient of intact coral surfaces. Clear squares indicate the specific absorption coefficients of freshly isolated symbionts as a function of pigment density. Red line represents the enhanced absorption resulting from the multiple scattering of the coral skeleton. Blue line represents the seasonal variation in coral calcification rates. The shaded area indicates limits of seasonal acclimation and the onset of coral bleaching response (Source: modified from Enriquez *et al.*, 2005).

Research project profile:

Title: Robustness trade-offs in coral symbioses

Funding: The Canon Fundation

Participants: Mónica Medina, Roberto Iglesias-Prieto, Hiroaki Kitano

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selecting for the best possible rapeseed

How using system biology models improves plant breeding

by Amine Abbadi and Gunhild Leckband

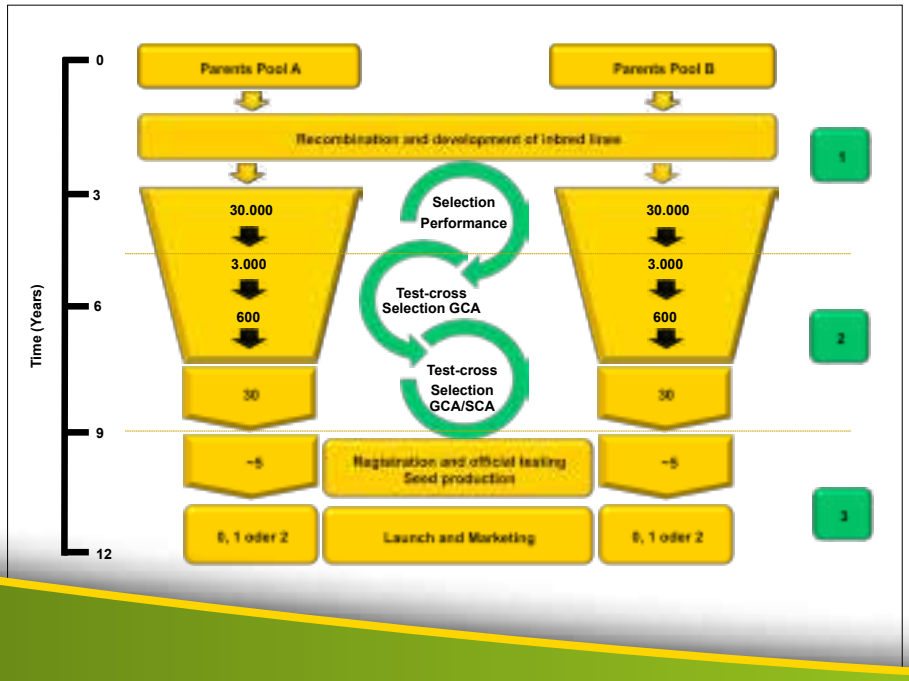
Plant breeding is an activity which has the characteristics of a type of "scientific art", as it combines scientific methods with a sense of what the requirements of the future may be. Together, identifying the genetic make-up of individual features, masked by varying environmental influences, and their wide-ranging beneficial combinations generate the "total work of art": new, high-performance plant varieties. Plant breeders have to make decisions regarding long-term developments at short notice and usually based on limited data. Systems biology opens new options for making this decision-making process more rational and efficient thanks to the use of a wider range of data and mathematical modeling.

Plant breeding is the starting point for agricultural value creation, and it lays the foundations for healthy nutrition and future energy sources. Rapeseed (*Brassica napus L.*) accounts for 38 million tons of the world's vegetable oil production every year, making it one of the most important sources of plant oil, both in Europe and internationally. However, in light of more stringent sustainability considerations and future climate changes that are almost impossible to predict, it is important to achieve continuous breeding progress in rapeseed with regard to yield and yield stability even in changing environmental conditions. At present, only hybrid varieties guarantee this.

Heterosis: the key to increasing yield

A hybrid is the result of cross-breeding two genetically different homozygous (purebred) parent plants, and it is distinguished by greater vigor than its parents. This leads to higher and more secure yields, and it is the outcome of the genetic phenomenon of **heterosis**. In plant breeding, heterosis is the effect by which **hybrid plants can be used to achieve considerably improved performance or higher yields than it is possible with the plant's parents.** The reasons behind this phenomenon could lie

Figure 1: Systematic chart showing the development of a hybrid rapeseed variety



Breeding a marketable hybrid variety of rapeseed normally takes 10 to 12 years and consists of three steps: (1) creating and selecting inbred lines; (2) test crosses for identifying general (GCA) and specific (SCA) combination abilities; (3) registering the candidates, subjecting them to officially approved tests and using managed pollination for seed production and launch of hybrids to the market (Source: NPZ Innovation GmbH).

Aerial view of a rapeseed breeding nursery (Source: NPZ Innovation GmbH).

with the additive effect of genes, the combination of dominant genes, and in the positive interaction between genes.

The breeding process to develop a marketable hybrid rapeseed variety generally takes ten to twelve years (Figure 1) and consists of three essential stages: (1) creating and selecting genetically diverse inbred lines; (2) generation of test crosses to identify the features of the hybrids and their parents; (3) using managed pollination to create the hybrids for the market. Due to the high number of available lines within a breeding program, it is, in practice, only possible to use a relatively small selection of the possible crosses between the inbred lines for field testing. Researchers therefore aim to establish heterotic groups ("pools") that contain a range of diverse, but mutually complimentary inbred lines. The molecular mechanisms behind heterosis and its expression under different environmental conditions are unknown, and despite its high agronomic and economic value, its prediction for improved efficiency of crop breeding still remains an elusive goal. As a result, selecting a hybrid's parents is a largely empirical process that can only use small sets of experimental hybrids and test crosses. On the one hand, this prevents the use of a broad range of genetic resources and, on the other hand, it is a major factor contributing to high development costs. Creating predictive models can substantially reduce the length of this drawn-out and therefore expensive development period. Mathematical modeling makes it possible to replace the "best guess" designs currently in use with focused, experimental designs. This gives breeders an opportunity to predict, even early in the breeding process, which hybrids might be able to generate the highest yield in the field.

Systems biology of heterosis: developing predictive models

The research consortium PROGReSs – "Systems biology approaches to predicting and modeling hybrid performance and yield increase in rapeseed" constitutes a nation-wide effort of sufficient scale and expertise to study heterosis and combine iterative modeling, based on the *ad hoc* production of new quantitative data, with the relevant aspects of genetic, developmental and environmental responses – a systems biology approach. To model genotype-phenotype-environment relations, large scale quantitative data of different complexity levels generated in a three-dimensional experimental design (genome/phenome/environment) are combined in iterative processes for modeling and validating a range of algorithms and interlinked accordingly (Figure 2). During these processes, high-performance mathematical models are validated using newly generated data, and they are checked for their suitability regarding three issues: breaking heterosis down into its causative genetic and molecular mechanisms; allowing predictions regarding hybrid performance and yield; and finally enabling the early selection (picking the best candidates) of promising parent genotypes during the breeding process.

Molecular models

It is widely accepted that the heterosis effect is based on the contributions of numerous genetic factors involving, at least to some extent, the combined action of heterozygous alleles. Initial models for predicting hybrid performance on the basis of single nucleotide polymorphism (SNP) profiles and phenotypes are available for rapeseed. By applying models that seem suitable, it is possible to make predictions about the characteristics of crossing parents based on certain sections of their genetic material, known as molecular markers, which are inherited together with important agronomical traits. However, these predictive methods remain limited in scope, as they cannot explain additive and dominant effects at the same time (Jan *et al.*, 2016). The considerable limitations of predictive methods based purely on molecular markers are probably due to the significant influence of epistatic effects, which can explain genes' interaction in collectively regulated heterosis-relevant gene networks. These insights have given rise to alternative approaches that incorporate gene expression profiles in order to decode this interaction between genes. Initial

results indicate that information about gene expression in the early stages of plant development provides valuable insights into the later expression of yield-relevant characteristics in field tests. Short RNAs (sRNA) are another quantifiable factor, which offer a lot of potential for representing the genetic make-up of complex genomes. They are directly connected to important regulatory mechanisms of the genome, such as the level of DNA methylation and the targeted alteration of epigenetic status. A link to hybrid effects can also be assumed from the considerable differences in the DNA methylation levels of hybrids and their parent lines (Zhao *et al.*, 2012). This illustrates the relevance and impact of these factors with regard to network modeling for heterosis in general and for the hybrid performance in particular. It is highly likely that a network model for predicting heterosis that incorporates DNA sequence variations, mRNA expression differences, DNA methylation and the impact of small regulating RNAs could deliver predictions for hybrid performance and associated epistatic effects for a large number of potential hybrid combinations.

Phenotype/genotype interaction models

Breeding hybrids requires the handling of a large number of genotypes that are mass-phenotyped in yield field trials at different locations and over several years. However, compared to other crop plants, relatively little research has been performed on the processes affecting the yield development of rapeseed due to the lack of objective field-based phenotyping. In recent years, different phenotyping methods have been developed for performing non-invasive analysis of plant morphology, growth dynamics and physiological conditions. For example, non-destructive techniques for identifying growth characteristics have made it possible to obtain new insights into the dynamics of root systems and the adaptability of plants to changes in spatial and temporal environmental conditions (Fiorani and Schurr, 2013). Miniaturized and cheaper sensors allow remote sensing measurement processes on a field-study scale and in high-throughput procedures. This way, it is possible to ascertain a number of biophysical and agronomic parameters directly using spectroscopic methods. While there are now a large number of highly diverse indices, these exploit the potential offered by spectral information only to a limited degree. New methods from the fields of chemometrics and machine learning have therefore been developed to cover the entire spectrum for the purpose of performing analyses. Using the whole spectrum for analysis yields more stable prediction power of vegetation parameters, resulting in models that are better transferable to other environments and perform much better than those using only vegetation indices.

Environment/genotype interaction models

Measuring environmental parameters (soil and weather) makes it possible to study the influence of these factors on the development of different genotypes at different locations. New geoelectric measurement techniques even enable us to gather information about soil characteristics layer by layer. Just as with the measurements taken by plant sensors, it is possible to use geographic information systems to assign geoelectric sensor measurements to a single, specific plot of soil. Soil moisture sensors and mobile weather stations can provide additional data on the influence exerted by the soil, the water available to plants and precipitation, changes in soil and air temperatures, and global radiation on the growth of different genotypes. A number of mathematical models can be used for quantifying environmental influences on the development, biomass formation and yield of rapeseed (Hammer *et al.*, 2010). When combined with genetic data, they can help us achieve a better understanding of genotype-environment interaction.

Integrative modeling: the systems biology of heterosis

Integrative modeling requires the models obtained from the three-dimensional levels (genome/phenome/environment) to be combined within a single model. Iterative processes are currently being developed to establish a direct connection between the physiologically oriented growth models and molecular biological data (Gu, 2013), and initial approaches are now under review. For example, it has been demonstrated that the use of one model within the context of plant breeding results in the substantial improvement of selected characteristics by deploying molecular, marker-based estimates of additive allele effects as the input data for these models. It is possible to use models of this nature in two different ways for characterizing gene-phenotype relationships. The first way breaks the model view of phenotypical expressions at the plant level down to a molecular biology scale in order to look for genetic explanations for phenotypical phenomena (“top down”). The second involves the integration of molecular biological knowledge, thereby the model can be used for quantifying the genetic architecture’s influence on the generation of characteristics at the phenotype level (“bottom up”). These methods are currently being reviewed in order to connect the complex relationships within the gene-phenotype system with the aid of a solid understanding of physiology and molecular biology studies. Hence, based on the abovementioned experience, our expectation is that an implementation of larger biological datasets, encompassing multiple biological dimensions spanning the genome, the mRNA and sRNA transcriptomes, the epigenome, the developmental phenome and also the influence of the environment, is likely to considerably improve the power of prediction models.

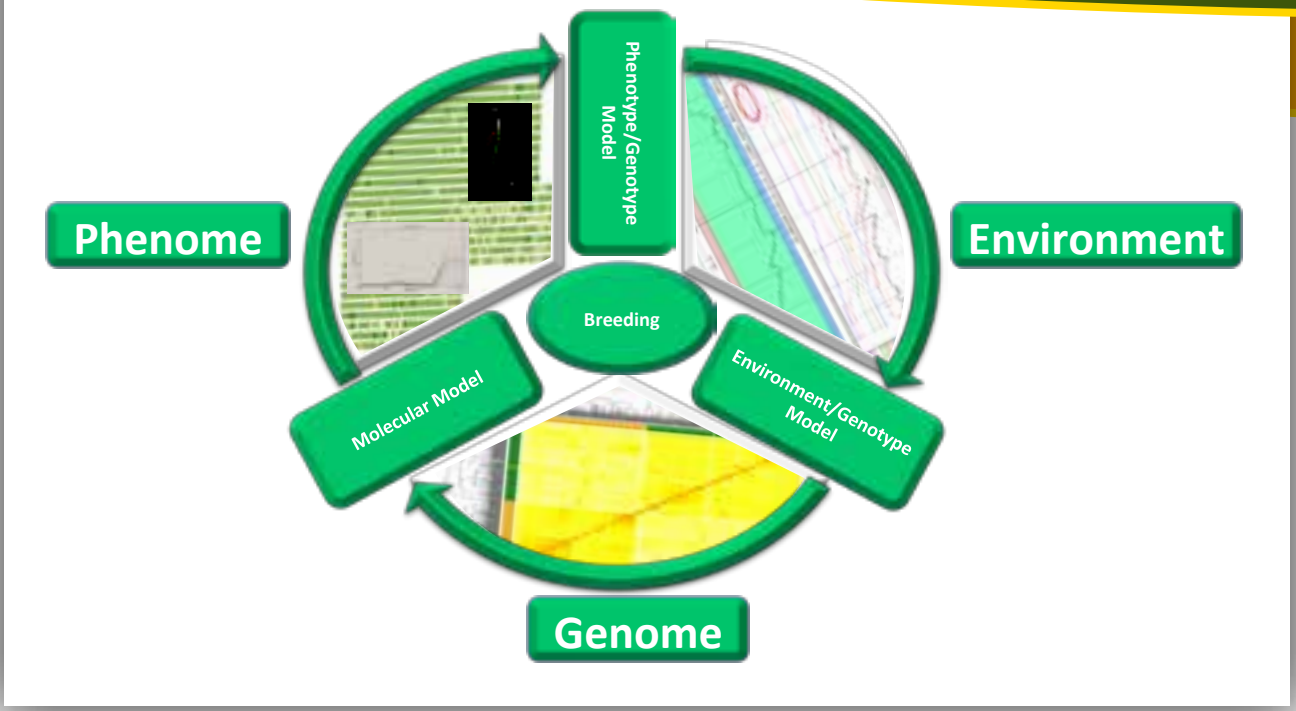


Figure 2: Using genome, phenome and environmental data for modeling as part of the breeding process (Source: NPZ Innovation GmbH).

Every year, plant breeders have just a short window of opportunity and data that is frequently limited in nature when they make decisions that can shape product development for the next ten years. Systems biology opens up new opportunities for simplifying the decision-making process for plant breeders in a more efficient manner by underpinning it with rational foundations and incorporating mathematical modeling. Modeling thereby plays a key role in increasing our knowledge on the influence of genetic and environmental factors on yield physiology in natural cultivation conditions, and it can be deployed in a targeted manner to support the selection process when breeding hybrid plants. We believe that the development of validated, stable predictive models will result in considerably faster progress in the breeding process.

Research project profile:

The German research ministry provides funding for the PROGReSs project as part of its e:Bio (innovation competition) program. Plant breeders, biologists, agronomists, bioinformatics specialists, mathematicians, modelers and technology developers from four SME companies and six research institutes are working within an interdisciplinary network to develop predictive models on genotype-phenotype-environment relationships and for prediction of heterosis. The predictive models are to find direct application in the breeding of high-yield hybrid crops.

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Photos: NPZ Innovation GmbH

from mice to humans

Computer models in clinical translation

by Ute Hofmann, Christoph Thiel, Ahmed Ghallab, Rolf Gebhardt, Jan G. Hengstler and Lars Kuepfer

The transition from the animal model to the patient represents a major challenge when developing new drugs as the transfer of experimental data and results from lab animals to humans is possible only to a limited degree. A comparative study between mice and humans has now been able to assess the benefit of pharmacokinetic computer models in cross-species translation. The computer models, which describe the distribution of an active ingredient within the body, were systemically compared for different substances. The study demonstrated the options offered by model-based extrapolation in pharmacokinetics, but also revealed the need for new conceptual approaches in the field of clinical translation.

At present, a new drug requires a development process of around ten years before it is ready for the market, and prices can rise to more than €1 billion. The early assessment of the therapeutic potential of a new product is therefore of tremendous interest, as this would make it possible to minimise financial losses if the development process ends ahead of schedule. Such an assessment needs to demonstrate the new substance's therapeutic effectiveness and at the same time consider safety-related aspects regarding potential side effects for patients. The individual phases of preclinical and clinical research play a particularly important role in providing ongoing support during the decision-making process (Figure 1).

In the field of pharmaceutical development, the transition from the preclinical phase to clinical phases I–III is a step of crucial importance as this is the point at which a new substance is tested on humans for the first time. On average, the development process for 37 % of active ingredients as potential drug candidates is terminated in the preclinical stage, but this figure rapidly increases to 52 % during clinical phase I (Cook *et al.*, 2014). Here, the identification of toxic side effects is the main reason (over 60 %) for discontinuing the development process for a new active ingredient (Cook *et al.*, 2014).

Genetic factors of drug metabolization in mice and humans

One reason for the high discontinuation rates during the transition from preclinical to clinical trials is the difficulty in transferring metabolic, pharmacokinetic and toxicological

Figure 1: Phases in the pharmaceutical drug discovery process

The transition from preclinical to clinical development (phase I) is shown in blue (Source: L. Kuepfer).

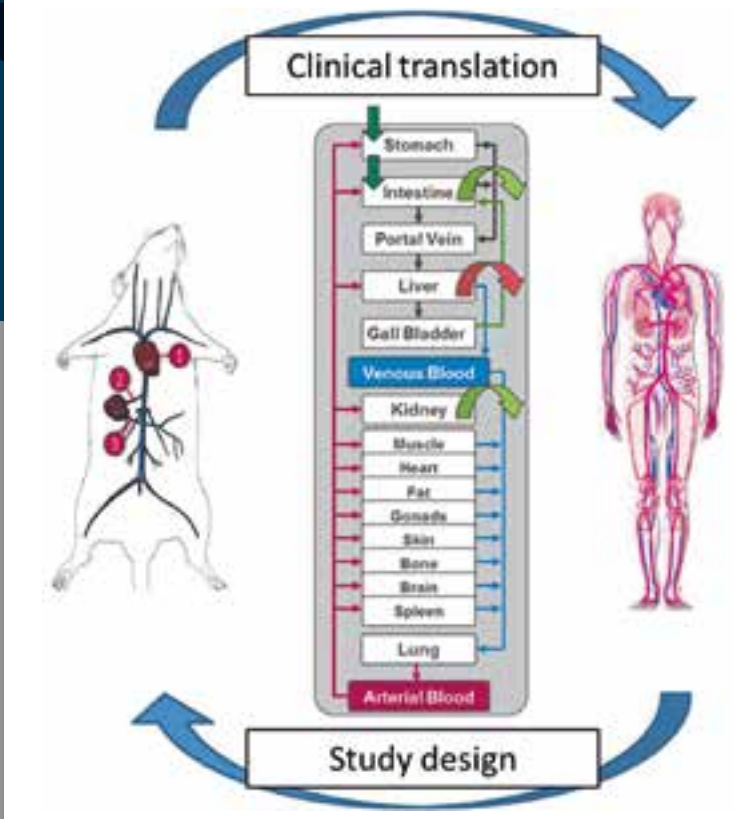

Figure 2: PBPK-based cross-species extrapolation
(Source: A. Ghallab and L. Kuepfer).

data from lab animals to humans. There are significant differences in how species metabolise drugs, and these can be largely traced back to differences in the metabolic enzymes found in animals and humans. In the body, drugs are metabolised in a complex series of reaction steps such as oxidation, hydrolysis and conjugation in order to facilitate their clearance. In the liver, drugs are metabolised and transported along several intermediate steps. Via blood sinusoids, they are exposed to hepatocytes, are absorbed and processed through several metabolic reaction steps and are afterwards excreted back into the blood or into the bile via bile ducts. The cytochrome P450 (CYP) family of enzymes plays a key role in drug clearance. Members of this family are found in all organisms, and they are highly conserved, i. e. large sections of their amino acid sequences have remained essentially unchanged throughout the course of evolution. Nevertheless, there are still noteworthy differences between species. A comparison between the murine and human genomes has revealed around 40 pairs of CYP genes which all descend from the same originator gene. Scientists assume that the metabolic functions of different species' orthologous genes, i. e. genes with the same line of descent, resemble each other. However, a CYP enzyme's substrate specificity can be altered by changing a single amino acid. Due to the variations in amino acid sequences between the species, there can be significant differences in metabolic activity as well as in metabolite patterns. This of course also applies to other enzymes which play a role in drug metabolization. For these reasons, it is often extremely difficult to directly extrapolate drug metabolization from lab animals to humans.

Computer models for cross-species knowledge extrapolation

Researchers from the "Die Virtuelle Leber" ("The Virtual Liver") programme funded by the German Ministry for Education and Research (BMBF) studied the potential benefits of using computer models in conjunction with specific experimental data for the cross-species extrapolation of pharmacokinetic findings from lab animals, such as mice, to humans (Thiel *et al.*, 2015). They assessed **physiology-based pharmacokinetic** (PBPK) models for ten drugs in mice and humans. By adjusting certain parameters, these models were used to predict the pharmacokinetic outcome of a substance for the other species. Generally, PBPK models enable a mechanistic description of the intake, distribution, metabolization and excretion of substances within the body as they map the body's physiology in detail. They explicitly describe organs and quantitatively describe physiological processes such as passive transport and accumulation of a drug within tissues by using generic equations (Figure 2). In this manner, PBPK models permit the simulation of pharmacokinetic profiles within the body and therefore the assessment of a specific organ's exposure to a given substance. It is then possible to deduce key information about the subsequent therapeutic effect, as well as potential toxic consequences.

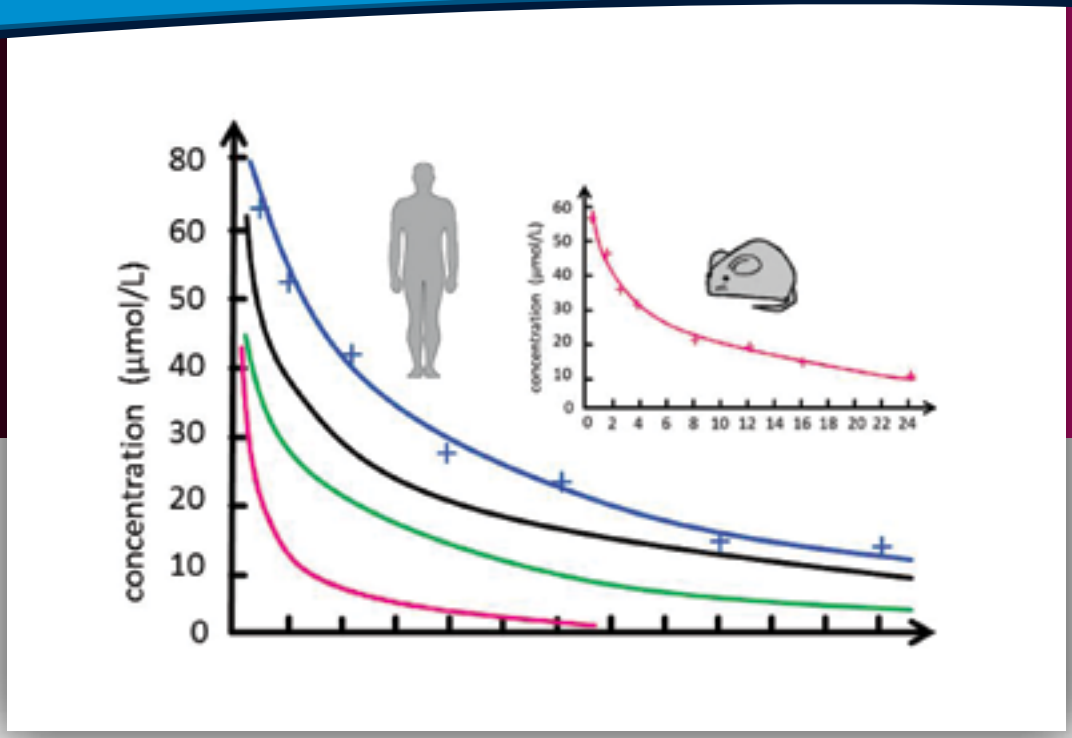

Figure 3: Concept of cross-species extrapolation of pharmacokinetic models. Based on a PBPK model in mice (box on top right, red line and crosses), an initial human model for a dosage level normalised for bodyweight is simulated (red line), which considerably deviates from the experimental data (blue crosses). The successive integration of additional physiological parameters (green and black lines) results in the most favourable situation a human PBPK model (blue line) that largely correlates with the experimental data (Thiel *et al.*, 2015) (Source: C. Thiel and L. Kuepfer).

Using PBPK models, the recently concluded study (Thiel *et al.*, 2015) evaluated the match between simulated and experimentally quantified pharmacokinetic profiles by successive adjustments to physiological model parameters (Figure 3). The study explicitly covered orthologous genes involved in metabolising the drug in question. The comparison of dosage levels normalised to bodyweight showed that the specific physiological factors make an essential contribution to the quality of the prediction. For certain combinations of model parameters, the PBPK simulations also generated good average matches with experimental data. The study was therefore able to show that computer models with specific experimental data can be used for the cross-species extrapolation of knowledge. This finding is of great interest for the pharmaceutical development process and for toxicological questions and received the 2016 Ebert Prize from the American Pharmacists Association (APhA) as the best article in the Journal of Pharmaceutical Sciences. The study also presented additional options for further improving cross-species extrapolation.

Perspectives for complex pharmacokinetic simulations

In recent years, computer simulations have been used for making predictions of therapeutic relevance. For example, the development of spatiotemporal metabolic models for normal and damaged livers has made it possible to predict a new therapeutic strategy that can be used for counteracting elevated blood ammonia levels, a common issue connected to liver conditions (Schliess *et al.*, 2014). Experiments subsequently validated these model predictions (Ghallab *et al.*, 2016), and, due to the application of computer simulations, this step required significantly less work than a purely experiment-based process. In the future, new experimental methods such as imaging techniques will enable an even better analyses of individual physiological processes and their simulation in models. The detailed description of physiological processes will therefore improve the transferability between different species. Nowadays, drug transport and metabolic processes can be visualized using *in vivo* two-photon microscopy and can be quantitatively monitored in organs using fluorescence correlation spectroscopy. This way, we will be able to quantitatively analyse physiological processes within living organisms, understand disease-related changes and visualise them in computer models. A follow-up project within the BMBF-funded LiSyM programme (a research network for systems medicine on the liver), for example, intends to study to which extent drug pharmacokinetics change due to liver illnesses such as steatosis, fibrosis and cirrhosis.

Computer simulations will play an increasingly important role in the pharmaceutical development process. On the one

hand, they can promote the rational structuring of studies to precisely and effectively answer specific questions (Figure 2). On the other hand, the inclusion of research findings in mechanistic computer models facilitates knowledge transfer between the individual development phases and ensures a sustainable application of the results obtained. This approach will enable the continuous evaluation of experimental findings during the development process in pharmaceutical research and increase the chances of success of new candidate medications in human studies.

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non-animal testing methods in toxicology

New approaches for predicting the toxicity of inhaled chemicals

by Jeannette Koschmann, Katherina Sewald and Sylvia E. Escher

Every day, chemical irritants enter our lungs when we breathe. These substances can cause potentially serious illnesses such as cancer or asthma. Until now, large-scale animal testing practices have been used when investigating harmful chemicals and the risks they pose to humans. The results observed in animals serve to identify the type of toxicity and the threshold below which toxic effects do not occur.

Risk evaluation processes are currently undergoing a paradigm shift. The increasing concern with animal welfare is prompting the replacement of animal testing with suitable models based on human cells and computer-assisted processes. Another objective of using models based on human cells and tissue is to generate more accurate predictions regarding toxic effects on people.

ExITox combines computer-assisted models with human pulmonary test systems

Within the scope of the ExITox project funded by the German education and research ministry, we looked at predicting toxicity levels following the inhalation of a chemical substance. It was a challenging undertaking. Our work entailed investigating a range of human cell and tissue-based models. As single human models only cover complex biological processes (i. e. inhalation, distribution, metabolism and excretion) to a limited degree, our intention was to evaluate how far a combination of relevant models would ultimately generate an integrated testing and evaluation strategy. This testing and evaluation strategy, often called IATA (*Integrated Approach to Testing and Assessment*), is performed to enable risk assessments that will avoid the use of animal testing to the greatest degree possible.

We selected nine substances that trigger pathological changes in the respiratory system, i.e. nose, larynx, pharynx and lungs, of lab animals. We then combined biologically relevant models and transposed the results first from simple cells to tissue, and then from tissue to the organism (Figure 1). The cells we used were human alveolar epithelial cells, a common type of lung cell. These cells are widely used in science and research, and they are excellent candidates for investigating mechanisms of action. We also made use of human lung tissue sourced from people which required the surgical removal of part of this organ. With the consent of these individuals, we were able to make use of this tissue for research purposes. From it, we obtained small slices of living tissue, also known as *precision-cut lung slices* (PCLSs). These PCLSs contain almost all of the cells normally present in the lungs. The cells are biologically active and communicate with one another. In addition, PCLSs reflect the natural three-dimensional structure of the lungs and therefore come very close to the composition and functions of the human organ (Sewald and Braun, 2013, Figure 1).

The biological point of reference – human or animal?

To judge if the results obtained from the cells and tissue were able to predict the toxicity of the chemicals, it was necessary to have reference data from living organisms, ideally from humans. However, chemicals are not normally tested on people, so we used data from toxicity studies on lab animals as the reference material for our project. These data existed before the start of ExITox, so it was not necessary to perform any additional tests on animals. Over the past few years, Fraunhofer ITEM has built up the RepDose (www.fraunhofer-repdose.de) database, which

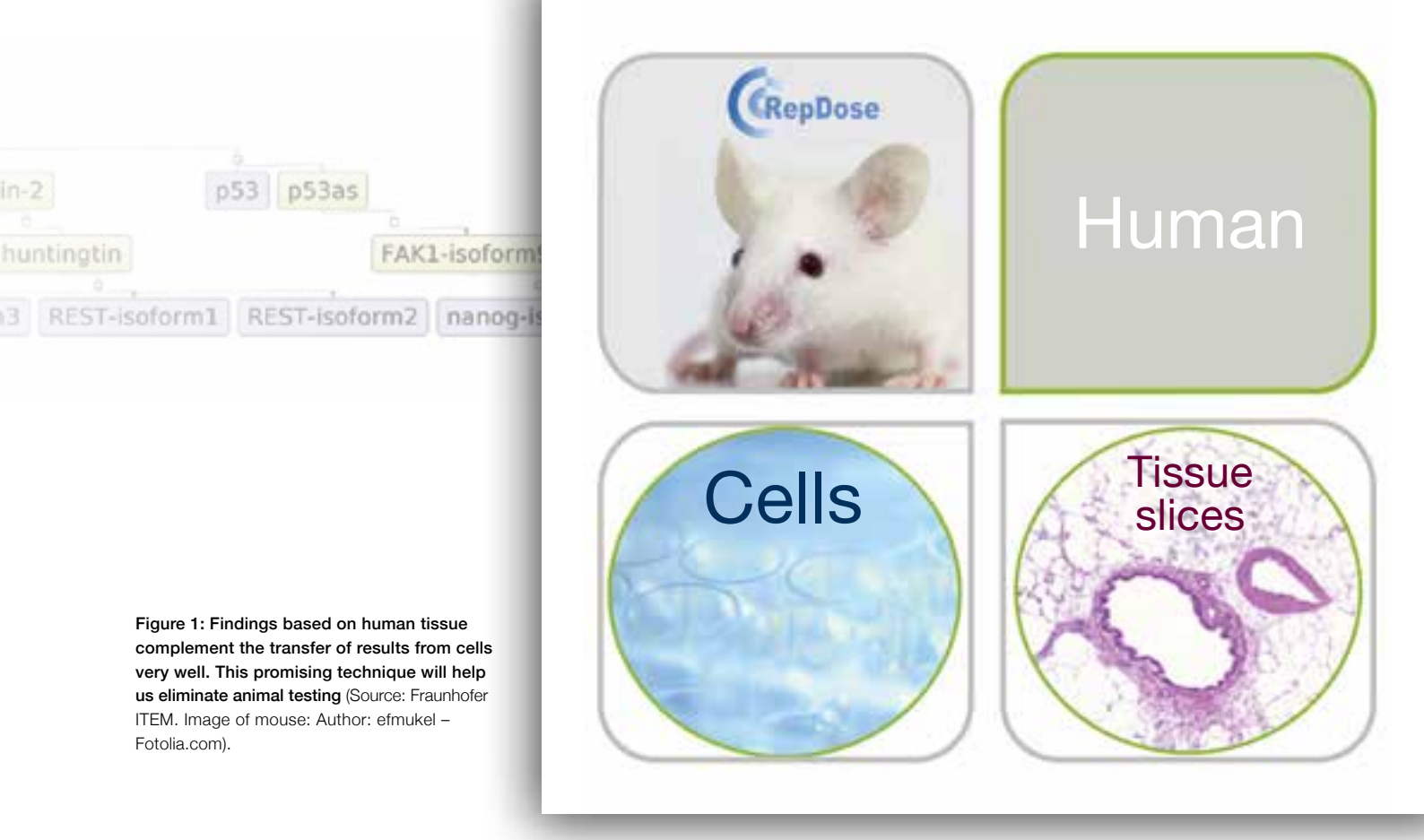

Figure 1: Findings based on human tissue complement the transfer of results from cells very well. This promising technique will help us eliminate animal testing (Source: Fraunhofer ITEM. Image of mouse: Author: efmukel – Fotolia.com).

contains approx. 3,000 published studies that mostly use rats and mice as their subjects (Bitsch *et al.*, 2006). Using the RepDose database, we selected three groups of chemicals with different mechanisms of action: naphthalenes, vicinal halides and vinyl esters. Naphthalenes were once used as an insecticide in mothballs, while vicinal halides were used as a solvent for lipids and wax in substances such as 1,2,3-Trichlorpropane. Vinyl acetate, a member of the vinyl ester group, is a component in the production of polymers such as the binding agent used in the manufacture of paints and varnishes or as a raw material in the adhesives, paper and textile industry. Within each group of chemicals, we selected three similar substances and put our faith in the basic toxicological rule that chemicals with similar structures often have similar toxic effects. Similarity in toxicity after inhalation was additionally confirmed by an analysis of shared toxic effects in the animal studies from the RepDose database.

Using the three different chemical groups, we attempted to answer the following questions:

1. **Are the alternative methods e.g. use of human cells and tissue or a combination thereof capable of predicting the toxicity of the three chemical classes?**
2. **Do changes in gene expression reflect different mechanisms of action exhibited by the three chemical classes?**

To answer these questions, we treated the epithelial cells and human lung tissue with our chosen chemicals and then measured the chemicals' toxicity. We found that, within a group, it is possible to make accurate predictions about the toxicity of the chemicals, as chemicals that were toxic within an animal were also found to be toxic to cells and tissue outside of the organism, and vice versa. We also identified differences between the three groups. Our study showed that the vicinal halides were less toxic than the vinyl esters, while the naphthalenes were highly toxic.

Analyzing gene expression

We also looked at changes which the chemicals had on the transcription of genes within the cells and tissue samples. We started by performing **microarrays*** and evaluating them using the geneXplain platform (www.genexplain.com) (Figure 2). The geneXplain platform is a bioinformatic collection of applications in the field of transcriptomics, proteomics, epigenomics, meta-bolomics, next generation sequencing, drug targets, pharma-cogenomics, etc., and it serves to help elucidating a host of different aspects relating to biology, medicine and genetics.

Figure 2: The geneXplain platform (Source: geneXplain).

We performed a quality analysis on the microarray data. Following this, we had a range of statistical tools at our disposal to identify the genes which revealed differences in expression in connection with the various substances. Gene expression is the term for the process in a cell by which an activated gene forms a gene product. The first step is always transcription into an RNA molecule, and mRNA molecules are then translated into proteins. A traditional bioinformatics analysis of the functions of expressed genes and their products consists of a matching with gene ontology, which facilitates the systematic assignment of gene functions. However, this approach was only of limited value in our case, as the number of differentially expressed genes per compound was small, which meant that it was not possible to achieve the necessary level of statistical reliability. We therefore analyzed both the transcription regulation of the genes and the signal transduction in addition. Based on a unique integrated promoter and pathway analysis on the geneXplain platform, we identified and compared "master regulators" for the different groups (Figure 3). Promoters are sections near genes which regulate the genes' activity by binding specific proteins. Complex signal cascades in turn control the effectiveness of these proteins. In the case of certain biological processes, these cascades are routed via a small number of important molecules called master regulators. This enabled us to differentiate the three groups of chemicals, which are subject to a host of regulatory processes, in terms of their mechanisms of action. We paid particular attention to lung-specific reactions and signal pathways.

Outlook

We designed the ExITox project as a "proof of concept" undertaking. We wanted to show that we could use chemical groups and relevant reference data from traditional animal tests to predict toxicity without the need for live testing on animals. In our ExITox project, we were able to prove that it is possible to predict the toxicity of groups of chemicals following inhalation without the use of animal testing. This revealed that it made sense to conduct a systematic, step-by-step prediction extending from simple cells to living tissue slices and then on to the organism. The PCLSs were particularly good when it came to displaying complex toxic changes resulting from the interaction between a range of cell types within an organism. Alongside gene expression data, we plan to include other "omics" technologies, such as next generation sequencing and proteomics, for evaluating the mechanisms of action in future studies. Predicting the level of impact in the organism was semi-quantitatively possible within the framework of this project, i. e. we distinguished less toxic groups of chemicals from more toxic groups, but we were unable to make predictions regarding the severity of an individual substance's effect. We therefore intend to use follow-up studies

*Microarray:

Microarrays are small chips that can be used for simultaneously measuring the activity of a large number of genes. One commercially available chip contains almost all of a human's genes (approx. 20,000). Using a special technique, probes for identifying the genes are "spotted" onto the chip. The actual analysis process uses fluorescence intensity to assess gene intensity (number of transcribed genes in the form of messenger RNA). Gene chips have long been in use in research and medicine to verify the existence of conditions and illnesses.

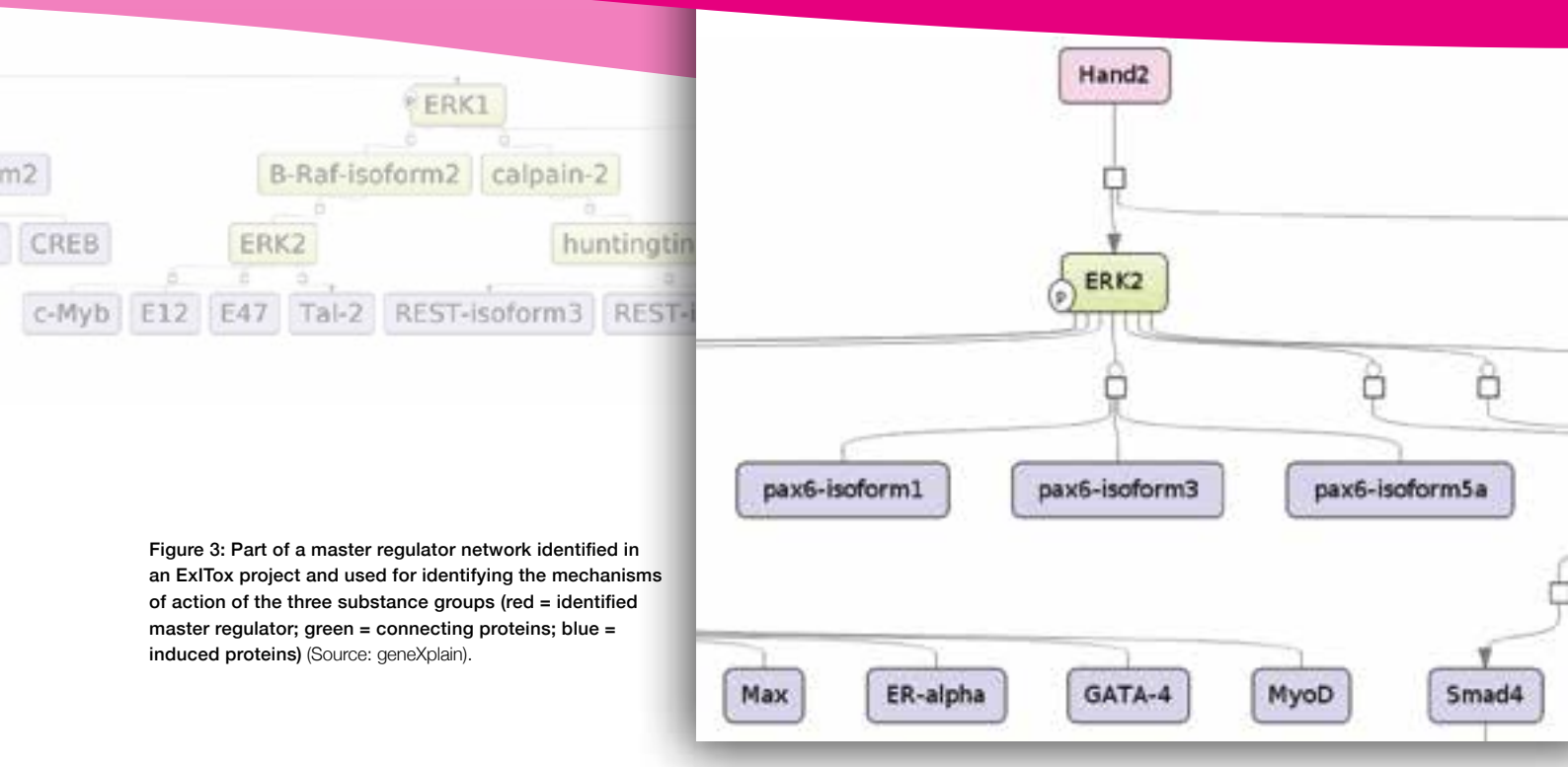

Figure 3: Part of a master regulator network identified in an ExITox project and used for identifying the mechanisms of action of the three substance groups (red = identified master regulator; green = connecting proteins; blue = induced proteins) (Source: geneXplain).

to investigate the uptake and breakdown of substances in the non-animal testing methods, and we want to apply or develop models for calculating bioavailability within the organism. Another objective is to study additional chemical groups and their mechanisms of action in order to generate information about the broad application of the concept developed here for risk assessment in humans.

Research project profile:

Project title: Development of an integrated testing strategy for the prediction of toxicity after repeated dose inhalation exposure: a proof of concept

Acronym: ExITox (Explain Inhalation Toxicity)

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a question of balance

An interview with microbiologist and genetics expert Bärbel Friedrich

Microbiologist Bärbel Friedrich is one of Germany's pioneering figures in genetic research, and her expert opinion is in high demand whenever the opportunities and risks of new technologies are being weighed up. Her ground-breaking research has appeared in over 200 publications. She has received numerous awards for her work, including the Arthur Burkhardt Prize and the Order of Merit of the Federal Republic of Germany. Friedrich is a member of Germany's Leopoldina National Academy of Sciences, where she held the position of vice-president for ten years. Since 2008, she has been the scientific director of Alfried Krupp Wissenschaftskolleg, an institute for advanced studies in Greifswald, and emeritus professor at Berlin's Humboldt University. In her interview with *systembiologie.de*, she speaks about the topics of gene editing, big data and modelling in systems biology.

Systembiologie.de: *Around the world, unimaginable quantities of data are generated every day in the fields of systems biology and systems medicine. So far, there have been virtually no generally binding standards and specifications for such data. How have researchers been handling this data and the analyses based on it?*

Prof. Dr. Bärbel Friedrich: The problem is that although data is collected, aggregated and stored, there are often no unified standards for how to format and explore it. This becomes particularly difficult if you want to merge information, for example data about the entire range of proteins, the proteome, and data concerning metabolic products, the metabolome. However, there are some exemplary undertakings such as the International Cancer Genome Consortium, one of the world's major interdisciplinary biomedical research projects into the molecular causes of cancer. This enables research scientists from around the globe to combine their findings in a single database. The information is noted in a standardised form so that it can be repeatedly used for evaluations in different contexts, even at a later date. A similar system could, in principle, be created for all genetic illnesses.

So far, there has been no across-the-board data management structure. What do you believe is the problem here?

Big data means first gathering an incredible quantity of data and information, and doing so maybe without yet knowing what we will one day be able to or want to study using it. Unfortunately, we are still behind the times when it comes to data processing and unified standards. This is the case all over the world, but we have clear shortcomings in Germany as well. Put simply, everyone has to join in: research scientists, doctors and clinics. They can't keep using messages in bottles to distribute their data. We have to catch up. This is also something everyone has experienced – if you change doctors, the new one normally runs all your tests again.

Data security is a big issue. On the one hand, people want to prevent the misuse of data, but gathering sensitive information for an individual patient is, on the other hand, necessary for personalised medical treatment. How difficult is it to achieve the correct balance?

Striking the right balance definitely isn't easy. However, look at how incautious people are when giving personal information away online. I think data protection is demonised, to a degree. Of course, everything needs to be done to ensure that patients' data is not misused but, at the same time, it must be possible to access a patient's data in the interest of their own well-being. If we impose too many limits on ourselves regarding data protection, we will also limit options and potential for research and innovations.

Despite the tremendous technological opportunities for collecting individual systems biology and systems medicine data (using omics techniques, for example), personalised medicine is still in its infancy when it comes to treating illnesses. Why is this so? What needs to be done to make progress?

Medical professionals can already use modern bioanalytical high-throughput processes to perform genome analyses and then deploy the results in their diagnoses. This applies, for

Bärbel Friedrich is the scientific director of Alfried Krupp Wissenschaftskolleg in Greifswald. From 1994 to 2013, she was professor of microbiology at Berlin's Humboldt University. She is a member of the Leopoldina National Academy of Sciences, where she held the position of vice-president from 2005 to 2015 (Source: Vincent Leifer/Alfried Krupp Wissenschaftskolleg Greifswald).

example, to conditions caused only by mutations in individual genes or by specific infectious diseases, such as the impairment of the immune system due to HIV. In oncology, it is possible to make statements about the characteristics of a tumour and the mechanisms of its metastasis. However, these results are too often looked at in isolation, like the separate pieces of a jigsaw puzzle. One of the major challenges will be how to standardise and store this complex array of personal information, generate reliable results from it and then identify steps in the treatment process. Above all, we need to keep up investment in research – simply collecting patients' data is not enough. We have to understand what it is that we are gathering.

How far are we still from the vision of personalised medicine and therapy, i. e. treatment tailored to the individual patient?

One advantage we already experience today is that we can group patients with certain illnesses, such as breast or intestinal cancer, into smaller subdivisions according to genetic characteristics and then give them certain drugs in a focused manner because we know they will respond to these drugs. Unfortunately, genetic conditions are extremely complex, in particular common illnesses such as diabetes and cardiovascular diseases. The number of conditions caused by a single genetic defect, such as cystic fibrosis, is increasing. For a very small group of cystic fibrosis sufferers, researchers have identified a specific mutation for which an effective drug has been developed. Other monogenetic illnesses are very rare and affect just a few patients on a worldwide scale. Nevertheless, we have been making progress regarding the treatment of these conditions as well, and if we make intelligent use of this information, we can expect further results. Cancer therapy, in particular, will soon experience a veritable quantum leap. In this field, it could soon be the case that a DNA analysis performed before a patient starts therapy delivers a detailed genetic image of the tumour and the structure of its surface. The doctor would then be able to see how aggressive the tumour is and what specific drugs and therapy strategies the patient needs.

Is personalised medicine something that healthcare systems can afford?

The affordability of our healthcare system isn't just a problem in terms of personalised medicine, but tailored therapy will probably be expensive starting out. However, in the long term, I think we will be able to save a lot of money. For example, tailored therapy will make it possible for a patient to receive medicine dosages that are right for their treatment, and they will not undergo any form of therapy that does not work for them. Furthermore, someone can undertake preventative measures when they know that they are predisposed to a certain illness. This will definitely be of financial benefit for society.

New techniques bring progress, but they also bring risks. At the Human Gene Edit Meeting in Paris in April, you discussed the opportunities and risks of gene editing with experts from around the world. What questions were covered? Did the experts come to an agreement?

Meetings such as the gatherings in Paris and the International Summit on Human Gene Editing in Washington show that there is a huge need for communication and discussion. Nobody wants to repeat the mistakes that were made with stem cell research and

green genetic engineering. There was no proposal for a moratorium to stop human gene editing at either of the two meetings, but everyone agrees that creating new human beings is not an objective. However, progress will continue on embryo research with the goal of acquiring basic information, which also covers the development of illnesses. All the same, we are extremely unlikely to see a single, international set of rules for governing this research any time soon. Similarly, due to the differences between national regulations, it will be difficult to develop a common policy in Europe.

What is the situation in Germany?

At present, we are unable to perform research on embryos in Germany. It is a punishable offence. The field of green genetic technology offers another example of a problem, as each German state takes a different approach to import regulations, for example concerning US seed lines intended for research. This lack of coordination greatly limits the freedom of research activities, while the prohibitions and scores of bureaucratic requirements hinder international cooperation.

What does this mean for research and research scientists here?

Research into certain issues is becoming increasingly difficult for German scientists. After completing my doctorate in 1975, I went to the USA, a training route taken by a lot of people. In the US, you move into another field of research in which you will later become independent. But what sort of prospects are there for research scientists if Germany's laws prohibit them from continuing with their projects? In my opinion, this constrains our freedom of research, something guaranteed to us by Article 5 of the German constitution. And it doesn't just impact on research with embryos, but also on plant breeding as research in this field normally requires experimental releases. It isn't possible to draw good enough conclusions based only on experiments in greenhouses. Though legal, the few experimental releases undertaken so far were sobering experiences for research scientists, as their fields were often destroyed by biased opponents. The research scientists were subsequently so frustrated that they didn't want to continue their work. You start writing a doctorate thesis, but all of your work gets trashed by attacks of this sort. Given this state of affairs, how are we supposed to encourage young people to work in this field?

What could happen if Germany continues to enforce these restrictions?

Today, our competitors are Asia, the USA and the UK. Foreign research scientists say they can no longer work with us because they don't want to conflict with Germany's laws. When stem cell research started out, we were one of the countries that led the field. Then the reins were tightened. We have to pay attention once more if we don't want to be left behind because overly stringent bans prevent research.

In a statement in 2015, the Leopoldina National Academy of Sciences, Germany's National Academy of Science and Engineering (acatech), the Union of the German Academies of Sciences and Humanities and Germany's DFG research association spoke of the "high scientific potential" of gene editing and of their "express support for a voluntary international moratorium on all forms of human germline intervention". What has become of this approach?

Some of my Leopoldina peers and I are involved in gene editing, but we are not reckless people who are easily swayed. All the same, we have also seen our opinions evolve – something that all scientists should have a right to. Today, I would suggest a multi-strand approach to a moratorium. For example, Germany should permit research using gene editing, including on early-stage embryos if necessary. We don't need to go as far as the Dutch, who generate embryos especially for use in research. I would reject that. However, fertility clinics have a large number of leftover embryos which are normally destroyed. With the consent of the donors, our researchers should also have the opportunity to use these embryos in their work. It isn't yet a matter of intervening in the germline – we are still a long way from that. As far as we currently know, we are not able to make clinical use of this technology, and anyone who does lacks ethical responsibility.

Why is the CRISPR-Cas9 method more efficient than existing gene editing techniques? What potential does this method have?

The CRISPR-Cas9 "molecular scissors" is a simple, elegant and cost-effective method of changing an organism's genetic material in a very precise and targeted way. It can be used universally, on plants, animals and human cells. I see nucleases such as CRISPR-Cas9 as a key form of technology that can be used in everything from green genetic engineering to biomedical research. It can permit us to study the most serious genetic diseases and, hopefully, one day treat them too, or perhaps even prevent them.

Some of Bärbel Friedrich's research focuses include the functioning and biosynthesis of metalloproteins, enzymatic catalysis mechanisms in metalliferous redox proteins, in particular hydrogenase and their biotechnological application. Her functional genome analyses concentrated on facultative lithoautotrophic bacteria. She has so far contributed her work to over 200 publications (Source: Vincent Leifer/Alfried Krupp Wissenschaftskolleg Greifswald).

German experts agree that the clinical use of CRISPR-Cas9 in egg cells, sperm and embryos should currently be forbidden due to its completely unexplored risks. However, is it even possible to assess risks without conducting research on embryos?

No. We have to perform research to find out what the risks are. How can we gauge the risks if we can't study them? We can't use animal models for every form of research activity. It is important to consider the balance between the risks and benefits.

This technology is already in use on embryos in other European countries. What would you like to see happen in Germany?

In two Chinese publications, research scientists have announced their more or less successful attempts to perform gene editing on embryos. The Chinese are not the only ones active in this field, and their findings won't be alone for long. Research scientists in the USA, the UK and Sweden have also received permission to perform research on early-stage embryos. My personal view is that we should permit basic research as it is essential for potential applications.

Using modern systems biology, systems medicine and genetic engineering techniques, what kind of world will we create for future generations?

It is my great hope that the ground-breaking insights in the life sciences will make a permanent contribution to the well-being of all people. One way would be to ensure that serious illnesses, if they cannot be avoided, can at least be diagnosed and treated reliably. Another way would be the intelligent and restrained use of genetic engineering processes to protect our ecosystem and enable us to provide enough food for the 11.2 billion people predicted to inhabit the planet in 2100. While these ideas might be illusions, they are probably the source of inspiration driving many of today's scientists in their search for new insights.

Interview conducted by Kristin Huettmann.

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Source: Vincent Leifer/Alfried Krupp Wissenschaftskolleg Greifswald

towards *in silico* design of selective kinase inhibitors

Company profile of Team SKI at the BioMed X Innovation Center

by Simone Fulle, Benjamin Merget and Samo Turk

Computational approaches are nowadays an integral part of drug discovery processes. This holds especially true for the initial steps such as the target selection and the identification and optimization of small molecules which inhibit the target(s) of interest. The design of novel drugs requires solving complex problems to reach optimal safety and efficacy. For instance, to reduce potential side effects, it is crucial that the compound does not interact with other signaling pathways in the body. The bioinformatics research group at the BioMed X Innovation Center is developing and applying computational methods that support the rational design of selective inhibitors, a crucial step for reducing undesired side effects. In line with a focus on protein kinases, the team is called internally “Selective Kinase Inhibitors” (SKI).

BioMed X

Selective kinase inhibitors provide opportunities for the treatment of various diseases

Protein kinases have been a major target of drug discovery programs for many years due to their central roles in signaling pathways involved in the formation and progression of human cancer, inflammation and Alzheimer’s disease. Most kinase in-

Figure 1: Phylogenetic tree of the human kinome with key targets

The image illustrates the phylogenetic tree of the human kinome including key targets for FDA approved drugs (red triangles) and kinases with a high druggability score (blue circles) (Volkamer *et al.*, 2015). Although kinases are established drug targets, most of the current drug discovery projects focus on a small set of kinases. The darts point to a subset of less explored kinases that could be further explored in drug discovery efforts. The picture was made using http://kinhub.org/kinmap/; the human kinome tree illustration is reproduced with courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com).

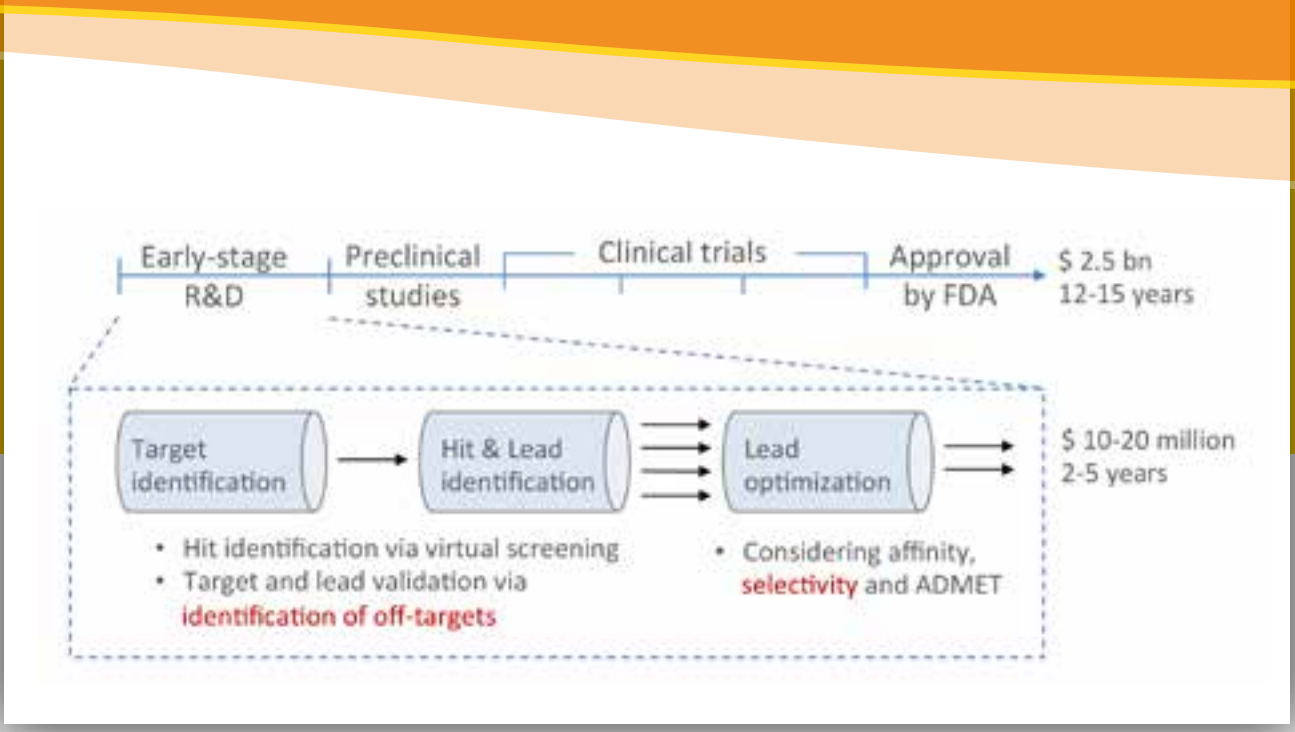

Figure 2: Pharmaceutical research and development process. To bring new drugs to the market it takes on average 12-15 years and costs approximately $2.5 billion. The early-stage research and discovery phase (R&D) costs tens of millions of dollars, but is crucial to reduce costly late-stage attrition. The arrows in the blue box reflect the relative numbers of targets and compounds resulting from the individual steps. The *in silico* tools developed by the bioinformatics team at BioMed X support the rational design of selective inhibitors by taking into account identified off-targets and selectivity determining features on the compound and target side (Source: Simone Fulle).

hibitors that are currently approved and in development bind to the highly conserved ATP-binding pocket of kinases. Given that more than 500 human protein kinases exist, this often leads to low selectivity for the defined target kinase and thus, to undesired side effects or failure in the research and development (R&D) of novel drugs. Optimizing the selectivity profile is therefore a crucial step when it comes to designing novel kinase inhibitors. Moreover, although kinases have been the focus of R&D for more than two decades, a large number of kinases are still unexplored and offer opportunities for novel, competition-free drug discovery projects (Volkamer *et al.*, 2015) (Figure 1).

Developed computational methods

A key aspect to rationally design safer drugs is detecting binding profiles across a wide range of potential off-targets and identifying unique features in the binding site of the key-target. To address these aspects, the bioinformatics team at BioMed X develops novel computational tools that allow I) profiling compound activity across the human kinome, II) identification of selectivity determining features, and III) virtual compound optimization (Figure 2). The developed techniques are grounded in bio- and cheminformatics, utilize the wealth of available profiling data and crystal structures as input, and employ concepts and techniques of machine learning and biophysics.

Step 1: Profiling compound activity across the human kinome

The promiscuity of most kinase inhibitors requires that a large number of kinases are considered when designing selective kinase inhibitors. However, experimental kinase profiling is still a costly process and publicly available bioactivity data are not extensive enough to train prediction models for the entire kinome. Our *KinSpectrum* technology contains reliable prediction models for ~250 kinases which are evenly distributed across the kinome tree and thus, allow the detection of potential off-targets in all kinase branches (Merget *et al.*, 2016). The high-quality prediction is obtained by employing machine learning techniques on a unique training set of bioactivity values for thousands of kinase inhibitors. Besides the detection of off-targets, *KinSpectrum* can support various hit identification tasks by identifying novel compounds for a given target (virtual screening) or by cross-linking already known compounds to novel targets (compound repurposing).

Step 2: Identification of selectivity determining features

The identification of selectivity determining features in the binding site of the target is obtained by mining the wealth of structural information of kinases as well as profiling data of compounds

(Figure 3). For instance, our *X-Grids* technology enables visually inspecting the differences of multiple kinase structures at once by highlighting target specific areas, and prioritizing compounds regarding their selectivity profile by assigning specific scores. *X-Grids* can be applied to a predefined set of 1-10 off-targets and, as no project specific training of the technology is required, can also provide insights into unexplored kinases (Volkamer *et al.*, 2016). The analysis of off-targets is complemented by analyzing available profiling data via machine learning technologies. Overall, this results into an improved understanding of structure-activity relationships of kinase inhibitors and enables the rational design of novel compounds with improved selectivity profiles.

Step 3: Virtual compound optimization

Lead optimization is a complex process aiming to improve several compound properties. This process can be rationally guided when having a precise understanding of binding determinants as well as structure-activity relationships (SAR). The latter can be obtained, for instance, by extracting so-called Matched Molecular Pairs (MMP) from compound series. An MMP is defined as two molecules that differ by only a small change in structure, but which have a significant difference in a particular property such as activity or selectivity. Deep learning algorithms allow processing complex information and have dramatically improved automatic speech recognition and visual object recognition (LeCun *et al.*, 2015) and have shown promise in other domains such as drug discovery. Combining MMP information with the deep learning approach, our *X-Hop* technology is able to extract relevant SAR information which in turn can guide compound optimization by designing novel compounds with improved properties.

Definitions:

- **Target:**
  Molecular structure in the body, such as a protein and nucleic acid, that upon modulation with a drug causes a clinical effect. The primary targets of drug design are called "key-targets" and the unintended ones "off-targets".
- **Lead compound:**
  Promising molecule that possesses biological activity and that has a potential to be optimized into a drug.
- **Lead optimization:**
  Improving the properties of lead candidates with the goal of increased efficacy and safety.

Table 1: Summary of developed computational tools

Tool name	Key aspects, application and/or outcome
KinSpectrum	Prediction models that are trained on a unique and large panel of diverse kinase inhibitors and allow predictions for ~250 kinases • Hit-identification and repurposing • Identification of off-targets
X-Grids	Fusion of molecular interaction grids of several targets to a user-friendly and content rich representation of target specific subpockets • Guidelines for modifying lead compounds • Prioritization of a large number of compounds via 'selectivity scores'
X-Hop	Matched Molecular Pair approach combined with deep learning • Prioritized list of improved and synthesizable compounds

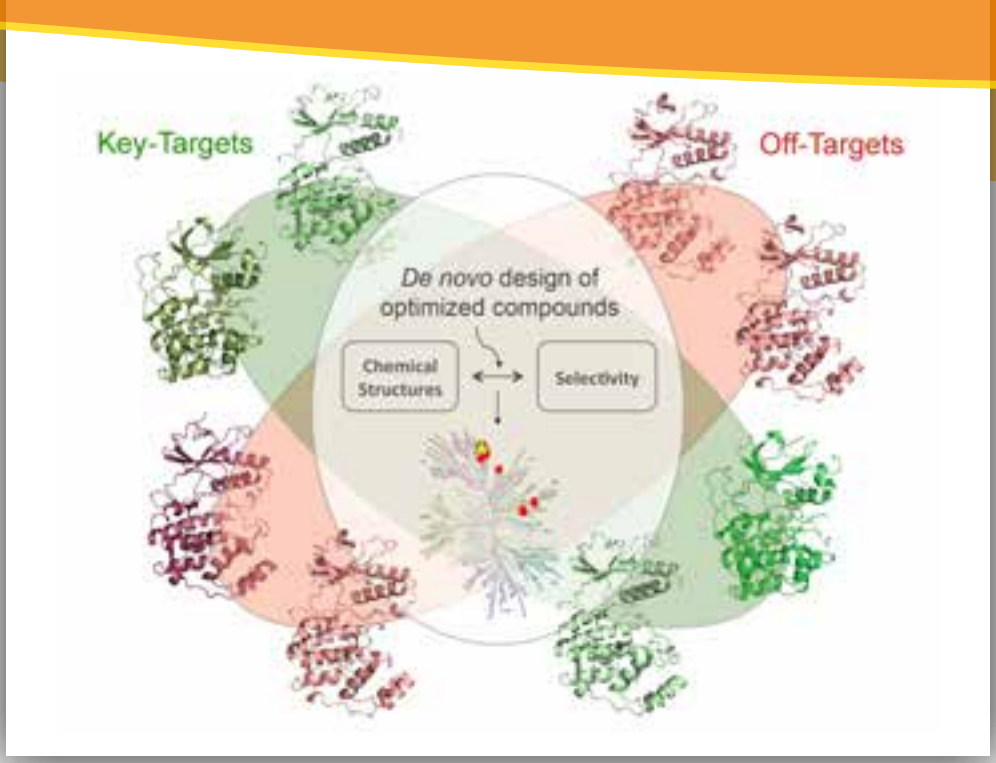

Figure 3: Rational optimization of compounds can benefit from varying aspects on the molecular target and compound side. The design of novel compounds can be guided, for instance, by a precise knowledge of selectivity determining features in the binding site of the key-target as well as structure-activity relationships. The kinome tree illustrates exemplarily the binding profile of lapatinib, a selective inhibitor of the kinases EGFR and ErbB2 (green triangle) (Source: Simone Fulle, Volkamer *et al.*, 2016).

- **FDA:**
  U.S. Food and Drug Administration (FDA) reviews and assesses drug candidates for marketing.
- **Kinome tree:**
  Phylogenetic tree of all human kinases.

About the bioinformatics team 'SKI' at BioMed X

The focus of the bioinformatics team at the BioMed X Innovation Center in Heidelberg is developing and applying computer-aided drug design technologies. BioMed X, founded in 2013, develops solutions for early R&D challenges at the interface between academia and industry (Betz and Tidona, 2015). To date, nine teams are working on different biomedical research areas including oncology, neuroscience, respiratory, diagnostics and consumer care. The bioinformatics team is currently in a preparation phase to spin off into an independent start-up company. The aim is to use the proprietary *in silico* tools in combination with classical methods of computer-aided drug design as a service to pharmaceutical and biotechnology companies.

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Photo: BioMed X GmbH

Federal Ministry
of Education
and Research

News from the BMBF

Education and research will be further strengthened in 2017

The budget of the Federal Ministry of Education and Research (BMBF) will once again see a substantial increase in 2017. It will rise by 1.2 billion euros to around 17.6 billion, indicating the key role which the Federal Government assigns to education and research.

The Ministry is investing around 5.8 million euros in institutional research funding in 2017 – an increase of three percent. Approximately 2.5 billion euros are being spent on funding additional university places. Sixty million euros have been earmarked for the third year of the National Programme to Improve the Quality of Teacher Training.

The new Federal Government-*Länder* agreements on institutions of higher education are another priority. They include the Excellence Strategy to strengthen cutting-edge university research, the establishment of 1,000 tenure track professorships, which will provide junior researchers with better career opportunities, and the "Innovative University" funding initiative, which supports smaller universities and universities of applied sciences.

The Federal Government is promoting and shaping digital transformation under its Digital Agenda. The BMBF is contributing a number of measures in the fields of innovation support and education. The 2017 budget also includes an increase in research funding under the Federal Government's new High-Tech Strategy. Fifty-five million euros have been made available for the targeted support of research at universities of applied sciences – an increase of around 15 percent over the previous year.

In addition, the BMBF has put together a package of measures in order to react promptly to the challenges resulting from the refugee situation. These measures apply key elements of the Federal Government-*Länder* joint integration strategy. The Federal Ministry of Education and Research is thereby focusing on providing language tuition and identifying competences and potentials as well as on providing access to vocational training and higher education. Funding is also being provided for research projects to enhance our knowledge of migration and integration.

www.bmbf.de/en/education-and-research-priority-areas-of-federal-government-policy-1410.html

Innovation as the motor for small and medium-sized enterprises

Small and medium-sized enterprises (SMEs) often lack both the capacity to conduct their own research and access to the latest research findings. Cooperation between industry, science and other partners is therefore essential for the emergence of innovations. The BMBF's "Innovation Forums for Small and Medium-Sized Enterprises" funding initiative supports the formation of networks which extend far beyond mere project work and lead to sustainable, strategic alliances.

In the words of Minister Wanka: "It is a great challenge for small and medium-sized enterprises in particular to accurately estimate the risks and opportunities which innovations involve. By establishing the Innovation Forums, we are making it easier for small and medium-sized enterprises to develop new business models. Furthermore, we are helping these companies to commercialize their ideas – even during the start-up phase."

Strong together: The central instrument of the funding initiative is a two-day innovation forum bringing together all the relevant stakeholders.

Source: Gaj Rudolf

The "Innovation Forums for Small and Medium-Sized Enterprises" are one element of the "Priority for SMEs" programme. The BMBF is boosting the innovative strength of SMEs with this ten-point programme.

www.bmbf.de/de/innovationsforen-mittelstand-3064.html

Three pacts for the institutions of higher education

The Federal Chancellor and the heads of the *Länder* have launched three programmes to strengthen institutions of higher education. These are the "Excellence Strategy", the "Programme to Promote Young Scientists" and the "Innovative University" funding initiative.

The Excellence Strategy deals with the promotion of excellence clusters and universities of excellence. In future, the Federal Government and the *Länder* will provide 533 million euros per year for this purpose. The universities of excellence are intended to strengthen universities and university alliances as institutions in the long term and expand their leading international role. The excellence clusters will provide project-related support for internationally competitive research fields at universities and university alliances.

The Tenure Track Programme provides young researchers with greater clarity and certainty when planning their careers in academia. Beginning in 2017, the Federal Government will provide one billion euros to fund 1,000 additional tenure track professorships. These positions will generally become long-term professorships following a successful probation phase lasting several years.

The new "Innovative University" funding initiative is aimed in particular at universities of applied sciences and small and medium-sized universities. It will support the research-based transfer of ideas, knowledge and technologies at German institutions of higher education and strengthen the strategic role they play in regional innovation systems. According to Federal Research Minister Johanna Wanka: " We want to ensure that good scientific ideas are translated into practice – in local government, in industry and in society. We are encouraging this transfer with the "Innovative University" funding initiative."

www.bmbf.de/en/innovative-hochschule-3367.html

Young researchers in Germany want more certainty when planning their careers. The Tenure Track Programme makes this possible.

Source: Thinkstock/Fuse

Using exhaust fumes to save the climate

The "Carbon2Chem" project shows how a climate killer can become a climate saver. Eight industrial companies are currently working with the Max Planck Society and Fraunhofer to develop a solution that can be applied worldwide to convert exhaust gases from blast furnaces into primary products for fuels, plastics and fertilizers. The hydrogen needed for this process is produced using surplus electricity from renewable sources of energy. The "Carbon2Chem" approach sets out to convert 20 million tonnes of Germany's annual carbon emissions from the steel industry into products that can be used commercially.

In the words of Federal Research Minister Johanna Wanka: "With "Carbon2Chem" we are demonstrating how to reconcile climate protection with a competitive steel industry through research and innovation in Germany. We are thereby safeguarding jobs in our country's steel industry."

Over the next ten years, the "Carbon2Chem" research project will develop a sustainable value chain, which will link various sectors. Climate protection is driving innovations across sectoral boundaries. The BMBF is providing the project with funding of over 60 million euros. The partners involved intend to invest over 100 million euros by 2025. They plan investments of over one billion euros for the project's commercial realization.

www.bmbf.de/de/mit-abgas-das-klima-retten-3044.html

Germany is becoming increasingly attractive for foreign researchers

Over 85,000 foreign researchers were teaching and conducting research at German institutions of higher education and non-university research institutions in 2014. At the same time, around 43,000 German researchers were working abroad. These impressive figures are contained in the report "Wissenschaft weltoffen 2016", which is published by the BMBF in association with the German Academic Exchange Service (DAAD) and the German Centre for Research on Higher Education and Science Studies (DZHW).

Compared with the figures for 2006, the number of foreign researchers at German higher education institutions had increased by 84 percent in 2014 to a total of 40,000. The number of foreign researchers at non-university research institutions had also increased to approximately 9,000. Foreign researchers now account for 20 percent of all research personnel.

Speaking about this development, Minister Johanna Wanka said: "Our scientific sector is interlinked with science throughout the world and is strong and attractive precisely for this reason. A cosmopolitan scientific sector is and remains essential for Germany as a key location for research and for German society as a whole. The "Excellence Initiative", the "Higher Education Pact", the "Pact for Research and Innovation" and all the other measures we have introduced to encourage internationalization over the years are paying off. Researchers from all over the world want to teach and conduct research in Germany."

A similar development can be seen among students. The number of foreign students in Germany rose once again last year. In 2015, there were 321,000 foreigners studying at German higher education institutions. The biggest increase in numbers of foreign students is to be seen in the field of master's degrees (+25 percent) and doctoral degrees (+3 percent). Sixty-five thousand foreign students are studying engineering sciences.

www.research-in-germany.org/en/

Projects in the field of Education for Sustainable Development

How can sustainability be firmly embedded in the structures of the German education landscape? Federal Education Minister Johanna Wanka and the President of the German UNESCO Commission, Verena Metze-Mangold, took advantage of the first national agenda congress on "Education for Sustainable Development" in Berlin to award prizes to 65 projects involving genuine examples of education for sustainable development.

Speaking at the congress, Johanna Wanka said: "Education for sustainable development must be tangible. It must be embedded in people's everyday lives. I am delighted that this is already the case in many places as demonstrated by the first awards under the World Action Programme." The Minister remarked on the impressive diversity of the 65 award winners.

She welcomed the fact that small and large cities alike had decided to adopt education for sustainable development as their guiding principle. They were anchoring this principle in their urban development strategies, in everyday life at nurseries and schools. She said that public meetings were taking up this topic as too were action groups which tackled Germany's transition to renewable energy at local level through urban gardening projects, for example, thus demonstrating that climate protection was fun.

www.bmbf.de/de/bildung-fuer-nachhaltige-entwicklung-535.html

Federal Minister Johanna Wanka (centre) with Margret Wintermantel, President of the DAAD (left), and Monika Jungbauer-Gans, Scientific Director of the German Centre for Higher Education Research and Science Studies.

Source: BMBF/Hans-Joachim Rickel

Researchers decode bone healing process using a 3D model

A broken bone usually heals within three months at the latest. This process is somewhat quicker for young patients than for older patients. In about ten percent of cases, however, the fracture heals far more slowly or does not heal at all. Doctors call this a fracture or bone healing disorder.

Researchers at the Charité university hospital in Berlin are developing a three dimensional model to simulate the bone healing process with the help of human cell material. They are focusing on the first phase of fracture healing, which is particularly susceptible to disruptive influences. Their goal is to improve their understanding of the initial mechanisms in the bone healing process in order to develop new therapies for patients with fracture/bone healing disorders.

The Charité model is also intended to help to reduce the number of animal experiments in fracture healing research. In the past, animal experiments in this area have usually involved mice and rats as well as larger animals. Veterinary surgeon Annemarie Lang from the project team is convinced that her research will not only prevent animals from suffering but will also provide more valid results. "Observations from animal studies often cannot be transferred to humans," she says. For example, the livers of rodents can break down cortisone far quicker than the human liver. It is therefore hardly possible to draw a comparison.

The Federal Research Ministry is supporting the interdisciplinary research team consisting of doctors, biotechnologists, biologists, veterinary surgeons and biochemists under the "Alternative Methods to Animal Experiments" funding programme. The project is receiving funding until the end of 2017. If everything goes according to plan, the model will then be ready for use.

www.bmbf.de/de/alternativen-zum-tierversuch-412.html

The majority of foreign vocational qualifications are recognized

Many companies, trades businesses, hospitals and care facilities depend on skilled staff from abroad. The Federal Government has therefore introduced the so-called Recognition Act as a new instrument to secure the supply of skilled staff in Germany.

Judith Yawa Aggor-Edorh is a trained dressmaker from Ghana. Thanks to the Recognition Act, she can now once again work in her profession.

Source: Portal „Anerkennung in Deutschland"/BIBB

Judith Yawa Aggor-Edorh from Ghana is a qualified dressmaker. Thanks to the Recognition Act, she can now work in her profession in Germany. Hers is just one of over 40,000 success stories. More than 44,000 applications for recognition were submitted between the introduction of the Recognition Act in 2012 and the end of 2014. The person's foreign qualifications were either fully or partly recognized in over 96 percent of the cases. An analysis of qualifications helps in this context. In cases where applicants cannot present important certificates and documents from their native country, they have to prove their ability by providing a practical example of their work – as in the case of Judith Yawa Aggor-Edorh.

The Recognition Act makes an important contribution to the integration of migrants both on the German labour market and in German society. Since education is the key to integration, the Recognition Act is an important instrument for the integration of refugees in Germany.

www.bmbf.de/en/recognition-of-foreign-professional-qualifications-1413.html

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IMPACTS OF CLIMATE CHANGE ON THE WADDEN SEA FOOD WEB

A systems level approach

by Harald Asmus, Ragnhild Asmus and Lisa Shama

The coastal ecology section of the Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung (AWI; Figure 1) has recently undertaken four projects dealing with basic research on coastal systems within the framework of the BMBF funded programs FONA, KÜNO and MARE:N. The main goal is to find and model indicators that describe changes to the ecosystem status, particularly in the Wadden Sea, in light of climate change. A unique feature of this research is the use of a holistic approach to find answers to urgent questions at the (eco-) system level. These projects integrate historic data from long term time series into model scenarios, use large-scale mesocosm experiments to simulate the impacts of future climate change on communities, and use quantitative genetics and genomics to investigate the evolutionary potential of local populations.

The development of coastal systems happens in the area of conflict between strong anthropogenic impacts and natural forces. Currently, we are observing a loss of function and biodiversity in coastal ecosystems, and urgent questions about how to evaluate the status and development of these systems are being raised. Intensive research on the (eco-) system level is needed to support decision making of stakeholders and politicians at local, regional and global scales.

Ecosystems encompass both the abiotic environment and the organisms living within. Some organisms affect the environment and alter its shape and function as ecosystem engineers. Within the community, organisms are interacting by producing or consuming, and each may be prey or predator to others. On an ecosystem level, these complex species networks are composed of bacteria and unicellular algae as well as higher organisms such as plants, fish, birds and mammals including man. The quantity and direction of energy flows between these ecosystem compartments describe the ecosystem status as a whole, and can tell us something about the future development of the system.

Our research focuses on the ecosystem of the Wadden Sea. External threats such as climate change are influencing the behavior and resilience of this system, but in what ways? To answer this, the AWI has conducted four projects highlighting different aspects of direct or indirect influences of climate change on Wadden Sea food webs: the change of sediment types, the growing impact of invasive species, the combined stress of warming and acidification on intertidal communities, and the role of (epi-)genetics in shaping the adaptive potential of populations.

ECOLOGICAL NETWORK ANALYSIS AT MULTIPLE SCALES: FROM MESOCOSMS TO WHOLE ECOSYSTEMS

Constructing models of food webs is done using Ecological Network Analysis (ENA). This modeling technique gives a snapshot of the total system at a certain time point and analyses the system properties and indicators. It can also estimate the resilience of the system to perturbation and the extent of specialization or generality that a system may develop.

ENA was used as the main investigation tool in a project aimed to assess the influence of invasive species at the ecosystem level (INFOWEB project). A Dutch – German team focused on three tidal basins of different types, thus different impacts of invasive species were expected for the three systems. A very abundant invasive species in the Wadden Sea is the Pacific oyster, originally introduced from Japan, which has been cultivated for aquaculture in Western Europe for decades. In the German Wadden Sea, Pacific oysters showed a direct and strong negative impact particularly on phyto-plankton and suspended particles, but also on other filter feeder species via competition for phytoplankton. Even more interesting was a negative impact of oysters on carnivorous birds and some benthic carnivorous fish (Figure 2). Together with other invasive species, Pacific oysters tend to control the energy flow of the ecosystem by their rapid biomass production. Yet, a sudden decrease in biomass of oysters as a consequence of a very hard winter in 2009/10 could not be balanced by native fauna, and the system reached an unstable stage the following year that increased its sensitivity to disturbance [1].

The Wadden Sea is an important resting site for migratory birds on the East Atlantik Flyway. An interdisciplinary team of geologists and biologists studied the distribution of different sediment types and its importance to foraging birds in the tidal flats of the Wadden Sea in another project (STopP). The AWI team investigated six bottom fauna communities, each representing a certain habitat type, and

Figure 1: a) Wadden Sea Station Sylt of the Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, **b)** the research vessel Mya II, and **c)** a section of the study area with foraging dunlins.

Sources: **a)** © AWI / GMSH; **b)** © AWI, Florian Lange; **c)** © AWI / Birgit Hussel

constructed a food web for each of these communities. Bird data for each community were then integrated into these food webs and analyzed using ENA. While seagrass beds and sand flats were the preferred foraging grounds for birds, other communities were less important, highlighting the role of habitat diversity for the functioning of the Wadden Sea ecosystem as a whole. Although the individual food webs themselves revealed a high stability and resistance to perturbation, the different bird components showed a distinct tendency to compete with each other, suggesting a feeding pressure exerted by birds, and indicating that the system is already close to carrying capacity for birds.

A third project assessed the impacts of ocean acidification on the (eco-) system level, based on large outdoor mesocosm experiments carried out within the project BIOACID (Figure 3). Physical and chemical environmental conditions such as temperature and pH are hard to control in the field, hence large scale mesocosms (experimental tanks) were installed at the Wadden Sea Station Sylt to carry out experiments about future climate scenarios [2]. The system tested was an intertidal mussel bed community that included not only mussels, oysters and seaweed, but also most of the dominant macrofauna associated with them. For each season, this community was exposed to warming (ambient temperature +5°C), a combination of warming and increased CO_2, and additionally treated with nutrients. The combination of increased temperature, CO_2 and nutrients had the largest impact on the community. This treatment combination resulted in massive growth of epiphytic algae while the growth of bladder wrack (*Fucus vesiculosus*) was strongly reduced. This was a deep impact at the base of the food web. A higher trophic level, consisting of grazing animals, was stimulated in spring and autumn, while high temperatures in summer limited their development and growth. These experiments gave the first insights into a system-level response of a community to climate change.

FROM (EPI-)GENES TO ECOSYSTEMS: INCORPORATING EVOLUTION INTO THE FUNCTION OF NATURAL SYSTEMS

Estimating ecosystem function has primarily been undertaken from an ecology perspective. However, evolutionary processes play a fundamental role in determining the capacity of organisms to respond to rapid environmental change. Changes to trait evolution and the genetic diversity of populations resulting from climate change will have direct effects on their evolutionary potential, in

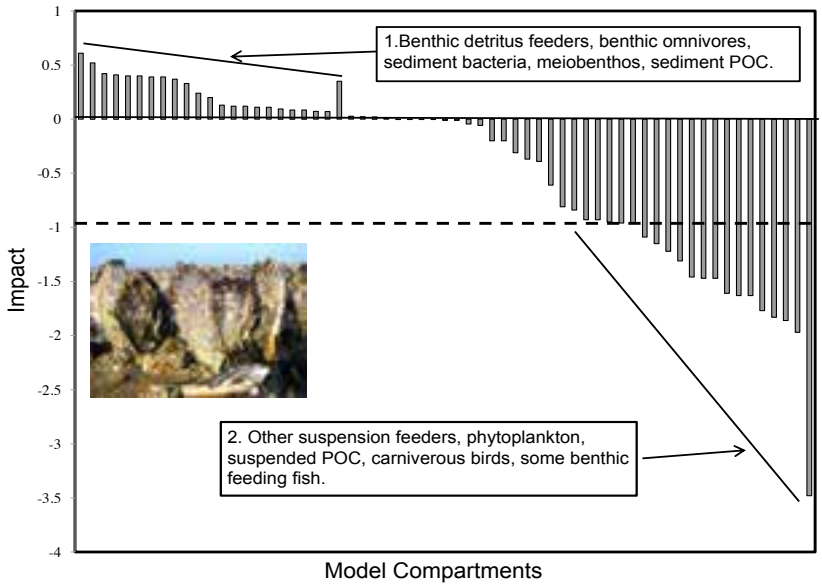

Figure 2: Impact of the Pacific oyster on the food web of the Sylt Rømø Bight. Each bar represents a specific influence on a particular component (species) of the food web. Positive bars represent a promoting influence, negative bars an inhibiting influence.

Source: © Modified after Figure 5 in Baird *et al.*, 2012 [1]; © Pacific oysters *(Crassostrea gigas)* Karsten Reise

that genetically depauperate populations are more prone to local extinction. Local extinction of particular species within an ecosystem will change how the remaining species interact, and this can alter the functioning of the ecosystem as a whole. Surprisingly, the interplay between evolutionary processes, species interactions, and ecosystem function is poorly understood. Additionally, the adaptive potential of populations may be influenced not only by genetic variation, but also by phenotypic and epigenetic variation. Yet, the role of these in population dynamics and resulting impacts on ecosystem function is not well known [3].

The same genotype can produce alternate phenotypes depending on the environment (phenotypic plasticity). Phenotypic plasticity allows organisms to respond adaptively to environmental change, and the resulting phenotypic variation can buffer the population from local declines. Importantly, plasticity can also occur across generations (transgenerational plasticity or TGP), whereby the environment experienced by parents can determine offspring phenotypes in future environments. One potential mechanism underlying TGP is epigenetic variation. Epigenetic variation arises from environmentally-induced changes to the function of the genome (e. g. changes to gene expression) without altering the underlying DNA sequence, and these epigenomes can be inherited across generations. Thus, changes to gene expression altering the response of individuals can scale up to changes in population dynamics, species interactions, and ecosystem function (Figure 4a).

In the Wadden Sea, surface temperatures are expected to increase by up to 4-5°C in the near future. Using the threespine stickleback (*Gasterosteus aculeatus*) as an evolutionary model, we showed that local populations harbor substantial genetic variation for several traits related to fitness (size, shape, survival), and that these traits are phenotypically plastic in response to warming (e. g. fish reach a larger size and have higher survival rates when reared at ambient (17°C) vs. elevated (21°C) water temperatures). TGP, in particular maternal and grand-maternal effects, can mediate some of the negative consequences of elevated temperature on offspring size and physiology. Specifically, offspring of mothers acclimated to 21°C reached a larger size when reared at 21°C compared to 17°C, and the underlying mechanism was optimized mitochondrial respiration inherited from mothers [4] (Figure 4b). These plastic physiological and growth responses of offspring can be traced back to heritable changes to the epigenome (transcriptome) of grand-mothers and mothers, providing a direct functional link between epigenetic and phenotypic variation [5]. The missing piece of the puzzle and area of future research is to estimate the impacts of this variation on changes to population dynamics and species interactions, and to incorporate these into an evolution-based ENA for the Wadden Sea, adding an evolutionary component to the functioning and resilience of (eco-) systems under climate change.

Figure 3: Sylt Mesocosms: Effects of climate change (i.e. increased temperature, CO_2 and nutrient concentrations) on intertidal communities of the Wadden Sea were tested in 12 tanks each holding 1800 l of seawater. Currents and tidal conditions were also simulated.
Source: © AWI/Claudia Pichler

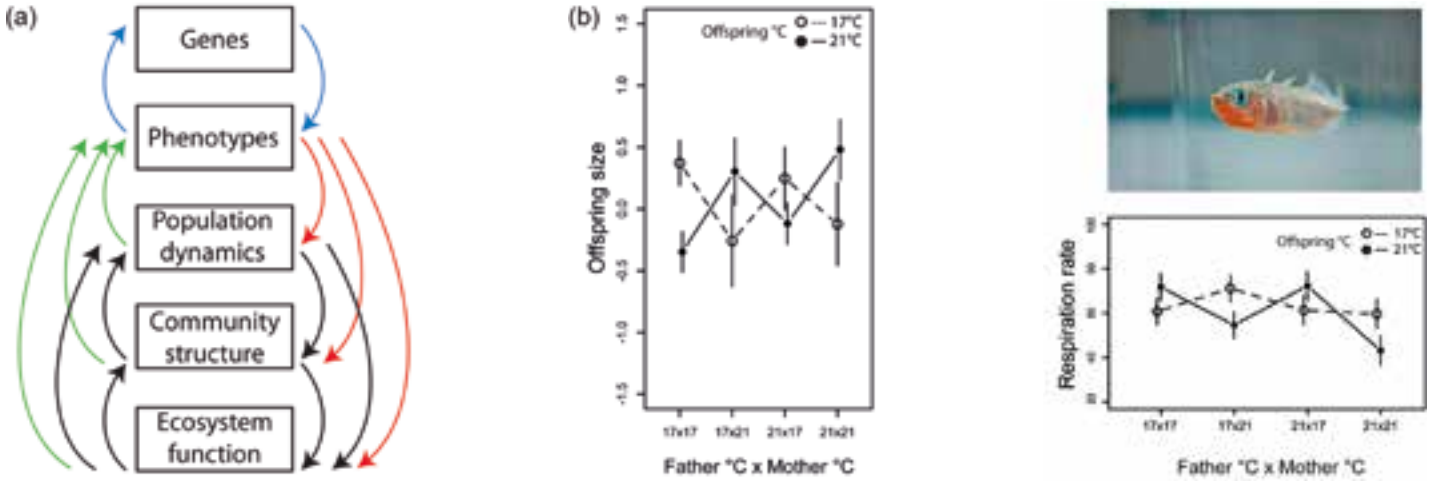

Figure 4: a) Eco-evolutionary dynamics: genes and phenotypes of organisms can influence population dynamics, community structure and ecosystem function – and vice versa, as ecological effects at the population, community and ecosystem level can feedback through plasticity or selection to influence phenotypic traits. **b)** Maternal transgenerational plasticity (TGP) in marine sticklebacks: offspring grow better via optimized mitochondrial respiration when reared in the same environment their mother experienced.

Sources: **a)** © adapted from Figure 1 in Hendry 2013 [3]; **b)** © Dario Fiorentino; **and** © adapted from Figures 2 & 3 in Shama *et al.*, 2014 [4]

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BRIEF DESCRIPTION OF RESEARCH PROJECTS:

INFOWEB: Influence of invasive species on the food web of the Wadden Sea. Bilateral Research Project (The Netherlands/Germany) of the Wadden Academy. Funded by NWO- BMBF. Partners are the Koninklijk Nederlands Instituut voor Onderzoek der Zee (NIOZ), Marine Research Department Senckenberg am Meer in Wilhelmshafen and the Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung (AWI). Leading Scientists are: Dr. Harald Asmus, Dr. Ingrid Kröncke and Dr. Henk van der Veer.

STopP: From Sediment to Top Predator. National joint research project in research program FONA/KÜNO, funded by BMBF. Partners are the Research and Technology Centre West Coast, Büsum (Prof. Dr. Stefan Garthe, Dr. Klaus Ricklefs), Christian Albrechts University Kiel (Prof. Dr. Klaus Schwarzer), the Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung (AWI; Dr. Ragnhild Asmus), and the Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig Holstein (Dr. Christian Reimers). The joint research project is coordinated by the Landesbetrieb für Küstenschutz, Nationalpark und Meeresschutz, Schleswig-Holstein (Kai Eskildsen).

BIOACID II: Biological impact of Ocean Acidification, Consortium 2: Responses of benthic assemblages to interactive stress, funded by BMBF. Partners are GEOMAR Helmholtz Centre for Ocean Research Kiel (Prof. Dr. Martin Wahl), the Leibniz Institute for Baltic Sea Research Warnemünde (Prof. Dr. Michael Böttcher), and the Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung Bremerhaven (Dr. Inka Bartsch and Dr. Lars Gutow) and Sylt (Dr. Ragnhild and Dr. Harald Asmus).

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Photo Lisa Shama: K. Baer, AWI; Photo H. Asmus and R. Asmus: Claudia Pichler, AWI

"ageing is an evolutionary trade-off"

An interview with Karl Lenhard Rudolph – gerontologist and systems biologist from Jena

How do people age? Is it possible to stop the process or at least reduce its impact? And if so, how do we do it? Gerontologists are looking for answers to these fundamental questions. Speaking to *systembiologie.de*, medical expert Karl Lenhard Rudolph from the Leibniz Institute on Aging talks about why our chromosome ends become shorter as we age and what systems biology can contribute to the field of gerontology. Some of the funding for his work comes from the German Federal Ministry of Education and Research's "GerontoSys – Systems Biology for Health in Old Age" program.

Systembiologie.de: *It looks like we will never achieve the dream of eternal youth. Despite this, what can gerontology achieve?*

Prof. Dr. Karl Lenhard Rudolph: It's true that we won't find the fountain of youth. The real issue is how we can stay healthy for as long as possible as we age, and this goal is certainly realistic. We have to decode and understand the mechanisms that cause dysfunction in old age and so increase the risk of illness. If we manage that, we can develop new forms of therapy that can contribute to improving the health of elderly people and, by extension, their quality of life.

How much progress have researchers made so far?

It isn't just one gene that is responsible for making us grow old. Instead, the ageing process is triggered by a host of factors. One is the way that telomeres, which form the ends of our chromosomes and stabilise them, cease functioning. Over the course of our lives, cells in our bodies undergo many divisions which see the telomeres become ever shorter and thereby gradually lose their protective function. This results in damaged DNA, which in turn promotes tumour formation. Experiments have now successfully been able to restore the function of telomeres. This can form the foundations for new forms of therapy.

What other processes underlie the ageing process?

An embryo's development within its mother's body is governed by means of signalling pathways. These provide every cell with precise instructions on what tissue it should form, i. e. bone or muscle. These signalling pathways remain active into adulthood, ensuring that the body remains capable of optimum performance for the purposes of reproduction and raising the next generation. What happens afterwards, ageing, can be described as an evolutionary trade-off. We think that the same signalling pathways which successfully manage physical processes up to the point of optimal fitness during reproductive age begin to go out of control as we age. This causes organs to change and lose some of their functioning capacity.

"Gerontology is making good progress"

You are currently studying the ageing of stem cells.

Stem cells are present in almost every type of human tissue, and throughout our lives, they contribute to maintaining and regenerating our organs. Blood stem cells are very important as they also form the cells of our immune systems. Today, we know that stem cells age too. One interesting discovery in recent years was the realisation that blood stem cells undergo mutations as we age. These developments cannot be identified before the age of 45. More recent research has shown that these mutations can be found in the blood of up to 50 % of 70-year-olds. The interesting fact here is that people with these mutations have lower life expectancy.

So are these mutations a biomarker for the ageing process?

Yes, but not just that. There is evidence that they also promote the development of illnesses, such as leukaemia and cardiovascular conditions. This is probably because the cells of the immune system are also generated by these mutated stem cells

and the mutations render these cells dysfunctional. It seems that, overall, the immune system plays a major role in disease development in the elderly as it is of systemic importance. The entire body depends on it. For example, we also know today that immune cells identify and remove aged cells. If the immune system no longer functions correctly, this could accelerate the ageing process itself by impairment with respect to the removal of damaged cells. We want to find out what causes these stem cell mutations and how this affects the immune response in old age.

When might we be able to use insights from gerontology to the benefit of patients?

Gerontology has made a lot of progress in recent years. We have clarified processes which play a crucial role in the development of illnesses and decline in organ function in old age. We also know so much about some of these processes already that we can think about therapeutic approaches for delaying the point when pathological changes begin to occur when we grow old. However, many years of research are still necessary before patients have access to these forms of therapy. We are still studying the fundamentals of these processes.

What exerts the greatest influence on how long we live, our genes or our lifestyles?

Genes account for some 30 %, lifestyle for about 70 %. We already know a lot about the impact of external conditions on the ageing process. For example, being overweight, smoking and excessive alcohol consumption shorten our lifespans. However, while people today lead much healthier lives than in the past, they still get compromised at old age. In other words, no matter how healthy my lifestyle is, there is still no way to escape ageing. This is the starting point for gerontologists' work. We are interested in the processes that underlie ageing and that affect us all regardless of how healthy we live.

People are living longer today. Is there a natural limit to life expectancy?

Average life expectancy in particular has increased. It has almost doubled in the past 150 years. In contrast, maximum life expectancy has remained virtually unchanged. In my view, this demonstrates the existence of an upper biological limit for the human lifespan. Gerontology can help us get gradually closer to this threshold, but I think that we will not be able to go far beyond it.

Professor Karl Lenhard Rudolph is the scientific director at the Fritz Lipmann Institute on Aging in Jena. He heads up a working group in a GerontoSys research project (Photo: Nadine Grimm/ FLI).

When does the ageing process start in the human body?

Ageing starts after the point when the human body achieves its maximum performance. For people, this is between the ages of 25 and 40. Subsequently, there is an increase in dysfunction and, concomitantly, disease development. This is slow at first but becomes faster later on.

What role does systems biology play in gerontology?

It is an important method in gerontology because we have to view the body's systems as a whole. You can't simply study one single type of cell in isolation but instead look at the entire organism if you want to decode the complex process of ageing.

Interview conducted by Melanie Bergs and Gesa Terstiege.

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understanding ageing in a systematic manner

GerontoSys initiative: the Sybacol research core in Cologne

by Martin Höhne

Changes in the age structure of our society pose major social, medical and economic challenges. The German Federal Ministry of Education and Research realized the seriousness of this at an early stage, and it has made an important contribution to Germany's scientific capacity by creating the GerontoSys initiative to promote systems biology projects in the field of ageing research. This platform has facilitated the successful integration of systems biology and the biology of ageing and secured long-term collaboration between the two fields. System-wide and cross-system research play a key role in enhancing our (molecular) knowledge, something that is particularly pertinent for processes that are as complicated as ageing. The Sybacol research core in Cologne is an excellent example of the GerontoSys initiative's success.

Systems biology and the biology of ageing in Cologne

The Sybacol ("Systems Biology of Ageing Cologne") research core in Cologne started receiving funding from the GerontoSys initiative in September 2011, and the program is to run until August 2017. Sybacol is a research association of scientists from the University of Cologne, University Hospital Cologne, the Max Planck Institute for Biology of Ageing and the Max Planck Institute for Metabolism Research. Its scientific coordinator is Prof. Thomas Benzing from the university hospital, and he is supported by Prof. Andreas Beyer from the university and Prof. Adam Antebi from the MPI for Biology of Ageing.

The ultimate objective of the Sybacol program is to expand our knowledge and understanding of the dynamics exhibited by the ageing process and age-associated illnesses on the level of molecular systems biology. Only when the molecular foundations are clear can we start thinking about the final step of "translation", i. e. making practical use of research findings in day-to-day clinical practice.

To achieve this goal, Sybacol selected two topics as the focal points for its work: (1) systems biology of *longevity* signaling pathways and (2) microRNA-dependent control of gene regulatory networks in metabolism and stress resistance.

(1) Systems biology of *longevity* signaling pathways

One of the most remarkable findings in ageing research in recent decades is that the manipulation of different molecular signaling pathways can dramatically extend various organisms' lifespans. Some examples include lowering the activity of the insulin signaling pathway, germline removal, diminishing mitochondrial activity, increasing HIF1-α activity (hypoxia signaling pathway), or reducing caloric intake. At Sybacol, research into these signaling pathways largely uses the nematode *Caenorhabditis elegans*, a very well-established model organism (Figures 1 and 2). This is possible because basic molecular signaling pathways are conserved during evolution, regardless of the huge differences between organisms such as nematodes and humans. In this research area, we have been looking at a range of topics, such as if (and how) the different signaling pathways are connected with each other and if these signaling pathways potentially activate the same effector mechanisms, i. e. if the activation or repression of a common set of effector genes is the outcome of the different *longevity* signaling cascades. Our work so far does indeed suggest that at least some of the *longevity* signaling pathways we have studied ultimately regulate a shared group of genes. For example, the

Figure 1: Only 1 mm long, the nematode *Caenorhabditis elegans* is what is known as a model organism, and its use in research has facilitated outstanding discoveries relating to biological questions. Its short generation time, inexpensive and straightforward breeding requirements and genetic manipulation options are why this small worm is so popular in the field of ageing research. The left-hand section of the image shows the culture plate with a bacterial lawn that serves as food. A normal pin serves to illustrate the animals' size. The middle section shows an adult worm and the point of the pin under the microscope. Worm paths through the bacterial lawn are clearly visible. A larger image of a worm is on the right (Source: Martin Höhne).

group headed by Adam Antebi has identified the transcription factor complex MML-1/MXL-2, which is part of the regulatory network into which a range of *longevity* signaling pathways are routed (Nakamura *et al.*, 2016).

Another focal topic in this research area is the link between DNA damage and ageing (Ribezzo *et al.*, 2016). There is no doubt that the accumulation of DNA damage plays a crucial role in the ageing process. Luckily, cells and organisms can deploy a range of repair mechanisms to deal with the continuously occurring DNA damage. Within the Sybacol team, a lot of research effort focuses on understanding these DNA repair mechanisms on a molecular level.

(2) MicroRNA-dependent control of gene regulatory networks in metabolism and stress resistance

In recent years, it has become increasingly clear that small RNA molecules known as microRNAs play an important role in gene regulation. Within the Sybacol consortium, microRNAs are being studied for their influence on the ageing process and changes to metabolic processes. For example, one subproject is looking at microRNAs' regulation of brown adipose tissue (BAT). This special type of fatty tissue can convert energy stored in the form of carbohydrates or fat into heat during the thermogenesis process, and it has only recently once again become the focus of scientific research. It has been shown that the quantity and activity of brown adipose tissue falls with increasing BMI (body mass index) and with increasing age. Sybacol research scientists have been able to show that using a pharmacological inhibitor to manipulate this microRNA-BAT loop can enhance "metabolic fitness", which is also relevant for ageing (Oliverio *et al.*, 2016). Other studies that are currently under way will now have to show if this promising result can also be used in clinical practices ("translation", see below).

In this Sybacol research area, researchers in different subprojects use different model organisms, such as *C. elegans*, the fruit fly *Drosophila melanogaster* and mice in their work. Fruit flies, for example, are used for studying networks connecting insulin and mTOR signaling pathways and their role in regulating metabolic and ageing processes (Essers *et al.*, 2016).

The close links between research scientists within the consortium make it very easy to compare and integrate data. This integration of experimental data from different organisms is beneficial and sometimes even necessary for understanding the general principles underlying the different signaling pathways.

Translation

There is no doubt that no single funding program can cover the entire period of time between fundamental research as performed at Sybacol and the application of our findings in the

treatment of patients. Nevertheless, we are doing everything we can to ensure that this transfer from research to clinical practice, called "translation", happens as soon as possible. The special drive spurring this on is last but not least the fact that the interdisciplinary research core is made up of not just biologists, geneticists and bioinformaticians, but it also includes clinicians. Stress resistance is of particular interest when it comes to translation. In model organisms, a longer lifespan is often connected on a cellular level with increased resistance to cellular stress. For example, using our findings from experiments on *C. elegans*, we were able to develop a mouse-based model for acute kidney failure in which the animals' kidneys displayed much better values than in the control group. This is a key first step towards clinical use, and we are working hard on the next stages.

Is the research worth the money?

Via the GerontoSys program, the German Federal Ministry of Education and Research invested just over EUR 50 million, a major sum of money, in a range of projects in the field of systems biology in ageing research. The Sybacol research core is one such project. Looking at the Cologne group, we can only say that the investment was definitely worth it. Our level of knowledge has increased in leaps and bounds. In addition, the impetus supplied by GerontoSys funding has now resulted in a permanent chair for systems biology in Cologne, and this has created new, long-lasting structures. As a result, even after the GerontoSys funding ends, research scientists in Cologne will be able to establish and conduct successor projects in the field of systems biology of ageing research.

Research project profile:

Systems Biology of Ageing Cologne (Sybacol) is a research initiative that is part of the GerontoSys2 program funded by the German Federal Ministry of Education and Research (BMBF). Research scientists from the University of Cologne, University Hospital Cologne, the Max Planck Institute for Biology of Ageing and the Max Planck Institute for Metabolism Research work on a single campus to understand the complex processes involved in the ageing process.

The interdisciplinary research core pools the expertise of biologists, physicians, physicists and bioinformaticians. Together with the Max Planck Institute for Biology of Ageing and the Cologne Cellular Stress Responses in Aging-Associated Diseases (CECAD) Cluster of Excellence, Sybacol is an integral part of a research environment that has taken up the challenge of studying the ageing process.

Participating partners: Prof. Adam Antebi, Prof. Thomas Benzing, Prof. Johannes Berg, Prof. Andreas Beyer, Prof. Jens Brüning, Prof. Christoph Dieterich, Prof. Joachim Krug, Prof. Michael Lässig, Prof. Linda Partridge, Prof. Björn Schumacher.
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Figure 2: One of the signaling pathways being researched in the Sybacol consortium is the hypoxia signaling pathway. To generate conclusive findings, the worms must be subjected to a precisely defined level of hypoxia (oxygen deprivation) for a certain period of time. As no suitable equipment is available for this, special hypoxia chambers were developed and constructed in close collaboration with research scientists and workshops at the University Hospital (Source: Martin Höhne).

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systems medicine and multiple myeloma

Hope for treatment in the form of personalized therapy

by Hartmut Goldschmidt

"CLIOMMICS – Clinically-applicable, omics-based assessment of survival, side effects and targets in multiple myeloma" is an e:Med consortium project that applies systems medicine approaches to the study of multiple myeloma. The aim is to take personalized therapy for myeloma sufferers to the next level so that a CLIOMMICS report will be available in the near future. Thanks in particular to the identification of myeloma-specific target structures, this will make it possible to customize treatment to each patients´ needs. Furthermore the understanding of molecular mechanisms and biological processes will be increased. Working groups from Heidelberg's university hospital, the National Center for Tumor Diseases (NCT) and the German Cancer Research Center (DKFZ) are all involved in the CLIOMMICS project.

Myeloma

Multiple myeloma is a genetically heterogeneous malignant disease of monoclonal plasma cells, and the proliferation of myeloma cells in the bone marrow is its hallmark. The disease's main symptoms are raised serum calcium, kidney damage, insufficient haematopoiesis and bone pain. In the latest guidelines, myeloma-defining criteria also include factors indicating threatening organ failure and/or bone damage. Multiple myeloma is the second most widespread haematological neoplasm in Germany, with some 6,000–7,000 new cases every year. Two-thirds of patients are above the age of 65 when diagnosed.

The condition develops only as a "monoclonal gammopathy of unknown significance" (MGUS), which is not a disease in itself but instead an irregularity in lab values. By definition, sufferers do not display any symptoms, and their illness is normally discovered by chance following a blood test. A "smouldering multiple myeloma" (SMM) is positioned mid-way between MGUS and multiple myeloma, and it too lacks the signs of illness typical of multiple myeloma.

In recent years, the introduction of new therapeutic substances has significantly improved the prognosis for patients with multiple myeloma. However, new drugs have also had side effects, most notably diseases affecting the peripheral nervous system and toxicity problems regarding the blood and blood-forming organs. Our work focuses on inducing long-term remission of over ten years in a subgroup of myeloma patients and effecting a cure for these people.

Necessary conditions for CLIOMMICS systems medicine in Heidelberg

Germany's education and research ministry has provided funding for the ambitious and large-scale project "CLIOMMICS – Clinically-applicable, omics-based assessment of survival, side effects and targets in multiple myeloma" at Heidelberg's myeloma center since 2013. The consortium, comprising the University Hospital Heidelberg, the NCT and the DKFZ, merges scientific data from molecular analyses (interphase fluorescence in situ hybridization, gene expression analyses, RNA sequencing, single-nucleotide polymorphism analysis) and imaging with clinical data. Within this context, CLIOMMICS interacts with IIT studies performed by the German-Speaking Myeloma Multicenter Group (GMMG), designed under the supervision of Hartmut Goldschmidt in Heidelberg and performed throughout Germany. Within the GMMG study group, 2,830 patients have been treated over the past 20 years as part of prospective phase II/III studies. The existence of a myeloma materials bank in Heidelberg is another factor that contributes to the excellent conditions for work on systems medicine issues within the CLIOMMICS program. Since 1996, cells and/or serum from people with plasmacellular diseases have been collected both within and outside of studies.

Figure 1: Heidelberg myeloma group in early summer 2016 (Source: University Hospital Heidelberg).

Since 2001, the malignant plasma cells which represent a relatively small subset of bone marrow cells, were enriched prior to analysis. As a result, translational research projects today have access to over 15,000 DNA, RNA and cell samples.

As long ago as 1992, work began in Heidelberg to document the clinical courses of the diseases of patients with MGUS, SMM and symptomatic multiple myeloma. The clinical data of 873 Heidelberg non-study patients who underwent an autologous transplant were continually documented for the Heidelberg myeloma register. Together with the data of Heidelberg patients participating in prospective GMMG studies, this information forms the basis for one of the world's largest myeloma register, and it has been used as a source of basic data for scores of systems medicine research undertakings.

For many years, researchers have also been collecting extensive molecular data on multiple myeloma, with information regarding genome and post-genome research (gene expression analyses, interphase fluorescence in situ hybridization, RNA sequencing, single-nucleotide polymorphism analyses) and immunological data becoming gradually more important.

What insights into multiple myeloma is systems medicine expected to deliver? What are the objectives?

The CLIOMMICS group is working on a special IT infrastructure and multi-stage data management concept so the project can evaluate the immense quantity of data coming from molecular and clinical projects. Mathematical models are used for combining these data with each other and for linking them to already

Research and clinical care for patients go hand in hand

Research and clinical patient care go hand in hand at the Heidelberg myeloma center (medical clinic 5 at the university hospital) and the National Center for Tumor Diseases (NCT). Whether they are outpatients or inpatients, everyone receives high-quality medical treatment that is tailored to suit their overall physical condition, age, previous forms of therapy and risk factors. Such treatment is generally undertaken within the framework of studies.

With 380 first contacts, 120 second opinions and approx. 300 written responses to questions from patients and medical peers, the Heidelberg myeloma center is a high-profile international reference center for doctors and patients alike. Contact with patients reveals clinical issues, and these, together with results from quality peer-reviewed research activities (DFG, European Union, German Federal Ministry of Education and Research, International Myeloma Foundation, Dietmar Hopp Foundation) are incorporated into new ideas for the group's experimental trial concepts with the objective of improving myeloma diagnoses and therapy (Raab *et al.*, 2016). Clinical studies that are linked to a biomaterials bank and a clinical cancer register are the cornerstones for the success of myeloma research in Heidelberg. "This is the only way to bring the translational strategy to life, i. e. make translation into clinics a success," says Prof. Dr. Hartmut Goldschmidt, head of the multiple myeloma team. He and PD Dr. Dr. Dirk Hose have been coordinators of the CLIOMMICS research group since 2013.

known and potential prognostic factors. Integrated statistical prediction models regarding the course of diseases are developed and deployed in routine clinical work for patient-specific decisions about therapy options. The results so far show that inherited germline variants increase familial risks of developing multiple myeloma. Researchers have now identified a total of 17 risk markers (what are known as single-nucleotide polymorphisms, SNPs) that heighten the risk of developing myeloma (Mitchell *et al.*, 2016). Together with other studies, these results confirm the model of multiple myeloma inheritance. Germline variation also influences a person's risk of developing polyneuropathy (PNP) if they suffer from multiple myeloma (Campa *et al.*, 2016) or a myeloma-linked bone disease (Johnson *et al.*, 2016). Researchers have been able to identify the responsible gene loci, but further studies and functional analyses are still necessary so that we can better understand the underlying biological processes.

It has also been possible to demonstrate that certain germline variations in MGUS patients lead to an increased risk of developing multiple myeloma (Weinhold *et al.*, 2014). In other words, the role of inherited risk variants does not come into play only when malignant transformation occurs. Instead, it has an impact on the myeloma's premalignant early stage, MGUS. In the event of early-stage multiple myeloma such as MGUS or SMM, new prognostic factors can permit us to make better predictions regarding if and how fast this pre-existing condition will develop into symptomatic multiple myeloma. Other results show the impact of, for example, translocations and gains in terms of overall survival for myeloma patients.

The long-term goal of CLIOMMICS is to include all therapy-relevant information available for a patient in a single report. This CLIOMMICS report will permit doctors to make a better prognosis and make it possible to deploy these technologies for the first time in clinical routines. This will have a twofold effect: the effectiveness of the therapy can be improved by the use of new forms of medication. And secondly, changing drugs can significantly help to reduce side effects. These options will represent a major step forward in personalized therapy for patients with multiple myeloma.

Research project profile:

e:Med (measures for establishing systems medicine) is a research and funding scheme supported by the German Federal Ministry of Education and Research (BMBF) to establish systems medicine in Germany. The name stands for the electronic processing and integration of medically relevant data from a range of knowledge sources within the systems medicine model. CLIOMMICS stands for "Clinically-applicable, omics-based assessment of survival,

What we consider systems medicine to be

The new buzzword, systems medicine, is playing an increasingly significant role in the discussion about the future of healthcare. One meaning of systems medicine is **personalized or customized medicine**, whereby approaches are based on mathematical models that combine prognostic factors and extensive genomic, postgenomic, immunological and clinical data from medical activities with methodologies drawn from the fields of mathematics and information sciences. Integrated statistical models to predict the course of an illness are developed, and doctors make use of these in their clinical routines to make therapeutic decisions based on an individual's data.

Systems medicine also has another meaning, connected to highly application-focused **translational concepts**. Myeloma research in particular has a lot of translational potential which is currently not exploited in full. There are several reasons for this, such as the high demands which trial concepts, structures and processes have to meet. Within the same trial various and large amounts of scientific and clinical data have to be collected at the same time. The data have to be evaluated and links between the different items of data have to be established and made usable.

Within systems medicine, the use of IT tools for medical objectives is seen as particularly important. Strategies comprise highly developed omics research methods, big data and mathematical modelling. Systems medicine can only succeed if the right infrastructural preconditions regarding the materials banks (standardized sample-taking, processing, storage of blood and bone marrow samples), data management, data interpretation and medical informatics have been securely put in place to ensure processes of sufficient quality. Systems medicine is the bridge between research and everyday clinical activities.

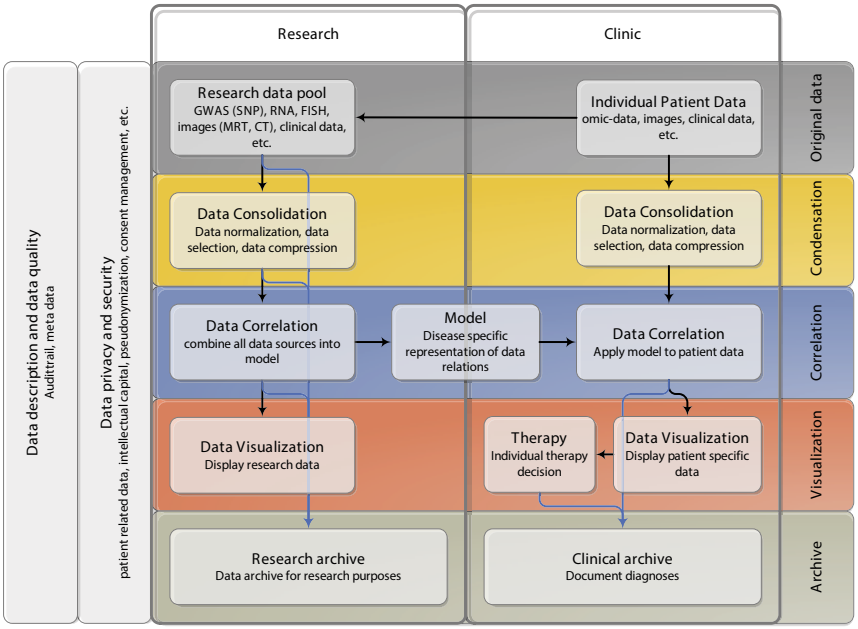

Figure 2: Data management and modelling.
Five-stage model for data management and building a comprehensive stochastic model for multiple myeloma. Using the group's existing data, researchers develop a model (left), to which they apply an individual patient's data during clinical care (right), thereby providing support for a decision regarding customized therapy (Source: University Hospital Heidelberg).

side effects and targets in multiple myeloma", which in turn represents improving options for preventing myeloma, and a comprehensive myeloma diagnosis and individually customized treatment plan for multiple myeloma patients. A consortium of the University Hospital Heidelberg, the National Center for Tumor Diseases (NCT) and the German Cancer Research Center (DKFZ) makes up the CLIOMMICS team, which is headed by Hartmut Goldschmidt and Dirk Hose. Petra Knaup (University Hospital Heidelberg), Kari Hemminki (DKFZ Heidelberg), Anja Seckinger (University Hospital Heidelberg), Annette Kopp-Schneider (DKFZ Heidelberg) and Thomas Hielscher (DKFZ Heidelberg) are senior partners in the undertaking.

Further information:
www.sys-med.de/en/consortia/cliommics/

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Photo: University Hospital Heidelberg

"germany provides me with the ideal setting for my work"

Interview with Michael Ziller, a member of the up-and-coming generation of e:Med researchers

Michael Ziller leads one of the eight groups of young scientists within the e:Med support program. He moved from Harvard University to the Max Planck Institute of Psychiatry in Munich several months ago to take up the position. Speaking to *systembiologie.de*, the bioinformatics specialist and physicist talks about the opportunities available to him in Germany and about the working conditions that shape young researchers' everyday activities in Germany and the USA.

Systembiologie.de: *After spending almost seven years in Harvard, you returned to Germany at the beginning of April. Why Germany, and why Munich?*

Dr. Michael Ziller: Germany is one of the world's leading locations for scientific research. I opted for Munich because the Max Planck Institute of Psychiatry provides me with an ideal setting for my work. In addition, the city is also home to three more institutions where countless world-class specialists work, people who could be very important for my research. The institutions in question are the psychiatric clinic of LMU Munich, Helmholtz Zentrum München and the biology faculty of the Technical University of Munich.

What motivated you to take this step at this moment in time?

My family and I had to make the decision whether or not to stay in the USA for five more years and build a lab there, or to return to Germany. We made our choice based on the attractive conditions for research here and our family connections to the country, though we had mixed feelings about saying goodbye to Cambridge, Massachusetts, and heading back to Germany. I would add that the same goes for most German research scientists in Boston. People often talk about the brain drain, but about 80 % of the German postdoctoral researchers I got to know in the US were also considering coming back.

Research structures in Germany have changed in the past ten years.

In my opinion, the scientific community has benefited a lot from these changes. As I see it, setting new objectives and creating organisational structures, for example excellence clusters, enhances everybody's ability to produce good work. For young research scientists like myself, there are lots of opportunities to establish our independence along with our own research programs.

To me, the sheer scope of this research landscape, with universities and major scientific institutions, represents a great advantage. There is also a lot of state funding, along with an excellent infrastructure and ready access to large items of equipment. In contrast to the situation in Germany, scientists in the USA have to secure almost all of their lab funding through external grants, including most of their own salary.

What advantages does the ministry's e:Med program for young researchers give you?

The program provides me with constant funding for five years and at the same time lets me choose my topic and research field. I can focus all of my attention on my research and don't have to worry about anything else. To me, this is the best possible situation. As an assistant professor in the US, my start-up package would have been enough for about three years. During this period, I would have needed the National Institutes of Health (NIH) to approve one, or better, two, major research applications, otherwise the lights would have literally gone out in my lab.

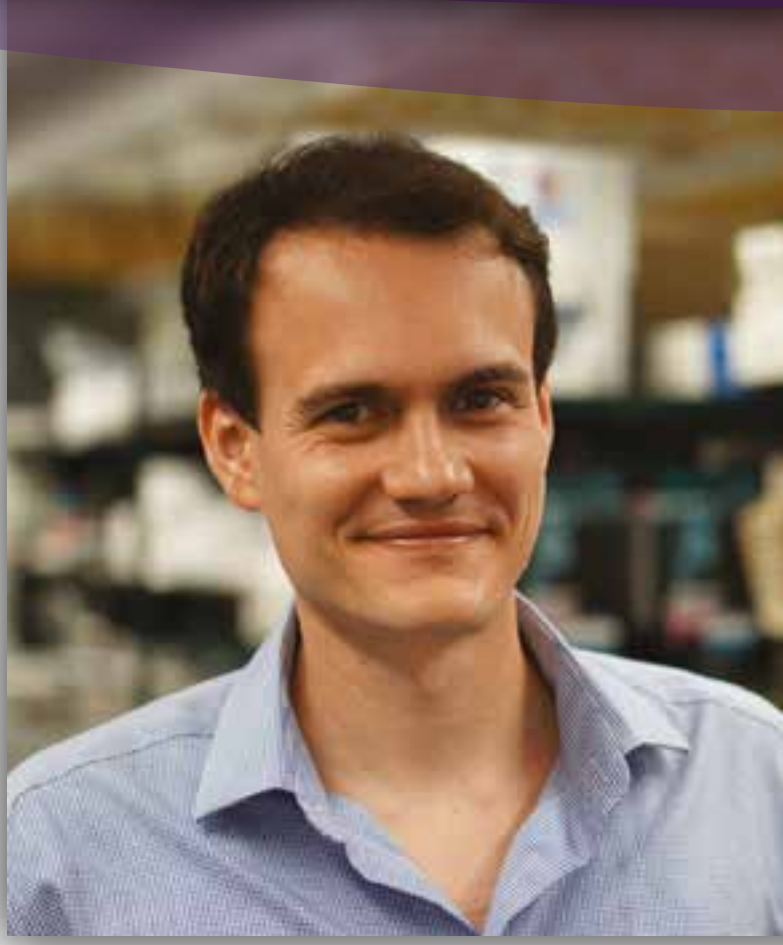
Long-term opportunities are often in short supply in Germany, particularly for young scientists. What does the country still need to do about this?

I think this is the most significant drawback about Germany's scientific landscape. There aren't enough institutional tenure track options with transparent evaluation. For example, I think it would be great if tenure options with government funding could be created for the junior researcher programs run by the education and research ministry, the DFG or Max Planck. These would give young scientists definite orientation for their future, and they would also eliminate the serious setbacks that affect a team when it has to regroup time and time again at different locations. At the same time, doing things in the long term would enable much better integration of groups of young researchers into their local research landscapes and promote continuity when it comes to collaboration.

Boston is seen as a city where different disciplines work in close cooperation with one another. Can you say the same about Munich?

The dense network of scientists, research institutes and infrastructure elements in Boston is unequalled. The extensive networking and sheer degree of collaborative work between the different, sometimes even rival, institutions are particularly impressive.

There are some similarities in Munich, as the city also has a large number of excellent groups and a local science cluster of international quality. There are great connections between scientists from the different institutes, and they take all part in meetings and symposia. For example, my group works with the LMU and Helmholtz Zentrum research groups. Links to biotech firms are also, in part, very extensive.



Michael Ziller heads up one of the eight groups of young scientists within the e:Med support program (Photo: Michael J. Ziller, MPI of Psychiatry).

You lead an e:Med working group. What advantages does this research and funding program confer?

The e:Med program lets me be part of a wider community that is interested in similar questions but that also studies different systems and illnesses. It provides institutional platforms for getting to know other scientists and exchanging information with them. There are, for example, annual meetings for all groups and topic-based project groups. These are valuable to me because they let me establish links throughout Germany and expand my network. This helps integrate me back into the local research scene and get to know interesting project partners. Lots of the computer-aided methods that we develop when researching schizophrenia can be applied to other complex illnesses and phenomena such as diabetes, multiple sclerosis and the ageing process.

Biologists, mathematicians and computer sciences work in tandem in your working group. Why is this important to you?

In my view, interdisciplinarity does not consist of each specialist group within an interdisciplinary research project focusing only on answering the part of a question that falls within its field. It should much more be a case of developing thought processes, questions and strategies in an all-encompassing manner from the get-go.

My lab is half wet lab, where we work with cell cultures and molecular biology, and half bioinformatics lab. My own background is in bioinformatics and physics, but I worked in a wet lab during my doctorate. The issues we work on are at the point where different fields intersect, so combining different approaches is the only way to address them. In contrast to the classic, reductionist paradigm, it is essential for us to understand not only how the individual components function, but also how they interact with one another. This requires measuring and describing the different elements, and in-depth biological expertise is a precondition for it to work. At the same time, complex computer models are necessary so that we can piece together the different items of information to form a meaningful whole. Using statistical learning processes to fill these models in turn brings them to life. This way, we combine the purely data-driven *bottom-up* approach to generating models with a host of biology-derived information. Lab researchers then use experiments to assess these models' predictions, following which the models undergo further refinement.

Interview conducted by Dr. Bettina Koblenz and Dr. Marco Leuer.

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Genomics of schizophrenia

Systems genomic approaches to research into complex illnesses

Mental illnesses represent some of the most serious medical challenges facing us in the 21st century. Schizophrenia alone affects about 1 % of the world's population. The causes of this illness are complex, as they are shaped by the combination of genetic predisposition and environmental factors, such as lifestyle and personal history. Decoding the molecular and genetic triggers has so far proven to be an extremely difficult task, but this undertaking is viewed as being particularly promising if we want to understand the causes of schizophrenia and find better and faster forms of treatment.

The genomes of several tens of thousands of schizophrenia patients were analysed during the course of large-scale genome wide association studies. Researchers have identified thousands of genetic variations known as single nucleotide polymorphisms (SNPs) that appear with greater statistical frequency in the genomes of people with schizophrenia than in people unaffected by the illness. However, we still do not know what role these SNPs play in the pathogenesis of the condition. One reason is because some 90 % of them are ***not*** located in the genes, but in the non-coded genome sections. The function of these sections is largely unknown, but a small fraction, the gene regulatory elements, serve as molecular switches controlling how and under what conditions genes are activated. Another reason for the lack of clarity is that schizophrenia-associated SNPs are often present in healthy people. Individual SNPs contribute virtually nothing to the risk of developing schizophrenia. It is only the combination of numerous small changes caused by SNPs and the different environmental factors that triggers the illness.

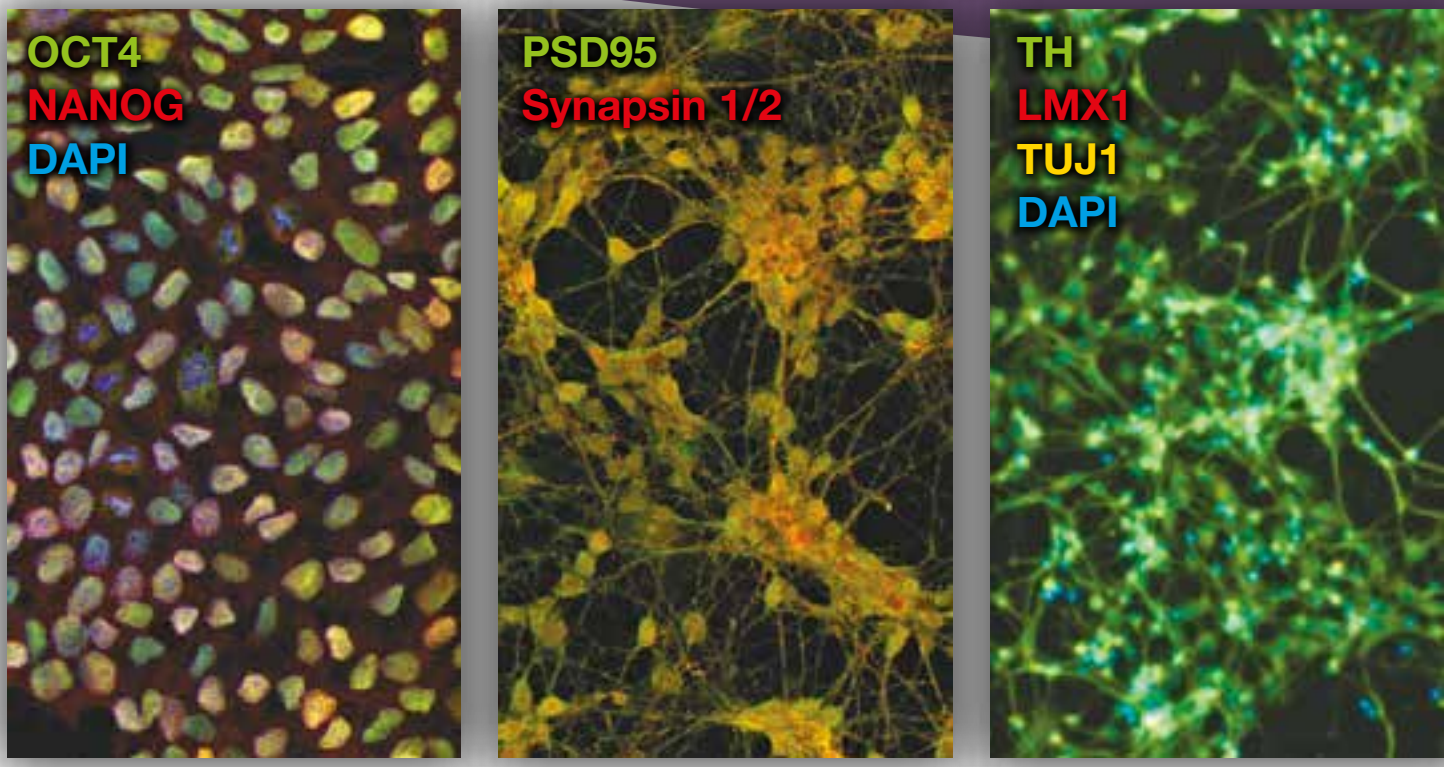

Figure 1: Creating different neurones with various characteristics from iPSCs. From left to right: Induced pluripotent stem cells (iPSCs) from patients with schizophrenia (SCZ) (green: OCT4, red: NANOG, blue: DAPI); cortical neurones generated from SCZ iPSCs form synapses (green: PSD95, red: SYNAPSIN 1/2); dopaminergic neurones generated from SCZ iPSCs (green: TH, red: LMX1, yellow: TUJ1, blue: DAPI) (Source: Michael J. Ziller, MPI of Psychiatry).

The goal of Dr. Michael J. Ziller's research group, which receives funding from the German Federal Ministry of Education and Research (BMBF), is to improve our understanding of the molecular causes of schizophrenia. For this, the function of the many SNPs in the genome's non-coded sections needs to be clarified. The underlying hypothesis is that the bulk of these SNPs do not influence gene function in itself, but instead regulate the intensity of gene activity. Scientists believe that the SNPs interfere with the normal workings of genetic regulators in brain cells and thereby increase an individual's chances of developing schizophrenia.

The research team uses human pluripotent stem cells that are transformed into different neural cell types in the lab (Figure 1), and members then apply different high-throughput processes to identify which SNPs actually have an impact on a molecular level. Using the latest computer-aided models, they can then combine the different SNPs' effects and mechanisms of action to predict the consequences for the entire cellular system. In a subsequent step, these predictions are tested in experiments that use induced pluripotent stem cells taken from schizophrenia patients. These correspond to embryonic stem cells and can be transformed into cells of every other type. At the same time, they possess the genetic makeup of the schizophrenia patient and are therefore suitable as testing systems for the disorder.

In a parallel step, Ziller's team also uses genome editing processes to artificially insert genetic variants into control stem cells. These genetically manipulated stem cells generated by schizophrenia patients are then transformed into different types of neural cells and undergo molecular and physiological analyses. Afterwards, they are compared with the cells of healthy individuals to permit a direct validation of the predicted effects. This process establishes links between the SNPs, the molecular changes and their physiological consequences, thereby helping to reveal the mechanisms behind the condition. The resulting insights can then be used to group patients with similar diagnoses but different molecular profiles. At the same time, the findings are the starting point for developing new pharmacological intervention strategies and customised therapeutic treatment.

learning about the regeneration of the liver

The first junior systems biology group in DFG's Emmy Noether Programme

by Stefan Hoehme

Since the beginning of the year, I have been the head of a new research group for computer science at Leipzig University. It is the first group working on systems biology to receive funding from the DFG's Emmy Noether Programme. As a computer scientist who follows an interdisciplinary approach, I have for many years focused on the computer-aided analysis of microscopy image data and the mathematical modelling of biological tissue based on this data. I was a researcher in the Virtual Liver project (2010–2015) and am currently involved in the LiSyM programme, a research network for systems medicine on the liver (since 2016). Both are funded by Germany's Federal Ministry of Education and Research (BMBF). Fascinated by the liver's ability to regenerate itself, my working group is now studying the underlying mechanisms of this process.

While the variety and dynamism of biological systems make them truly compelling, these factors also make studying biological processes correspondingly difficult as individual elements interact with one another on extremely different scales in terms of size and time. Researchers need to factor in processes between individual molecules, look at the interaction between the cellular and tissue levels, and consider the functions of entire organs. In the case of cancers, for example, it only takes the slightest, often genetic, malfunctions in the cells' control mechanisms to trigger uncontrolled growth, the impact of which can be life-threatening if on the level of an organ.

Among the issues that my Leipzig research group is looking at, we are conducting in-depth research into the regeneration of the liver following damage due to toxic substances, and into the healing process following the removal of part of the organ, for example due to a transplant or tumour removal. The liver has the fascinating and unique trait of being the only internal organ that can fully regenerate itself, even after serious damage. Understanding this amazing ability better is one of the central objectives of the new working group in Leipzig. The cross-scale regulation of cell division and movement plays an important role in liver regeneration, but our understanding of it is so far merely fragmentary.

Several years ago, in a joint undertaking with colleagues working on experiments, we were able to show that capillary blood vessels in the liver, called sinusoids, play a far larger role in coordinating cell division and movement than had previously been thought (Hoehme *et al.*, 2010). At the time, I was still doing my postdoc in Dr. Dirk Drasdo's working group at INRIA (Institut national de recherche en informatique et en automatique), which had been conducting research into tissue modelling for decades. The model-based predictions had been verified by experiments performed in the lab of Prof. Jan Hengstler at the Leibniz Institute in Dortmund. The results have since been independently confirmed several times by other groups. Today, close interdisciplinary collaboration with experiment-conducting groups, such as Prof. Jan Hengstler's or Prof. Ursula Klingmüller's teams at the German Cancer Research Center in Heidelberg, is still essential for my working group's systems biology research. After all, combining and analysing experimental data is the only way to create conclusive, three-dimensional tissue models.

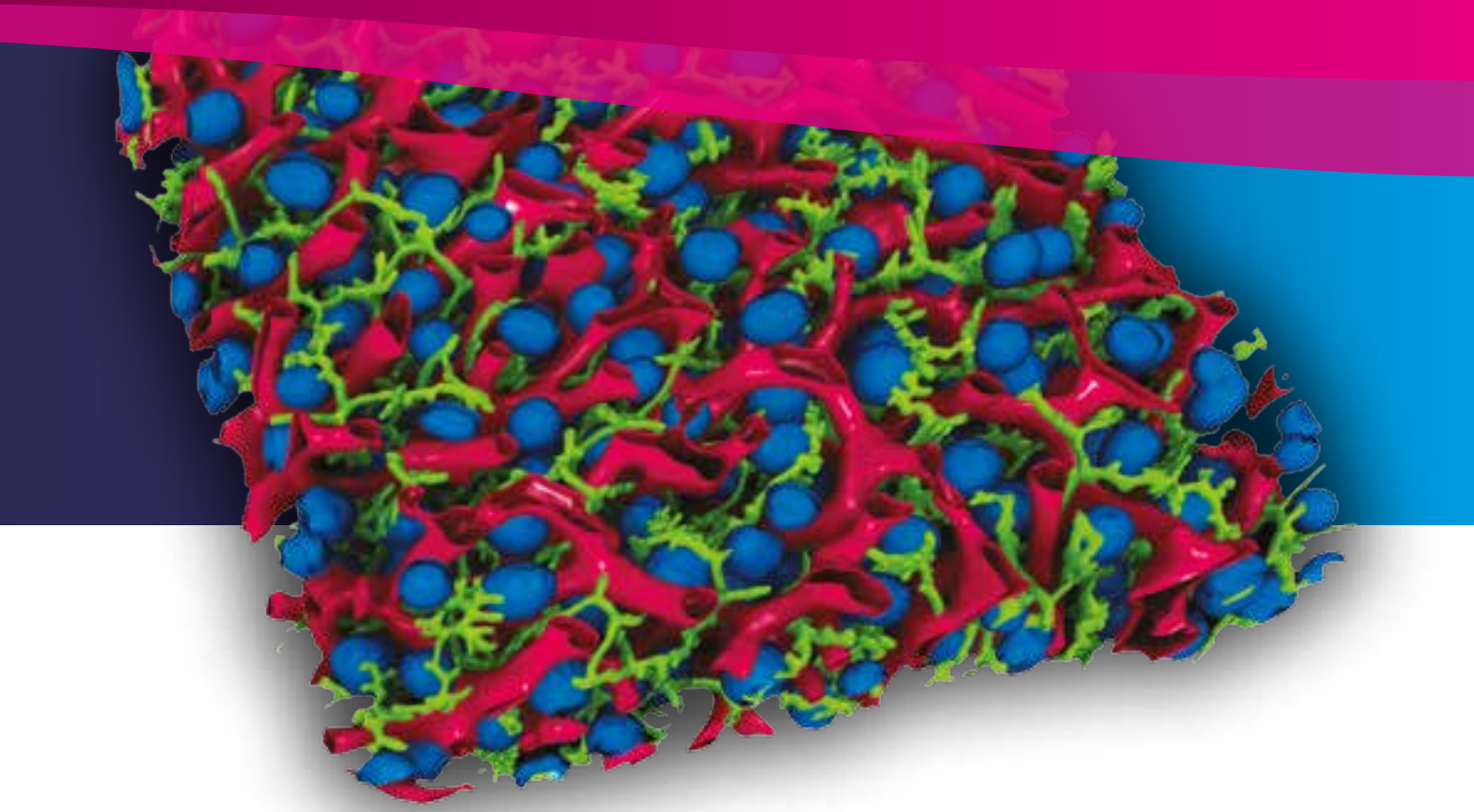

Figure 1: Three-dimensional representation of a segmentation showing a typical confocal microscopy image of murine liver tissue. The illustration shows cell nuclei in blue, blood vessels (sinusoids) in red and bile canaliculi in green. TiQuant was used to generate the segmentation image (Source: Adrian Friebel).

Understanding microscopy

Analysing visual data as acquired using confocal microscopy plays a particularly significant role in this process, as processing and quantifying these data are the first steps towards mathematical modelling. With this in mind, one focal issue for our research is the reconstruction and quantification of all kinds of tissue structures, such as cells or blood vessels, from microscope images (Schliess *et al.*, 2014). In recent years, there has been tremendous progress regarding the methods used for this and the associated options for processing and analysing images. There are scores of new techniques, for example for identifying the precise three-dimensional shape of cells and lobules in the liver without the use of special dyes. While very problem-specific "processing chains" consisting of a host of known algorithms have often been used for such tasks in recent years, methods based on machine learning and artificial intelligence will become steadily more important in the future. Major advances have been made in recent years in particular, despite the fact that the essential techniques have been available for several decades. As long ago as 1950, Alan Turing, one of the most influential early informatics researchers, proposed the "Turing test" for comparing the intellectual capacities of people and machines. For several years now, computer programs have been able to outperform humans in specific tasks that are generally associated with higher cognitive functions. Large companies such as Google and Facebook use machine learning methods for speech and face recognition, and the results over the past few years have been impressive. Today, machines can effortlessly beat human opponents at chess, jeopardy or the Chinese game of Go, described as the world's most complex board game.

The goal of my group is the application of these machine learning techniques to the analysis of microscopy image data to make it easier for doctors without a background in computer science to analyse and quantify this kind of data. Machine learning enables users to incorporate expert knowledge in a much more straightforward manner than was previously the case. Classic techniques meant using a multitude of often complex, predetermined parameters that could be difficult to understand if you weren't a computer science specialist, but machine learning makes interactive training possible. For example, it takes just a few clicks on the microscope images to "teach" the computer the difference between cells and blood vessels, or between healthy and diseased tissue. This process makes the transfer of expert biological and medical knowledge to computers so much

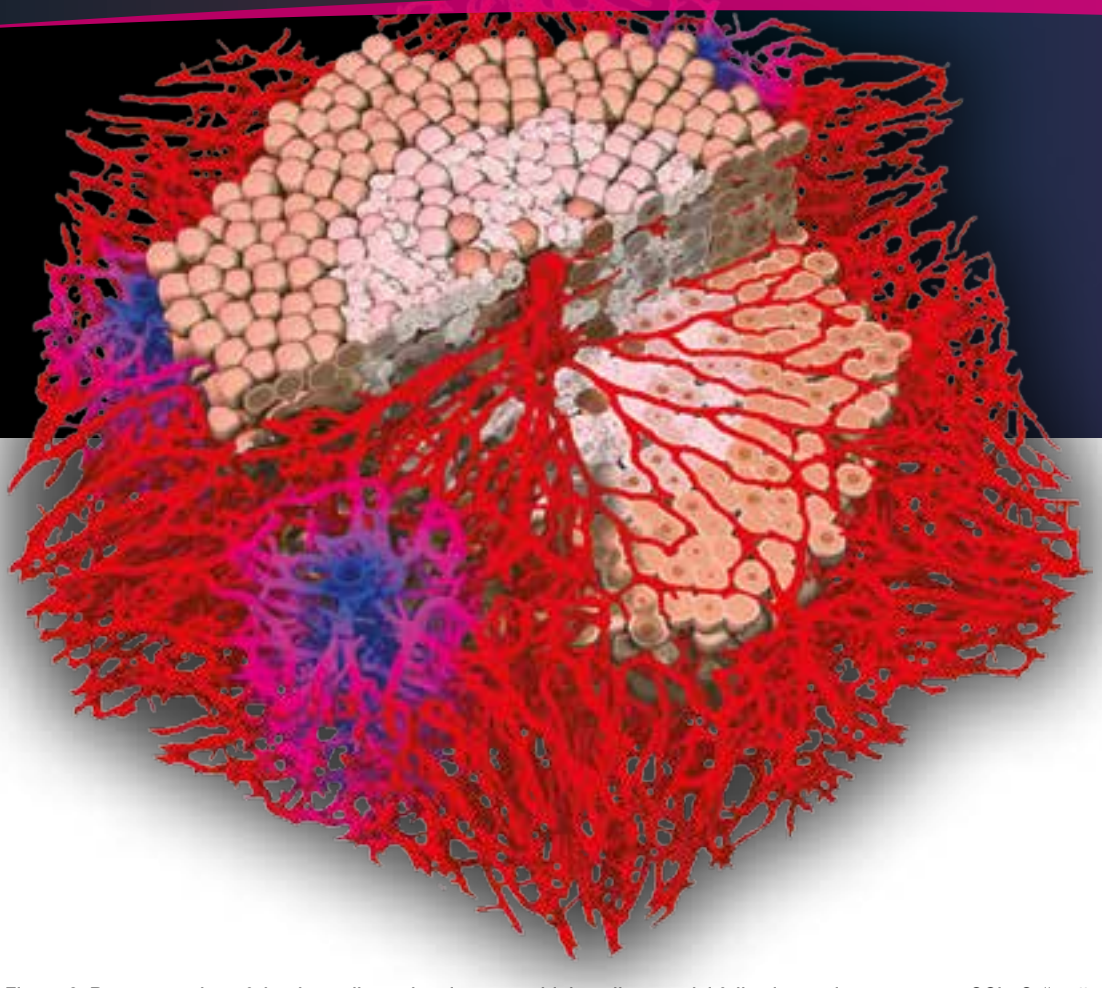

Figure 2: Representation of the three-dimensional systems biology liver model following toxic exposure to CCl_4. Cells affected by the toxin in the centre of the lobule are coloured white, while unaffected cells are shown in light brown. The blood vessel system is labelled in red, and blue marks the portal veins (Source: Stefan Hoehme).

simpler. We want to give a large audience access to this information and the associated analytical opportunities. In order to achieve this, for example, we are collaborating closely with Dr. Drasdo's working group to develop a free software resource, TiQuant (Friebel *et al.*, 2015, www.hoehme.com/software).

From image to model

Using the information obtained from analysing experimental data, it is now possible to create a multi-scale tissue model, which often incorporates a range of submodels verified by experiments. This form of integration has already been successful for a range of signal transduction processes that take place within individual cells, or for biophysical interactions between different tissue components. Step by step, these integrated systems biology models are now being tested in additional experiments, and they are being improved and expanded. During this process of iteration, interdisciplinary cooperation is once more of crucial importance, as modelling can often suggest the most informative experiments and studies, which our partners then run in their labs (Drasdo *et al.*, 2014). For example, during the course of the BMBF-funded Virtual Liver project, we worked with our peers to demonstrate that certain growth factors such as HGF can assume a key function for cell division control during liver regeneration. The systems biology tissue model was able to predict the possible details of how liver regeneration works, i.e. where, when and why liver cells undergo cell division and where these cells migrate during regeneration. The model eliminates a number of hypotheses that are, in principle at least, biologically possible, and this substantially accelerates the experiment-based search for the actual processes. Hypotheses describing the interaction between different biological components in healthy and diseased tissue are often extraordinarily elaborate, so using the right models is the only way of effectively grasping them. In this way, computers can help to better understand complex systems biology processes.

In a last but very important step, the resulting tissue models should be made available to the scientific community. We are working with Dr. Drasdo on this topic, and our free TiSim modelling software will be available shortly. However, three-dimensional tissue modelling still faces one basic problem that our field has not yet solved. There is currently no standardised means of describing tissue models. For example, the SBML standard has long been used for intracellular signal transduction

Figure 3: The new Emmy Noether group in Leipzig (from left to right): Stefan Hoehme, Adrian Friebel, Tim Johann and Johannes Neitsch (PhD co-supervision) (Source: Stefan Hoehme).

modelling and can be considered widely accepted in this field. A comparable development for tissue modelling is still largely in its infancy. In response, an international workshop funded by the BMBF via VLN took place in Leipzig in March 2016. During the workshop, participants had in-depth discussions regarding options and methods for solving this problem. We are confident that we will see progress in the near future, for example in the form of a collaboration with Prof. Peter Hunter in Auckland aiming for an extension of model description languages CellML and FieldML, and participation in the work of Los Angeles-based Prof. Paul Macklin on developing a standard named MultiCellDS. I am involved in this project in my capacity as a member of the review board of this new standard. Creating a widely accepted model description language for tissue modelling will be an additional significant boost to their usability in systems biology and systems medicine. This would bring us much closer to our goal of making direct use of tissue models for the benefit of the patient.

Research project profile:

A systems biology and interdisciplinary research group consisting of computer scientists, physicists and mathematicians focusing on processing and analysing microscopy images and constructing three-dimensional tissue models based on them. Funded by DFG and the BMBF, the working group is currently still taking shape. Vacancies are listed at www.hoehme.com/open-positions.

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computer models for customized cancer treatment

Business profile: Alacris Theranostics GmbH

by Christoph Wierling and Bodo Lange

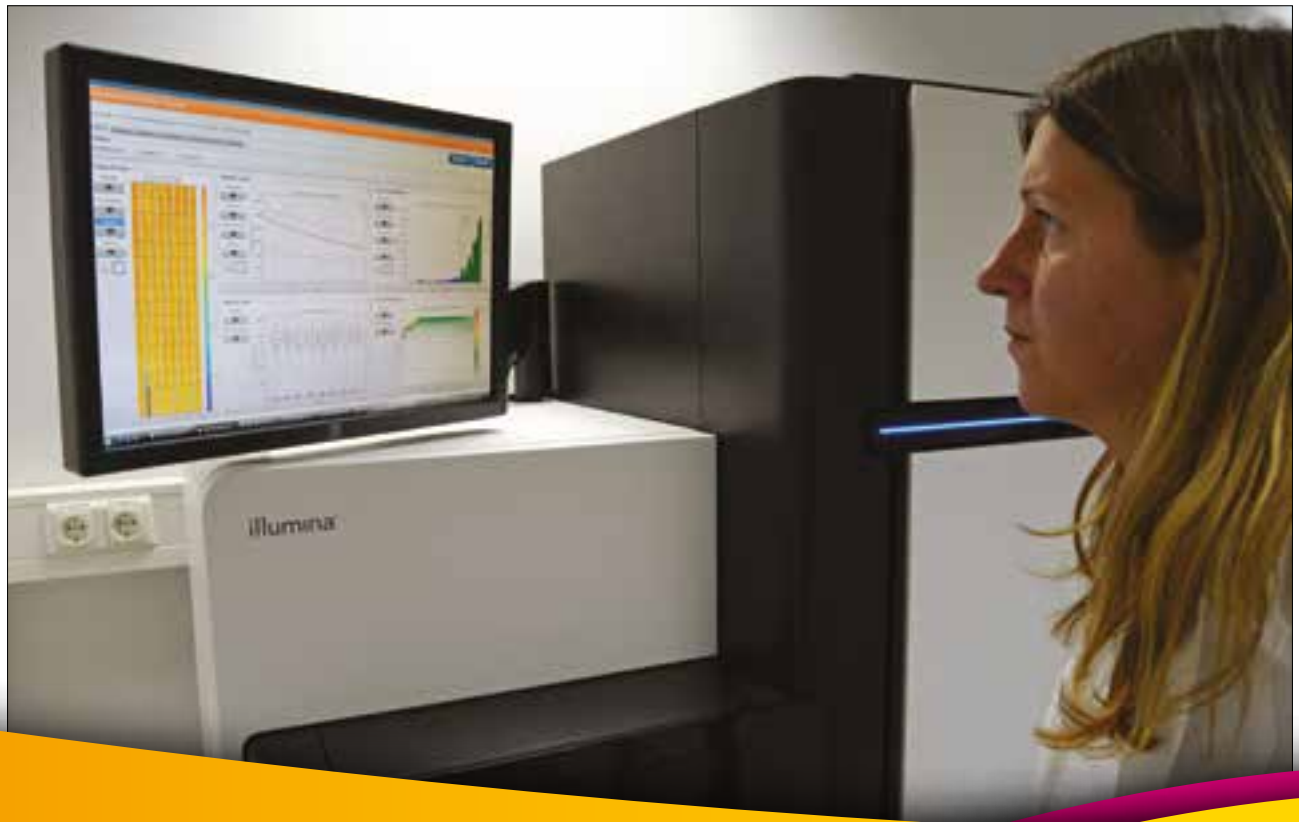
The rapidly growing quantity of data sourced from the sequencing of patient and cancer genomes gives us an opportunity to understand how cancers develop and devise more focussed treatment methods for patients. Access to greater processing power and new insights into the basic molecular processes in cancers now enable us to create and deploy mechanistic models that predict the effects of a specific drug and how cells will behave. Computer models are already in use in other fields such as crash testing and flight simulation. They make it possible to run initial tests for a whole range of scenarios on a computer, thereby minimising potential risks.

Cancer – one of the leading causes of death

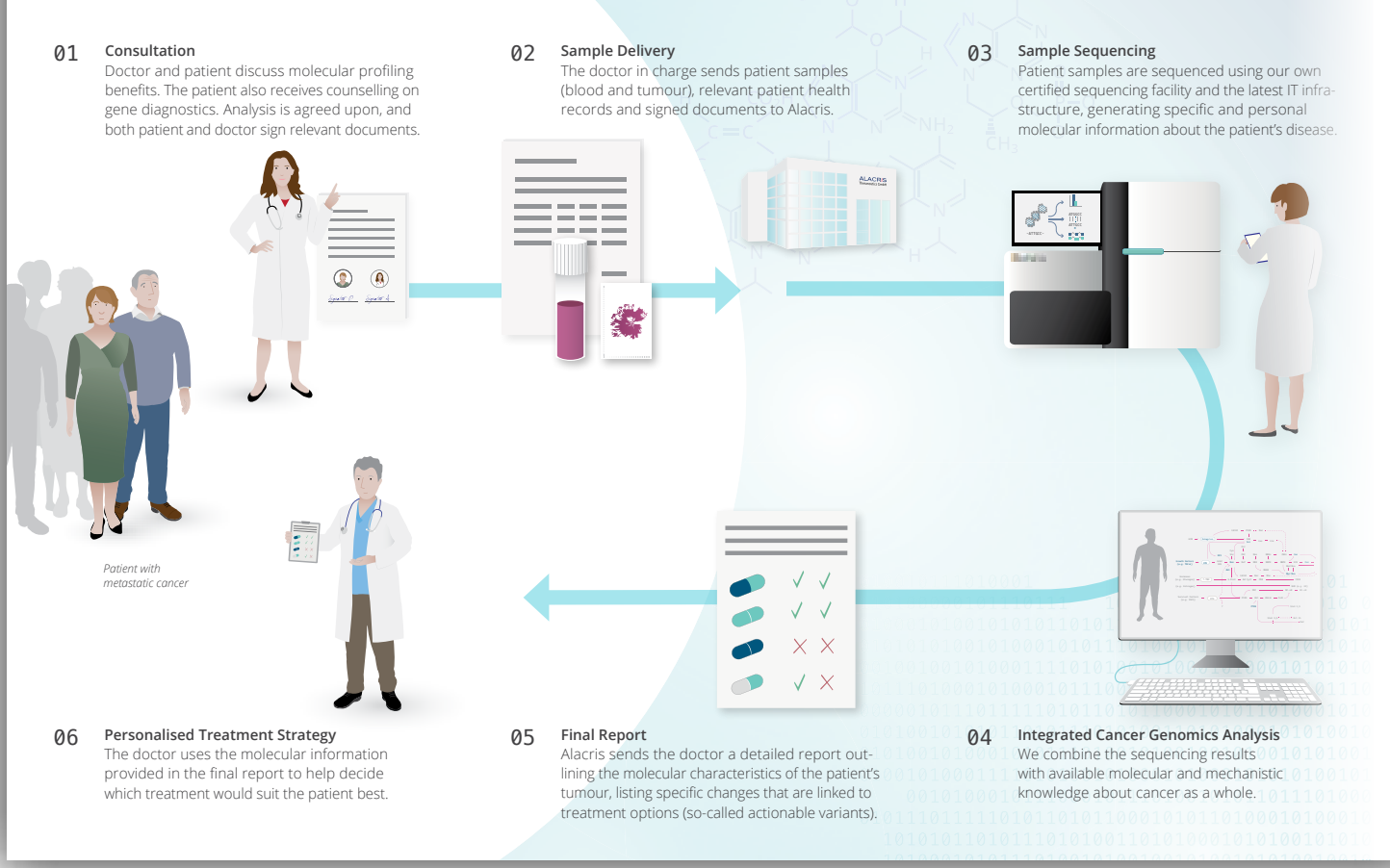
Despite intensive research and health care efforts, cancer is still one of the most common causes of death worldwide. In Germany alone, each year there are 500,000 new cases and 224,000 cancer-related deaths (Federal Statistics Office, 2015). Cancer is a complex disease with a multitude of causes, which makes it highly difficult to provide successful treatment options with standard therapies. However, new opportunities are now arising from our ability to analyse the tumour genome and transcriptome in

ALACRiS Theranostics GmbH

Modern sequencing methods (next generation sequencing, NGS) facilitate the fast and comprehensive identification of genetic changes within cancer tissue taken from a patient.



Source: Alacris Theranostics GmbH



Customized treatment options based on molecular tumour-specific characteristics and integrated analysis methods (Source: Alacris Theranostics GmbH).

great molecular depth thanks to high-throughput methods such as next generation sequencing (NGS). These methods help to give us an enhanced understanding of cancer and make finding new, targeted forms of therapy possible. Alacris is exploring new approaches within this context. We have created mechanistic computer models and can use these to describe and better understand the molecular processes that occur in healthy and cancerous cells. In the future, doctors will be able to use these models for support when analysing tumours on a molecular level and selecting the best possible drugs for treating cancer patients.

Many causes behind the development of cancer

Douglas Hanahan and Richard A. Weinberg use the term "hallmarks of cancer" to summarise the main factors behind carcinogenesis. They include a host of external influences (environmental, chemical and biological signals, i. e. growth hormones, immune reactions, etc.) along with intrinsic genetic and epigenetic cell characteristics (Hanahan and Weinberg, 2000, 2011). The appearance of these factors is connected with mutations in the cancer genome that change the function or activity of regulatory proteins. This can apply to oncogenes whose functions are interfered with by a specific mutation. For example, in the oncogene BRAF, the amino acid valine is replaced at position 600 by glutamic acid (mutation V600E), triggering constitutive activity of this protein. BRAF is part of the MAP kinase cascade, a multi-stage signalling pathway, which under healthy conditions mediates the cell's response to a host of external growth factors and thereby influences cell division. Constitutive activity in this signalling pathway caused by the mutated protein BRAF-V600E leads to a lack of response to external growth factors, which can promote the development of cancer. It is therefore obvious that an in-depth understanding of the molecular changes and regulatory mechanisms that cause cancer are essential if we want to devise effective treatment approaches.

Next generation sequencing permits the detailed analysis of cancer genomes and transcriptomes

Decoding the human genome at the start of the century laid the foundations for improving our understanding of the molecular causes of carcinogenesis. The sequencing of the first human genome was carried out by the Human Genome Project over a period of more than ten years, and at a cost of around USD 3 billion. Today's sequencers utilizing next generation technology (NGS), make it possible to sequence up to 18 genomes in parallel, with a per genome price of approximately USD 1,000. NGS enables us to perform very precise analyses of the genome and transcriptome (expression patterns) of tumour and patient samples. This allows us to identify individual mutations within tumours, study differences between the expression patterns of genes and gauge the heterogeneity of the tumours in question.

Alacris therefore makes routine use of an integrated molecular analysis, comprising exome analysis (high coverage of all protein-encoding areas) and genome analysis ("whole genome sequencing", WGS with low coverage) of tumour biopsies and blood (germline information), in addition to transcriptome analysis (RNASeq) of the tumour biopsy. This enables us to clearly identify key functional changes in the genome in addition to structural changes in the transcriptome (e. g. fusion genes, gene expression).

Cellular processes can be reconstructed on computers

Today, biologists make extensive use of computer modelling as they attempt to learn more about complex biological networks. For example, simple Boolean models permit the simulation of gene regulatory networks, and differential equations contribute to quantitative simulations of cellular reaction systems. Techniques such as these also make it possible to describe cellular signalling pathways and regulatory processes, such as the MAP kinase signalling pathway. This in turn lets us develop models of specific functional changes that a mutation triggers in an oncogene, e. g. BRAF-V600E, and we can also simulate what individual drug is the most effective in the presence of the mutation (Wierling *et al.*, 2012).

ModCell – a computer model for cellular processes

The human genome comprises more than 20,000 protein-encoding genes, many of which are involved in regulatory processes of cells in different ways. They are part of an extremely large cellular signalling network that will become disrupted due to a disease as complex as cancer. At Alacris, we developed the ModCell computer model as a tool for gathering more information about this intricate network. ModCell is built around cellular signalling pathways that are closely associated with cancer, and these pathways can be assembled in a piece-by-piece manner to form larger, more complex models. Along with the signalling pathways, we also create modules that describe the functional changes linked to specific mutations and that integrate the molecular binding partners of target-specific drugs. We have also developed a software solution for ModCell that supports the modular development of models and automates large simulation studies. At present, ModCell includes 47 signalling pathways and about 780 genes, along with the associated proteins, protein complexes and protein modifications (such as phosphorylations).

ModCell in action – simulating cancer cells and the impact of medication

No two patients have the same response to a drug, and no two tumours are identical. Even if tumours of the same tissue origin are classified as being at the same developmental stage due to pathological criteria, they often display different molecular characteristics. As a result, every tumour is unique and needs to be treated in a unique way. Using the ModCell computer model, our simulations at Alacris attempt to identify the drugs that are particularly suitable for each individual patient. To do this, ModCell requires "customisation" based on the data from NGS analysis of a specific patient and their tumour. Analysing this virtual patient model for molecular biological details, the software looks for the right drugs that would increase the chances of therapeutic success (Wierling *et al.*, 2015).

Virtual clinical trials are an important application field for ModCell, as they make it possible to test the effects that a drug can have on different types of cancer even though it has not been approved for use. An individual model is developed based on NGS molecular data from different cancer types and simulations are run with single or multiple drugs to reveal which type of cancer could display a noticeable reduction in cell division.

Computer models have long been in use in other fields relating to everyday life (crash tests, flight simulations) in order to assess thousands of options first in the computer, thereby reducing possible risks. ModCell is also intended as a tool to learn more about carcinogenesis first on the computer and then to identify suitable treatment approaches, find targets for new drugs and explore the potential benefits of using approved drugs to fight other cancers, and other diseases too. A key advantage of ModCell is that drugs still in development can first be tested on virtual patient cohorts before they move to real, focussed clinical trials. For pharmaceutical companies, this can deliver major advantages in the form of reduced testing times and costs when developing drugs.

Alacris Theranostics GmbH profile:

Based in Berlin, Alacris Theranostics was founded in 2011 and is a spin-off from Prof. Lehrach's department at the Max Planck Institute for Molecular Genetics. We are dedicated to developing mathematical models and software for personalized medicine

Developing mathematical models of cellular signalling pathways on the computer (Source: Alacris Theranostics GmbH).

and tumour analysis with the aid of NGS. With a staff of 20 people, we offer a full analysis service that covers sample preparation, genome and transcriptome sequencing, bioinformatics analysis, including identification of mutations and therapeutically relevant gene variants, and the simulation of the possible impact of target-specific drugs on the tumour. The ModCell software is undergoing further refinement and validation within the scope of national and international research projects. For example, clinically orientated aspects are being developed through the Treat20+ project funded by the German Federal Ministry of Education and Research. This undertaking involves researchers from the Max Planck Institute for Molecular Genetics, the Dahlem Centre for Genome Research and Medical Systems Biology and doctors from the Comprehensive Cancer Centers at Berlin's Charité hospital and other clinics.

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Source: Alacris Theranostics GmbH

"outsmarting brain tumors systematically"

A profile of e:Med junior research alliance head Christiane Opitz

by Kristin Huettmann

Christiane Opitz loves unconventional approaches, and this helps with the investigation of the strategies used by malignant tumors.

It all began under a blanket when she was seven years old. Christiane Opitz was lying and waiting on the back seat of her parents' car. She couldn't see anything, and she was told not to move. The car was parked on the premises of a Californian company bought by the German pharmaceutical company Bayer in the 1970s. Her parents – both biologists – were working there. "My parents often had to feed cells on the weekend, so we would make a family outing of it," Opitz recounts. However, children weren't allowed on the site. They had to make sure the guard didn't see her, and that's where the blanket came in. "It was probably that sense of mystery that got me fascinated in research."

Today, Opitz is 37 years old and says that she didn't fully realize the significance of her new-found fascination at the time. "Until I was 16, I actually wanted to be a farmer." Her biography, however, paints a different picture. When she was eight, she presented her first experiment at a science fair at her school in the USA, demonstrating that peas submerged in plaster generate sufficient force to crack the plaster. Back in Germany, at the age of 17, she discovered what causes water to bead on nasturtium leaves, winning third prize in a national young scientist competition.

The rest of her academic achievements also contribute to an exemplary scientific CV. She left school with excellent results, studied medicine, found time on the side to do a master's in cell biology, and spent time abroad in Sweden, Switzerland and the USA. Then came a doctorate, publications in high-profile journals and a host of prizes. Today, she heads a junior research group and coordinates the activities of two research consortia. On paper, she comes across as impressive, ambitious and possibly even flawless. However, meeting Opitz in person gives a different impression. She may be a person who knows exactly what she wants, but she is where she is today precisely because she doubts, questions and keeps peeking out from underneath that blanket.

Figure 1: Dr. Christiane Opitz using HPLC to check metabolites

Source: Silke Argo/e:Med

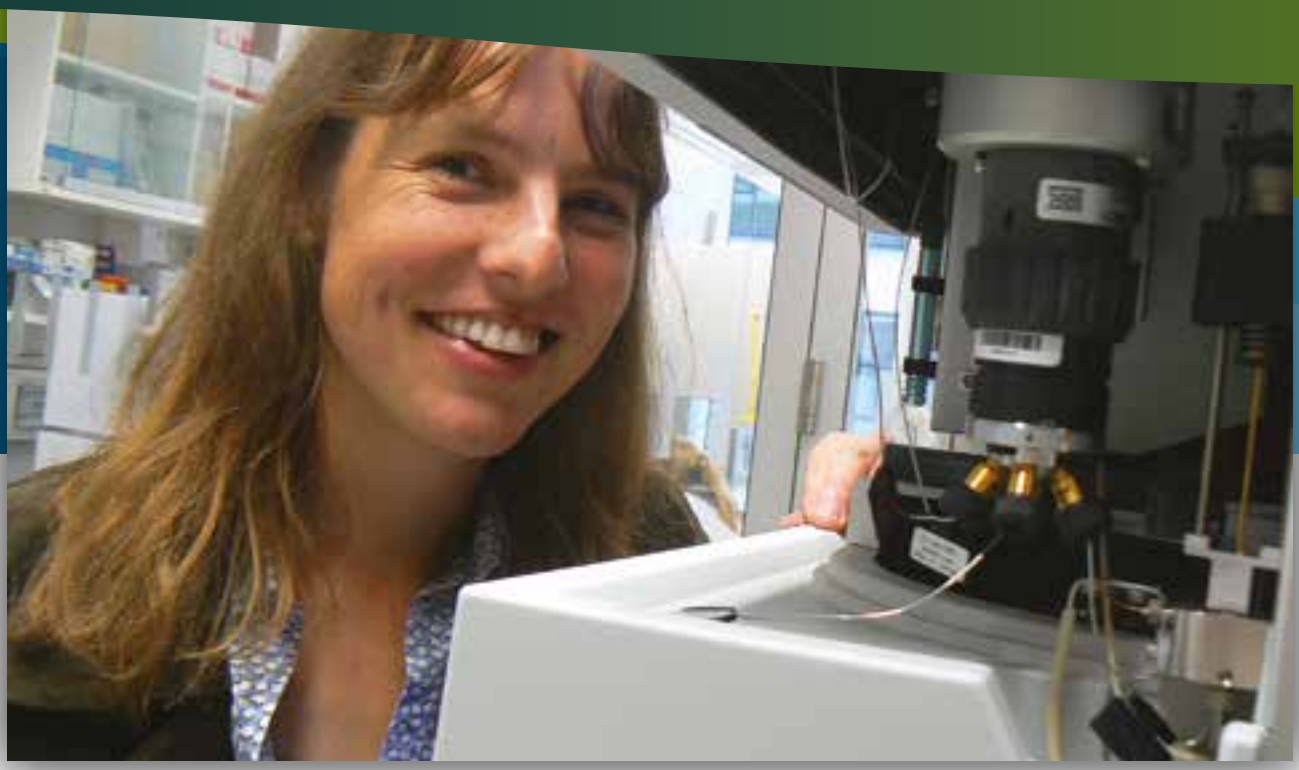

Figure 2: Dr. Christiane Opitz analyzing metabolic products by means of high performance liquid chromatography at her DKFZ lab in Heidelberg (Source: Silke Argo/e:Med).

Network research in the GlioPATH junior research alliance

Opitz has not chosen an easy field for herself – cancer research, specializing in brain tumors. She works at the German Cancer Research Center (DKFZ) in Heidelberg, which is not only Germany's flagship cancer research institute, but also one of the leading international centers in this field. She leads the Brain Cancer Metabolism group, and is also a resident at the university hospital, where she is training to be a neurology consultant.

She has lead the GlioPATH ("Comparison of central metabolic routes and signaling pathways in IDH mutant and wildtype gliomas") junior research alliance since February 2015. This alliance is part of the e:Med research program established by the German Federal Ministry of Education and Research (BMBF). This systems medicine network provides support for a total of nine junior research alliances, as well as offering young researchers from a range of fields the chance to carry out innovative research in interdisciplinary teams. This enables the young researchers to collaborate with scientists outside their specialities, foster scientific expertise and establish themselves in the field of systems-oriented medical research.

Under Opitz's leadership, four young research groups, from Heidelberg, Oldenburg, Jena and Leipzig, have come together to form GlioPATH. The researchers' goal is to gain a clearer understanding of the connection between brain tumors' metabolic and signaling pathways. Growing at frightening speed, glioblastomas are the most lethal form of brain tumor, with patients surviving on average just over one year after diagnosis. Current treatments consist of surgery, chemotherapy and radiotherapy, but these treatments fail to eradicate the tumor cells, causing the cancer to recur. For this reason, there is a great medical need for new therapy options.

GlioPATH's researchers, from the fields of medicine, biology, chemistry and bioinformatics, study these tumors' metabolic and signaling networks to reveal their role in cancer progression and potential implications for therapy. Just like healthy cells, cancerous cells need oxygen and nutrients for their growth, so they stimulate the formation of new blood vessels or attract existing blood vessels. Signaling pathways are harnessed to create these new blood vessels. Current therapies often target one of the tumor's signaling pathways, but there is a problem here. "If a drug blocks one pathway, the tumor simply uses another one and keeps growing," says Opitz. Because of this, the GlioPATH team is focusing above all on the interactions between the signaling pathways and metabolic processes. This angle has been overlooked in the past, but the research scientist thinks it could be very promising. "Our hope is that if we use the tumor's metabolism as a therapeutic target, the tumor will not be able

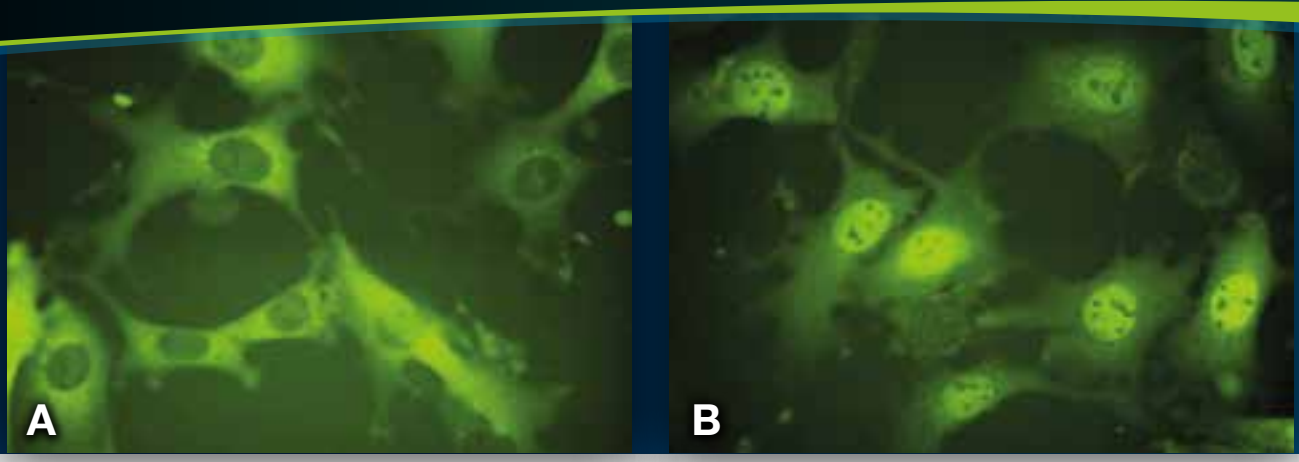

Figure 3: A: In unstimulated cells, the dioxin receptor (also known as the aryl hydrocarbon receptor) is located in the cytoplasm. **B**: Following stimulation with certain tryptophan metabolites, the dioxin receptor translocates to the nucleus and regulates gene expression there. The photos are taken from films of live cells (live cell imaging) (Source: Saskia Trump, Helmholtz Centre for Environmental Research, GlioPATH).

to evade the treatment." she says. This would lengthen patients' lives. The research team is also looking for markers in blood or urine that would improve the ability to predict which forms of therapy are best suited for a specific tumor.

Looking for new treatment approaches

One issue of particular interest to the junior research alliance is tryptophan metabolism. Opitz has long been studying this amino acid, and her DKFZ junior group is now working on it too. In 2006, she conducted research into tryptophan metabolism in human mesenchymal stem cells, the progenitor cells of connective tissue. However, as peers at the same lab were working on glioma cells, she decided to take a closer look at the metabolic processes of these cells as well. This lead to significant findings, which she published in a 2011 article in renowned science journal Nature. The research revealed that gliomas also display changes in tryptophan metabolism, and that the molecule kynurenine is produced when tryptophan breaks down in tumor cells. Kynurenine not only suppresses the immune system, but it also boosts tumor cells' activity, thereby enabling them to penetrate cerebral tissue more aggressively. Working with UK biotech company Proteome Sciences, she developed a method for measuring tryptophan and its metabolites simultaneously in up to ten samples, thereby increasing the accuracy of measurements.

Though one of the project's objectives is to develop therapy approaches to target glioblastomas that could, at some stage, be tested in clinical studies. Opitz however warns against overly high expectations. "Previous forms of therapy have not been able to cure tumors, only delay their growth," she points out. In her opinion, few things are as difficult as treating brain tumor patients, and this makes research into basic issues all the more important. "This will at least give us something we can use as a foundation for developing drugs we can use on patients."

She believes that GlioPATH's interdisciplinary cooperation is an excellent setting for making progress on basic research into glioblastomas. The German Federal Ministry of Education and Research (BMBF) is supplying the junior research alliance with funding of some €1 million over three years. "I would really love to be able to continue with this work if there was some follow-up funding," Opitz says, explaining that it had not been possible to devote the three years purely to research. "Though everyone in the junior research alliance comes from similar fields, it still takes some time to coordinate our processes and put work structures into place in our respective labs." Another problem is that "as a young researcher, you spend a lot of time writing grant applications and cannot focus primarily on your research topics." Luckily, however, she knows exactly when to invest energy in bureaucratic processes – and when to avoid them. Take her office, for example. The walls are bare. No pictures at all. "If I wanted one, I'd have to fill in an application. Then someone comes and hangs the pictures." Instead, she has simply placed them around the room – on the cupboard, on the cabinet, on the windowsill. An empty soft drink bottle stands in the corner, and her rucksack, taken off in a hurry, is on the table.

Three jobs, a family and a yard full of animals

This refreshing pragmatism and beaming smile have accompanied her throughout her life, and they help her deal with setbacks and difficulties. A paper of hers was turned down just tonight, but she can already smile about it. "If I couldn't cope with this, I wouldn't have become a researcher." She'll submit it to another journal.

Her high tolerance for frustration also helps her as she works with brain tumor patients at the university hospital's neurological outpatient unit. "The way I see it, my main duty is to attend to patients and to talk to them, because I know that

Figure 4: Partners of the GlioPATH junior research alliance
From left to right: Mirja Tamara Prentzell, Patricia Razquin Navas, Dr. Saskia Trump, Dr. Ines Heiland, Dr. Kathrin Thedieck, Dr. Christiane Opitz, Dr. Sascha Schäuble, Stefanie Loth (Source: Sascha Schäuble, Jena, GlioPATH).

our arsenal of therapies is very limited," she says. "These conversations are difficult, so I am glad that I can move back and forth between the lab and the clinic."

With roles at DKFZ, e:Med and the university hospital, strictly speaking, Opitz holds three jobs. Then there are her two children, her husband and a menagerie of animals at home. Most families have a dog or a cat, or perhaps a hamster. Not the Opitzes: their household is home to water snails, baby newts, an axolotl, fish and a couple of rabbits.

Just as during her own childhood, questions about fundamental knowledge are part of conversation when the family sits down for dinner. "My daughter recently asked me how brain tumors develop, and what they are made of," Opitz says. "Of course, I'd like to be able to answer that question." You can be certain that she won't stop trying to get to the bottom of this issue, and that she won't necessarily follow the tried-and-tested scientific paths either. "The art to research is developing a sense for which unexpected results to pursue, and which ones to drop," she states.

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www.sys-med.de/en/young-investigators/junior-research-alliances/gliopath/

Source: Silke Argo/e:Med

Research project profile GlioPATH

GlioPATH ("Comparison of central metabolic routes and signaling pathways in IDH mutant and wildtype gliomas") is an e:Med junior research alliance that investigates the interface points and interactions between metabolic and signaling pathways in brain tumors. As part of the project, models are developed (Dr. Sascha Schäuble, Jena University) that link tryptophan and NAD metabolism (Dr. Christiane Opitz and international GlioPATH partner Prof. Dr. Ines Heiland, Tromsø University, Norway) with the mTOR network (Prof. Dr. Kathrin Thedieck, Oldenburg University) and the dioxin receptor signaling path (Dr. Saskia Trump, Helmholtz Centre for Environmental Research, Leipzig).

model-based treatment optimization in haematology

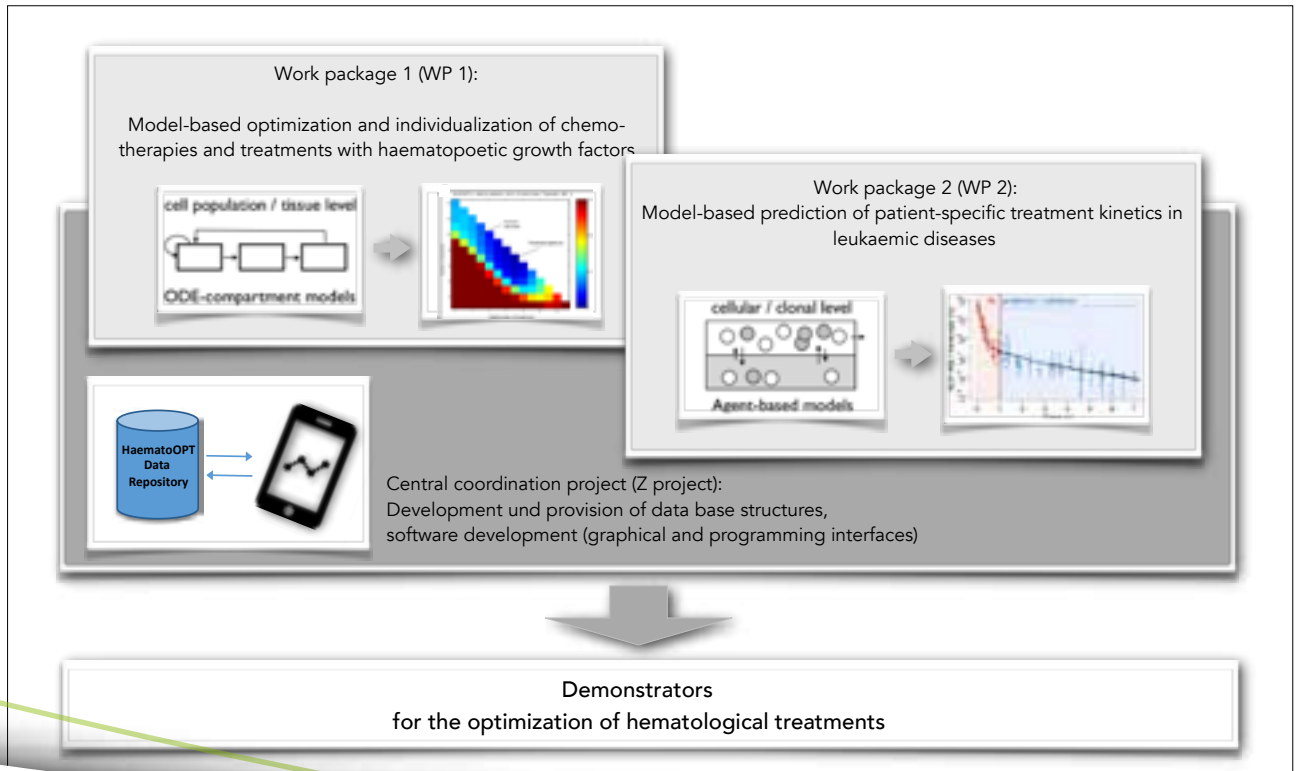
e:Med demonstrator project *HaematoOPT* develops strategies for model-based improvements to haematological therapies

by Ingmar Glauche, Markus Scholz, Markus Loeffler and Ingo Roeder

The development of personalized therapies has long been advocated as systems medicine's most promising application. Especially the increasing availability of "omics" technologies calls for systematic approaches to access and interpret the wealth of data. However, systems medicine goes far beyond the bioinformatics-based description of high-dimensional and clinical data. In particular, an understanding of functional relations and interactions between different components of a tissue provides essential information for describing pathogenesis and therapy effects on the systemic level. The HaematoOPT consortium has been designed to demonstrate the application of such predictive, mathematical models in haematology in a practical, pre-clinical context.

There has been a long tradition of applying mathematical models in haematology, especially for study design, data analysis and statistical modelling. At the same time, there was and still is a range of different mathematical modelling approaches for describing haematological phenomena from a dynamic and functional perspective. Of particular importance are, for example, the "classic" works of Michael Mackey (Mackey *et al.*, 1978) or Markus Loeffler (Loeffler *et al.*, 1980) on the modelling of dynamic processes concerning blood formation in healthy and diseased

Figure 1: Overview of the HaematoOPT consortium's structure



Source: Institute for Medical Informatics and Biometry, TU Dresden

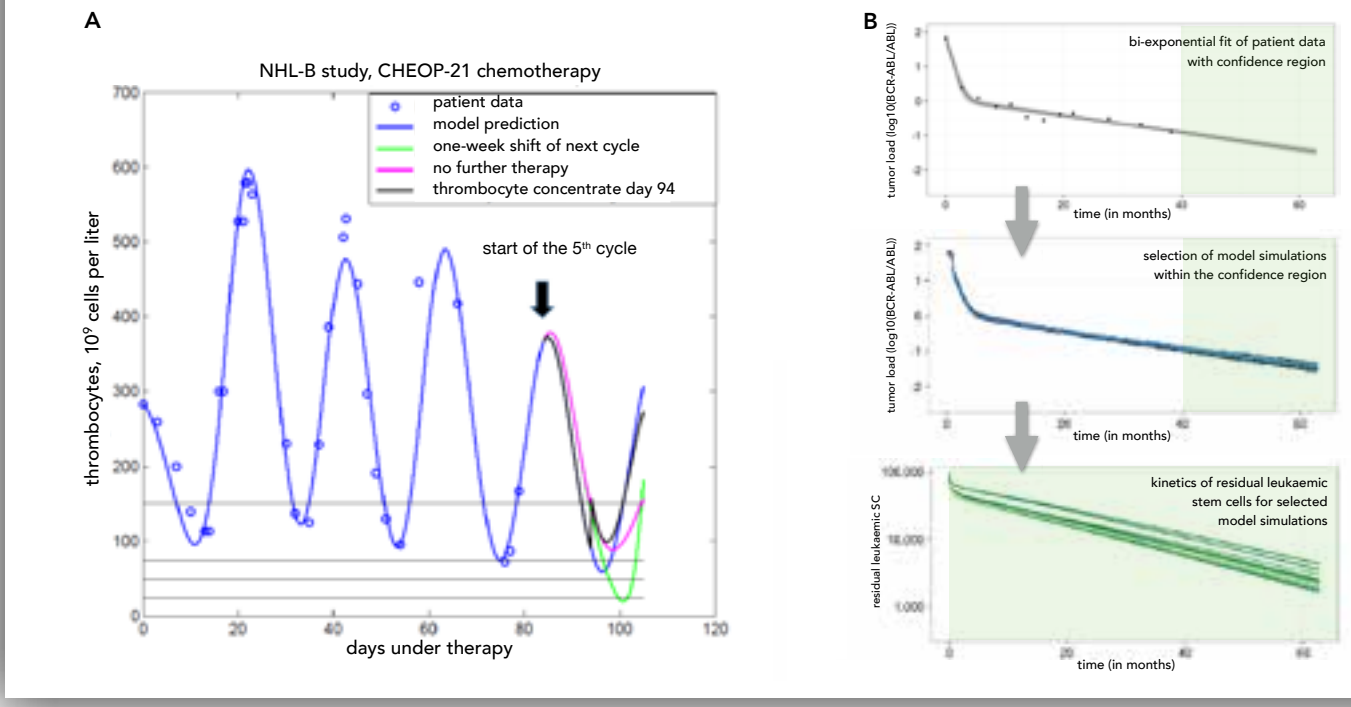


Figure 2: Individual predictions of platelet dynamics during chemotherapy
A: Our biomathematical thrombopoiesis model allows predictions regarding individual patient responses to further treatment. Here we present data of a patient treated with CHOEP-21 chemotherapy (blue). Based on observations from the first four chemotherapy cycles, the model predicts platelet dynamics of the fifth cycle (green area). One can also predict the results of therapy adaptations such as no further chemotherapy (magenta), one-week postponement of chemotherapy (green line) or supportive administration of a platelet concentrate (black). Black lines represent grades of thrombocytopenia.
B: To generate predictions for CML remission kinetics during treatment with tyrosine kinase inhibitors, we use a bi-exponential model for parameterizing an individual patient's history. Additionally, we estimate the data variability (grey confidence area). Based on this information, we select the parameter sets that predict remission kinetics within the scope of the customized confidence estimation from a pool of available simulations (blue lines, predictions in green areas). Using the selected simulations, it is possible to make further predictions about the residual disease level (green lines).
(Source: Institute for Medical Informatics and Biometry, TU Dresden)

organisms. These and other publications contributed to the development of a conceptual understanding of basic haematopoietic processes in the 1970s and '80s. Despite the existence of these conceptual works, the successful application of mathematical predictions to optimize therapy options for direct clinical integration and application (such as model-based prediction and clinical application of improved therapies of Hodgkin's lymphoma, Diehl *et al.*, 2003) remained the exception rather than a general practice.

Introduction of mathematical models into clinical contexts

The central goal of the interdisciplinary HaematoOPT consortium ("model-based optimization and individualization of haematology treatment strategies", Figure 1) is to demonstrate the practical applicability of mathematical models for describing normal and leukaemic haematopoiesis for clinical decision-making. The project is based on mathematical models developed by the participating research groups within the past years. While one focus of the consortium is on the regulation of the three main differentiation lines of haematopoietic cells (i.e. erythropoiesis, granulopoiesis and thrombopoiesis) by growth factors, the other focus is on the regulation and deregulation of haematopoietic stem cells. All models have been developed and consolidated over the course of the past ten years by joint efforts of modellers, biologists and clinicians. Today, some of these models are sufficiently advanced and validated, such that introduction into clinical decision-making is a realistic perspective for the near future. To permit this, existing models are further developed to explicitly cover patient heterogeneity with respect to pathogenesis and treatment response. These extended models are a key issue to analyze individual therapy plans and to assess the risks of modified therapy options.

The HaematoOPT project brings together mathematicians and bioinformatics specialists with clinical haematologists and biologists. They joined up to establish "demonstrators" showing applicability and benefits of mathematical and bioinformatical tools (e.g. algorithms, models, software) for optimized therapeutic strategies. In this respect, we understand the term "demonstrator" as elaborated and practice-based examples that show the application of theoretical and/or computational models

Figure 3: Participants of the HaematoOPT consortium meeting in Leipzig in August 2016 (Source: D. Ludwig).

in therapeutic settings. In particular, the consortium focuses on two types of "demonstrators": one for optimizing cytokine scheduling for patients with disturbed haematopoiesis (work package 1), and the second one to optimize tyrosine kinase inhibitor (TKI) application for the treatment of leukaemia (work package 2).

Work package 1: Model-based optimization and individualization of chemotherapy and haematopoietic growth factors

Work package 1 of the HaematoOPT consortium focuses on the application of mathematical models to optimize chemotherapy and supportive treatments with haematopoietic growth factors to suit individual patients' needs. This approach is based on models of erythropoiesis, granulopoiesis and thrombopoiesis during cytotoxic chemotherapy (Schirm *et al.*, 2014; Scholz *et al.*, 2010). Although haematopoietic growth factors are currently in routine clinical use to minimize cytotoxic side-effects during chemotherapy, there are only a few attempts to systematically study how the therapies could be improved, risk-adapted or individualized. It is still current standard to follow fixed schedules irrespective of individual patient's risks and therapy responses.

The goal of our research is to improve this situation. We adapt and apply our models to support the development of risk-specific chemotherapy and growth factor administrations. We aim at minimizing chemotherapy-induced declines in blood cell numbers (neutropenia, anaemia and thrombocytopenia) while maintaining the effectiveness of chemotherapy. Similarly, as another field of application, we study anaemia occurring in patients with chronic kidney disease (CKD). Here, we pursue the development of optimized long-term treatment with EPO and iron in dependence on individual risk factors and the course of disease.

Figure 2A shows examples of individual therapy predictions regarding thrombocytopenia during the course of chemotherapies. Given initial observations, our thrombopoiesis model can be used to predict a patient's response to further therapy cycles and allows simulations of the effects of possible therapy adaptations such as postponement of therapy or platelet transfusions. Thus, clinical users can test different therapy adaptations *in silico* and select the most promising option for the specific patient. We currently analyze the reliability of these model predictions and collaborate with clinical partners regarding the development of user-friendly tools allowing an introduction of our approach into clinical practice.

Work package 2: Model-based prediction of patient-specific treatment kinetics in leukaemia therapy

The second work package focuses on application and refinement of single cell-based models of leukaemia and how they can be used to optimize treatment and assess the risk of therapies. We use chronic myeloid leukaemia (CML) as the showcase example to demonstrate how existing models can be expanded to reflect patient and treatment heterogeneity and to include new drugs such as second-generation tyrosine kinase inhibitors (TKIs). In an analogous manner, we are attempting to establish a similar computational model of NPM1 positive[1] acute myeloid leukaemia (AML) during chemotherapy and following stem cell transplantation.

The ratio of oncogenic (BCR ABL-positive[2]) vs. normal mRNA in the peripheral blood cells of CML patients has been established as a reliable measure of tumor burden. Concomitantly, we developed a mechanistic, mathematical model that describes the disease as a competition between leukaemic and normal haematopoietic stem cells (Roeder *et al.*, 2006; Horn *et al.*, 2013).

[1] A special mutation that occurs in roughly one third of AML patients.

[2] An oncogene causally linked to CML and present in almost all patients.

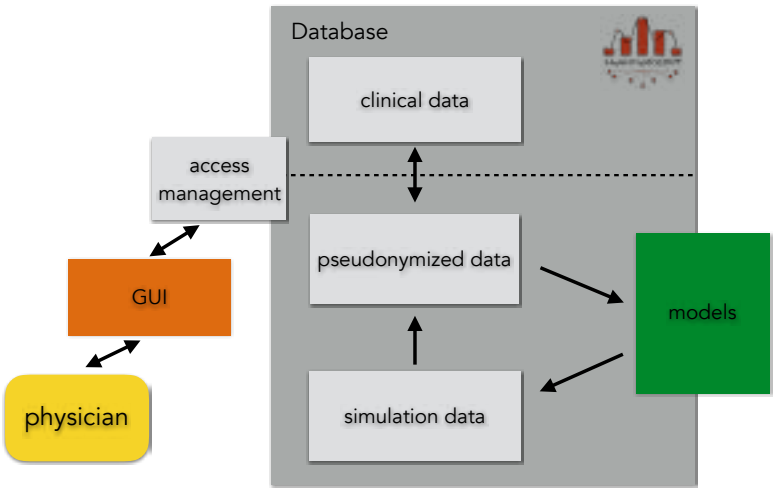


Figure 4: Structure of HaematoOPT's data management (Source: Institute for Medical Informatics and Biometry, TU Dresden).

By adjusting the resulting model kinetics according to individual records of patients' data, we aim to deduce information about the number of remaining leukaemic stem cells, which are associated with "minimal residual disease" (MRD) often causing a relapse.

Uncertainty in predicting stem cell kinetics mainly depends on measurement uncertainty, and on the other hand, on uncertainties regarding the model structure itself, i. e. different stem cell dynamics result in the same or similar peripheral blood dynamics. To account for these uncertainties and to allow for reliable predictions, we are establishing methods to supplement the "optimal" model result with a confidence region to estimate the degree of reliability for this particular prediction. Figure 2B outlines our target procedure.

Prerequisites for establishing a "demonstrator"

How well our "demonstrators" can be applied in the clinical context largely depends on the availability and accessibility of the underlying data (i. e. the database solution) as well as on the usability and practicality of the application environments (i. e. simulation software and graphical user interfaces). The HaematoOPT project addresses both aspects by developing several illustrative use cases. The project's central challenge is to provide clinical patient data along with customized simulation results in a unified environment that practically supports decision-making. For this purpose, several aspects have to be taken into consideration: From a technical point of view, simulation results must be available in real-time ("on the fly") to support decision-making. This possibly requires that time-consuming model simulations must be pre-calculated and made accessible in a corresponding database. Alongside technical aspects, data protection and data security are of equal importance and pose additional challenges on data handling and access rights.

While pseudonymized data are sufficient for modelling purposes (i. e. estimating parameters and performing simulations), clinical decisions require a direct connection with individual patient data. Figure 4 shows an overview of the data flow that will meet all of these criteria. Other topics covered by our demonstrator project include the reproducibility of results and the documentation of underlying processes. These features are of particular importance for demonstrating the future clinical applicability of predictive mathematical models (e. g. via software certification).

HaematoOPT partners and their activities:

Prof. Dr. Thomas Benzing (University Hospital Cologne)
Preparation and analysis of clinical patient data, advice on clinical issues, software testing in the clinical context
Prof. Dr. Martin Bornhaeuser (University Hospital Carl Gustav Carus, TU Dresden)
Preparation and analysis of clinical patient data, advice on clinical issues, software testing in the clinical context
Dr. Ingmar Glauche (Institute for Medical Informatics and Biometry, TU Dresden)
Leader of work package 2, model development, data management/analysis
Prof. Dr. Andreas Hochhaus (University Hospital Jena)
Preparation and analysis of clinical patient data, sequencing, molecular quantification, advice on clinical issues, software testing in the clinical context

Prof. Dr. Markus Loeffler (Institute for Medical Informatics, Statistics and Epidemiology, Leipzig University)
Deputy consortium coordinator, data management, model implementation/software development
Prof. Dr. Ingo Roeder (Institute for Medical Informatics and Biometry, TU Dresden)
Consortium coordinator, Z project management, model development, data management/analysis
Prof. Dr. Karl Lenhard Rudolph (Leibniz Institute on Aging, Fritz Lipmann Institute)
Phenotypical and molecular classification of cells (stem and progenitor)
Prof. Dr. Markus Scholz (Institute for Medical Informatics, Statistics and Epidemiology, Leipzig University)
Leader of work package 1, model development, data management/analysis
Prof. Dr. Christian Thiede (University Hospital Carl Gustav Carus, TU Dresden)
Preparation and analysis of molecular data (NGS), advice on clinical issues

Further information:
www.haematoopt.de
www.sys-med.de/de/demonstratoren/haematoopt

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Image source: Institute for Medical Informatics and Biometry, TU Dresden and Institute for Medical Informatics, Statistics and Epidemiology, Leipzig

twelve years of systems biology funding

What has been achieved so far, and where are we today?

by Project Management Jülich: Business Area Life Sciences, Health, Universities of Applied Sciences

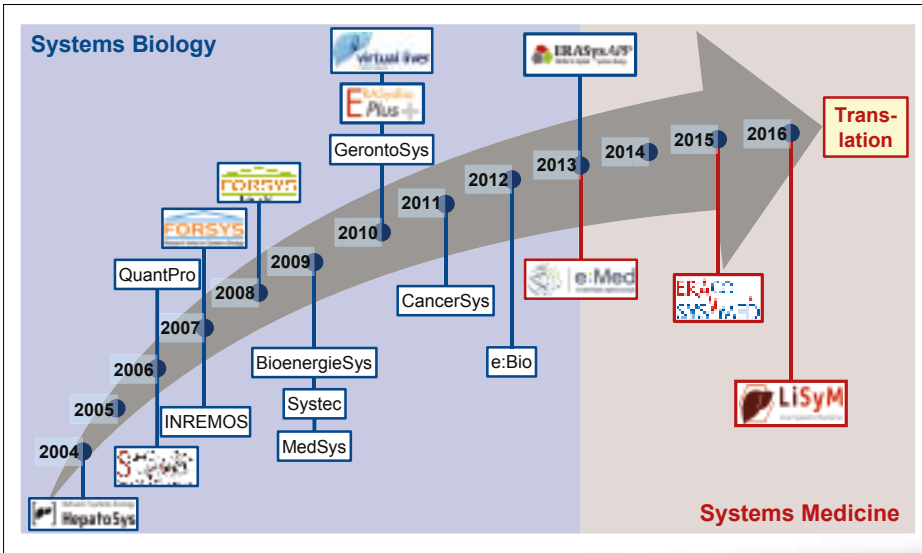
With its continuous funding of systems biology, the German Federal Ministry of Education and Research (BMBF) has made a vital contribution to raising the profile of this research field in Germany and Europe. By pursuing a needs-based funding policy with different but complementary initiatives, Germany has seen the formation of a scientific community of international repute. This community is dedicated to applying the systems biology approach to its research, which is based on interdisciplinary cooperation and a common language that embraces all research disciplines involved. Systems biology has laid the foundations for systems medicine, and the first fruits of this strategy can be seen with regard to clinical research activities.

The widespread use of mathematical models for describing biological processes was still nothing more than an idea at the start of the millennium. Nevertheless, there was an urgent need to develop mathematical models, which have been proven in the Engineering fields and adapt them to the life sciences so that researchers could gain a better understanding of biological systems and their complex, dynamic processes. Similarly, the required interdisciplinary cooperation between lab-based experimental researchers and mathematicians or modellers was not well-established at that time.

The BMBF recognised at an early stage that combining mathematical techniques and computer simulations with classic life science methods could help improve our understanding of biological processes. By providing extensive funding over recent years for this research approach, the ministry has paved the way in order to tap into new potential for innovations in the life sciences, and has played a major role in shaping the term "systems biology" as such.

Putting the systems biology strategy into place required new research structures, and the BMBF has supported this process by funding a series of interlinked initiatives (Figure 1). For example, forming centres dedicated to systems biology research

Figure 1: BMBF funding measures focusing on systems biology and systems medicine



The chart shows all of the funding measures in Germany and Europe that have received or are receiving BMBF funding (Source: Project Management Jülich).

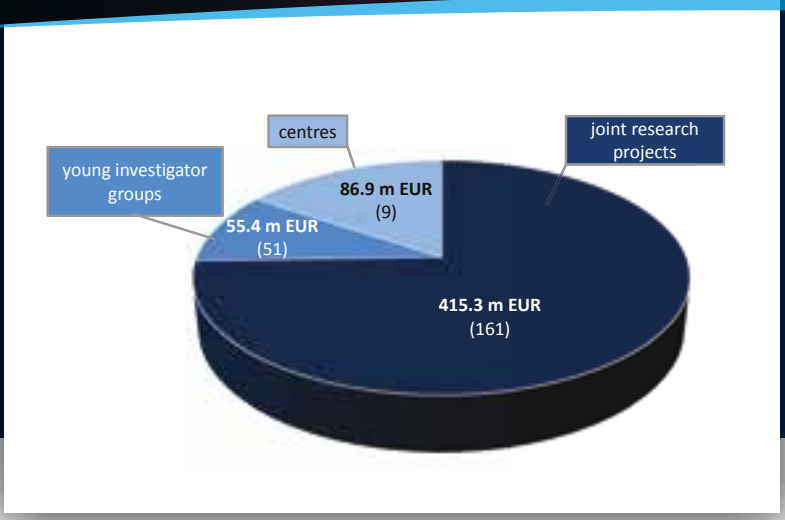


Figure 2: The different funding instruments used by the BMBF for the establishment of systems biology in Germany. The chart shows the funding budget together with the number (in brackets) of projects in each group (Source: Project Management Jülich).

was one element in creating the necessary research infrastructure, while support for young researchers in various funding programmes has driven the training of future systems biology experts. Funding measures have also established the systems biology approach in different fields such as medicine, plant research, white (industrial) biotechnology and technology development. Today, systems biology has become part of clinical processes with the help of application-oriented initiatives.

The total budget for the BMBF's funding activities up to 2021 comes to €560 million, an impressive sum that has helped systems biology assume a central role in the life sciences. Tremendous commitment to the development of this research field, both on a national and an international level, has enabled Germany to attain a leading position in this field in Europe.

Establishing systems biology in the long run

In order to make sure that systems biology is in a position to deliver long-lasting innovations for research activities, the BMBF has focused on providing funding for interdisciplinary research groups, centres and young investigators (Figure 2).

The aim of funding centres dedicated to systems biology research was to pool core competencies in the same place and promote the development of training structures. The BMBF spent almost €87 million on funding nine such centres all across Germany, and this helped trigger the founding of other research centres. Degree programmes in systems biology have now been established at these locations, which demonstrates how successful the targeted support actions have been. In parallel, other educational structures were also created by various institutions, such as the DFG's support for graduate schools and colleges, resulting in an extensive network of training options in the field of systems biology in Germany.

With a budget of around €55 million in total, 51 young investigator groups were funded which has made a decisive contribution to the sustained accumulation of expertise in the field of systems biology. It has given many of the next generation of scientists their first opportunity to form their own working groups and thus laying the foundation for their academic career. Today, 13 of the young investigators who received funding have accepted professorships, and ten have taken over a working group at an institute. 27 researchers continue to work on BMBF-funded projects.

Furthermore, extensive funding (€415 million) for a total of 161 joint projects covering a host of different research topics has helped achieve the goal of encouraging the widespread application of systems biology research techniques. By these measures, all funding incentives have thus proved to be a success.

A range of cross-cutting activities has also prompted the development of platforms that encourage the members of the new scientific community to create networks and exchange information. Examples include the systembiologie.de website and magazine, the international Systems Biology of Mammalian Cells (SBMC) conference and different workshops covering topics of relevance to systems biology.

Implementing the systems biology approach in different fields of application

Systems biology has gradually established itself in every biotechnological field. Looking at the distribution of the substantive prioritisation of the funded projects, we can observe that about three-quarters, i. e. the majority, are related to "red" or medicine-oriented biotechnology (Figure 3). A smaller number of projects are focused on the fields of white (industrial) and green (plant) biotechnology.

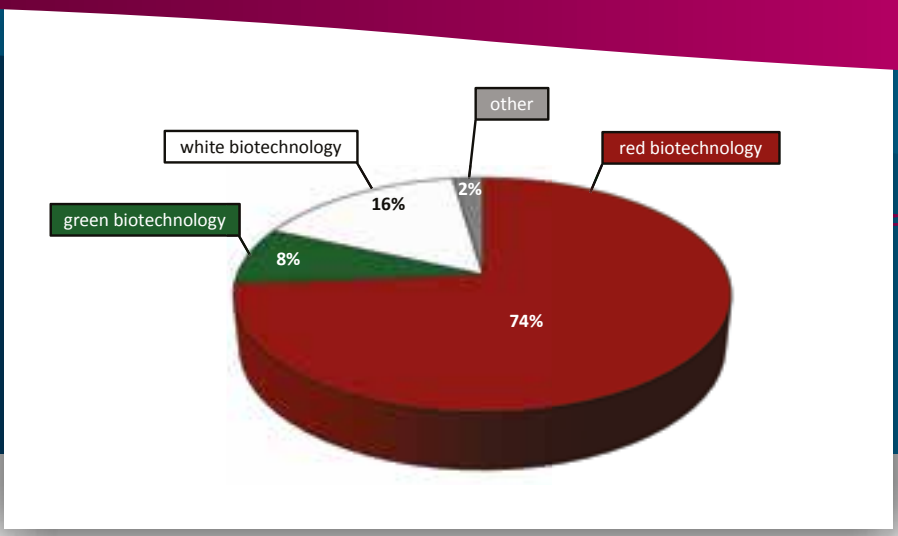


Figure 3: Percentage distribution of BMBF-funded systems biology projects according to their respective application in the biotechnology field (Source: Project Management Jülich).

Although the BMBF promoted this distribution by targeted funding of medicine-focused research programmes, the same breakdown is also noticeable in open-topic calls. The evaluation of the ministry's largest initiative, e:Bio (an open-topic call), also revealed that three-quarters of the projects receiving funding were driven by biological questions and future medical applications. Collectively, these insights reveal the great need for funding in the field of medicine-oriented systems biology.

The interdisciplinary nature of systems biology represents one of its key components as well as a major challenge, particularly with regard to collaboration between researchers working in the separate fields of theory and experiments. Every funding activity achieved balanced participation from these two groups. On average, 40 % of working groups came from a theoretical background.

Funding of systems biology research has contributed considerably to enhanced interdisciplinary collaborations in the life sciences, a view corroborated by the statements of well-known research scientists. Prof. Ursula Klingmüller from the German Cancer Research Centre in Heidelberg, says with regard to the LungSys project, which was coordinated by her *"A major advantage was the inclusion of the participating project partners' complementary expertise, which ranged from basic research to clinical and industrial applications."* Prof. Steven Dooley from University Hospital Mannheim, adds, *"BMBF's support for systems biology projects has [...] strengthened interdisciplinary research in Germany."*

Germany's systems biology research: internationally visible and a European leader

The ministry's support has been crucial for the establishment of a large number of long-lasting systems biology working groups, research associations and centres whose work is respected around the world and which can compete on an international level. This is particularly noticeable from the high number of articles that German systems biologists publish every year in peer-reviewed magazines. With BMBF support, this figure went from 20 in 2004 to approximately 250 a year in 2011, which represents a more than a tenfold increase (figures sourced from Web of Science (2016)). In addition, Germany has become a hub for scientific exchange and networking since 2006, with international conferences in the country being one of several contributing factors.

The work of German systems biologists is also highly regarded outside of Europe, as can be confirmed with positive statements and appraisals of scientists with international profiles. One example that reveals the attention paid to German systems biologists by the transatlantic community, particularly in the USA, is the decision of the American Pharmacists Association to award its prestigious Ebert Prize to the Leverkusen-based Bayer group, headed by Dr. Lars Küpfer, in recognition of its outstanding work on predictive physiology-based pharmacokinetic models (Thiel *et al.*, 2015).

The BMBF however is not just involved in extensive funding on a national level. It is also a member of the European Commission's seventh Framework Programme for Research and Technological Development (FP7) and current Horizon 2020 programme, giving it a role to play in key transnational systems biology activities such as SysMO, ERASysBio+ and ERASysAPP, as well as in funding measures such as ERACoSysMed and CASyM which build on them. These are all managed from Germany. Providing 37 % of the total funding budget for all activities, the BMBF is the most active supporter of systems biology in Europe (Figure 4).

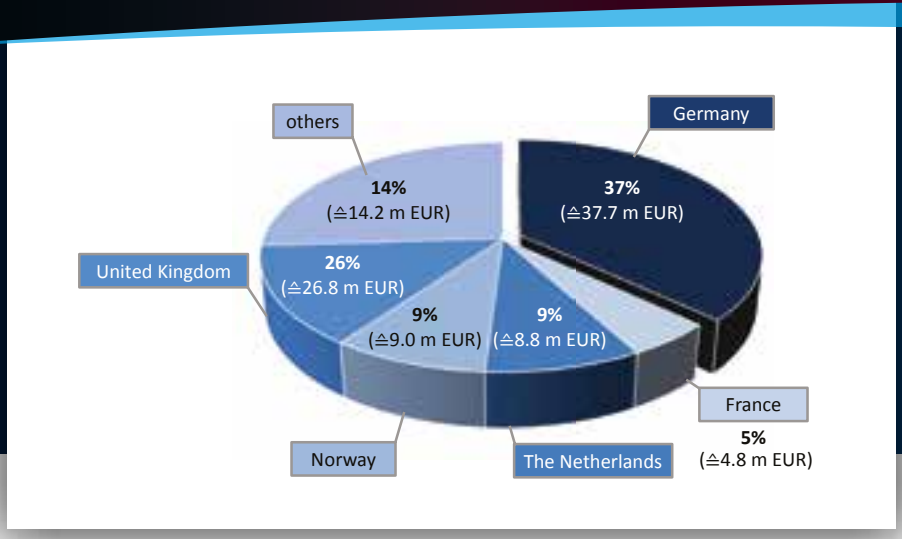


Figure 4: Contribution of European countries to transnational systems biology funding measures (FP7 and Horizon 2020) (Source: Project Management Jülich based on data from the national life sciences contact offices).

The following comparison demonstrates that systems biology in Germany has benefited from these investments: For every euro that the BMBF has contributed to transnational measures, the European Commission has given about €2.10 back to German projects in the field of systems biology or systems medicine within FP7 or Horizon 2020 funding (Figure 5).

Systems biology: setting the stage for systems medicine

The systems biology approach is particularly well-established in medically-oriented research. In a range of BMBF-funded projects, researchers have successfully applied systems biology strategies to results and methods drawn from basic research, thereby developing applications for clinical use.

In this way, members of the Virtual Liver network designed a computer model that facilitates a highly realistic simulation of the effect of drugs in the liver (announcement Pt-LG, 2014). This model not only makes it possible to virtually track how a drug is distributed and absorbed, it can also simulate potential interactions with other drugs and provide information about the progress of liver injury. The technique offers potential for designing individualised treatment plans for people with liver damage, and it could prove to be a useful tool for pharmaceutical companies in their drug development activities.

Members of the same network have also created a software platform that scientists can use to develop substance-specific physiology-based pharmacokinetic (PBPK) models (Kuepfer,

Members of the Business Area Life Sciences, Health and Universities of Applied Science at Project Management Jülich who are involved in systems biology programmes on behalf of BMBF (not all depicted)

Photo: Ralf-Uwe Limbach, Forschungszentrum Jülich GmbH

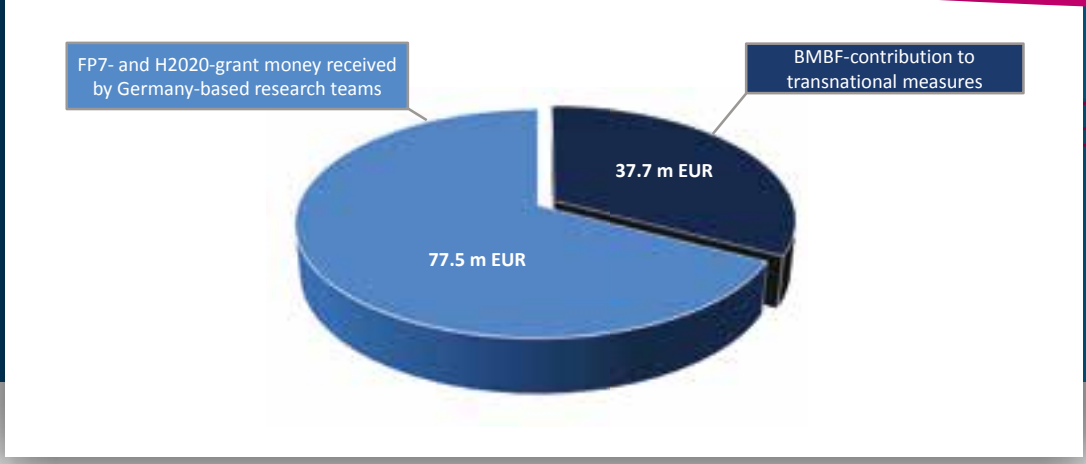


Figure 5: BMBF-contribution to transnational funding measures versus FP7 and H2020-grant money received by Germany-based research groups. Ministry funding (navy blue) covers the following activities: SysMO1/2, ERASysBio+, ERASysAPP1/2 and ERACoSysMed. Commission funding via the FP7 and Horizon 2020 programmes (light blue) covers German systems biology/systems medicine projects (Source: Project Management Jülich based on data from the national life sciences contact offices).

2013). These permit the simulation of the distribution and breakdown of drugs within the body. They have been used, for example, in the BMBF-funding activity FORSYS-Partner to simulate the physiological distribution of therapeutically active proteins for treating lung cancer. This has enabled researchers to describe drug enrichment in specific tissue types in quantitative terms. The potential of PBPK models as a method for drug development is increasingly acknowledged by regulatory authorities.

Use of the systems biology approach has also helped score successes in HIV-therapy optimization (Reinberger, 2016). The HIVCellEntry project has developed a mathematical model that predicts HIV's development of resistance to widely used drugs. This model is superior to the traditional methods used for identifying resistance-promoting mutations as it also takes into account the interaction of genetic changes to the virus's DNA. In the future doctors could routinely use resistance models in clinical practice to select the best combination of drugs for each HIV patient and thus ensure effectiveness of treatment for as long as possible.

Other examples of systems biology's successful deployment in medical research are outlined in the BMBF brochures ("Systems biology – understanding life's networks" and "Systems medicine: new opportunities for research, diagnoses and therapy").

In 2012, the ministry announced its e:Med research and funding concept for the implementation of systems medicine as an innovative field of research, thereby laying the groundwork for the establishment and advancement of the field of systems medicine in Germany. At EU level the first systems medicine-oriented ERA-Net (ERACoSysMed) was announced last year with the objective of establishing the systems medicine approach within research activities throughout Europe. A total of 14 European funding agencies are involved in this new ERA-Net, and BMBF has once more taken the lead in managing this European research endeavour.

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events

Conference report

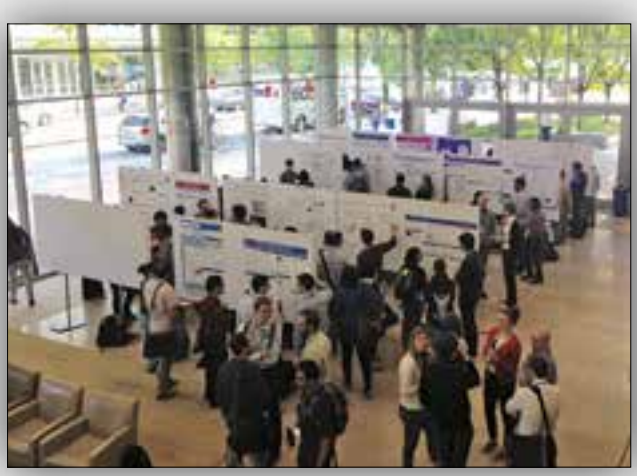
9th International Conference on Systems Biology of Human Disease – SBHD 2016

LAYING CELL BEHAVIOR BARE WITH HIGH-RESOLUTION MICROSCOPY AND MATHEMATICAL MODELLING

by Cornelia Depner

From 14 - 16 June 2016, 190 scientists from twelve countries discussed their latest findings from the field of systems biology at the International Conference on Systems Biology of Human Disease (SBHD), held at the Broad Institute in Boston, USA. This year's conference was organized by the team headed by Prof. Peter Sorger from Harvard Medical School with support from the *Harvard Program in Therapeutic Science*, the *Synthetic Biology Center @ MIT*, applied biomath and the Swiss systems biology research initiative *SystemsX.ch*. The conference's co-chair was Prof. Roland Eils, who has worked with Sorger for almost ten years as the series of SBHD conferences alternates between Boston and Heidelberg.

The 2016 conference saw scores of speakers give interesting talks, along with two poster sessions with presentations on selected posters. These discussions focused in particular on systems biology techniques for developing new diagnostic and therapeutic strategies to treat diseases in humans.



Source: Lucy Dilworth

Poster presentations in the foyer of the Broad Institute

Alongside systems biology research in medicine, presentations also included work on genetic network reconstruction, analyzing single-cell transcription and protein, and microbiology. A lively panel discussion with representatives from the companies AstraZeneca, Pfizer and Genentech about systems biology and industrial uses complemented the program.

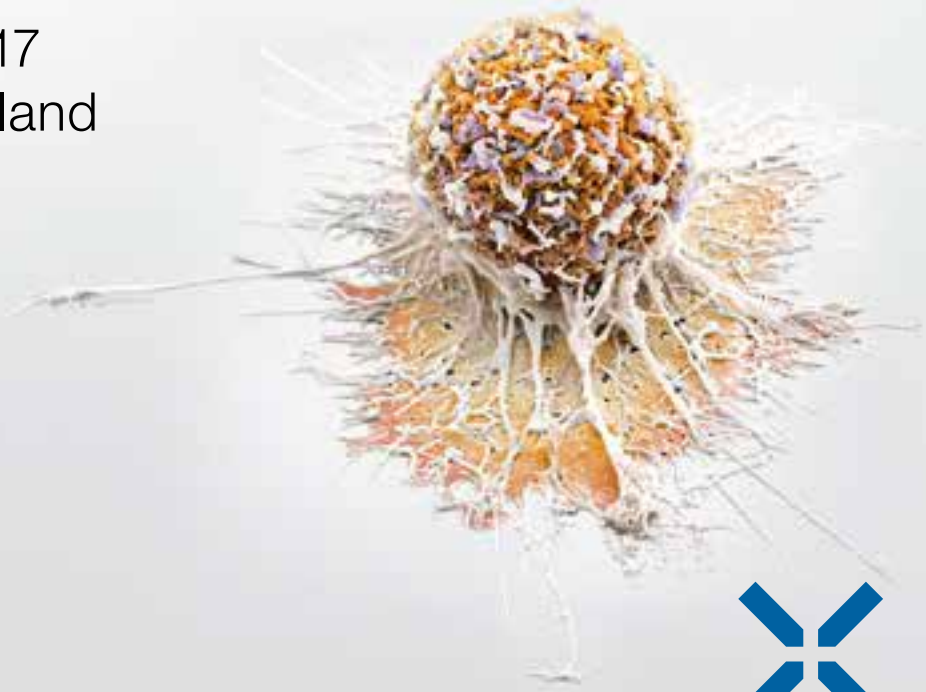
Professor Hari Shroff from the NIH received the Anne Heidenthal Prize for Fluorescence Research, sponsored by Chroma Technology Corp., for his innovative microscope work. With the help of *two-photon instant structured illumination microscopy* (SIM) and *light-sheet microscopy*, he captures high-resolution images of multi-layered objects and cells in motion, for example in live-cell microscopy. With its new calculation processes, *inverted selective plane illumination microscopy* (iSPIM) even enables detailed observation and cell identification of the nervous system of the nematode *Caenorhabditis elegans* during embryonic development. He demonstrated his process in a talk that impressed also by its visual data.

Dr. Matthew Thomson from the University of California in San Francisco received the CSB2 Prize in Systems Biology, sponsored by Merrimack Pharmaceuticals, for his work on cell regulation networks. He investigated cell populations and cellular mechanisms in tissue structures and the immune system. When performing his analysis, he combined mathematical modelling and statistical studies of high-throughput gene expression data with single-cell RNA analyses to ascertain how cells in tissue can differentiate, regulate and repair themselves.

We are looking forward to the tenth SBHD conference that is taking place on 5 – 7 July 2017 at the German Cancer Research Center (DKFZ) and the BioQuant center in Heidelberg.
Further information about the conference is available at
www.sbhd-conference.org/2017

3rd International SystemsX.ch Conference on Systems Biology

September 4 – 7, 2017
ETH Zurich, Switzerland



Keynote Speakers
James Ferrell Stanford, USA
Chris Sander Dana-Farber Institute, USA

International Speakers
Ido Amit Weizmann Institute, Israel
Martha Bulyk HMS, USA
Peter Campbell Sanger Institute, UK
Raymond Goldstein Cambridge, UK
Eran Segal Weizmann Institute, Israel
Sander Tans AMOLF, Netherlands
Barbara Treutlein MPI Leipzig, Germany
Kevin Verstrepen KU Leuven, Belgium
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Conference report

6th Conference on Systems Biology of Mammalian Cells – SBMC 2016

INSIGHTS INTO CURRENT SYSTEMS BIOLOGY RESEARCH

by Anna Sacher and Fabian Theis

The 6th Conference on Systems Biology of Mammalian Cells (SBMC) took place in Munich on 6–8 April 2016. Since 2006, the German Federal Ministry of Education and Research (BMBF) has funded this event. In 2016, the conference was again under the patronage of Minister Prof. Johanna Wanka. The series of annual conferences started in Heidelberg and, at that time, the HepatoSys research consortium formed the basis for the gatherings. HepatoSys was succeeded by the Virtual Liver Network consortium in 2010, and it was partially reconstituted in early 2016 within the framework of the LiSyM consortium. Parallel to the beginning of LiSyM's activities, the SBMC 2016 took place in Munich and was organized by Prof. Dr. Dr. Fabian Theis, head of the Institute of Computational Biology and professor at the Technical University of Munich, and by Prof. Dr. Ursula Klingmueller, head of the team working on systems biology in signal transduction at the German Cancer Research Center and professor at Heidelberg University. During the three-day meeting, which took place at the 'Klinikum rechts der Isar', 270 scientists from 19 different countries were discussing about new approaches in systems biology and systems medicine, and they also exchanged experiences about new technologies and methods used in these fields.

The program reflected the entire range of issues covered by current systems biology research. This included classic modelling strategies, new technologies such as single-cell analyses,

SBMC 2016: Prizes

The 2016 SBMC also featured a ceremony for the MTZ®-Award for Medical Systems Biology, presented every two years as part of a joint undertaking between MTZ®-Foundation, the BMBF and Project Management Jülich. The prize is awarded for outstanding dissertations by young researchers in the field of medically-oriented systems biology. (From left to right: Dr. Gisela Miczka (PtJ Jülich), Prof. Dr. Dr. Fabian Theis, Dr. Frank S. Heldt (winner), Dr. Klaus-Peter Michel (BMBF Berlin), Dr. Joern M. Schmiedel (winner), Dr. Franziska Witzel (winner), Thomas und Monika Zimmermann).

Source: HMGU

Plenary talk by Prof. Chris Sander (Dana Farber Cancer Institute and Harvard Medical School, Boston) on "Systems Biology in Action: Design of Cancer Combination Therapy" (Source: HMGU).

image-based systems biology, systems medicine approaches and their applications in clinical activities and the pharmaceutical industry. In almost 40 presentations a multitude of methodological approaches and sample applications were covered. The program was divided into the following seven sessions: image-based systems biology, single-cell systems biology, signaling modelling, metabolism, multi-scale approaches, systems medicine and systems pharmacology, systems medicine and genetic/epigenetic mechanisms. During two poster sessions with more than 150 posters, participants had the opportunity to learn more about specific research activities and to exchange knowledge and ideas.

The welcoming speeches were given by Prof. Fabian Theis, Dr. Alfons Enhsen, managing director for the Scientific-Technical Infrastructure at Helmholtz Zentrum München, Dr. Klaus-Peter Michel from the BMBF and Prof. Peter Jansen from the LiSyM consortium. In his opening speech, Theis referred to the complexity of systems biology research. He in particular focused on the need for research approaches that address questions concerning human health and pave the way for personalized (systems) medicine. One of the conference highlights was the plenary talk given by Prof. Chris Sander from the Dana Farber Cancer Institute and Harvard Medical School on the topic of "Systems Biology in Action: Design of Cancer Combination Therapy". In his talk, he discussed the ability of cells and organisms to adapt to changing external conditions and perturbations, and how this causes problems even when target-oriented medication against cancer is applied. He especially pointed out the core scientific challenges that can contribute to developing combined cancer therapies and thus result in improved treatment for patients.

Furthermore, the awardees of the MTZ®-Award for Medical Systems Biology were announced in the context of the conference. The MTZ®-Award for Medical Systems Biology is jointly presented every two years by the MTZ®-Foundation, the BMBF and Project Management Jülich. The prize is awarded to young researchers with outstanding dissertations in the field of medically oriented systems biology.

The winners were:

- **Franziska Witzel**
  Charité Universitätsmedizin Berlin: "Robustness of MAPK signaling"
- **Jörn M. Schmiedel**
  Charité Universitätsmedizin Berlin: "MicroRNAs decrease protein expression noise"
- **Frank S. Heldt**
  University of Oxford: "Models of Influenza A Virus Infection: From Intracellular Replication to Virus Growth in Cell Populations"

At the end of the conference, the three best posters were selected:

- **Giovanni Dalmasso**
  German Cancer Research Center: "Agent-based modelling characterizes the effect of localized versus spread damage among mitochondrial population"
- **Michael Seifert**
  TU Dresden: "Importance of rare gene copy number alterations for personalized tumor characterization"
- **Vito Zanotelli**
  University of Zurich: "Investigating Microenvironment-to-cell Signaling in 3D Spheroids through Imaging Mass Cytometry"

Of course, also social events were part of the SBMC program, giving the attendees the opportunity to exchange scientific ideas in an informal setting. On the first evening, there was a welcome reception as part of the poster session. The real highlight, however, took place on the second evening with a gala dinner at Munich's spectacular *BMW Welt* building. The positive feedback from the conference participants and the stimulating scientific discussions covering diverse topics with enormous potential makes everyone look forward to the 2018 SBMC in Bremen.

Human Cell Atlas initiative starts in London

A catalogue of all human cell types in the making

by Isabel Goehring and Jan Eufinger based on material from the Human Cell Atlas initiative

Creating an atlas of human cells, a "map" that describes every kind of cell in the body, is the goal of the Human Cell Atlas project. Having such a database would revolutionize medical research. To mark the official start of this undertaking, researchers and scientists gathered in London on 13 - 14 October 2016. As the first project of this type, the Human Cell Atlas aims to map mRNA molecules and their regulation in all human body cell types, thereby generating a "reference map" of the healthy body that researchers can use for their work. The mission is as ambitious as the Human Genome Project, an international research campaign that ran from 1990 to 2003 and identified the first complete human genome sequence.

"The cell is the key to understanding the biology of health and disease, but we are currently limited in our understanding of how cells differ across each organ, or even how many cell types there are in the body. The Human Cell Atlas initiative is the beginning of a new era of cellular understanding, as we will discover new cell types, find out how cells change across time, during development and disease, and gain a better understanding of biology." says Sarah Teichmann, head of the Cellular Genetics unit at the Wellcome Trust Sanger Institute in Cambridge, UK.

Group photo of participants of the first meeting of the Human Cell Atlas initiative in London (Source: CC-BY Thomas Farnetti/Wellcome).

Organized by the Broad Institute in Boston and the UK's Wellcome Trust Sanger Institute and Wellcome Trust Foundation, the October meeting brought together international experts to discuss the core issues relating to the work of creating the Human Cell Atlas. The issues discussed included how large the atlas will be, where the human samples will be processed, what technological tools will be used, how the data and information generated will be made available and visualized.

"We believe that a successful description of all cells in the healthy human body will impact almost every aspect of biology and medicine in the decades to come. We now have the tools to understand what we are composed of, which allows us to learn how our bodies work, and uncover how all these elements malfunction in disease. By creating this atlas through an open, international effort, we are building a new research tool for the whole community." says Aviv Regev from the Broad Institute.

Until now, diagnostic and research activities have used mixtures composed of a large number of cells for microscopic and molecular analyses. This process often entails difficulties. For example, it is hard to analyze the results for healthy and diseased cells independently of each other when studying tumor biopsies. With new single-cell profiling techniques, we can now analyze thousands of individual cells at the same time and as such produce precise comparisons of the characteristics of healthy and diseased cells.

"By using single-cell genomics, we expect to greatly improve our understanding of how an organ's overall topology changes during the course of an illness, and how these changes affect the regulation of individual cells in that organ," says Roland Eils (Heidelberg University and German Cancer Research Center), who participated as a German representative at the inaugural meeting. Eils outlined the potential benefits a human cell atlas

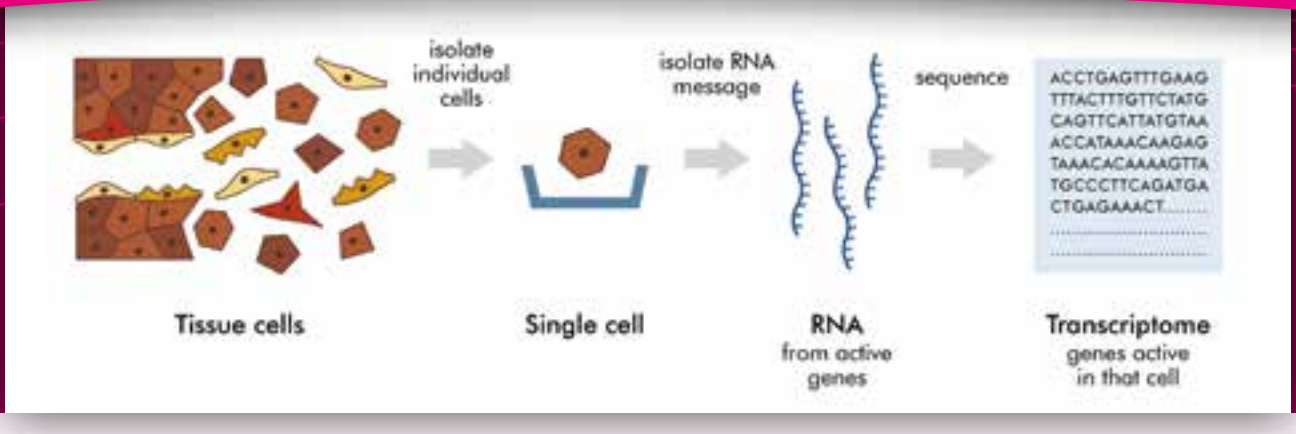


Figure 1: Single-cell genomics consists of reducing tissue samples to single cells, and then isolating these cells. By sequencing the RNAs they contain, researchers can identify the active genes present in each cell (Source: Sanger Institute / Genome Research Limited).

will have for cancer research. "By classifying each cell type individually, we will uncover a wealth of detail about the interaction between degenerated cells and their environments. This will allow targeted identification of specific tumor cell characteristics that, for example, prevent the patient's immune system from attacking the tumor. Understanding these processes will help us develop better treatment options."

The research community will benefit from the many recent advances made in the field of genome sequencing and single-cell sorting. Innovative technological approaches such as single-cell genomics make it possible to separate and sort individual cells taken from tissue and organ samples. This in turn permits measurement of the transcriptome, i.e. the totality of all synthesized RNA molecules, and other molecules within a single cell. The transcriptome helps assigning each cell its own identity and distinguishing it from other cell types within the body. Just a few years ago, it would have been impossible to quantify this complex and vast information in an individual cell.

Pilot projects are already up and running that will provide insights into efficient biopsy techniques and analysis strategies. These projects will include performing measurements on cells from the immune system and the brain, along with single-cell analyses of epithelial tissue cells and tumor cells taken from cancer patients.

The Human Cell Atlas initiative has ambitious goals. The atlas is planned to be freely available to all scientists to form a basis for generating new knowledge about humans' physical development, along with the origins and processes of illnesses such as asthma, Alzheimer's and cancer.

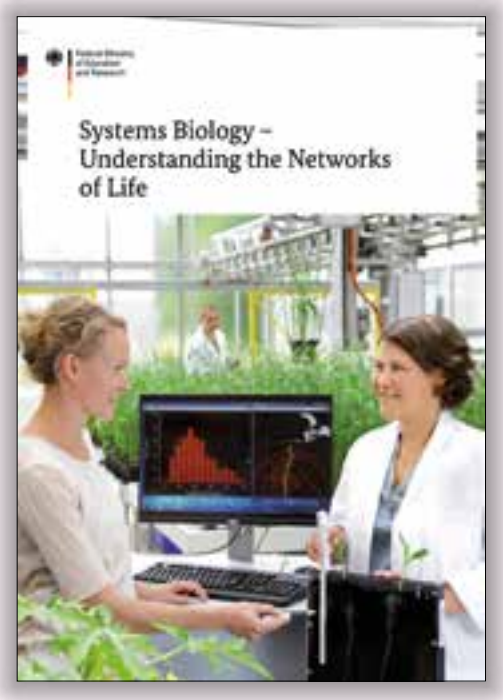
Further information on the Human Cell Atlas:
www.humancellatlas.org

Publication of new brochure on systems biology research

The Federal Ministry of Education and Research has brought out a new brochure about support and research relating to the field of systems biology in Germany.

It contains a host of reports about fascinating research projects in medicine, biotechnology and plant research, profiles of scientists and researchers, plus an interview with systems biology pioneers in Germany. You can also learn more about the background behind support measures for systems biology and this young discipline's international networks.

Brochures are available at
www.bmbf.de/en/information-material.php



Source: FZ Jülich/R. U. Limbach

Current developments at the German Network for Bioinformatics Infrastructure – de.NBI

The German Federal Ministry of Education and Research (BMBF) continues with the enlargement of de.NBI and strengthens Europe-wide bioinformatics cooperation

by Yvonne Pfeiffenschneider

There have been quite a few changes at the de.NBI (German Network for Bioinformatics Infrastructure) support program, founded in March 2015. de.NBI offers comprehensive, top-quality bioinformatics services for specialists in the fields of life sciences and biomedicine. Since the project started, it has seen a number of dynamic developments that nobody had predicted at the beginning. Certain topics had previously not been covered, and these gaps have now been filled by new partner projects joining the scheme. de.NBI has joined ELIXIR, the European research network for life science data and information. In addition, its computer problems will soon be resolved thanks to a cloud created especially for the undertaking.

A lot has happened at de.NBI, the German Network for Bioinformatics Infrastructure, over the past two years. The program has seen a comprehensive range of outstanding bioinformatics services established for users active in the life sciences and biomedicine. At scores of training events, scientists from the bioinformatics network have shown experimental researchers how to use their data effectively and have given them support.

It quickly became clear that the network, which consists of eight service centers (23 sub-projects), did not cover certain topics and issues, but moves were made to rectify these shortcomings in February 2016, when a tender was posted for de.NBI partners. From a cohort of 39 proposals, eight partner projects (covering 17 sub-projects in total) were selected to augment the network since November 2016. These groups are working on epigenetics, metaproteomics, systems biology modelling, protein structure data (enzymology), RNA sequencing, metabolomics, lipidomics and image analysis.

In August 2016, Germany joined the Europe-wide ELIXIR (European Life Sciences Infrastructure for Biological Information) initiative, an association currently made up of 19 partners from across the continent. The activities of the de.NBI network are very similar to those of the European group, guaranteeing that the two bodies will work closely with each other in the future. Joining ELIXIR has benefited both sides. The German researchers round out and augment the resources and expertise available within the ELIXIR network, and in return, being ELIXIR members gives German scientists access to European funding schemes, infrastructure, knowledge and a well-organized bioinformatics platform. Johanna Wanka, Germany's research minister, says, "By joining the scheme, we have provided a double boost to the life sciences in Germany. Being part of an international initiative raises Germany's profile as a base for research activity and enhances our competitiveness. Furthermore, now that Germany is part of the European steering committee, the country can add its national interests to the agenda and contribute to shaping the European information infrastructure." de.NBI was established as a country-level hub within the ELIXIR system in 2017.

In another welcome development, the education and research ministry has acted to resolve the lack of adequate computer capacity in the network by creating a cloud specifically for de.NBI. At

TABLE: SELECTED PROJECT PARTNERS

ACRONYM	FIELD	ORGANISATION	NAME
LIFS	Lipidomics	ISAS (Leibniz Institute for Analytical Sciences)	Robert Ahrends
			Albert Sickmann
		Borstel Research Center, Leibniz Center for Medicine and Biosciences	Dominik Schwudke
		Max Planck Institute of Molecular Cell Biology and Genetics	Andrej Shevchenko
MASH	Metabolomics	Leibniz Institute of Plant Biochemistry	Steffen Neumann
EnzymeStructures	Protein structure data (enzymology)	Hamburg University	Matthias Rarey
de.STAIR	RNA sequencing	Leipzig University	Steve Hoffmann
		Freiburg University	Wolfgang Hess
		Rostock University	Olaf Wolkenhauer
NBI-ModSim	Systems biology modelling	Heidelberg University	Ursula Kummer
		Max Planck Institute for Dynamics of Complex Technical Systems	Steffen Klamt
DAIS	Image analysis	Max Planck Institute of Molecular Cell Biology and Genetics	Eugene Myers
de.NBI-epi	Epigenomics	German Cancer Research Center (DKFZ)	Benedikt Brors
		Max Delbrück Center for Molecular Medicine (MDC)	Altuna Akalin
		Freiburg University	Björn Grüning
		Saarland University	Jörn Walter
MetaProtServ	Metaproteomics	Magdeburg University	Dirk Benndorf
			Gunter Saake

the end of last year, the network has received EUR 5 million in funding just for hardware. The BMBF will also finance salaries for six experts entrusted with the task of creating and operating this cloud between now and the end of 2020. The sites selected to handle this undertaking were ones that had previously been involved in creating cloud solutions. They already have the basic technology in addition to experts in the field. Combined, these factors will ensure that the de.NBI cloud equipment is put into place and designed for long-term operation. Heidelberg, Bielefeld, Gießen, Freiburg and Tübingen are the locations that best fit the bill, so all de.NBI sub-project partners agreed that hardware should be concentrated at these sites. Experts are on hand to help make the service center tools used at the other sub-projects cloud-ready.

Total funding from the BMBF for de.NBI comes to some EUR 31 million over a period of five years. A lot has been achieved since the bioinformatics network's inception, but the researchers involved in it still have a lot ahead of them. Their mission not only includes the tasks and challenges mentioned here, but maintaining this infrastructure following the end of the scheme is another major undertaking that they have to address.

Further information and an overview of de.NBI's training activities can be found at
www.denbi.de

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Photo: FZ Jülich/R. U. Limbach

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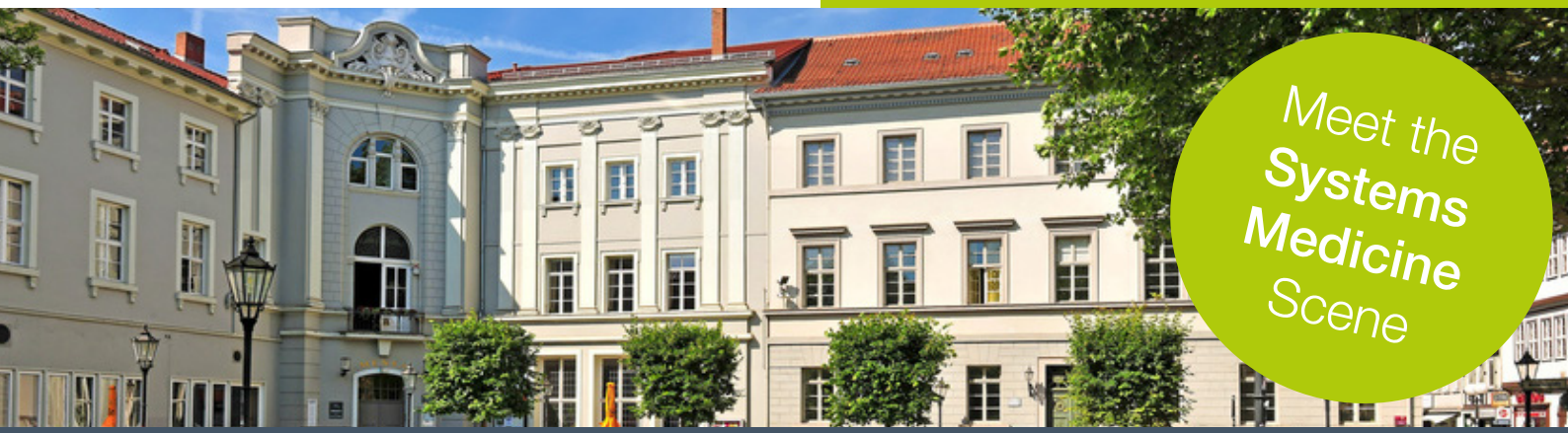
Karriere in der Systemmedizin



Registration and Abstract submission: www.sys-med.de/de/meeting

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systembiologie.de would like to make the success of German systems biology accessible to a wider public in an illustrative way. The magazine, which is published alternately in German and in English, is produced jointly by the Helmholtz Association, Cross Program Topic Systems Biology and Synthetic Biology, Liver Systems Medicine, e:Med Systems Medicine, German Aerospace Center (DLR) and Project Management Jülich (PtJ). It is financed by the Helmholtz Association and by the German Federal Ministry of Education and Research (BMBF).

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EMBL 2017

Conferences

30 AUG - 1 SEP | EMBO Conference
The Nucleosome: From Atoms to Genomes
I. Berger, K. Bystricky, A. Flaus, H. Kurumizaka, K. Luger, T. Schalch
EMBL Heidelberg, Germany

6 - 9 SEP | EMBO Conference
Protein Synthesis and Translational Control
J. Cate, F. Gebauer, R. Green, M. Hentze
EMBL Heidelberg, Germany

13 - 16 SEP | EMBO | EMBL Symposium
The Non-Coding Genome
D. Bartel, E. Izaurralde, J. Rinn, J. Vogel
EMBL Heidelberg, Germany

24 - 27 SEP | EMBO Conference
Centrosomes and Spindle Pole Bodies
S.Dutcher, I.Hagan, L.Pelletier, G. Pereira, E. Schiebel
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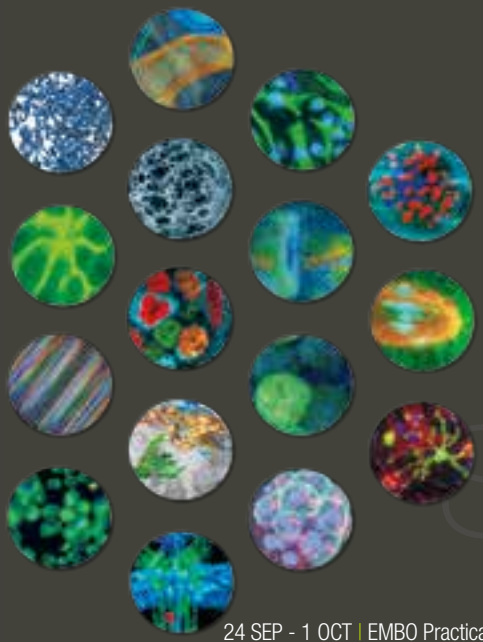

24 SEP - 1 OCT | EMBO Practical Course
Synthetic Biology in Action: Programming Bacteria to Do Amazing Things
V. de Lorenzo, J. Dreyer-Lamm, K. Patil, N. Typas
EMBL Heidelberg, Germany

26 - 28 SEP | EMBL Course
Bioinformatics Resources for Protein Biology
S. Morgan
EMBL-EBI Hinxton, UK

2 - 6 OCT | EMBL Course
Metagenomics Bioinformatics
L. Emery, R. Finn, A. Mitchell
EMBL-EBI Hinxton, UK

3 - 6 OCT | EMBL Course
Whole Transcriptome Data Analysis
V. Benes, R. Calogero
EMBL Heidelberg, Germany

9 - 12 OCT | EMBL Course
Introduction to Next Generation Sequencing
T. Hancocks, J. Randall, M. Rosello
EMBL-EBI Hinxton, UK

4 - 7 OCT | EMBO | EMBL Symposium
Seeing is Believing - Imaging the Processes of Life
J. Ellenberg, J. Lippincott-Schwartz, A. Miyawaki
EMBL Heidelberg, Germany

11 - 14 OCT | EMBO | EMBL Symposium
The Mobile Genome: Genetic and Physiological Impacts of Transposable Elements
O. Barabas, J.F. Brennecke, J.V. Moran
EMBL Heidelberg, Germany

24 - 27 OCT | EMBL Conference
Mammalian Genetics and Genomics: From Molecular Mechanisms to Translational Applications
P. Avner, T. Gunn, M. Hrabe de Angelis, M. Justice, L. Siracusa, F. Spitz
EMBL Heidelberg, Germany

2 - 4 NOV | EMBO Conference
Quantitative Principles in Biology
A. Aulehla, J. Garcia-Ojalvo, R. Phillips
EMBL Heidelberg, Germany

5 - 8 NOV | EMBL Conference
Cancer Genomics
P. Campbell, J. Korbel, N. Lopez-Bigas, M. Meyerson
EMBL Heidelberg, Germany

12 - 14 NOV | EMBO | EMBL Symposium
From Single- to Multiomics: Applications and Challenges in Data Integration
N. Krogan, U. Sauer, J. Zaugg
EMBL Heidelberg, Germany

16 - 17 NOV | EMBL Conference
Revolutions in Structural Biology: Celebrating the 100th Anniversary of Sir John Kendrew
S. Cusack, C. Müller, J. Thornton, M. Wilmanns
EMBL Heidelberg, Germany

5 - 7 DEC | EMBL Conference
Lifelong Learning in the Biomedical Sciences
C. Brooksbank, M. Hardman C. Janko, C. Johnson, L. Wight
EMBL Heidelberg, Germany

Courses

9 - 13 OCT | EMBL Course
Liquid Biopsies
J. Dreyer-Lamm, A. Ståhlberg
EMBL Heidelberg, Germany

15 - 20 OCT | EMBO Practical Course
Humanized Mice in Biomedical Research
K-D. Bissig, N. Dear, C. Münz, J.J. Schuringa, L. Shultz, B. Soper, R. Stripecke
EMBL Heidelberg, Germany

18 - 20 OCT | EMBL Course
Software Carpentry
T. Hodges, M. Sharan, G. Zeller
EMBL Heidelberg, Germany

23 - 27 OCT | EMBL Course
Structural Bioinformatics
T. Hancocks, G. Kleywegt, C. Orengo
EMBL-EBI Hinxton, UK

30 OCT - 2 NOV | EMBL Course
Metabolomics
R. Salek
EMBL-EBI Hinxton, UK

6 - 10 NOV | EMBO Practical Course
Analysis of High-Throughput Sequencing Data
G. Rustici
EMBL-EBI Hinxton, UK

6 - 10 NOV | EMBL Course
Next Generation Sequencing: Enrichment Based Targeted Resequencing
V. Benes, J. Dreyer-Lamm, A. Heim
EMBL Heidelberg, Germany

8 - 9 NOV | EMBL Course
Microinjection into Adherent Cells
R. Pepperkok, S. Stobrawa, S. Terjung
EMBL Heidelberg, Germany

13 - 16 NOV | EMBL Course
Next Generation Sequencing: Amplicon Based Targeted Resequencing
V. Benes, J. Dreyer-Lamm, A. Heim
EMBL Heidelberg, Germany

13 - 17 NOV | EMBL Course
Plants and Plant Pathogens
P. Kersey
EMBL-EBI Hinxton, UK

14 - 16 NOV | EMBL Course
Exploring Biological Sequence Data
T. Hancocks
EMBL-EBI Hinxton, UK

20 - 24 NOV | EMBO Practical Course
The Fundamentals of High-End Cell Sorting
D. Davies, A. Filby, D. Ordonez, M. Paulsen, S. Schmitt
EMBL Heidelberg, Germany

29 NOV - 1 DEC | EMBL Course
Next Generation Sequencing: Whole Genome Sequencing Library Preparation
V. Benes, J. Dreyer-Lamm, A. Heim
EMBL Heidelberg, Germany

4 - 7 DEC | EMBL Course
Next Generation Sequencing: RNA Sequencing Library Preparation
V. Benes, J. Dreyer-Lamm, A. Heim
EMBL Heidelberg, Germany

10 - 15 DEC | EMBL Course
High-Accuracy CLEM: Applications at Room Temperature and in cryo
R. Mellwig, M. Schorb
EMBL Heidelberg, Germany

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