INTERNATIONAL EDITION

systembiologie.de

THE MAGAZINE FOR SYSTEMS BIOLOGY RESEARCH IN GERMANY

ISSUE 12 MAY 2018

special: systems medicine

from page 8

tickling the retina – how the retina processes visual stimuli

page 13

signals that never fall silent – how signaling pathways regulate liver metabolism

page 30

interviews with

Hamid R. Noori

Carsten Marr

Mike Snyder

Jeffrey Moffitt

page 18, 54, 71 and 84

ISSN 2191-2505

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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.

greetings

Dear Reader,

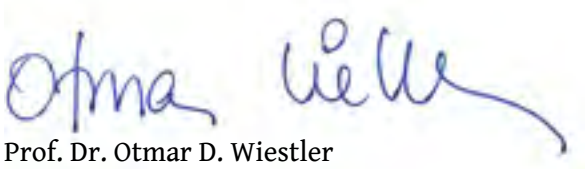
Thanks to exceptional medical care and improved living conditions, life expectancy continues to rise in Germany. According to estimates, girls who are born this year will live to an average age of 93 years, while boys can also look forward to living up to the age of 90. But higher life expectancy brings with it new challenges: the number of people suffering from diabetes, cancer and cardiovascular diseases will also increase. Understanding the complex relationships between diseases and putting the many factors together to create an overall picture is a major challenge for the discipline of systems medicine.

Researchers from the Helmholtz Association are making a huge contribution in this forward-looking area of research and combining experimental methods with mathematical models. The German Center for Neurodegenerative Diseases (DZNE), for example, is adopting approaches from systems medicine in order to better understand complex diseases of the central nervous system. Since March 2017, around 500 experts have been united under one roof in Bonn in one of Germany's most impressive newly constructed buildings (page 50), where an area of around 35,000 square meters is home to basic research, clinical trials and population research. Additional sites conduct healthcare research. The DZNE is dedicated to dementia and other neurodegenerative diseases in all their facets. It is a prime example of translational research.

Another new feature of the Bonn site is the recently founded platform for single-cell genomics and epigenomics (PRECISE, page 51). This joint venture between the DZNE and the University of Bonn will in future bundle the expertise of both institutions in sample processing, automation, sequencing, data preprocessing and data analysis, thus covering the entire workflow of systems biology approaches on the basis of genomic data.

The physicist Carsten Marr is also working on individual cells. At the Institute of Computational Biology at the Helmholtz Zentrum München, he is attempting to understand how individual cells develop with the aid of innovative, computer-aided simulations and intelligent image recognition. In the future, this could play a key role in understanding and even predicting the complex processes involved in the development of diseases such as skin cancer and leukemia (page 54).

The current issue of systembiologie.de shows how systems medicine is accelerating the integration of new research findings into clinical application and develops new preventive measures and therapies for patients. It takes a fascinating look at the various organs in the human body in the context of systems medicine. I hope you enjoy reading this issue!



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

Photo: Steffen Jänicke / Helmholtz Association

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foreword

Dear Reader,

For more than two decades now, systems biology has been an integral part of the life sciences. In addition to the more basic research-oriented systems biology, application-oriented systems medicine has also developed as a major branch of research. Systems medicine takes a holistic view of human diseases in order to study the molecular and cellular processes that cause them. The aim is both prevention and diagnosis, as well as studying and improving upon the therapies available for these diseases.

In doing so, systems medicine investigates the genetics, cellular biology and physiology behind diseases, using a quantitative approach. The data from high-throughput approaches known as "omics" technologies can be analyzed via computer-aided processes and the creation of mathematical models. This data can then be used to create a prognosis for the effect and success of therapies.

In this issue, we would like to give you an insight into the latest research findings from the world of systems medicine and take a look at various organs in the context of a wide variety of diseases. The topics include widespread diseases such as cancer and coronary disease, as well as pneumonia, metabolic diseases of the liver, intestinal diseases, and also innovative fields of research such as bionic sight (from page 8).

One exciting development is the recently founded international science initiative known as the Human Cell Atlas (HCA). The HCA aims to map and analyze each individual cell in the body. Thus, a cell atlas will be created to describe each type of cell in the body. In future, the HCA could be of unimaginable value in the analysis of cell mutations that turn normal cells into diseased cells within their tissue environment (page 88).

The continued development of single-cell analysis offers a plethora of potential applications in the field of biomedical research. Scientists Carsten Marr and Jeffrey Moffitt have both chosen to focus on the analysis of individual cells. In order to understand how individual cells function, Carsten Marr develops algorithms and computer-aided simulations at the Helmholtz Zentrum München. One of his goals is to understand how stem cells develop (page 54). Jeffrey Moffitt develops new microscopic imaging processes in order to depict thousands of RNA molecules within a cell (page 84).

As you will find out in this issue, the methods used in systems medicine will become essential in clinical research and will help us to better understand fundamental molecular and cellular processes.

I hope you enjoy this insight into the world of systems medicine!

Yours, Roland Eils

Editor in Chief

Photo: Tobias Schwerdt / DKFZ

index

systems-based analysis of drug resistance in melanoma

Analysis of survival and death signaling pathways in melanoma using Dynamic Bayesian Network approach

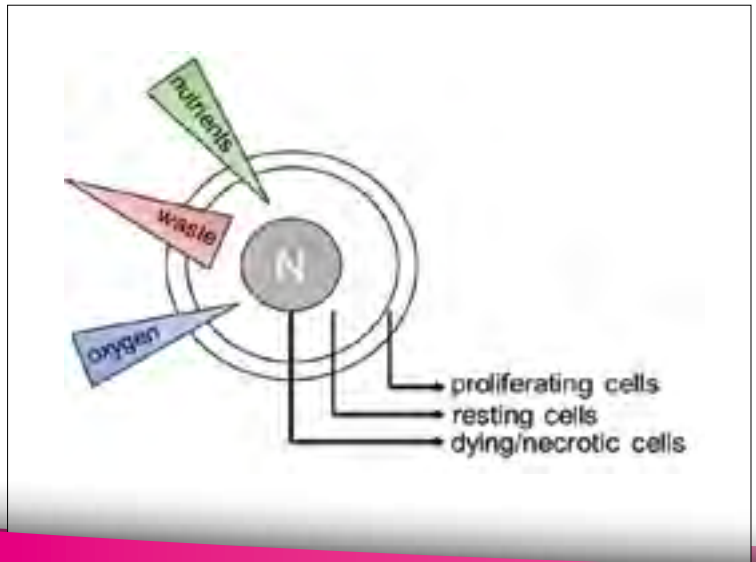
by Philippe Lucarelli, Greta Del Mistro, Ines Müller, Sébastien De Landtsheer, Friedegund Meier, Roland Kontermann, Markus Rehm, Thomas Sauter and Dagmar Kulms

Malignant melanoma, also called black skin cancer, is one of the most aggressive forms of this cancer type. The number of melanoma patients continues to increase and prognosis for the often early onset metastatic stage is extremely poor. The dysregulation of two major mitogen activated pathways (RAS-RAF-MEK-ERK and PI3K-AKT-PTEN) are key drivers of melanoma development and progression (Lawrence *et al.*, 2013). The development of targeted kinase inhibitors (vemurafenib, dabrafenib for BRAF mutated; trametinib for NRAS mutated melanoma) could not fulfill the promised therapeutical benefit, leading to relapse in the majority of patients, mostly resulting from reactivation of the MAP kinase pathway. Accordingly, combination of BRAF and MEK inhibitors, only delays development of resistance (Long *et al.*, 2014). Using a systems approach the e:Med demonstrator consortium "Melanoma Sensitivity" currently addresses whether inducing cell death by tumor-selective death ligands as single or co-treatment option may be beneficial.

A novel 2nd generation TRAIL-receptor agonist

Activation of the tumor-selective death ligand TRAIL (Tumor necrosis factor-related apoptosis-inducing ligand)-induced apoptosis pathway holds promise as a novel treatment option for cancer. Binding of TRAIL to cognate cell surface receptors induces a coordinated multi-protein coded cell death program referred to as apoptosis (Johnstone *et al.*, 2008). Since conventional trimeric TRAIL and receptor-agonistic antibodies as single agents failed in clinical trials, novel 2nd generation TRAIL-receptor agonists are being developed within the e:Med

Figure 1: Schematic overview of tumor architecture



Within avascularized tumor spheroids or metastasis gradients of oxygen and nutrients influx exists, while cellular debris accumulates in the necrotic center (Source: Vörsmann, H. *et al.*, 2013).

The number of melanoma patients is steadily increasing and prognosis for the often early onset metastatic stage is extremely poor (Source: Dan Race – Fotolia.com).

melanoma sensitivity consortium. IZI1551 is a fully human TRAIL-Fc-fusion protein consisting of two single-chain TRAIL molecules fused covalently to the Fc-part of human IgG, which can activate TRAIL receptors independent of FcγR-driven clustering. IZI1551 is significantly more potent than other agonists, and has passed safety studies in mice (Hutt *et al.*, 2017). In order to understand the potency of IZI1551 in melanoma treatment, we aimed to investigate the impact of lethal but also sublethal IZI1551 doses on melanoma cell migration, invasive potential and cell death using a combination of integrated experimental and mathematical studies.

The architecture of tumors and the drug dose applied may influence the therapeutic outcome

The architecture of solid tumors or metastasis represent with cellular heterogeneity due to a differential distribution of oxygen, nutrients and cellular waste within the tumor, starting with a proliferating cell population at the outer rim being located close to the vascularization. With increasing distance from the source of oxygen and nutrients and concomitant accumulation being of cellular debris a subsequent layer of quiescent cells is constituted, followed by a center being composed of necrotic and apoptotic cells, respectively (Figure 1).

Accordingly, responses to drugs may vary within the tumor tissue depending on the final dose and cell subpopulation reached by the drug. In order to gain a holistic understanding of the signal transduction network being affected by different IZI1551 doses we conditioned the melanoma cell line A375 to different IZI1551 doses to be investigated with a Dynamic Bayesian Network, called FALCON (Fast Contextualization of Logical Networks; https://sysbiolux.github.io/FALCON/) newly developed within the e:Med melanoma sensitivity program.

Developing a systems biological tool to predict melanoma sensitivity

FALCON is a Matlab-based computational toolbox which allows for a quantitative contextualization and analysis of large steady-state datasets of regulatory and signaling networks within a few minutes. First a context specific network is generated in an optimization process based on a generic starting network and context specific experimental data. The toolbox facilitates different model analysis methods such as parameter sensitivity analysis, systematic knock-outs, as well as differential activity analysis which allows to directly compare the differences between cell lines or experimental conditions (Figure 2). This enables the analysis of the properties of an optimal network (De Landsherr *et al.*, 2017).

The FALCON toolbox was applied to identify the optimal combination of targeted molecules to decrease survival and enhance cell death of melanoma cells. Therefore, we established a comprehensive network model containing those signal transduction pathways that are relevant in melanoma development and may

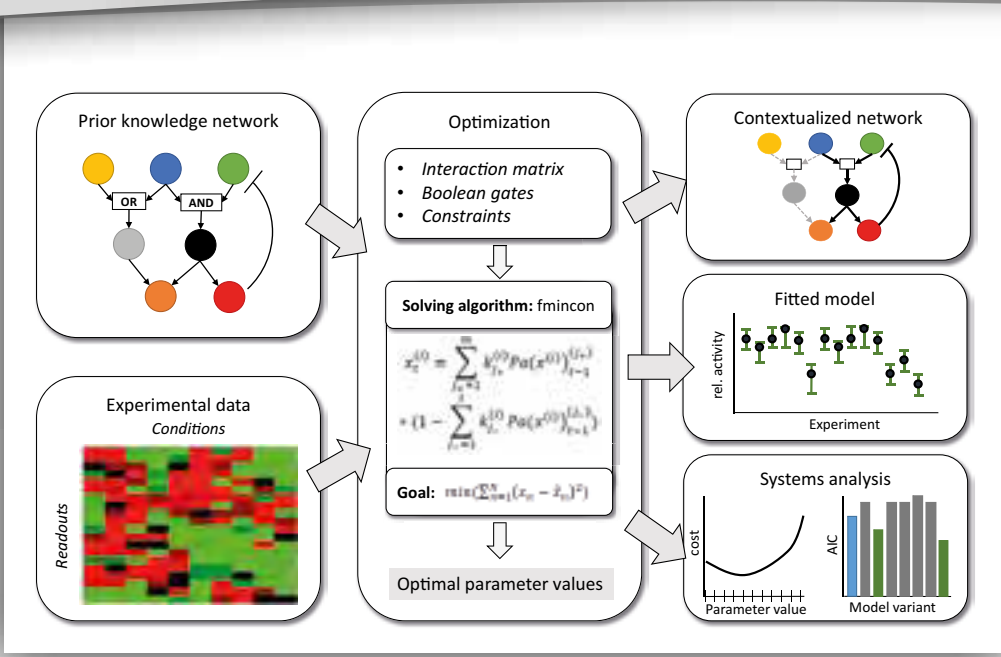


Figure 2: The FALCON pipeline. Prior knowledge network and experimental data are combined to generate a network optimization problem. After the optimization process, the properties of the optimal network are then analyzed (Source: De Landtsheer, S. *et al.*, 2017).

interfere with treatment regimes. Furthermore the crosstalk to TRAIL (IZI1551)-dependent signaling was monitored based on literature information obtained from different sources. This covers the MAPK pathways, NFκB signalling and apoptotic signal transduction pathways (Figure 3). By applying the FALCON toolbox we aimed to identify mechanisms responsible for survival as well as metastatic migration and invasion.

Melanoma cells that have acquired resistance to sublethal IZI1551 doses show increased migration/invasion in an NFκB-dependent manner

Conditioning A375 melanoma cells for 6 months to sublethal doses of IZI1551 revealed cells to stay resistant against apoptosis induction with a lethal IZI1551 dose. Conditioned cells revealed severe modifications in total protein levels and the activation status of survival proteins including NFκB, IκBα, AKT, ERK and JNK, anti-apoptotic, BCL-2 and FLIP, as well as pro-apoptotic proteins like Caspase-3 and Caspase-8. This new phenotype presented with newly acquired resistance only against the specific drug used for conditioning (IZI1551), while responses to alternative drugs (cisplatin, targeted kinase inhibitors) remained unaffected. Above this, conditioned melanoma cells presented with a significantly increased migration and invasion potential as revealed by using a newly developed 3D migration/invasion assay within the e:Med melanoma sensitivity consortium. Enhanced migration/invasion was shown to be dependent on constitutive activation of the transcription factor NFκB and correlated with upregulation of cell surface proteins MelCAM and αVβ3-integrin. These proteins have previously been shown to play a major role in cell adhesion during migration, thus the identified phenotype may mimic conditions metastatic spreading.

Predictive power of the FALCON toolbox

Predictive tools that specify dysregulation within cancer cells and focus on individual molecular targets are demanded to identify responders to selected combination therapies while sparing unnecessary treatment burden for non-responders. Quantitative protein measurements as well as apoptotic rates and cell proliferation were integrated into the FALCON network model. Optimal cell line comparison was achieved by different novel regularized algorithms in order to obtain cell type specific parameters and were applied to compare and identify cell type specific reactions between parental and IZI1551 conditioned A375 cells. Model analysis revealed that mainly reactions linked to NFκB and apoptosis signaling were strongly upregulated in the resistant cells. In addition, to target the essential nodes of both cell types, a systematic *in silico* knockout analysis of each protein in the network was performed. Whereas in the parental A375 cells nodes identified as essential mainly encompassed proteins linked to apoptosis, the essential regulators in the conditioned cell lines were identified to be mainly pro-survival proteins.

Subsequently, we aimed to identify optimal drug combinations which could be applied to render resistant melanoma cells sensitive towards apoptotic cell death thereby also preventing metastatic outgrowth. To identify the optimal treatment com-

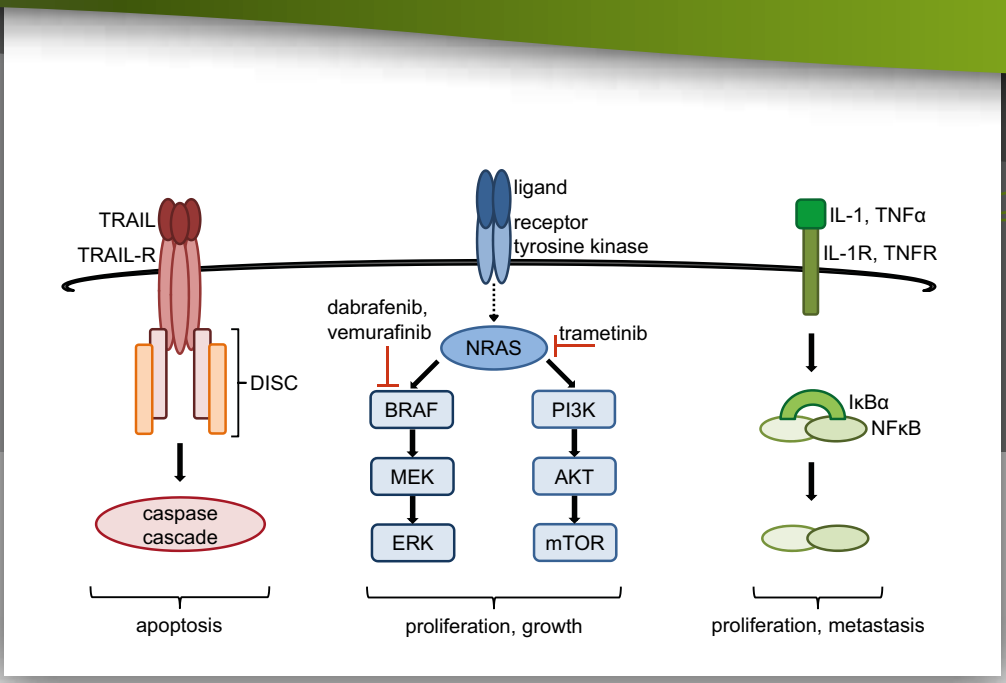


Figure 3: Cellular signal transduction pathways that may influence melanoma progression and treatment. MAPK-signalling pathways downstream of NRAS are mostly deregulated to induce melanoma progression. Both, apoptotic TRAIL-dependent signaling as well as pro survival NFκB-dependent pathways have been shown to interfere with MAPK signal transduction in malignant melanoma and therefore may potentially serve as therapeutic targets (Source: Ines Müller, TU Dresden).

bination, an *in silico* drug screen was performed and all available drug combinations were tested. The new drug targets and combinations identified *in silico* will consequently be validated in selected melanoma models *in vitro*.

Taken together, by combining cell and molecular biological research with systems biological modelling we have identified NFκB signaling to be dysregulated in response to sublethal drug doses. Constitutive activation of NFκB as a consequence results in upregulation of adhesion molecules that favor metastatic outgrowth. One could assume that sublethal drug doses – in the process of diffusion through the tumor – may cause survival and metastatic regrowth of individual tumor cells in an NFκB-dependent manner, thereby, inducing tumor relapse.

Research project profile:

The e:Med demonstrator consortium "Predicting individual sensitivity of malignant melanoma to combination therapies by statistical and network modeling on innovative 3D organotypic screening models (melanoma sensitivity)" consist of five partners that have a high quality long standing record in the fields of apoptosis, systems biological and translational cancer research involving TRAIL-induced as well as MEK-dependent signal transduction in malignant melanoma. As part of the research and funding concept "e:Med – measures establishing systems medicine", the Federal Ministry of Education and Research supports projects that can make an important contribution to personalized medicine.

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Prof. Friedegund Meier, Dermatologic Oncology, Department of Dermatology, TU Dresden
Prof. Roland Kontermann, Institute of Cell Biology and Immunology, University of Stuttgart
Prof. Markus Morrison, Institute of Cell Biology and Immunology, University of Stuttgart
Prof. Thomas Sauter, Life Science Research Unit, Université du Luxembourg

These different but intersecting viewpoints have merged to determine a molecular mechanism conferring resistance against conventional treatment approaches by combining cell- and molecular biological studies with systems biological analysis of signal transduction networks (FALCON toolbox). In particular we:

- Developed melanoma specific hexameric human TRAIL-Fc-fusion protein with significantly increase bioactivity which was shown to kill melanoma cells more potently than conventional targeted kinase inhibitors.

- Could show that conditioning of melanoma cells to a specific drug – mimicking therapy resistance – fosters metastatic outgrowth and proliferation in 2D and 3D models.
- Developed a Matlab-based computational toolbox (FALCON) which allows for a quantitative contextualization and analysis of large steady-state datasets of regulatory and signaling networks to identify alternative drug targets.

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tickling the retina

Source: Ramona Heim - Fotolia.com

A research team from Tuebingen examines a visual stimulus processing system of the retina to improve bionic vision

by Daniel L. Rathbun and Zohreh Hosseinzadeh

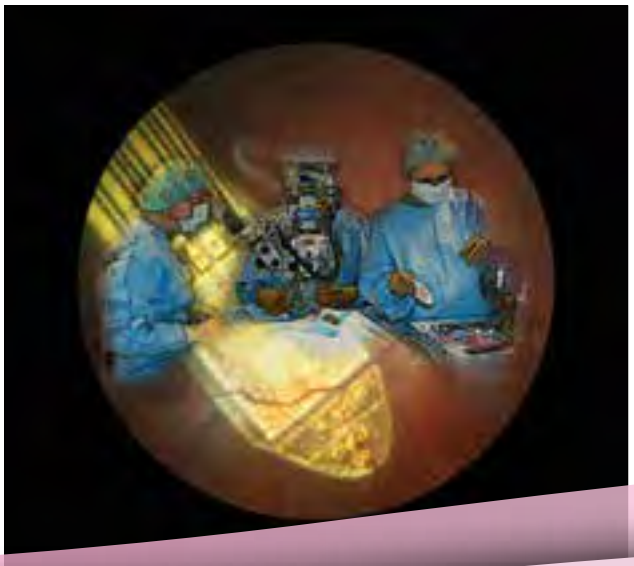
As human life expectancy increases, blindness and visual impairment due to degeneration of the light responsive cells of the eye – photoreceptors – becomes ever more significant. Thousands of laboratories are pursuing solutions to this problem. To date, one of the most successful approach has been the implantation of devices – like Tuebingen's Alpha-AMS implant – to electrically stimulate the remaining neuronal cells of the eye. Responding to input from an electronic camera these neurons then communicate visual information to the brain. Thus the natural vision that has been lost to blind patients is replaced with bionic vision. But the pivotal signals are far from being all decoded.

The thin network of neurons lining the inner surface of the eye is called the retina. It is organized into three primary layers of photoreceptor, bipolar, and ganglion cells – with horizontal and amacrine interneurons completing the network. The retina has many parallel information processing channels. As a result, the ganglion cells which pass information to the brain consist of dozens of differ types. All the ganglion cells of a single type constitute a visual information channel; and each of these channels carries a different message about the visual world to the brain (Figure 1). Ensuring that bionic retinal implants fully exploit this diversity of information channels has been a long-term goal of bionic vision research. To pursue this goal, we have applied the methods of linear system analysis to electrical stimulation of the retina. Although common in visual neuroscience, this approach is new to bionic vision.

White noise and the electrical input filter

In the field of bionic vision, a traditional approach is to take an electrical stimulus waveform (e.g. rectangular-, triangular-, sine-wave, etc.) and vary the parameters of this waveform to identify the best stimulus for a visual neuron (Figure 2). In one such study, we have assembled a database of thousands of mouse ganglion cell responses to rectangular voltage pulses varying in amplitude and duration (Jalligampala *et al.,* 2017). In another, we varied the space separating two such pulses (Hosseinzadeh *et al.,* 2017). This parameter-based approach is most effective when such parameters independently affect responsiveness, but has the disadvantage that the potential waveform space is functionally infinite. An alternative approach is to randomly sample from a large portion of this waveform space using a random noise stimulus. This noise-based approach allows each neuron to tell us which stimulus it prefers simply by responding with an action potential (also called a "spike") whenever a part of the stimulus resembles the neuron's preferred stimulus. These selected stimuli are

Retinal Implant Chip



Source: Graphic from Regina Ebenhoch, Tuebingen University

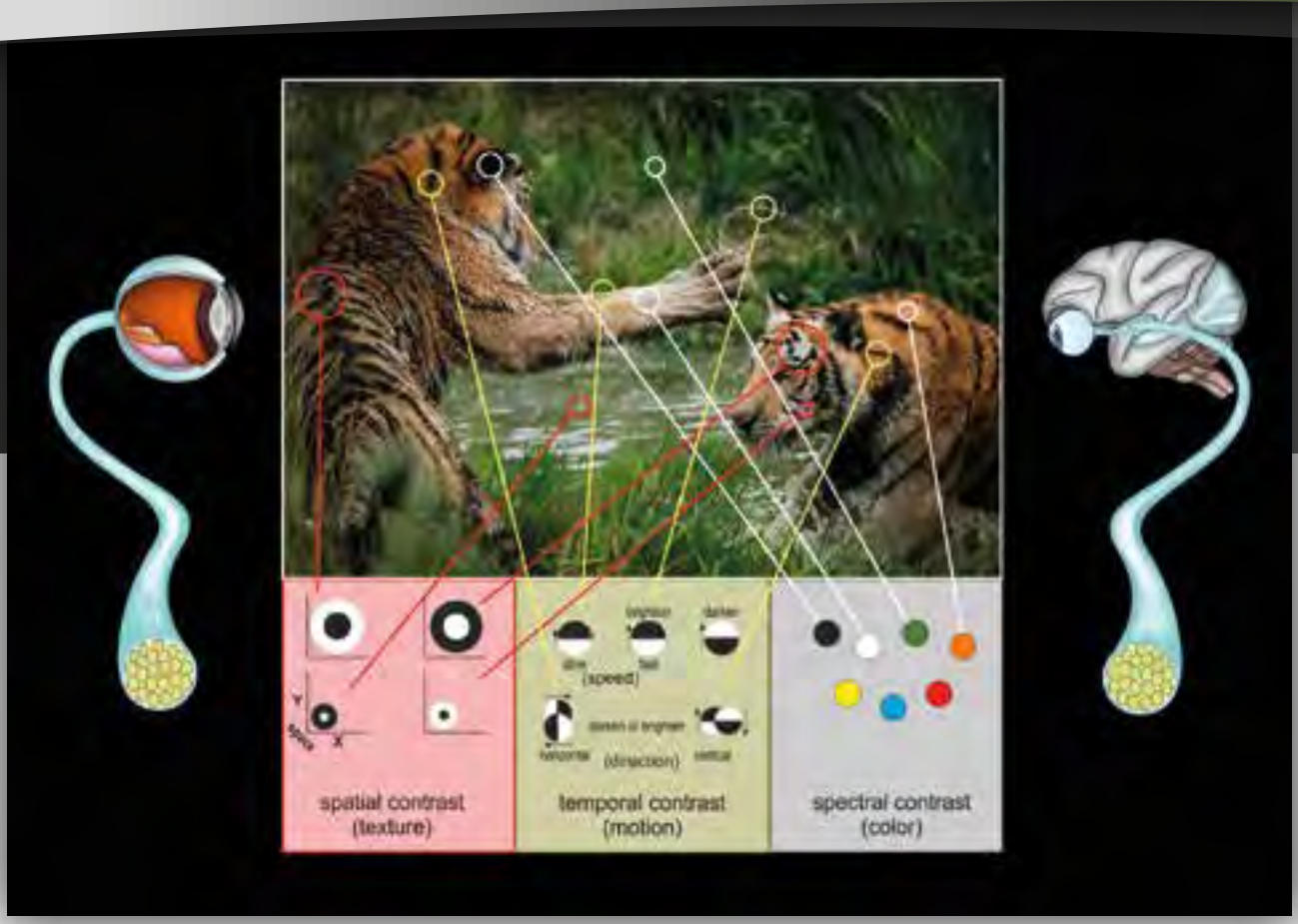


Figure 1: The retina sends many different channels of visual information to the brain. (Source: Regina Ebenhoch and Daniel Rathbun / Photo by Frida Bredesen on Unsplash.com).

then averaged together in a process called spike-triggered averaging. The resultant average stimulus is a good model of the filter applied by that neuron to electrical inputs. Stimuli which match this *electrical input filter* well will activate the cell, whereas poorly-matched stimuli will not.

Tickling the retina

In our particular application of white noise stimulation and spike-triggered averaging, we have used a series of short pulses similar in duration to those already used in retinal implants (Figure 3; Sekhar *et al.,* 2016). The amplitude of these pulses, however, was varied according to a noise sequence. Thus, we could investigate which sequences of pulses best activate the retina. We found that a sequence of pulses with amplitudes well below the single-pulse stimulation threshold could reliably cause retinal ganglion cells to spike (Figure 3). This discovery points to the possibility that, in order to send information to the brain, future implants might "tickle the retina" with low-amplitude pulse patterns instead of "hammering on the retina" with large single pulses. As a side effect of this approach, we were simultaneously able to measure the stimulus-response function of ganglion cells to pulse voltages under conditions similar to those used in human implant patients. Thus, in addition to revealing the electrical input filter, the white noise method is an efficient way of probing the large space of potential stimuli.

A unique filter for every visual channel?

One of our most surprising discoveries was that the electrical input filters differ in shape for different types of retinal ganglion cells (Sekhar *et al.,* 2017). Two broad classes of ganglion cells are ON cells which respond to increasing light and OFF cells which prefer decreasing light. We found that electrical pulse voltage typically increased before ON cell spikes, whereas it typically decreased before OFF cell spikes (Figure 4). This means that these two populations of ganglion cells can be selectively activated, simply by choosing between either a pattern of increasing pulse amplitude or decreasing amplitude. Therefore, light (ON) and dark (OFF) can both be quickly transmitted to the brain by splitting the visual information into these two channels. In current implants, the quick transmission of lightness information comes at the cost of slower information about darkness. While these results are promising, it remains to be seen whether ganglion cells which encode the many other visual features (e. g. color, motion speed, motion direction, stimulus size, stimulus orientation, etc.) have similarly distinct preferred electrical stimuli.

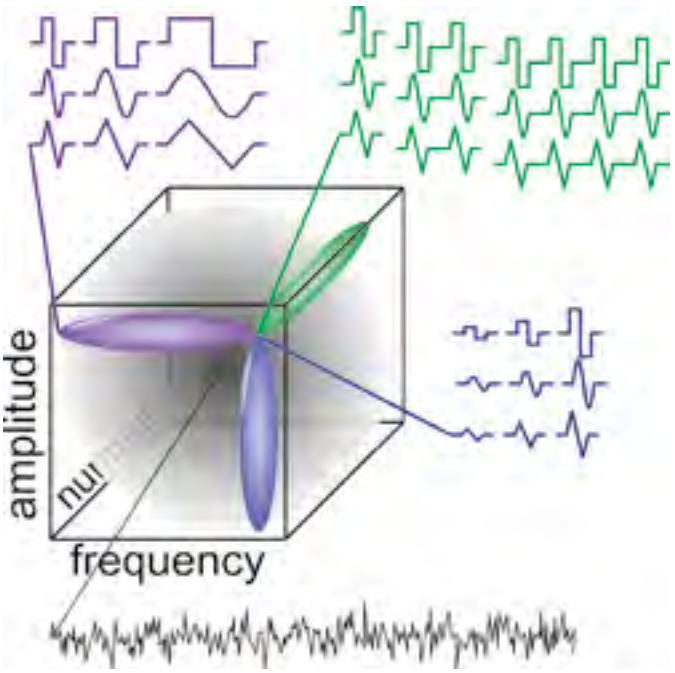


Figure 2: Electrical stimuli are drawn from a high-dimensional space of parameters. Three such parameters are pulse amplitude, pulse fundamental frequency, and pulse number. White noise stimuli draw from this parameter space randomly (Source: Daniel Rathbun and Zohreh Hosseinzadeh).

Linear system analysis for systems biology

The research we have described here appears very different from most research carrying the label of systems biology. Nevertheless, neurobiology like ours has much in common with other systems biology research. In fact, some of the earliest examples of a systems biology approach can be found in the Hodgkin-Huxley model of action potential generation and Denis Noble's model of the heart pacemaker. Because we study the retinal visual processing system in order to improve bionic vision, we largely disregard the individual cells of the retinal network, and instead model how inputs and outputs to this system are related in time. Happily, such a "black-box" modeling approach is common across many systems biology enterprises. What we also share in common with more mainstream systems biology approaches, is an emphasis on computational tools and explicit mathematical models to understand our data and to design future experiments.

Because neural coding is inherently concerned with the flow of information over time, the associated tools are particularly well-suited to understanding network dynamics. We suggest that these tools could also benefit other systems biology efforts. While a common focus in "omics"-style systems biology is on identifying which elements interact with each other at discrete points in time, such interactions are often quite dependent on timing. Therefore, the linear system approach we have applied for electrical stimulation of the retina could also be applied to gene expression, protein interaction, cellular interactions, ecosystem dependencies, etc. We have shown here how a time-varying noise signal can be used to probe the temporal filtering of a dynamic system. A corollary method that is likely to be more applicable to most systems biology projects is to present the system with an impulse or "click" stimulus in which the input is presented in a single quick pulse (e.g. Verotta 1996; Szabó *et al.,* 1985). The output of a linear system to such a stimulus is called the impulse response and closely resembles the input filter we have described here. As with the input filter, an impulse response compactly describes the temporal filtering properties of a system to the degree that the system has a linear input/output relationship. In conjunction with well-established tools for dealing with simple system nonlinearities, such noise or impulse probing may prove to be a powerful unifying tool across the broad diversity of systems biology projects.

Outlook

One of the obstacles to bionic vision has been a limited understanding of the visual information encoded by each of the retinal ganglion cell types. However, recent research by colleagues at the University of Tuebingen has taken great strides in delineating the 30 or so different information channels of the mammalian retina (Baden *et al.,* 2016). We plan to build on this basic research by determining the unique electrical input

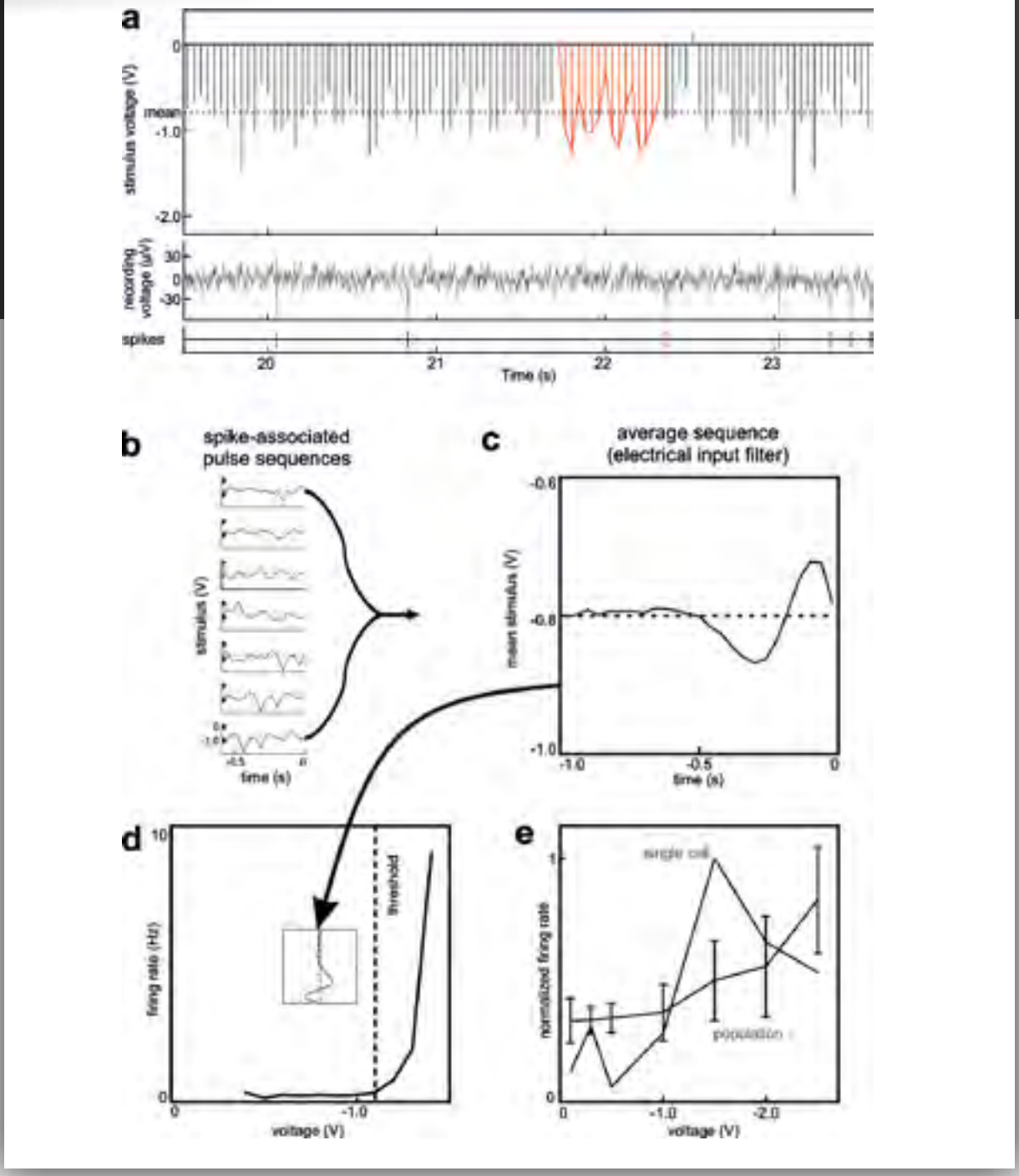


Figure 3: White noise stimulation and reverse correlation. (a) A series of pulse amplitudes is presented in a random order and neuronal spikes are recorded. (b) Pulse sequences that lead to a spike are averaged together. (c) The spike-triggered average sequence reveals the electrical input filter of the neuron. (d) Sorting the responses to single pulses out of the noise sequence also reveals the voltage-response function in greater detail than is typical for parametric experiments. (e) For comparison, the voltage-response functions calculated from parametric experiments are shown for both a single (different) cell and a large population. (Source: Daniel Rathbun, Zohreh Hosseinzadeh and Sudarshan Sekhar).

filters of these different channels. In doing so, we hope to soon deliver on the promise that bionic retinas will one day restore naturalistic vision to the blind.

Research project profile:

SysRetPro (SYStems neuroscience in service of the next-generation RETinal PROsthesis) uses linear systems analysis and explicit (visual and electrical) stimulus-response models to better understand how both light and electrical stimulation are encoded by the mouse retina. These models are used to improve the visual experiences of retinal implant patients. By altering the functioning of retinal implants to generate more natural spike patterns for the brain, we seek to perfect the simulated visual world of bionic vision recipients. The Federal Ministry of Education and Research supports the project as part of the innovation project “e: Bio”.

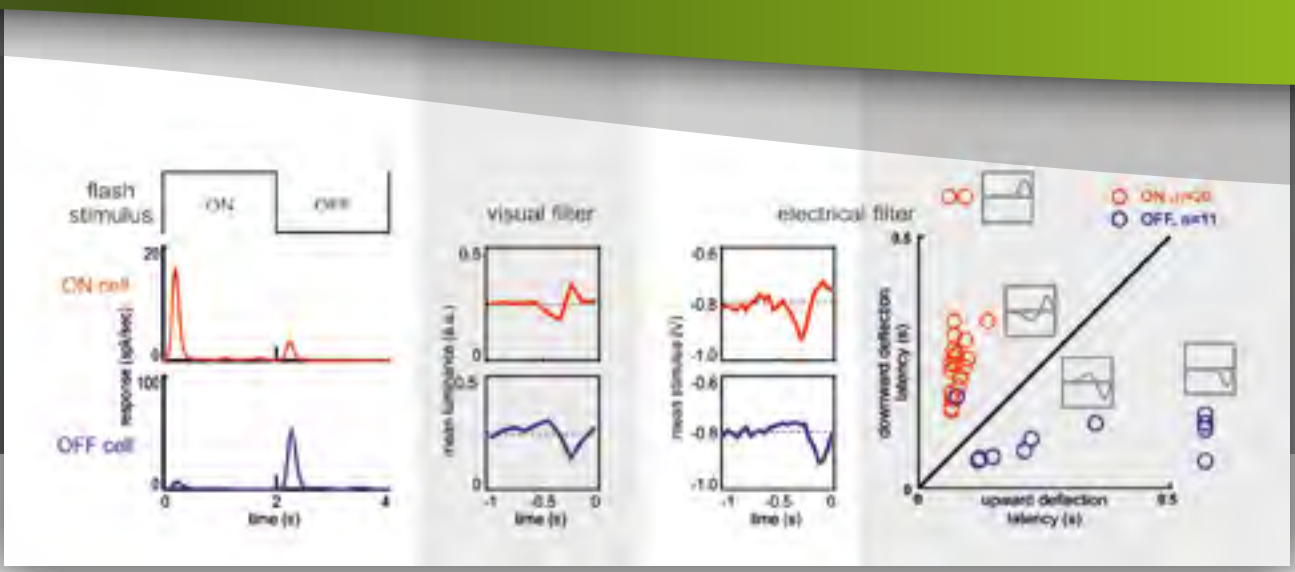


Figure 4: Visual and electrical responses of ON and OFF cells. (left column) Typical ON- and OFF- cell responses to a flashing light stimulus. (second column) Visual input filters for these cells. (third column) Electrical input filters are well matched to visual input filters. (last column) Latencies of the upward and downward electrical input filters for a population of cells. Some cells have monophasic filters while others are biphasic (Source: Daniel Rathbun and Sudarshan Sekhar).

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"the different disciplines speak different languages"

Interview with e:Med junior scientist Hamid R. Noori

Hamid R. Noori is the head of one of the eight junior research groups within the e:Med initiative. In this interview with *systembiologie.de*, the mathematician, physicist and medical scientist talks about interdisciplinarity and his passion for neuroscience.

Systembiologie.de: *The Heidelberg Academy of Sciences and Humanities awarded you the Manfred Fuchs Prize this year. Congratulations! Would you please briefly elaborate for the readers on why you were awarded?*

Dr. Dr. Hamid Noori: The Heidelberg Academy of Sciences and Humanities highly appreciates and encourages interdisciplinary research. It awards the Manfred Fuchs Prize to experienced researchers who have dedicated their careers to interdisciplinary research. This year, I received the prize for my "pioneering and interdisciplinary work in the field of biocybernetics and neurobiology".

Your research centers on the field of neuroscience. What do you find fascinating about your work?

The nervous system is a multi-scale system of enormous complexity. From a mathematical perspective, it is extremely interesting. In addition, there are a total of 600 neuropsychiatric diseases, of which none are currently curable. This provides great impetus for research scientists. Furthermore it is a highly interesting field to approach from a philosophical point of view. How do we think? What biological mechanisms govern our consciousness? How do we perceive the world? These questions motivate me to conduct research in this field.

In addition to medicine, you also studied physics and mathematics.

I'd put it slightly differently in that I also studied medicine in addition to mathematics and physics. I initially worked on pure mathematics and theoretical physics before becoming interested in neuroscience.

In what way do you benefit from your education today?

Mathematics defines my way of thinking and the way in which I approach various hypotheses. In particular, it enables me to put dynamic processes in a more abstract way and to generalize – thus enabling me to identify fundamental problems.

Physics, on the other hand, is the language of nature. It enables me to identify patterns; something that is necessary to systematically investigate natural processes. And my experiences in medicine and experimental neuroscience are necessary to have a realistic view on the right questions.

Systems medicine thrives on this interdisciplinary approach. Theory and practice work together, hand in hand. But this also results in problems.

The most obvious problem is one of language. The different disciplines speak different languages. And by that I am not only referring to their scientific terminology, but rather their way of thinking. These differences can lead to major misunderstandings. For example, theoreticians and experimental scientists often have an inaccurate impression of what the other group is capable of.

Figure 1: Hamid R. Noori at the Max Planck Institute for Biological Cybernetics (Source: Vahid Bokharaie / Hamid R. Noori).

Could you provide us with a practical example?

Theoreticians often develop and utilize mathematical models, which are brought to life by numerical simulations. Depending on the model and simulation method, this can enable us to investigate a large number of variables within a short space of time, and also to manipulate the system as necessary. It gives rise to the impression that there are no limits to these manipulations. For instance, it is possible to simulate the effect of a variety of drugs in different doses on a theoretical neural network. Such cocktails of drugs would be harmful or even fatal for all life forms, but the computer will survive. This may lead the experimental scientists to believe that everything is possible with this kind of modelling. But that is definitely not the case.

On the other hand, the theoreticians often lack an understanding of the actual hypothesis. They see what they want to see. Theoreticians often "recognize" analogies to other processes that appear similar to the hypothesis at hand in one way or another. But detailed knowledge is required in order to distinguish the fine differences.

Do these misunderstandings hinder this area of research?

No, I don't think they really hinder it. I view them as challenges.

And how do you approach these challenges within your research group?

My research group is interdisciplinary in the truest sense of the word. Neurobiologists, physiologists and behavioral researchers work alongside physicists, mathematicians and engineers on a common agenda. By discussing the issue, we are able to establish a common language. But that alone is not enough. Over time, my colleagues learn to recognize the methods and limitations of the other disciplines. In my group every modeler learns how to handle laboratory animals correctly and how to design and conduct experiments. The biologists in turn learn about modern data analysis and modelling methods, as well as how to correctly apply them. In this way, we can all find the common denominator – not just regarding jargon, but also in our way of thinking.

You have developed a mathematical model to improve the classification of mental illnesses.

I have been working on my model for 14 years now. It is now able to predict the systemic effect of neuropsychiatric drugs on a rat's brain. Rats are frequently used as animal models; they are often used in preclinical research to test the efficacy of a drug. The model is very accurate at predicting which neurotransmitters can be influenced with the application of a drug. Combined with our databases, the model can improve the current classifications of neuropsychiatric drugs.

Why is that necessary?

Just like psychiatric illnesses, psychiatric drugs have generally, until now, been classified according to clinical observations and patient reports, i. e. categories such as antidepressants, antipsychotics, etc. If you look at the diverse and highly heterogeneous clinical profile, you can see that the existing classifications are problematic. A disease is described as a sum of its symptoms. The drugs used to treat the disease influence one or more symptoms. But the problem is that the symptoms are not specific to a type of disease, i. e. they may also be components of other diseases. One drug therefore may not just influence depressive symptoms, but also anxiety. From this perspective, it would be better to identify quantifiable biological dimensions in order to better describe the diseases and the effects of medications. We are trying to achieve this. But our methods go far beyond the aforementioned model. In fact, we combine neurochemical and electrophysiological data with functional imaging and mathematical models in order to address the issue. This unique combination of mathematical modelling and animal experiments enables us to validate these predictions directly and even to generate new hypotheses.

You are the head of an e:Med junior research group. What advantages does that afford you?

The biggest benefit is independence. On an international level, we work with a number of high-profile institutions in order to achieve our research goals. Independence in research does not mean that we isolate ourselves. Rather, it means having the possibility to pursue our own ideas, take risks and to learn how to successfully address scientific issues despite the difficulties.

This interview was conducted by Dr. Bettina Koblenz and Dr. Marco Leuer.

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Methods for developing psychiatric medications

Psychiatric disorders remain very difficult to treat. For many patients, drug-based therapies do not lead to the desired effects. This is because the neurochemical effect of the drug is often unknown. The development of active substances is derived from the classification of psychiatric diseases, and this classification is only based on clinical observations and changes in symptoms.

The field of neurochemistry plays a crucial role in being able to develop effective medications.

Modern psychiatry is therefore searching for new ways of classifying psychopathologies based on both observable behaviors as well as neurobiological measures. To this end, the US *National Institute of Mental Health* has launched its "Research Domain Criteria" project. This project aims to translate progress in basic research from the fields of neurobiology and behavioral research into an improved and integrated method for more accurately classifying psychological disorders. This marks a paradigm shift that has also taken hold in the European research community. The "Roadmap for Mental Health Research in Europe" (ROAMER), a project set up by the European Commission, wants to set a new precedent in mental health research over the coming years.

The aim of our research group is to make a significant contribution to this new development. To do this, we are thereby pursuing two strategies:

1) We combine theoretical and experimental approaches in order to create functional, neurochemical and physiological "fingerprints" for psychiatric drugs.

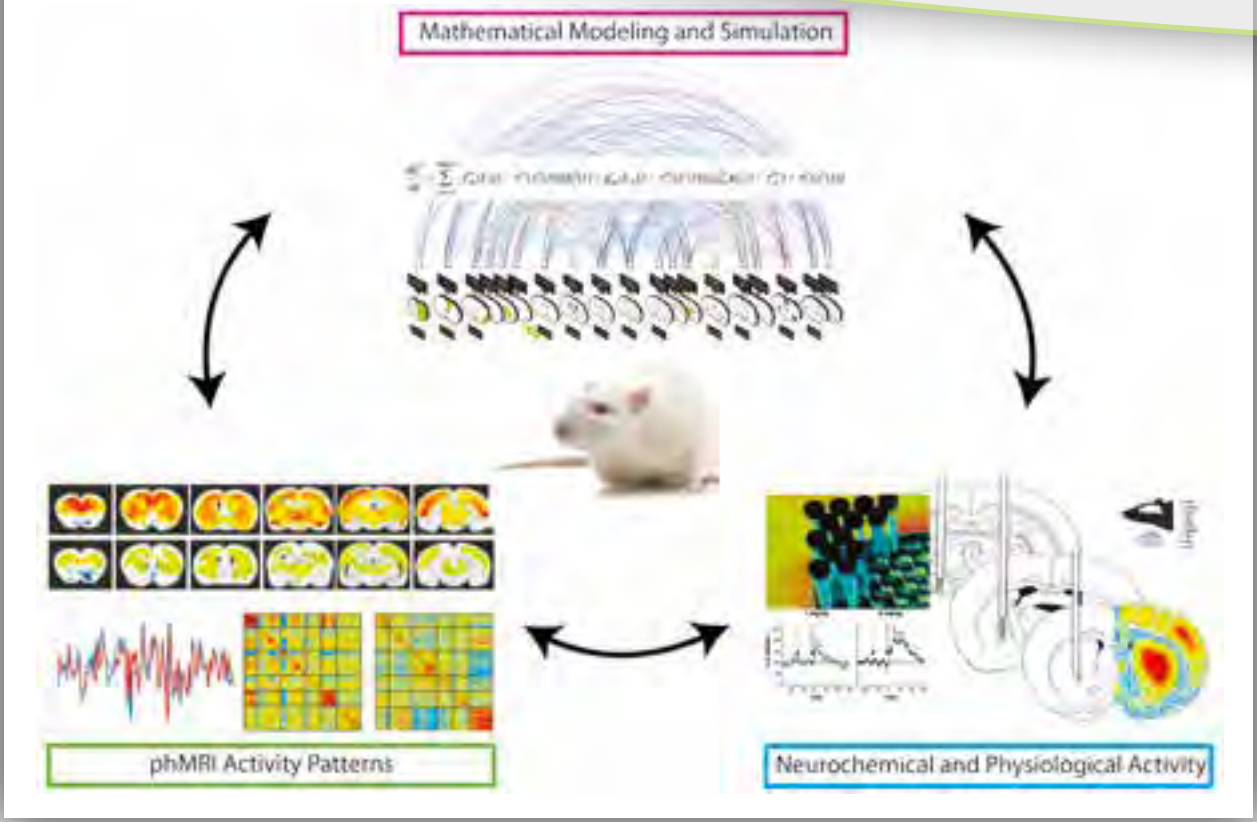

Figure 2: Schematic illustration of multidimensional neurochemical, functional and physiological measurements in a rat, converged into a single unit using mathematical modelling and simulation (Source: Hamid R. Noori).

Mathematical modelling and simulations represent the causal relationships between neurochemical and functional responses in the rat brain under the influence of the drug. The basis for these experiments is the existing databases (www.syphad.com, www.chemnetdb.org).

In addition, we are developing a method that combines the following three parameters concurrently:

a) Measurement data on changes in transmitter concentration

b) Recordings of neural activity

c) Functional MRI responses

This research strategy will not only lead to a better understanding of the neurobiological foundations of psychiatric disorders, but will also open up new opportunities in terms of *in-silico* applications for drug development.

2) We also combine data on neuronal processing with clinical observations in order to identify measurable parameters for mental illness. To this end, we have developed a highly sensitive monitoring system that permits high-resolution recording of locomotor activity, drinking, eating, grooming, playing, body temperature and, in future, also metabolic components within a stress-free social environment. At the same time, wireless electrodes record neuronal activity.

With the help of this approach, we will in future be able to establish paradigms for predicting the progression of various diseases and identify early-warning signs of obesity, drug addiction and neurological and psychological diseases, for example.

systems medicine of atrial fibrillation

From cohorts to the laboratory, from laboratory to the clinic

by Julia Krause and Tanja Zeller for the symAtrial consortium

The "symAtrial – Systems Medicine of Atrial Fibrillation" consortium conducts research on cardiovascular disease – in particular atrial fibrillation, using systems medicine approaches. The interdisciplinary project combines the fields of cardiology, epidemiology, molecular biology, bioinformatics and statistics. The aim is to obtain a better understanding of the molecular mechanisms underlying atrial fibrillation and to identify new approaches for risk prediction and therapies.

Atrial fibrillation

Atrial fibrillation represents the most common type of cardiac arrhythmia and affects approximately 2.2 % of the population. While the disorder itself is not directly life-threatening, the impaired conduction results in a higher risk of stroke and other cardiovascular diseases. The prevalence of atrial fibrillation is expected to double in the near future as a result of an aging population and the increase in cardiovascular risk factors.

Atrial fibrillation can be unnoticed by patients, but it can also manifest with a wide spectrum of symptoms, including palpitations, shortness of breath or chest pain. Depending on the time and duration of the episodes, atrial fibrillation is classified into three different types: paroxysmal (= sudden attack), persistent (= exists for more than seven days), permanent (= ongoing). From a pathophysiological point of view, atrial fibrillation depicts a heterogeneous disease. Affected individuals show one or more predisposing factors, such as high blood pressure, diabetes or advanced age illustrating the complexity of the disease and the difficulties associated with early diagnosis.

The molecular mechanisms behind atrial fibrillation are not fully understood yet. On a cellular level, numerous signalling

Figure 1: The symAtrial network

The interdisciplinary symAtrial consortium integrates knowledge from statistics, bioinformatics, epidemiology, cardiology and molecular biology (Source: Tanja Zeller, Julia Krause, Renate Schnabel; University Medical Center Hamburg-Eppendorf (UKE)).

Hamburg working group of Genomics and Systems Medicine and the clinical cohort studies (Source: University Medical Center Hamburg-Eppendorf (UKE)).

pathways associate with ion channel modulation, inflammation and fibrosis. In addition to classical cardiovascular risk factors, a genetic contribution to atrial fibrillation susceptibility exists. In this respect, studies revealed numerous mutations in diverse genes encoding for ion channels, transcription factors (*PITX2, ZFHX3*), structural proteins (*Cx40*) or proteins involved in inflammatory processes (*eNOS*) (Christophersen *et al.*, 2017). However, the extent to which these mutations are pathophysiologically relevant remains unclear. Furthermore, those variants only make a minor contribution to patients' risk assessments. A better understanding of the underlying mechanisms is therefore of central importance and could have major consequences for the early detection of the disease and individual therapies. To this end, the existing clinical and epidemiological understanding needs to be linked to molecular data sets and experimental findings.

symAtrial – systems medicine of atrial fibrillation

Since 2015, the symAtrial (Systems Medicine of Atrial Fibrillation) project has been funded by the German Federal Ministry of Education and Research (BMBF) as part of the e:Med programme. Within symAtrial, multiple groups have joined their expertise in an interdisciplinary consortium (Figure 1). symAtrial brings together scientific data from major epidemiological cohort studies, molecular *omics* data (*genomics, transcriptomics, proteomics* and *metabolomics*) and clinical knowledge. In order to manage and analyse an enormous amount of data, a central IT infrastructure has been set up, along with a management system for data storage and data exchange. In terms of both content and methods, symAtrial interacts with other groups in the e:Med programme such as the German Centre for Cardiovascular Research (DZHK) and the EU-wide BiomarCaRE consortium. Finally, the scientific results of symAtrial will contribute to a greater understanding of the pathomechanisms underlying atrial fibrillation and might lead to an improved individual risk prediction for atrial fibrillation.

symAtrial – an interdisciplinary and translational approach to systems medicine

The systems medicine approaches applied in symAtrial are based on large population-based and disease cohorts, for which detailed epidemiological phenotypic data and biobanks are available (Zeller *et al.*, 2014). Since several years, a whole range of consistent clinical parameters ranging from cardiovascular risk factors (age, sex, weight, waist circumference, blood lipids, blood pressure and smoking status) to atrial fibrillation events has been collected. In addition, various biomaterials have also been collected from the respective patients, which can be linked to the phenotypic data. Moreover, international population-based data are available for symAtrial, such as the EU-wide BiomarCaRE cohort studies, in which the incidence of cardiovascular diseases, including atrial fibrillation, was observed over a long time period. Over 8,000 new atrial fibrillation episodes have already been recorded.

Of great importance is the availability of *omics* data generated by modern high-throughput technologies. To identify new biomarkers and molecular signalling pathways, bioinformatics and mathematical models are used (Ojeda *et al.*, 2016). For instance, in order to identify pathophysiological signalling pathways, *gene set enrichment analyses* (GSEA) are performed. Here, groups of genes or proteins representing specific biological processes are identified (Schäfer *et al.*, 2015).

Identified biomarker candidates and signalling pathways are further investigated with regard to their function and role in atrial fibrillation using molecular biology approaches. A wide range of experimental methods (e. g. polymerase chain reaction, enzymatic reactions and staining) and models (cell cultures, tissue models) are used. Moreover, the clinical usefulness of identified biomarkers for an improved risk prediction can be tested by applying new mathematical algorithms (Schnabel *et al.*, 2009) (Figure 2).

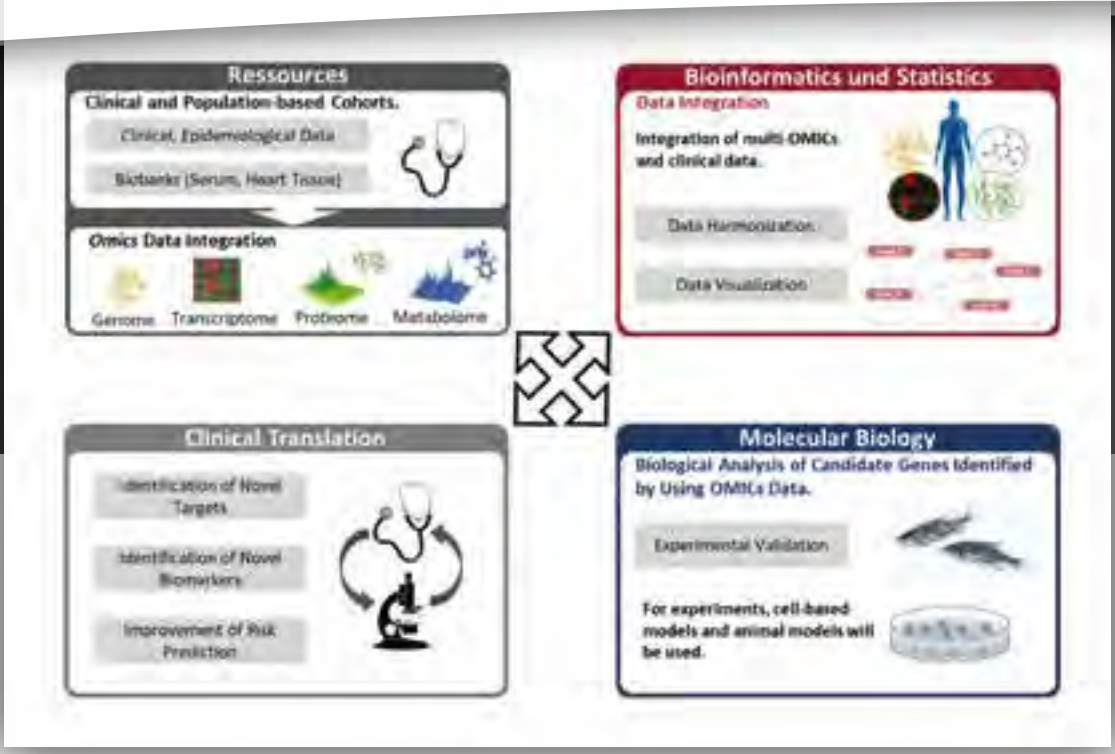

Figure 2: Translational approaches in atrial fibrillation. symAtrial aims to gain a better understanding of the cellular interactions between DNA, RNA, proteins and metabolites in atrial fibrillation using multi-level-*omics* data and subsequently, to translate the findings into clinical application (Source: Tanja Zeller, Julia Krause, Renate Schnabel; University Medical Center Hamburg-Eppendorf (UKE)).

Findings from the symAtrial consortium

Several circulating metabolites have been identified in association to atrial fibrillation. *Tissue engineering techniques* are used for the experimental validation and the functional analysis of these metabolites. The basis of *tissue engineering* is the construction of three-dimensional cardiac muscles. So far, there have been no constructs suitable for investigating the physiology of the atria. In this context, the symAtrial consortium established an atrial model in cooperation with the Institute of Pharmacology and Toxicology at the University Medical Center Hamburg-Eppendorf (UKE) enabling experiments in the context of atrial fibrillation. Additionally, further analyses identified non-coding RNAs (ncRNAs) associated with atrial fibrillation. ncRNAs are RNA molecules which seem not to be translated into proteins but rather regulate gene expression at various levels Novel identified ncRNAs with unknown functions are initially characterized by using cloning experiments for example.

Furthermore, the symAtrial consortium addresses gender differences in the development and progression of atrial fibrillation on the epidemiological level occurring less commonly in women than in men. The aim is therefore to develop gender-specific prevention strategies.

Future directions

The findings from molecular, bioinformatic and epidemiological analyses are considered to improve existing clinical risk prediction models and therapy strategies. The knowledge gained in symAtrial will be transferred to other cardiovascular diseases, such as heart failure and myocardial infarction. To this end, partnerships with other e:Med-funded projects have been established.

Research project profile:

The symAtrial consortium is funded by the German Federal Ministry of Education and Research (BMBF) as part of the e:Med programme. The symAtrial consortium consists of interdisciplinary working groups from the Helmholtz Institute in Munich (bioinformatics), the Institute of Medical Biometry and Statistics in Lübeck (mathematics and statistics) and the University Heart Center in Hamburg (cardiology, epidemiology and molecular biology).

Five subprojects of symAtrial:

SP1 Infrastructure of data management and data exchange
SP2 *Omics*-analyses and longitudinal gene expression analysis
SP3 Regulatory networks and computational systems biology
SP4 Molecular characterization of atrial fibrillation candidate genes, pathways and translation
SP5 Genomic Epidemiology of Atrial Fibrillation

Further information:

https://www.uke.de/kliniken-institute/kliniken/allgemeine-und-interventionelle-kardiologie/forschung/schwerpunkte/forschung_ag_zeller.html

http://www.sys-med.de/en/young-investigators/junior-research-alliances/symatrial

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CAPSyS – systems medicine in pneumonia research

Predicting progression and new therapy concepts for community-acquired pneumonia

by Peter Ahnert, Martin Witzenrath, Bernd Schmeck, Markus Scholz, Norbert Suttorp and Markus Löffler

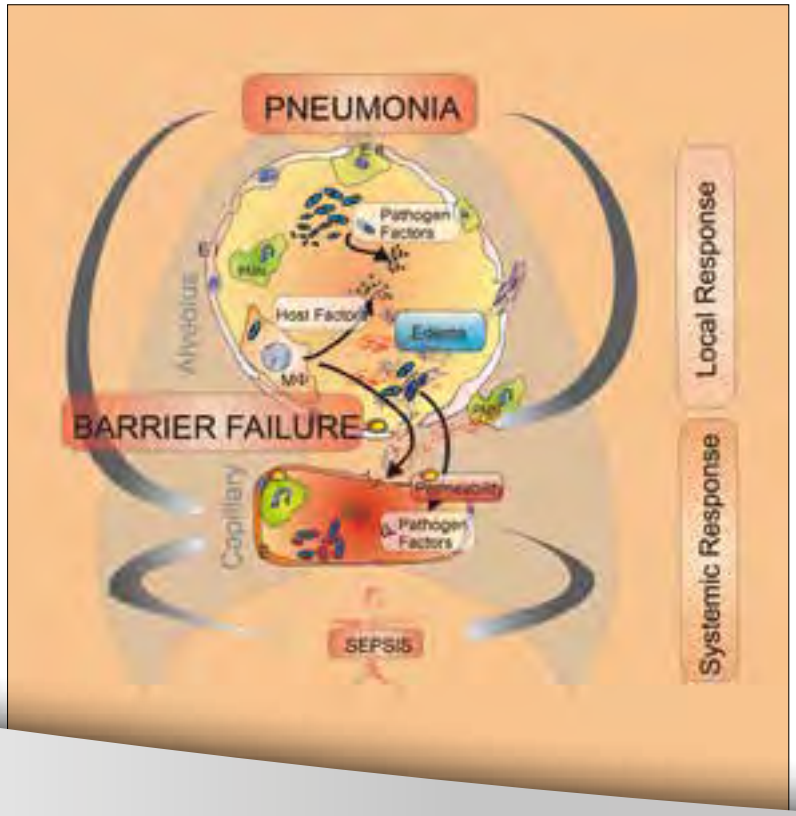
Pneumonia is caused by an infection of the lung – it can strike at any age. Over a quarter of a million patients are hospitalized in Germany every year. When the barrier between blood vessels and pulmonary alveoli fails, a severe reaction throughout the body, sepsis, can lead to organ damage – the patient must receive intensive care. Despite treatment with antibiotics, overall mortality rate has remained at around 13 % for many years. The CAPSyS consortium is investigating clinical and molecular processes involved in pneumonia using strategies from systems medicine for better prediction of critical cases and for creating a basis for more effective therapies.

The medical challenge

Community-**a**cquired **p**neumonia (**CAP**) is a common illness, which led to 290,740 hospital admissions in Germany in 2015 – more than for myocardial infarction or stroke (Gemeinsamer Bundesausschuss, 2016). As a result of demographic change, this trend looks set to continue. Although CAP poses the greatest risk to children and older people, it can affect people of any age – predisposition seems to vary greatly between individuals. Despite likely exposure to pathogens, some people do not contract CAP, while others become ill repeatedly.

CAP is the most common infectious disease in the world and has a high mortality rate. For patients for whom outpatient treat-

Figure 1: Local and systemic host reactions in pneumonia



Source: Holger Müller-Redetzky, Charité – Universitätsmedizin Berlin

If the body is not able to contain an infection in the lung and limit it to a localized area, barrier dysfunction leads to pulmonary edema on the one hand and, on the other, to a systemic spread of localized reactions and, thus, to sepsis. Barrier dysfunction of this kind triggers uncontrolled and systemic activation of mechanisms important for local immune response. In the worst-case scenario, this can lead to irreversible, life-threatening damage to other organs. If pathogens are detected, alveolar macrophages (MΦ), epithelial cells (E I) and endothelial cells (E) react first. Other components of the immune system are triggered later. Usually, these processes are finely tuned for overcoming a localized infection. However, if infection or immune response become systemic, the interaction of these processes may lead to worsening of the condition and even to organ failure.

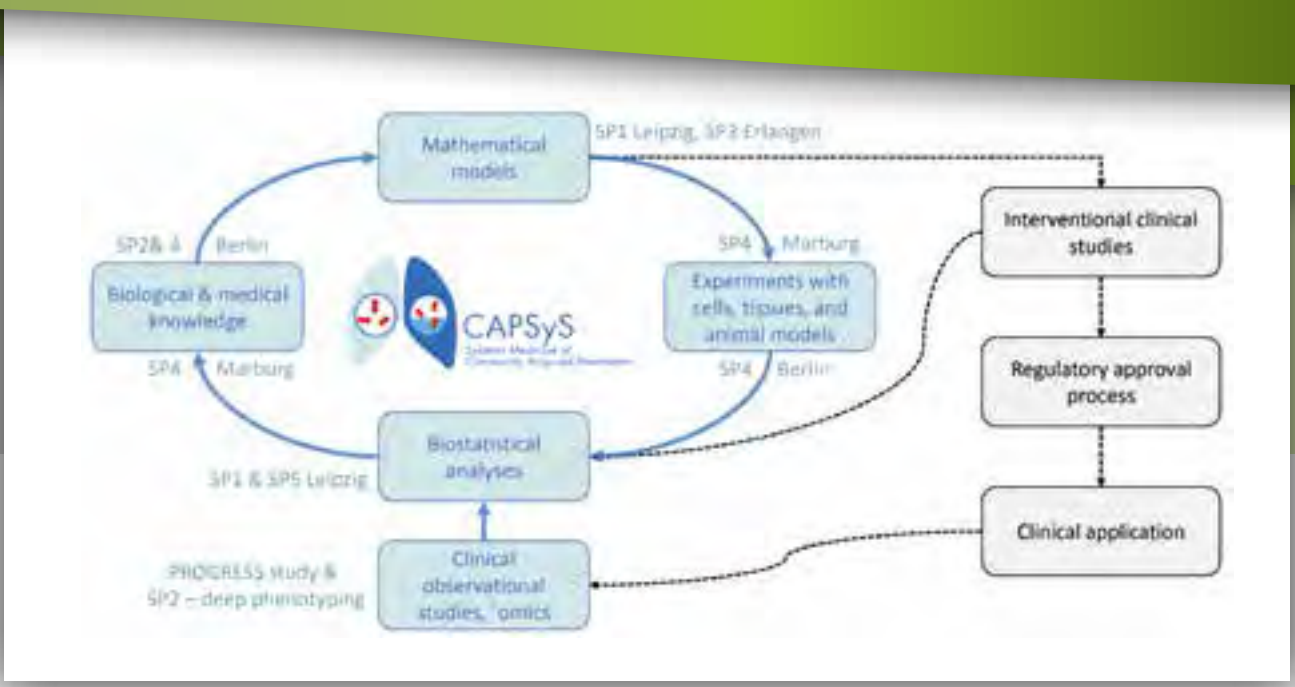


Figure 2: Cycle of systems medicine for pneumonia research. Left (in blue) referring to sub-projects of the CAPSyS consortium. Right, the path towards clinical application (grey, dotted arrow) (Source: Peter Ahnert, Leipzig University).

ment appears sufficient, this rate is around 1 %. Patients requiring hospitalization have a mortality rate of around 13 %, and for patients requiring intensive care, this rate rises to about 34 %.

Individual differences in disease progression

Variations in the progression of CAP in different patients can not only be attributed to the particular pathogen, therapy, or general health status of a patient. Individual characteristics of a patient's immune response also play a major role here – can the infection take hold in the first place? And if so, are the body's own defenses strong enough to limit the infection to an area of the lung without damaging it further? Or does the immune system overreact and cause additional damage? These factors make the progression of CAP very hard to predict. Uncomplicated CAP, which only affects limited areas of the lung, may for some patients progress to severe CAP with respiratory distress and imminent barrier dysfunction, while others may go on to develop severe CAP with sepsis. This may lead to lung failure (pulmonary edema) or even the failure of remote organs (Figure 1), which can be life threatening. The interaction of underlying biological processes and conditions eventually leading to systemic problems with multiple organ failure, due to an initially local infection and the body's reaction to it, are not fully understood and, therefore, are a major focus of research in CAPSyS.

Clinical objective

Currently, the danger of barrier dysfunction in the lung cannot be diagnosed early. Apart from antibiotics, specific therapeutic options are not yet available. New methods for reliably gauging prognosis early on and new non-antibiotic treatment strategies able to modulate immune response are urgently needed. Improving the prediction of disease progression and the treatment of severe progression of CAP will require extensive efforts. Clinical and molecular parameters for risk stratification, classification of disease severity, and prognostic markers and scores will have to be identified. Within the CAPSyS consortium, it is our aim to develop simple yet innovative blood tests that can detect imminent or existing barrier dysfunction reliably and early on in the course of CAP. We also expect that the systems-oriented approach within CAPSyS will lead to the identification of previously unknown diagnostic markers and therapeutic approaches.

Systems medicine in pneumonia research

Applying a systems medicine approach to pneumonia research promises a much more comprehensive understanding of the dynamics of mechanisms pertinent to pneumonia, particularly with regard to factors contributing to barrier dysfunction in the lung. Models are being developed to predict the relevance of new diagnostic and prognostic tests, and to gauge the efficacy of new, targeted therapeutic strategies. These models should eventually enable the development of new prospective interventional studies for diagnostic and therapeutic approaches. We believe that this is an important contribution towards the development of innovative therapeutic targets and strategies, as well as their translation into clinical practice (Figure 2).

The research program

The CAPSyS research program combines data from human clinical trials with functional experiments on cells, tissues, and animal models using methods of biostatistics and mathematical

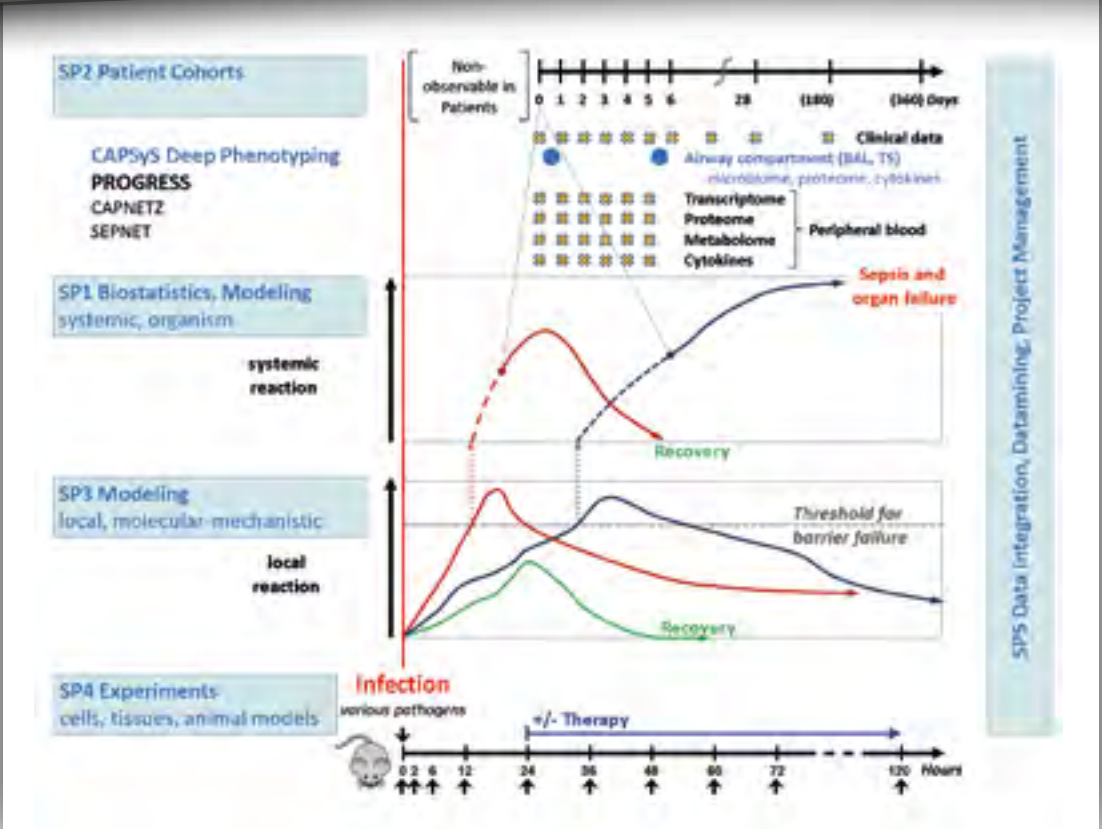


Figure 3: Research program of the CAPSyS Consortium (Source: CAPSyS Consortium).

modelling (Figure 3). Functional tests under controlled experimental conditions are often only possible for cells and tissues. They lead to important new insights, the relevance of which is often not fully clear within the substantially more complex clinical environment, shaped by interindividual characteristics (such as genetic predisposition, lifestyle factors, diseases) and various external factors. Therefore, it is essential to also consider data from clinical observational and interventional studies. CAPSyS can draw on the PROGRESS study, which investigates the body's systemic reaction to community-acquired pneumonia in hospitalized patients (Ahnert *et al.*, 2016). In order to make biomaterials from the lung and data on barrier dysfunction in the lung accessible, the CAPSyS deep phenotyping protocol has been developed to broaden the scope of the PROGRESS study. Using bronchoalveolar lavage (BAL), samples are taken from the lungs of CAP patients requiring mechanical ventilation. Barrier dysfunction and the microbiome are assessed in alveolar materials. Clinical studies on infectious diseases have one limitation: the exact time of infection is usually unknown. Therefore, the essential first few hours after infection cannot be observed. Additionally, it is often difficult to determine the causative pathogen. The gap between experiments on cells or tissues and clinical studies on humans can be bridged by preclinical studies on murine models offering control over the experimental setting.

Achievements so far

The CAPSyS deep phenotyping protocol has been established in the PROGRESS study. Despite the significant effort required for study sites, 50 patients have already been recruited, with a total target of 100. At the same time, a comprehensive preclinical study in mice was completed, which not only tested various antibiotic protocols but also innovative therapies – for example, substances stabilizing the barrier. Building on these data, a systems biology-based mathematical model of murine pneumonia was built, which describes the dynamics of the immune reaction and progression of the disease (Schirm *et al.*, 2016). The model enables first simulations of alternative treatment approaches and is currently being honed to take into account the effects of new therapeutic drugs and to describe their interaction with antibiotic treatment. Extensive biostatic analyses of clinical and molecular data of the PROGRESS study lead to in-depth insights into molecular disease mechanisms that are currently being translated into models. This work made a significant contribution towards the development of the publicly accessible data analysis tool known as "The Virtual Macrophage"[1]. This became the basis for the successful development of an experimentally validated mathematical model for the activation of alveolar epithelial cells in bacterial pneumonia (Schulz *et al.*, 2017). Thus, the CAPSyS consortium is on track towards significantly expanding our understanding of the dynamics behind the development of pneumonia using the systems medicine approach.

[1] https://vcells.net/macrophage/

Research project profile:

The research consortium "CAPSyS – systems medicine in community-acquired pneumonia" is funded by the German Federal Ministry of Education and Research (BMBF) as part of its "e:Med – measures for establishing systems medicine" research and development concept. The aim is to identify new diagnostic, prognostic, and therapeutic options in community-acquired pneumonia through consistent use of methods from systems medicine. For successfully tackling the biological, medical, methodical, and technical challenges of systems medicine, the CAPSyS consortium draws on expertise from seven sites. Partners in the consortium include: Prof. Dr. Markus Löffler (Leipzig University, Institute for Medical Informatics, Statistics, and Epidemiology (IMISE), spokesman for the consortium), Prof. Dr. Markus Scholz, (Leipzig University, Institute for Medical Informatics, Statistics, and Epidemiology (IMISE), Dr. Hans Binder (Leipzig University, Interdisciplinary Centre for Bioinformatics (IZBI)), Dr. Peter Ahnert (Leipzig University, Institute for Medical Informatics, Statistics, and Epidemiology (IMISE), scientific project coordinator), Prof. Dr. Norbert Suttorp (Charité – Universitätsmedizin Berlin, Clinic for Infectious and Respiratory Diseases, vice-spokesman for the consortium), Dr. Petra Creutz (Charité – Universitätsmedizin Berlin, Clinic for Infectious and Respiratory Diseases), Prof. Dr. Martin Witzenrath (Charité – Universitätsmedizin Berlin, Clinic for Infectious and Respiratory Diseases), Prof. Dr. Julio Vera-González (Universitätsklinikum Erlangen), Prof. Dr. Trinad Chakraborty (Giessen University, Institute for Medical Microbiology), Prof. Dr. Uwe Völker (University of Greifswald, Department for Functional Genomics), Dr. Dr. Michael Kiehntopf (University Hospital Jena, Institute for Clinical Chemistry and Laboratory Diagnostics), Prof. Dr. Bernd Thomas Schmeck (Philipps-Universität Marburg, Institute for Lung Research).

Further information:

www.capsys.imise.uni-leipzig.de/en/index.jsp
www.sys-med.de/en/consortia/capsys

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signals that never fall silent

How morphogenic signaling pathways regulate liver metabolism

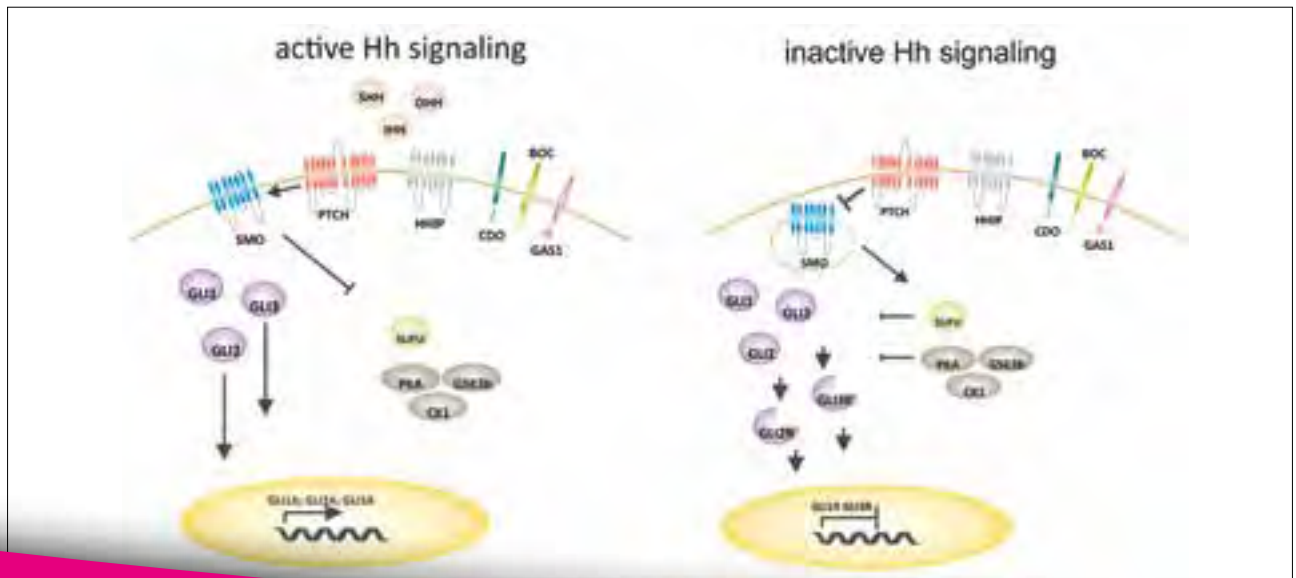
by Madlen Matz-Soja

Just a few signals are all that are required for highly complex structures and organs to arise from a single gamete over the course of our development. For a long time, people believed that these morphogenic signals fell silent after development was complete. But thanks to intensive research over the past few years, it has been shown that many of these cascades also control processes in adult organs. The extent to which the metabolism of the adult liver is regulated by morphogenic signals is the focus of research for our junior group, which is part of the "Systems Medicine of the Liver" (LiSyM) research association.

Morphogenic signaling and its relevance to the liver

As one of the body's largest metabolic organs, the liver is characterized by oxygen, hormone and metabolite gradients. For a long time, these gradients were seen as the reason for the "**metabolic zonation**" of the liver, according to which the expression of key enzymes behind most metabolic processes dominated in either the periportal (PP) or in the pericentral zone (PC) (Figure 2). Although many factors influencing metabolic zonation have already been researched, there are still some aspects of liver zonation that remain unclear. In 2002, it was shown that the pericentrally expressed enzyme glutamine synthetase, which acts as a catalyst for the formation of glutamine from glutamic acid and ammonium ions, is subject to the regulation of β-catenin, a protein of the morphogenic Wnt/β-catenin signaling pathway. Another morphogenic signaling cascade, whose importance to liver metabolism remained completely unknown until only a few years ago, is the **hedgehog (Hh) signaling pathway**. This signaling pathway was discovered in 1980 by future Nobel Prizewinners Christiane Nüsslein-Volhard and Eric F. Wieschaus thanks to their research into *Drosophila melanogaster*. This research showed that the Hh signaling pathway plays a major role in organogenesis and left–right asymmetry during embryogenesis. Papers identifying hedgehog orthologues in mammals appeared for the first time in 1993. This was followed by rapid progress in this field. Here, too, the initial focus was on investigating their influence

Figure 1: The Hh signaling pathway in its active and inactive state



Source: Madlen Matz-Soja, Rudolf Schönheimer Institute of Biochemistry, Faculty of Medicine, Leipzig University

Figure 2: Liver lobes and their disposition into the periportal and pericentral zones (Source: Madlen Matz-Soja, Rudolf Schönheimer Institute of Biochemistry, Faculty of Medicine, Leipzig University).

on embryogenesis, which resulted in the signaling pathway being identified and described as a decisive factor in pattern formation and cellular differentiation for almost every organ (Figure 1).

In mammals, the canonical Hh signaling cascade has three glioma-associated oncogene (GLI) transcription factors and their level of expression determines the activation or inactivation of the Hh target genes. In adult organisms, we know that the Hh signaling pathway regulates the homeostasis of various organs such as the stomach, intestines and pancreas. First of all, it was shown that Hh activation in the adult liver plays a major role in regenerative processes. The subject of these experiments is often the non-parenchymal fraction of liver cells (Kupffer cells, hepatic stellate cells, cholangiocytes and progenitor cells), which, in contrast to hepatocytes, have a much higher expression of the individual Hh genes. Having said that, our intensive experiments in this field of research revealed that even a low expression would be enough to control major metabolic processes of the liver.

Tracking the influence of Hh signaling on the liver's fat metabolism

During my doctorate, which I completed in the working group under Prof. Dr. Rolf Gebhardt at the Rudolf Schönheimer Institute of Biochemistry, Faculty of Medicine, Leipzig University, and within the "Virtual Liver" research group of the German Federal Ministry of Education and Research (BMBF), we were able to show that the Hh signaling pathway plays a major role within the liver's lipid metabolism (Matz-Soja *et al.*, 2014; Matz-Soja *et al.*, 2016). Thanks to a close interdisciplinary partnership with bioinformatics specialists, we were able to discover a specific signature for the GLI transcription factors, which is absolutely crucial to the development of non-alcoholic fatty liver disease (NAFLD) (Figure 3).

This disease is one of the most common pathologies in industrialized countries and its progression into non-alcoholic steatohepatitis (NASH) is associated with very high mortality rates. In addition, there are also a whole range of diseases associated with NAFLD, such as polycystic ovary syndrome (PCOS) and metabolic syndrome (MetS). Although plenty of predisposing factors have already been identified for NAFLD (obesity, type 2 diabetes), we still know very little about the initial key factors that lead from the simple accumulation of lipids in the liver to the clinically relevant disease profile of NAFLD. Early diagnosis in the initial stage of NAFLD is still very difficult and requires a combination of non-invasive methods (measurement of liver enzymes in the blood, MRI, ultrasound) because a liver biopsy is often not indicated for patients with obesity or metabolic syndrome. For this reason, the Virtual Liver Network (VLN) was very interested in identifying the initial key processes of NAFLD in a systems biology context.

Based on these findings, we now want to work with our junior group in the Systems Medicine of the Liver (LiSyM) research network to characterize more precisely the role of these signaling cascades during progression from benign steatosis to NAFLD

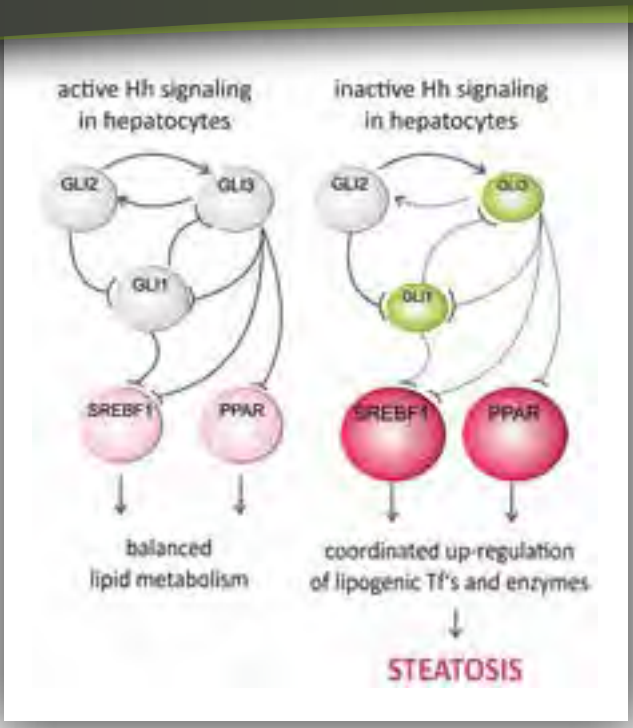


Figure 3: Regulation of the hepatic lipid metabolism by the Hh signaling pathway. When the Hh signaling pathway is active, the regulation of lipogenic transcription factors (TFs) and enzymes via the GLI factors is normal and the expression of lipogenic TFs (SREBF1, PPAR) in the lipid metabolism is balanced. If the Hh signaling pathway is deactivated, the GLI-TFs lose their equilibrium, which leads to an overactivation of lipogenic TFs and enzymes. Ultimately, this results in hepatic steatosis (Source: Madlen Matz-Soja, Rudolf Schönheimer Institute of Biochemistry, Faculty of Medicine, Leipzig University).

and NASH using patient material and models. In this way, we want to lay the foundations for the clinical application of new diagnostic markers and target genes in order to better predict the risk of NAFLD in individual patients and to be able to take preventive action sooner in its progression to NASH. In cooperation with the modeling partners from LiSyM, we aim to generate systems biology prognosis models to provide information about (pathological) Hh activity in human material to be used in a medical setting.

Morphogens and metabolic zonation

Investigating the zonation of elementary metabolic processes in the liver is a central aim of our junior group thanks to the growing understanding of the interaction between Wnt/β-catenin and the Hh signaling pathway in adult tissue. Based on the fundamental findings made by Kurt Jungermann in the 1980s, we are now looking at the significance of the role played by morphogenic signaling pathways in determining metabolic zonation. In 2002, it was shown that, for the Wnt/β-catenin signaling pathway, the most clearly zonated enzyme of the pericentral zone, glutamine synthetase, is subject to the regulation of β-catenin, a protein within the Wnt/β-catenin signaling pathway (Gebhardt and Matz-Soja, 2014). This heralded the initiation of many research projects that went on to show that the Wnt signaling cascade also plays a major role in regulating nitrogen and glucose metabolism within the liver. In order to be able to now answer the question as to the role played by the Hh signaling pathway in liver zonation, we have developed a wide range of methods that enable the representation and analysis of the relevant proteins in the liver material. Through the interdisciplinary partnership with bioinformatics specialists from the working group under Stefan Höhme, we aim to gain an understanding of the metabolic processes within the liver and to be able to better predict them by using a model of the zonation of these cascades.

Communication with peripheral organs

The fact that the liver as a central metabolic organ is in a constant interaction with peripheral organs is now indisputable. However, there is often a lack of understanding as to the basic mechanisms underpinning this interaction. To this end, our working group was able to show that comorbidities associated with NAFLD, such as polycystic ovary syndrome (PCOS), are also correlated with the activity of the Hh signaling pathway in the liver. It was revealed that a modulation of the signaling cascade affects the expression and translation of central genes in hepatocellular steroidogenesis and also the reproductive capacity of female mice. PCOS is one of the most common hormonal disorders and can lead to the complete loss of reproductive capacity in women. Approximately 5–10 % of women of childbearing age suffer from this disease, with 50 % of patients with an initial NAFLD diagnosis also showing the characteristic symptoms of PCOS. Intensive analyses in this area showed that the activity of the Hh signaling pathway in the adult liver has a significant influence on the quantity of testosterone in the blood, which, in turn, controls fertility (Rennert *et al.*, 2017).

Hedgehog in oncogenesis

The Hh signaling pathway plays a central role in carcinogenesis. For this reason, enormous efforts have been made in recent years to develop specific Hh inhibitors and to test them in clinical trials. Recent reports from these studies show, however, that unexpected side effects often occur after these inhibitors are administered, which is why there is a very high discontinuation rate. As a result, the Committee for Medicinal Products for Human Use has only awarded preliminary approval for these inhibitors. This highlights how little we still know about the functions of the Hh signaling pathway in adult organisms and the consequences that inhibiting the cascade could have on the metabolism as a whole. In light of this, our investigations also aim, based on a systems biology approach, to gauge the possible risks of reducing Hh activity as part of cancer therapy.

Figure 4: Matz-Soja junior group from right to left: Robert Lehmann, Erik Schröder, Peter Taufmann, Vivien Teßmar, Doris Mahn, Christiane Rennert, Madlen Matz-Soja (Source: Madlen Matz-Soja, Rudolf Schönheimer Institute of Biochemistry, Faculty of Medicine, Leipzig University).

Research project profile:

Research group: Junior group in the "Systems Medicine of the Liver" (LiSyM) research association

Project title: The Hedgehog Signaling Pathway – A New Regulator for Liver Metabolism

Financing: German Federal Ministry of Education and Research (BMBF)

Contents: The aim of the LiSyM project is to identify key processes responsible for causing liver disease. In doing so, it aims to pursue a systems biology approach that draws on the interaction between biological experiments and mathematical modeling for application-relevant liver research.

Partners:

Prof. Dr. Jochen Hampe, University Hospital Dresden

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Prof. Dr. Hermann-Georg Holzhütter, Charité – Universitätsmedizin Berlin

Prof. Dr. Damjana Rozman, University of Ljubljana

Dr. Stefan Höhme, Leipzig University

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systems medicine for rare tumor types

MAPTor-NET – personalized therapy for pancreatic neuroendocrine tumors

by Christine Sers and Kathrin Thedieck

In recent years, personalized tumor therapies have become a reality for many patients. Specific (epi)-genetic changes are recorded using high-throughput sequencing and give immediate indications on therapeutic target molecules, or can be used as predictive markers for therapy response. For common tumor types, mutation profiles and related treatment regimens are validated in clinical trials and are used routinely in diagnostics and for therapy decisions. For rare tumor types, implementing such approaches is much more difficult due to limited clinical cohort sizes. Therefore, underrepresented, potentially fatal diseases call for integrative approaches in research and treatment, offered by systems medicine.

Neuroendocrine tumors (NET) are classified as a rare tumor type, and affect two to four out of 100,000 individuals in Germany (Begum *et al.*, 2012) (http:// www.netregister.org/wDeutsch/ne_tumore/allgemein/index. php?navanchor= 1110006). The incidence of NETs has risen in recent years, due to demographic change in Western societies, but also improved detection methods. NETs display characteristics of both endocrine (secretory) and neuronal cells, and most commonly occur in the lung, gastro-intestinal (GI) tract, and the pancreas. Pancreatic NETs (pNETs) are a very heterogeneous and the most common NET subgroup, comprising around 30 % of all NETs. In a comprehensive systems modeling approach, MAPTor-NET integrates genetic, signaling, and clinical data into dynamic mathematical models specifically designed for pNETs.

Key aims of the MAPTor-NET project

The MAPTor-NET project is based on the hypothesis that a significant improvement in treatment response for pNET patients can be achieved via a focused, systems medicine approach that integrates patient-specific activation profiles for mutation, expression and signaling pathways.

Extensive pNET sequencing analyses (Jiao *et al.*, 2011; Sadanandam *et al.*, 2015; Scarpa *et al.*, 2017) provide evidence for the

Figure 1: The human pancreas (yellow), a central organ in the gastrointestinal (GI) tract

Source: blueringmedia@rtf123.com

Figure 2: mTOR and MAPK – a complex signaling network (Source: Evgeny Gromov@123rtf.com).

significance of the oncogenic mTOR (mammalian target of rapamycin) signaling network, DNA repair and telomere control mechanisms along with epigenetic control processes, in the progression of pNETs.

There has been awareness of mTOR activation and defects in telomere stability in pNETs for several years now, but the total number of deleterious mutations in mTOR or DNA repair genes is lower in pNETs than in other solid tumors (Di Domenico *et al.*, 2017). mTOR inhibitors, such as Everolimus, are a standard treatment in pNETs (Yao *et al.*, 2011) but the success rates remain low as many patients develop therapy resistance. However, to date no clinical trials have been published that consider the patients' genetic profile or molecular defects in the mTOR signaling network for therapy guidance.

Through a systems medicine approach, MAPTor-NET aims to predict the individual treatment response of pNET patients and thus improve therapeutic outcome. In order to simulate therapy response, molecular changes (mutations, gene expression and activation of signaling pathways) in individual pNET patients are represented using mathematical models, designed to reflect the dynamic interaction of the oncogenic mTOR and MAPK (mitogen-activated protein kinase) signaling pathways. The rationale behind this strategy is that Everolimus not only inhibits mTOR but also affects MAPK signaling, and that the treatment outcome is defined by the response of the networks directly targeted by a drug. In addition, we correlate genetic changes and differential expression of genes and proteins with dynamic mTOR-MAPK signatures, and with the individual therapy response.

Mathematical MAPK-mTOR models – simulating signaling pathway interaction and therapy response

Both the mTOR and MAPK signaling networks are highly dynamic and display complex topologies with multiple feedback and activation loops. Mutations as well as different expression levels of mTOR and MAPK network components in individual patients could result in altered network dynamics, which cannot be intuitively predicted or captured by two-dimensional schemes. Since network dynamics play a major role in therapy response, it is currently difficult to rationally predict whether a patient will respond to a drug. This continues to make therapy decisions a process shaped by trial and error.

Computer-based modeling has high potential to improve this situation: integrating the complex, pNET-specific topologies and dynamics of oncogenic signaling networks and simulating therapy response. The MAPTor-NET partners have previously developed dynamic models of the MAPK and mTOR networks in tumor cells (Dalle Pezze, 2012; Dalle Pezze, 2016; Sonntag, 2012; Klinger *et al.*, 2013; Fritsche-Günther *et al.*, 2011; Riemer *et al.*, 2017). These models are now being combined and specifically parameterized for pNETs. As a result, dynamic, pNET-specific models will allow us to simulate mTOR and MAPK-dependent, tumor-relevant phenotypes such as cell proliferation and survival. In order to test and improve the models' ability to predict therapy response, the signaling pathways are perturbed *in silico* and experimentally in iterative cycles and the response is tested at the level of signaling and cancer phenotype. Models are initially parameterized based on pNET cell line data and then gradually enriched with data from expression and sequencing analyses in xenografts and patient samples with known therapy

response. This will enable us to compare the model predictions with the actual therapy success, and to record and enhance the performance of our models.

Individual genetic fingerprint – identifying key genetic modifications for personalized therapy

All human tumors carry genetic alterations. Major international sequencing projects have created characteristic mutation profiles for the majority of human tumors. For NETs of the pancreas, lung and GI tract, many genetic modifications have also been identified. But which of these mutations influence the individual therapy response and could serve as biomarkers for customized therapies? Within the MAPTor-NET project, we use targeted sequencing of all major genes in the mTOR and MAPK network in order to analyze both individual, non-synonymous base pair mutations and gene copy-number variation. We also investigate which modifications correlate with therapy response to Everolimus to integrate key mutations into the mathematical models.

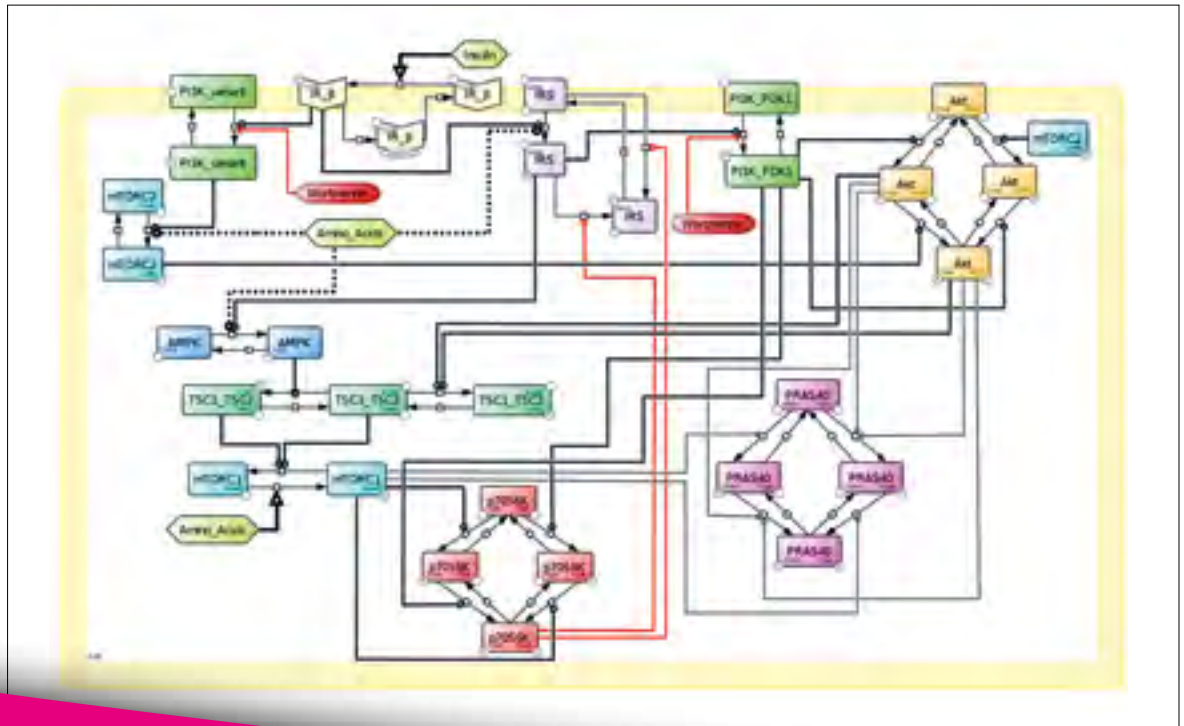
mTOR and Everolimus – where is the Achilles heel of the pNET signaling network?

In addition to sequencing and modeling the mTOR-MAPK network, we also investigate components that may represent a potential, as-yet unknown Achilles heel in the context of an active mTOR network in pNETs. To ask whether there are genes whose inactivation sensitizes pNET cells to Everolimus therapy, we conduct a genome-wide CRISPR/Cas9 screen in a well-characterized Everolimus-resistant pNET cell culture model. In parallel, we test Everolimus-dose-dependent cell growth and cell death. Genes essential for cell survival under Everolimus will be promising candidates, whose role in Everolimus resistance we will study in depth, to integrate them into our mathematical models.

The MAPTor-NET consortium

Our consortium unites expertise of scientists from Berlin, Oldenburg and Newcastle, who jointly implement an iterative, combined experimental, clinical and theoretical/bioinformatics strategy. At the Charité University Clinic in Berlin, Prof. Marianne Pavel is a leading clinical NET expert, represented in the team by Dr. Katharina Detjen, with many years of experimental experience in NET research. Prof. Christine Sers and Prof. Nils Blüthgen are an experienced team in practice and theory, whose key interest focuses on oncogenic signaling pathways in tumors, with a particular emphasis on MAPK signaling. Prof. Kathrin Thedieck from Oldenburg University is an expert in metabolic signaling networks, with a focus on mTOR-phosphatidyl-3-kinase (PI3K) signaling. Together with Dr. Daryl Shanley from Newcastle University, who supports MAPTor-NET

Figure 3: Graphical model of the mTOR signaling network activated by insulin and amino acids



Source: Dalle Pezze P. *et al.*, 2016

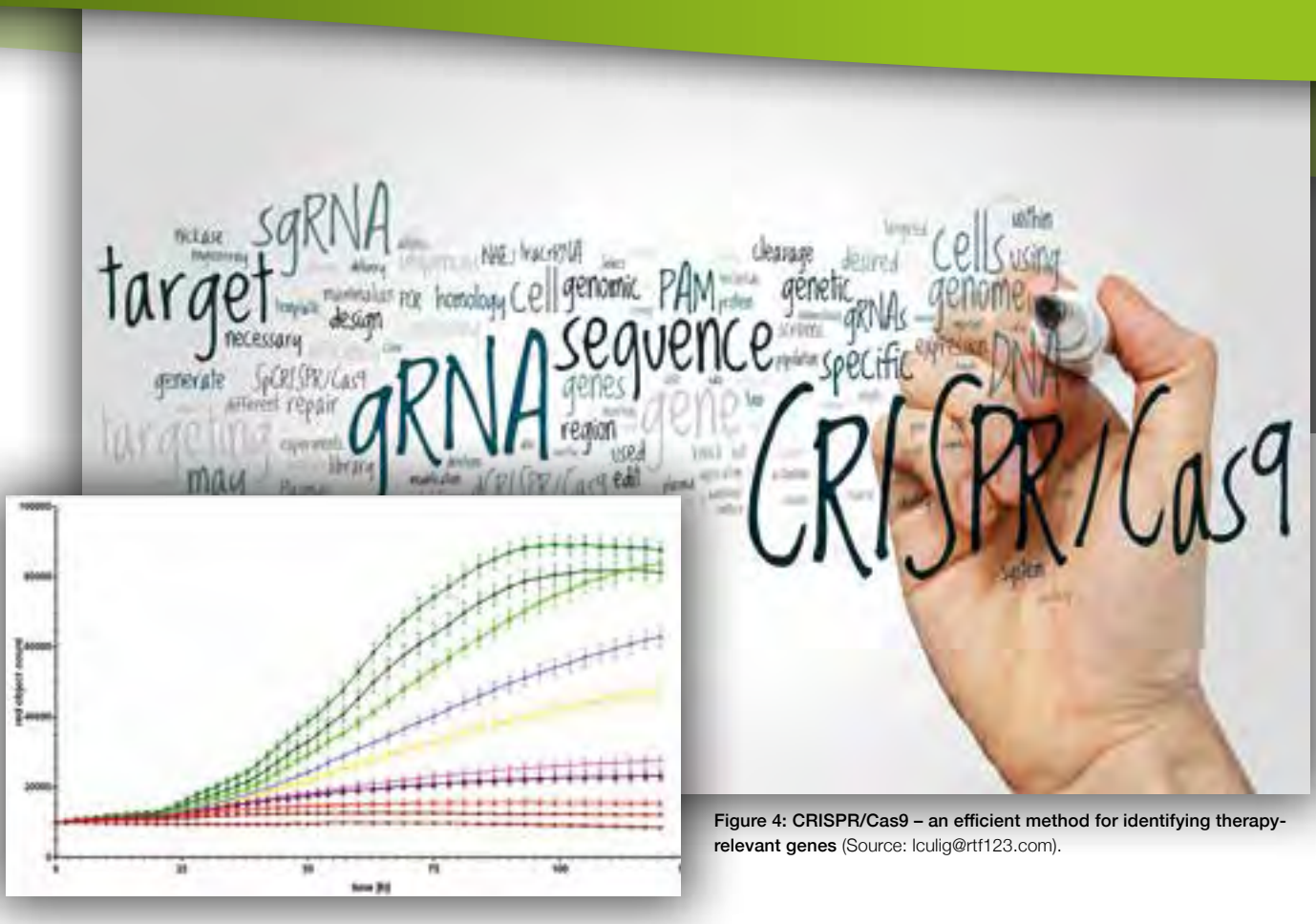


Figure 4: CRISPR/Cas9 – an efficient method for identifying therapy-relevant genes (Source: lculig@rtf123.com).

as an external partner, Kathrin Thedieck has established mathematical models of the PI3K-mTOR network, which are now being linked to the MAPK network and developed for pNETs. The team is complemented by Prof. Ulf Leser at Berlin's Humboldt University, with core expertise in data analysis, management and integration. His contribution primarily focuses on the integration of data sets at mRNA and protein level. The project is funded by the German Federal Ministry of Education and Research (BMBF) as part of its "e:Med – measures for establishing systems medicine" research and development concept.

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Figure 5: The MAPTor-NET consortium with Slim Khouja, Nils Blüthgen, Mathurin Dorel, Raik Otto and Pamela Riemer (last row from left to right), Laura Corbett, Kathrin Thedieck, Markus Morkel and Katharina Detjen (middle row from left to right), Tincy Simon, Christine Sers, Julia Hoffman, Lisa Dilz and Ulrike Bosch (front row from left to right). Not pictured are: Ulf Leser, Marianne Pavel and Daryl Shanley (Source: MAPTor-NET).

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dynamic, flexible and robustly self-renewing?

A Leipzig-based research team develops computer models of the self-organization of adult intestinal stem cells

by Maria Herberg, Torsten Thalheim, Marianne Quaas and Jörg Galle

The **INDRA** research group, headed by Jörg Galle, is working at the Interdisciplinary Centre for Bioinformatics (IZBI) at Leipzig University since 2015. The experimental-theoretical group aims at establishing the basis for a comprehensive systems biology model of the organization of the intestinal epithelium. On major objective is to contribute to a better understanding of the robustness and flexibility of tissue regeneration and of the changes being associated with tissue aging.

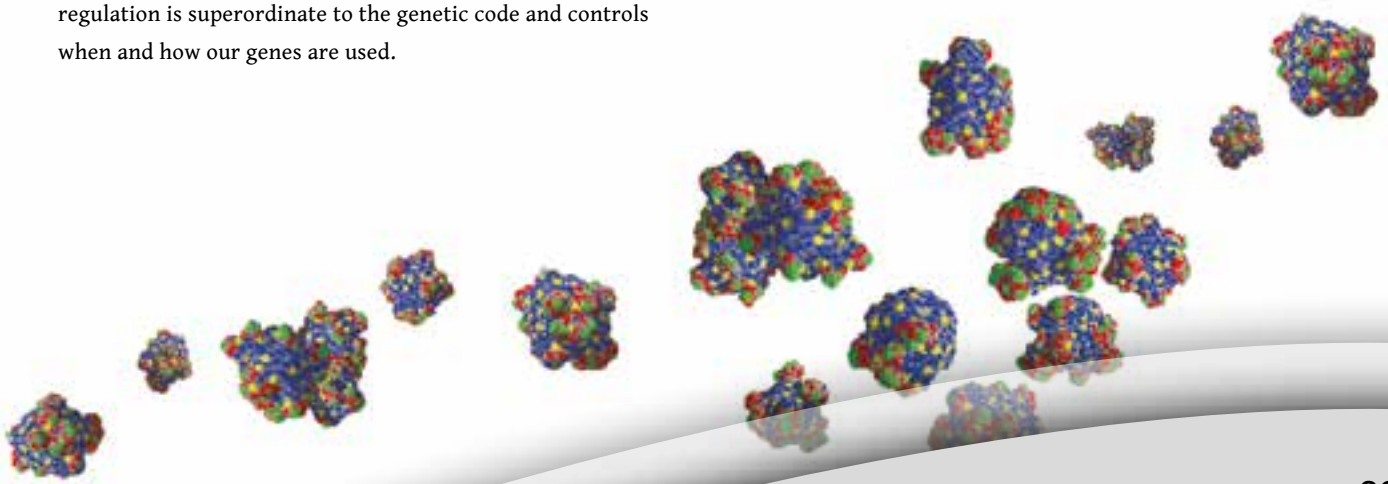
For more than 20 years, research scientists at the Faculty of Medicine at Leipzig University aim to understand how stem cells organize themselves within the body in order to reliably guarantee the stability and the regeneration of tissues and why this capability appears to decline with age. The main focus is thereby on the decision processes of stem cells during differentiation, and, in particular, on their regulation.

During early days of modern stem cell research, a strictly hierarchical picture of the differentiation process was prevailing. Findings from recent years indicate a much more flexible behavior. In particular, it has been shown that stem cells and their progeny can actively adapt to their environment by changing the expression of specific genes. This flexibility is primarily based on epigenetic mechanisms that trigger inheritable, albeit reversible, modifications of the chromatin. This epigenetic regulation is superordinate to the genetic code and controls when and how our genes are used.

Beside experimental and technological innovations, mathematical concepts for the generation and simulation models for the validation of hypotheses as well as the development of bioinformatics methods for the quantitative analysis of complex data supported this paradigm shift. In Leipzig, first computer-aided simulation models for the organization of stem cells in the blood, the skin, and the intestinal epithelium were developed under the direction of Prof. Markus Loeffler. These models propagated the new stem cell concept and used them successfully. The **INDRA** experimental-theoretical research group, being funded by the German Federal Ministry of Education and Research (BMBF) as part of its e:Bio research and development concept, develops and validates computer models of the self-organization of adult stem cells in the intestine.

Targeting the protective mechanisms of the intestinal epithelium

Physiologically, the intestinal epithelium regenerates faster than any other tissue. This regeneration is enabled by intestinal stem cells (**ISCs**), which are located on the bottom of the intestinal crypts (Figure 1). While stem cells of other tissues are often inactive even for weeks, ISCs divide on a time scale of one or a few days. This characteristic can be both a blessing and a curse. On the one hand, ISCs can effectively replace old or damaged cells. On the other hand, this regeneration potential is associated



Source: Maria Herberg, IZBI Leipzig

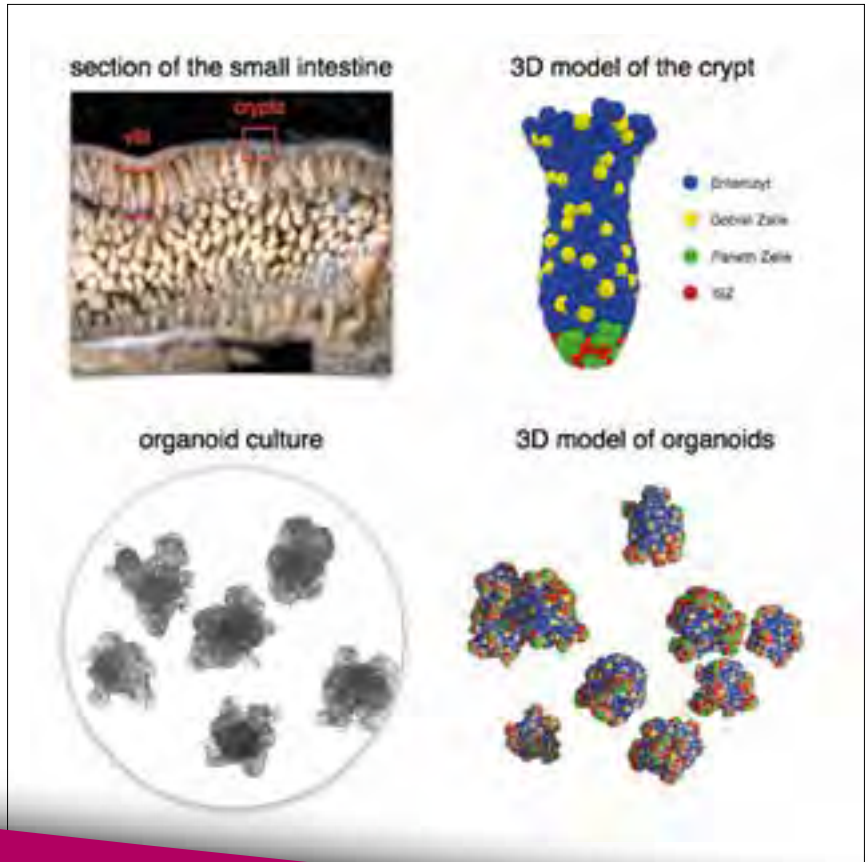
with a high risk of accumulation of DNA mutations, and, thus, potentially with the development of tumors. In **INDRA**, we try to identify mechanisms that prevent accumulation of such damage in the intestine and investigate whether these mechanisms are also active in culture.

Our research started in 2009, when scientists from the Hubrecht Institute in Utrecht successfully grew an intact mini gut in a Petri dish for the first time. For this purpose, crypts or individual ISCs were isolated from a donor intestine, embedded in an extracellular matrix and then supplied with specific growth factors and signaling molecules. The developing 'organoids' comprise all *in vivo* cell types of the small intestine and form typical crypt-villus units (Figure 1). These mini guts are currently used to investigate principles of developmental biology and disease profiles, and to foster regenerative therapies. However, efforts aiming at understanding the effects of the long-term culture on the molecular dynamics of these organoids are still rare. One aim of **INDRA** is to identify molecular changes associated with organoid culture and to analyze the related risks for the therapeutic use of the cells with the aid of computer models.

Competition at a cellular level

In cooperation with partners from Utrecht, we established a first 3-D computer model of the intestinal crypt in the run-up to **INDRA** (Buske *et al.*, 2011). Over the past three years, this model has been developed further, now enabling to trace the progeny of individual ISCs in simulations, i.e. to follow the long-term development of their clones, and to analyze related dynamics from a statistical point of view (Thalheim *et al.*, 2016). Thus, we are capable of investigating phenomena, such as 'monoclonal conversion', in a systematic way. Monoclonal conversion describes the fact that, after a certain period of time, all the cells in a crypt are part of the clone of a single ISC. All other ISCs and their progeny have been eliminated meanwhile. In a murine model, this process takes between four to six weeks, while the conversion period in humans is as yet unknown. A mutation can only manifest in the tissue if the mutated ISC wins this clonal competition. Assuming competition among similar stem cells, i.e. neutral competition, our 3-D computer model predicts that more than 85 % of all mutations are eliminated by this competition (Figure 2). Even if a stem cell is at an advantage, it does not always succeed in the clonal competition due to the spatial or-

Figure 1: Intestinal tissue and its 3-D models



Our model of the intestinal crypt assumes a defined shape of the crypt. Cell composition and distribution are self-organized. In the organoid model, the shape is self-organized as well. Photo of the intestine with the kind approval of H. Schneider (Clinic for Dentistry and Periodontology, Leipzig University).

Source: Maria Herberg, IZBI Leipzig

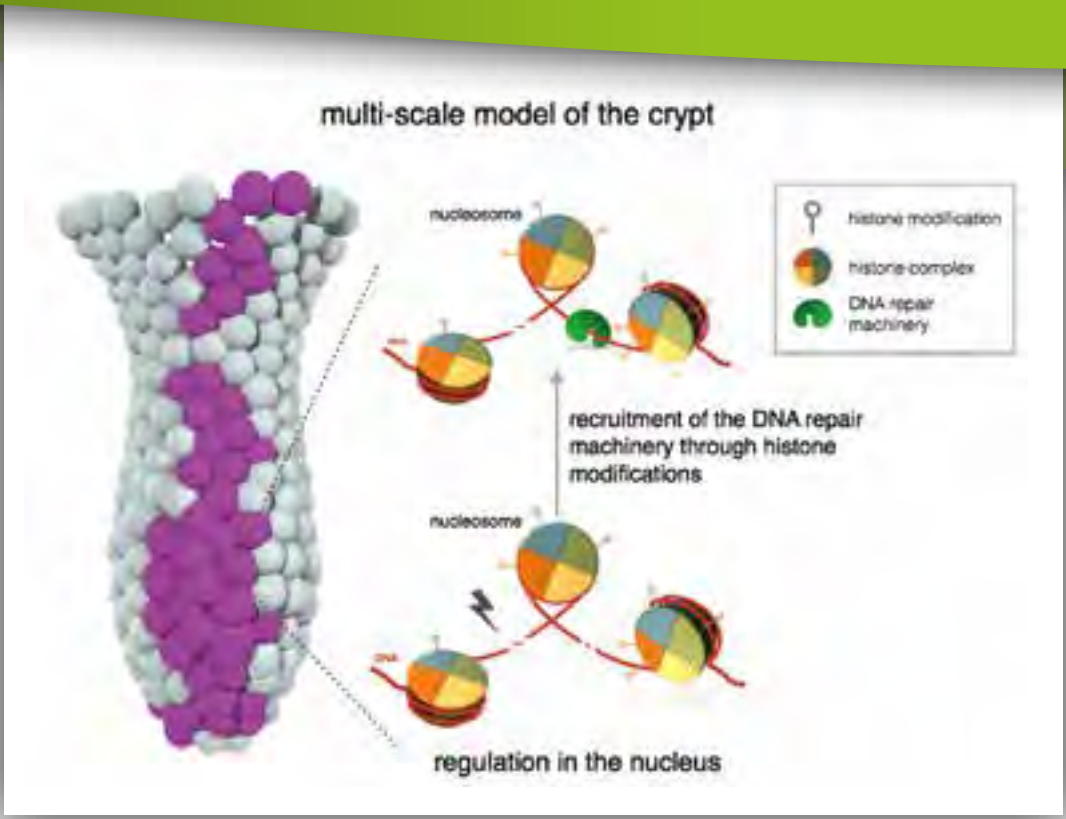


Figure 2: Mechanisms to protect the intestine from mutations. Cellular level: As a consequence of the competition, the progeny of a single ISC (clone, pink) will overtake the entire crypt. Molecular level: The recruitment of various DNA repair mechanisms to damaged sequences requires specific modification profiles of the associated histones. Our multi-scale models integrate both levels of regulation (Source: Maria Herberg, IZBI Leipzig).

ganization of the crypts. With other words, the architecture and cellular dynamics of the crypts can prevent the accumulation of mutations. But are these mechanisms also effective *in vitro*? We are attempting to find the answer using the world's first 3-D computer model of growing organoid cultures (Figure 1). This model allows us to simulate organoids under various conditions and thereby to perform a systematic analysis of clonal development within this system.

Repair requires specific conditions

Stem cells are also equipped with a plethora of mechanisms for direct DNA repair. However, recruitment of many of these repair mechanisms to the affected gene requires specific properties of the nucleosomes associated with the gene, in particular, of their histones. The tails of the histones need to be biochemically modified in a particular way in order to enable repair (Figure 2). In INDRA, we aim to investigate how these processes change in organoid culture.

In cooperation with a research project on the systems biology of hereditary colorectal cancer (HNPCC-Sys), we have already shown that age-related tissue changes take place much faster in organoid cultures than *in vivo* (Keyßelt *et al.*, 2016). In addition, strong expression changes in organoids indicate that also histone modifications and accordingly the recruitment of repair mechanisms vary compared to *in vivo*.

Together with the working groups of Prof. Gabriela Aust (Research Labs for Surgery, Medical Faculty of the University of Leipzig) and Prof. Michal-Ruth Schweiger (CMMC, University of Cologne), we currently measure genome-wide histone modification profiles and characterize their changes following short-term and permanent DNA damage. As a first result of these efforts, we found that, even after transient damage the modification changes induced by the repair are not fully reversible. For permanent damage or following repeated repair with increasing age, this 'deficiency' can result in loss of cell function. In **INDRA**, we develop computer models to describe these processes as well (Thalheim *et al.*, 2017). For the first time, these models combine cellular regulation based on epigenetic mechanisms and gene regulation networks.

More than individual components

Clearly, the regulation of stem cell organization in the intestine also involves other biological scales. So, how are epigenetic regulation processes influenced by the cellular dynamics of an organoid? Or, how do epigenetic changes influence the growth dynamics of organoids? In order to be able to investigate such interactions, we not only need sophisticated experimental strategies, but also suitable multi-scale models that enable a consistent interpretation of molecular, cellular and tissue-specific data. In a first step, we combined our crypt model with one of our epigenetic regulation models. This extension is also

Figure 3: Team INDRA from left to right Marianne Quaas, Torsten Thalheim, Jörg Galle and Maria Herberg (Source: Maria Herberg, IZBI Leipzig).

planned for the organoid model. With the aid of these combined models, we will simulate the complex interactions of intrinsic and extrinsic stem cell regulation and, based on the results, generate hypotheses on their interrelations. Systematic simulation studies of this kind quickly push technology to its limits. The availability of the Bull HPC Cluster Taurus at the Center for Information Services and High-Performance Computing (ZIH) in Dresden was therefore a prerequisite of our studies.

In **INDRA**, we will demonstrate the feasibility of such studies for the intestinal epithelium by the end of its term in 2019. Currently, other tissues have already come under scrutiny. As a result, we are striving for analog simulations e.g. for the epidermis. To this end, we are already collaborating with colleagues from the Max Planck Institute for Biology of Ageing in Cologne (Dr. Sara A. Wickström), the Cluster of Excellence in Cellular Stress Responses in Aging-associated Diseases (CECAD) at the University of Cologne (Prof. Carien Niessen) and the Leibniz Research Institute for Environmental Medicine (IUF) in Düsseldorf (Prof. Petra Boukamp).

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the future of chemical safety assessment

How systems toxicology may help to avoid animal testing

by Angela Mally

Chemicals are an integral part of modern life. In order to protect humans and the environment from chemical hazards, chemicals have to undergo toxicity testing. This is primarily done through internationally agreed regulatory tests in laboratory animals. Systems biology approaches are opening up new opportunities for more efficient, animal-sparing approaches to safety assessments.

Safety assessment of chemicals

Humans are exposed to a multitude of chemicals in all areas of daily life. In order to identify and assess health risks from chemicals, these have to be tested for toxicity. The current concept for assessing the safety of chemicals, which dates back to the 1930s, primarily relies on regulatory tests in animals. While this approach is widely considered to provide an acceptable level of health protection, societal pressure and scientific reasoning call for a fundamental change in the way toxicity assessment is carried out. These include an increasing demand for testing of new and existing chemicals under the EU's REACH regulation, legal restrictions for use of animals, high costs/low throughput, limited predictivity, and uncertainties associated with extrapolation from animal studies to humans. The US Environment Protection Agency (EPA) estimates that testing a single pesticide requires USD 5–6 million, 4,000 rodents, 80 rabbits and 70 dogs. The number of chemicals requiring toxicological testing and evaluation under REACH is estimated to range between 68,000 and 101,000 substances (Rovida and Hartung, 2009). Based on current regulatory test guidelines, this accounts for 54 million experimental animals (just vertebrates!) and costs amounting to EUR 9.5 billion (Rovida and Hartung, 2009). These figures illustrate that comprehensive toxicology testing of all chemicals is neither feasible nor ethically justifiable. In light of this, development of alternative, animal sparing test methods is legally required (Directive 2010/63/EU) and promoted through targeted funding schemes.

Paradigm shift in toxicological testing and risk assessment: moving away from animal tests towards mechanism-based animal-free solutions

Previous European initiatives aimed at reducing animal testing for toxicological tests primarily focused on replacing individual guideline studies by animal-free or animal-sparing methods. While this has led to the successful development and implementation of test guidelines to test for local adverse effects (e.g. corrosion or irritation of the skin and eyes, phototoxicity, skin sensitization), replacement of methods for complex health effects such as chronic systemic toxicity constitutes a major scientific challenge. Prompted by a report by the US National Research Council, Toxicity Testing in the 21st Century: A Vision and A Strategy (Tox21) (NRC, 2007), toxicology is currently undergoing a paradigm shift in the way toxicity testing and risk assessment is carried out – away from tests in animals towards mechanism-based *in vitro* methods. While in the past, tests for systemic toxicity primarily relied on histopathology and clinical-chemistry to identify adverse effects of chemicals and to characterize their dose-response relationships, innovative systems toxicology approaches build on mechanisms and latest technological advances to analyze and model the complex series of events that occur *in vivo* – from absorption of a substance to an adverse effect – using *in vitro* and *in silico* methods, and to utilize this information for chemical safety assessment (Figure 2). The adverse outcome pathway (AOP) concept underlying this strategy is based on the recognition that toxic effects are the result of a sequence of causally linked key events (KE), starting with the chemico-biological interaction (molecular initiating event, MIE) through subsequent cellular

Figure 1: Chemicals are an integral part of our lives. In order to protect humans and the environment, chemicals need to be tested for hazardous properties (Source: A. Mally).

effects down to an adverse outcome on the organ or organism level (Vinken, 2013) (Figure 2). There is scientific consensus that AOPs present an instrumental, mechanistic basis for the development of *in vitro* test batteries that may at least in part replace regulatory toxicity studies in animals. Moreover, quantitative *in vitro-in vivo* extrapolation, which allows for the calculation of no observed adverse effect levels (NOAELs) in animals or humans based on the no observed effect concentration (NOEC) determined *in vitro* (reverse dosimetry), presents an essential component of future test strategies designed to assess human health risks (Figure 2).

Opportunities and challenges of new test strategies

It has been estimated that new animal-sparing test strategies that efficiently combine information from various complementary test systems with reverse dosimetry modeling may reduce the need for traditional toxicity tests in animals to 15 % of all chemicals (Thomas *et al.*, 2013). However, it is clear that such fundamental paradigm shift in safety testing and assessment of chemicals can only be brought about through world-wide concerted efforts. Particularly for systemic effects of chemicals the situation is highly complex. It is important not only to systematically capture mechanistic knowledge relevant to a given hazard endpoint in a formal description of AOPs, but also to identify data gaps and to fill them through targeted mechanistic research. Establishing *in vitro* test batteries requires development and validation of innovative, high-throughput *in vitro* models that better reflect the structure and function of organs *in vivo*. Advancement and implementation of efficient methods for quantitative *in vitro-in vivo* extrapolation is also of key importance in order to account for absorption, distribution, metabolism and excretion as critical determinants of systemic toxicity (Rotroff *et al.*, 2010; Wetmore, 2014). Finally, implications for regulatory decision-making derived from *in vitro* assays need to be evaluated against risk assessments based on conventional toxicity tests to assess the confidence in risk assessment based on *in vitro* data.

Proving feasibility: Risk IT

Within the frame of Risk IT, a public-private collaborative project funded through the "Innovative systems toxicology for alternatives to animal testing" joint funding scheme by ZonMw and the German Federal Ministry of Education and Research (BMBF), scientists are attempting for the first time to implement the AOP strategy as the basis for the development of new alternative methods for systemic toxicity testing. Focusing on the kidney as an exemplary key target organ of toxicity, Risk IT aims to provide a proof-of-concept for the successful integration of new mechanism-based *in vitro* methods and toxicokinetic modeling into a tiered testing strategy that is fit-for-purposes of regulatory decision making. The project builds on expert mechanistic knowledge and systems-toxicology data generated within previous projects in order to develop a systems-toxicology-based mechanistic framework for drug/chemical-induced nephrotoxicity in line with the AOP concept, and to use this mechanistic framework to derive suitable endpoints that can subsequently be measured *in vitro* in renal epithelial cells using modern high-throughput approaches. Quantitative *in vitro-in vivo* extrapola-

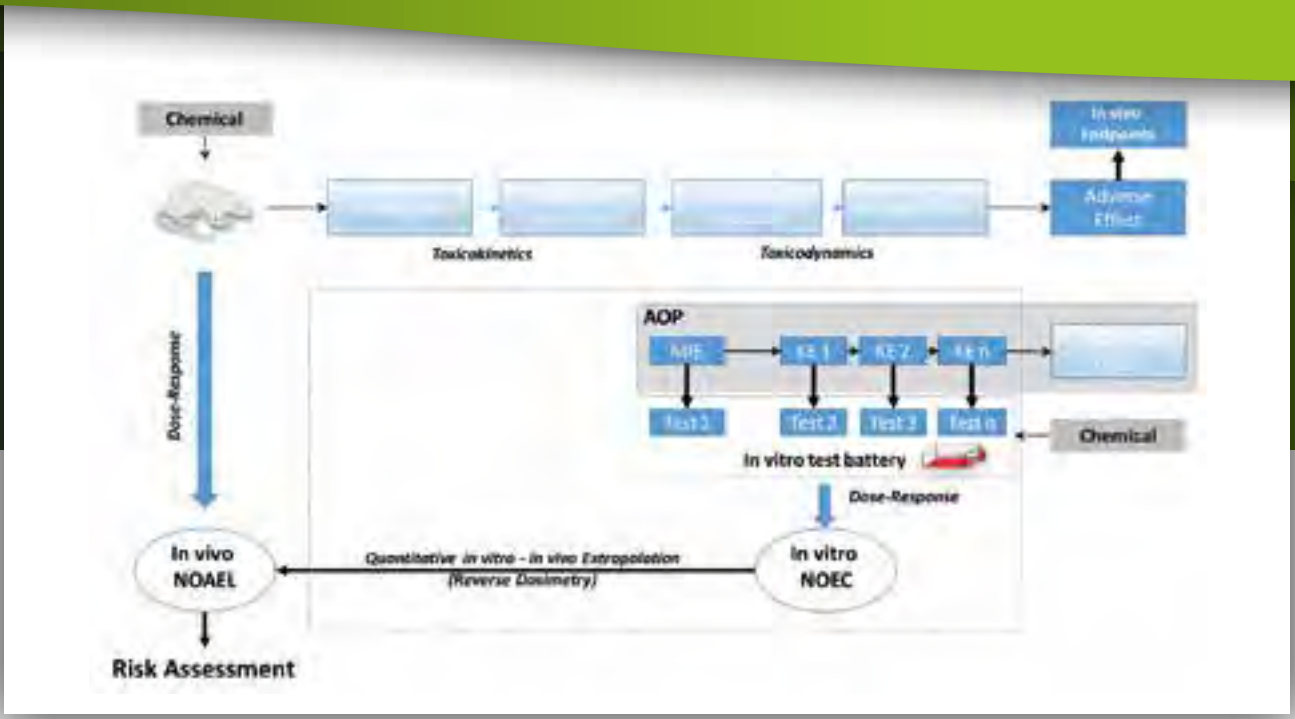

Figure 2: Traditional and innovative approaches to toxicology testing and chemical safety assessment. In contrast to determining adverse effects in animals and characterizing their dose-response relationships, new strategies focus on measuring key events (KE) using mechanism-based *in vitro* tests combined with reverse toxicokinetic modeling to derive equivalent *in vivo* doses. AOP: Adverse outcome pathway; MIE: Molecular initiating event; KE: Key event; NOAEL: No observed adverse effect level; NOEC: No observed effect concentration (Source: A. Mally).

tion QIVIVE is then used to transform *in vitro* concentration to *in vivo* oral dose to generate a point of departure for human risk assessment. A key aspect of the project is to assess the confidence in risk assessment based on *in vitro* data compared to current approaches, as well as to identify limitations and sources of uncertainty.

Research project profile:

Project name: Integrating mechanisms and quantitative *in vitro-in vivo* extrapolation (QIVIVE) modeling for risk assessment based on *in vitro* testing (Risk IT)

Participating partners:

University of Wuerzburg (Angela Mally)

Utrecht University, Netherlands (Nynke Kramer)

Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) (Stefano di Fiore, Bernhard Ellinger)

BASF SE (Bennard van Ravenzwaay, Barbara Birk)

Coordinator:

Angela Mally (University of Wuerzburg)

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Federal Ministry
of Education
and Research

News from the BMBF

European XFEL – the world's highest performance X-ray laser

Laser light is part of everyday life: DVD players, barcode readers, laser medicine, fibre optic transmission and many other applications depend on this special light source.

The European XFEL is a huge particle accelerator facility in Hamburg which generates laser flashes in the hard X-ray range. This X-ray laser enables researchers to decode the atomic structure of materials, biomolecules and entire viruses. The European XFEL generates short laser flashes of less than 0.0000000000001 seconds, thus allowing chemical reactions to be "filmed". It provides unprecedented deep insights into the micro and nano cosmos as a basis for developing new materials, production methods and medicines. The facility has a length of 3.4 kilometres and reaches out to Schenefeld in Schleswig-Holstein. Research operation started in September 2017.

Federal Research Ministry provides roughly 760 million euros

Germany as the host country covers roughly 58 per cent of the construction costs of 1.225 billion euros (2005 prices), thus enabling the German science community to take the international lead in this research area. Construction is financed with federal funds as well as funds from the *Länder* of Hamburg and Schleswig-Holstein. The Federal Ministry of Education and Research (BMBF) contributes roughly 760 million euros. In addition, the BMBF supports collaborative research projects involving German universities and research institutions to develop new instruments and technologies for the European XFEL.

A total of eleven countries are participating in the construction and operation of the facility. The X-ray laser is a milestone in European basic research and forms part of the BMBF's Roadmap for Research Infrastructures.

Further information is available at:

www.bmbf.de/de/european-xfel-2569.html

The accelerator tunnel of the European XFEL.

Sources: DESY 2015 (above) and Heiner Müller-Elsner / European XFEL (below)

Better treatment thanks to medical informatics

The BMBF's Medical Informatics Initiative sets the stage for effective digital patient services. It aims to pool the increasing amounts of data – from X-rays to genetic analyses – in a national infrastructure in order to create new knowledge for improved research and healthcare.

After consulting an international panel of high-level experts, the BMBF decided to include four consortia of 17 university hospitals and roughly 40 further partners in the four-year development and networking phase of the Medical Informatics Initiative. The BMBF will provide approximately 120 million euros for this purpose over the coming four years.

Data protection is a top priority

In January 2018, these consortia started to build up data integration centres which enable networking and the exchange of data. Data protection and data security are top priorities. The eligibility for funding depends on compliance with the stringent data protection standards and conditions applicable in Germany. The consortia will demonstrate the added value of digital networking for patients through various medical applications ranging from personalized cancer therapy to treatment of MS and intensive care.

Doctors and researchers around the globe are interlinked in the digital age. They generate new data and information every day. However, digital medicine is still a kind of web without search engines: The enormous amounts of data are most difficult to exploit.

Digital networking in the health system is a joint national task. It is therefore important to ensure that further university hospitals and partners involved in the conceptual phase can continue to participate in the initiative. The BMBF will provide additional funding of up to 30 million euros to achieve this aim. "We have increased the amount of funding from 100 million euros to over 150 million euros in order to strengthen the national character and impact of this forward-looking measure," said Minister Wanka.

Digital media are becoming more important in healthcare.

Source: Canstockphoto / Productionperig

The preceding nine-month conceptual phase of the initiative involved the participation of 28 of Germany's 33 university hospitals and many other partners. They worked together in consortia to plan the development of a networked national infrastructure for the use of digital health data and established a forum for dialogue with major players in public health, patient representatives and data protection officials as well as industry and health insurance funds.

Further information is available at:

www.medizininformatik-initiative.de/en/start

Intestinal inflammation treatment without side-effects

Patients suffering from intestinal inflammation are often reluctant to talk about it. An attack involves bloody diarrhoea, abdominal cramps, loss of appetite and nausea. The longer the duration of chronic inflammation, the greater the patients' weakness and fatigue because the illness causes deficiency and anaemia. Moreover, chronic inflammatory bowel diseases such as Crohn's and Ulcerative Colitis are incurable and ultimately increase the patient's risk of developing colon cancer. Approximately 400,000 people suffer from intestinal inflammation in Germany.

We still do not know the exact causes of these diseases. What we do know is that they involve a malfunction of the immune system. Normally, the immune system recognizes endogenous cells. Harmful bacteria or viruses entering the body trigger an immune response. This causes an inflammation. As a rule, the immune system calms down again after successful defence. Specific messenger substances play an important role in this process. This complex system is impaired in the case of chronic inflammatory disease, which leads to an overreaction of the immune cells and messenger substances. Healthy tissue is damaged and patients suffer from permanent inflammation of the affected areas.

Treatments for Crohn's Disease and Ulcerative Colitis aim to restore the immune balance. They use active agents which block inflammatory messenger substances like Interleukin 6 or TNFα in order to regain control of the excessive defence. But these treatments have a serious disadvantage. "They reduce not only the inflammation but also the patient's immunoprotection," explains Stefan Rose-John from the Biochemical Institute of Kiel University. "This means that patients run a higher risk of contracting harmful infections." This type of immunotherapy may trigger an outbreak of latent infections like tuberculosis or Hepatitis B.

Fighting inflammation without increasing the risk of infection

A team of experts at the universities of Kiel, Magdeburg and Düsseldorf is working to improve the situation with support from the Federal Research Ministry. They studied the messenger substance Interleukin 6 in order to understand how this protein works and what molecular processes are involved in its effect. "We found that the messenger substance is involved in two signal paths, one of which can have a positive and the other a negative impact on the body," says Rose-John. "We used this difference to develop an active ingredient which blocks the disease-promoting signal path without interfering with the protective properties of Interleukin 6." This means for patients that inflammation is reduced without increasing the risk of infection.

Patients suffering from chronic intestinal diseases are currently being treated with this new substance as part of a clinical trial at the university hospital in Kiel. The preceding phase I trials showed no serious side-effects. The new substance may be placed on the market in a few years if the current trials are successful. The team of researchers is already cooperating with a big pharmaceutical company.

But this will not mean the end of their work. Says Mr Rose-John: "Our ultimate goal is the development of a personalized anti-inflammatory treatment. Different patients need different doses depending on their genetic disposition." The newly developed substance may also be used to treat other diseases such as rheumatoid arthritis.

Further information is available at:

www.gesundheitsforschung-bmbf.de

Diarrhoea, cramps and nausea are symptoms of chronic inflammatory bowel disease.

Source: Nerthuz / Thinkstock

An app which indicates the onset of depression

Each year, more than five million people in Germany suffer from a depressive episode. The symptoms may be manifold: Patients experience a lack of pleasure and motivation, have trouble concentrating and sleeping and suffer from anxiety and self-doubt. They seldom succeed ridding themselves of their negative thoughts on their own – yet professional help is not easily available and waiting times of several months are not uncommon. Researchers and IT experts in Leipzig and Dortmund are therefore developing an app which patients can use to document the course of their disease. The STEADY system will use these data to warn patients of a depressive phase at an early stage. The Federal Research Ministry supports the development of this system with funding of approximately 1.7 million euros.

Depression has many faces: Its causes, symptoms and course vary from one patient to the next. Depressive episodes are the most frequent form of the disease. There may be symptom-free periods between two depressive episodes, which experts call unipolar depression. Patients may also experience alternating periods of depression and extreme excitement, which is known as bipolar depression. The researchers developing the STEADY app want to help patients realize at an early stage when they are heading for a depressive episode.

System reveals changes in health status

STEADY interacts with other mobile apps and wearables such as biosensor trackers. The system can thus record heartbeat, skin temperature and location as well as changes in speech or sleep patterns. Patients can add their own information on how they feel. The aim is to support patients with information about changing symptoms which is more accurate than their own feelings. The app can show specific patterns which precede changes in health status and provide recommendations for prevention. Patients can then actively combat specific causes.

However, all of this cannot replace medical advice. “Our aim is to support treatment,” says Katrin Rothmaler at the Institute for Applied Informatics of Leipzig University. Patients are free to share their data with their family doctor or medical specialist, their health insurance fund or a research institution. They remain the owners of their data which is passed on by the system in an encoded form. “We want to close information gaps to support diagnosis and treatment. It may thus be possible to increase the intervals between therapy sessions for stable patients,” explains Ms Rothmaler. This will benefit the entire health system. Specialists and psychologists would have more information about their patients, which would improve the quality of medical care and psychotherapy.

The many faces of depression: Its causes, symptoms and course vary from one patient to the next.

Source: Thinkstock

Further information is available at:

www.bmbf.de/de/app-soll-vor-depression-warnen-4535.html

BMBF-Newsletter:

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FROM BUILDINGS AND GENOMICS PLATFORMS TO MEMORY DRIVEN COMPUTING

New structures at the German Center for Neurodegenerative Diseases to promote systems biology

by Joachim Schultze and Dirk Förger

The burden posed by neurodegenerative diseases on society is increasing. The number of people with Alzheimer's disease is already estimated at 1.6 million in Germany alone. In order to address this challenge, the German Federal and State Governments decided ten years ago to establish the German Center for Neurodegenerative Diseases (DZNE). The DZNE also uses systems medicine approaches in order to better understand complex diseases of the central nervous system. The new DZNE building in Bonn, which was opened in March 2017, houses cutting-edge genomics technologies and a completely innovative high performance computing system known as memory-driven computing (MDC). MDC has already been installed at the DZNE for its new platform for single-cell genomics and epigenomics (PRECISE).

THE NEW DZNE BUILDING ON THE VENUSBERG HILL IN BONN

The new DZNE site stretches out on the campus of the University Hospital of Bonn (UKB), high up on the Venusberg hill. The DZNE staff in Bonn were previously spread across different locations, but now they are united under one roof. Over 550 people from more than 50 different countries work here. The buildings in Bonn house various departments: basic research, clinical research and population research. In addition, the central administration for all of the DZNE's nine sites is also based here. Special communication areas in the links between the three buildings aim to boost dialogue between the different areas of research. The location of the DZNE on the UKB campus is also ideal for promoting close collaboration with the University Hospital. The DZNE is linked via a tunnel with the UKB's Center for Neurology, Psychiatry and Psychosomatic Medicine (NPP).

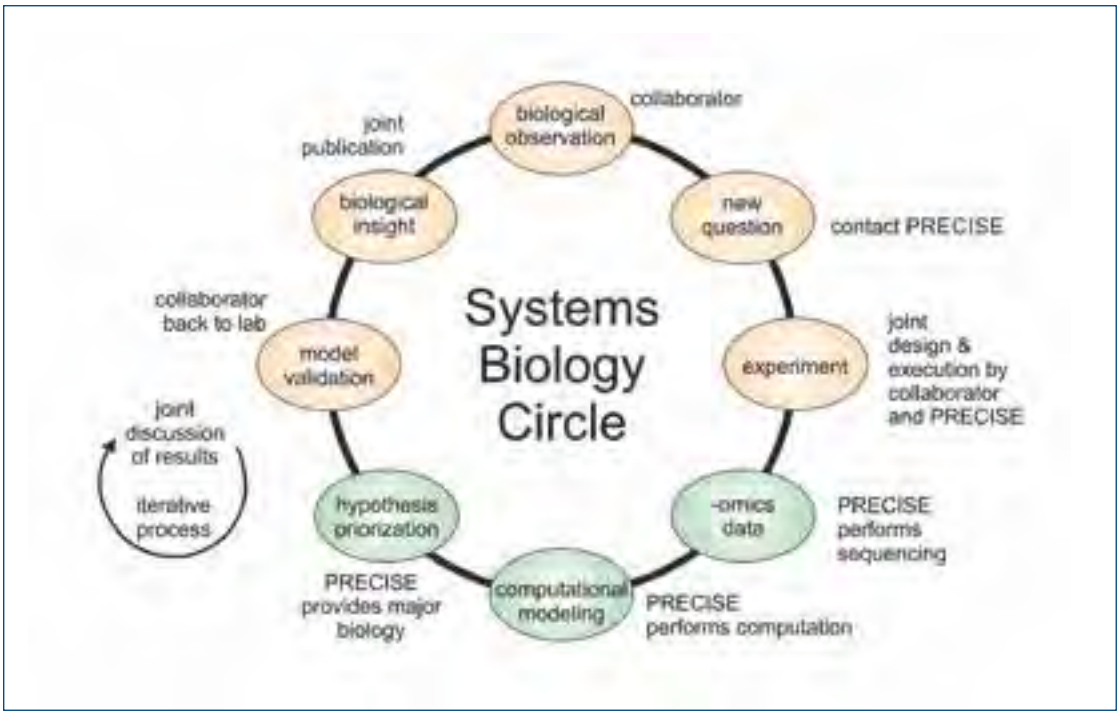

Figure 1: Systems biology circle
A systems biology circle used at PRECISE in projects concerning neurodegeneration, neuroinflammation, chronic inflammatory disease and systems immunology.

Source: German Center for Neurodegenerative Diseases (DZNE)

Figure 2: The new DZNE building in Bonn

Source: German Center for Neurodegenerative Diseases (DZNE) / Laubner

Beyond basic research, the DZNE is committed to investigating every facet of neurodegenerative disease via clinical trials, population studies and healthcare research. The various activities are strategically managed in order to support the application of scientific findings. A total of over 1,100 staff work at ten sites throughout Germany. Bonn is the largest of these and also home to the Management Board and Administration. The Bonn site also coordinates various clinical trials that the DZNE carries out at various sites in accordance with uniform standards. This approach expands the number of potential trial participants and boosts the statistical significance of the results.

The new DZNE building was designed by the architects wulf architekten GmbH (Stuttgart) and is one of the biggest new research centers in the state of North Rhine-Westphalia. The costs of building and equipping the site came to €126.8 million. Two thirds of the funding came from the state of North Rhine-Westphalia (Ministry for Innovation, Science and Research, MIWF) and one third came from Federal resources (German Federal Ministry of Education and Research (BMBF)). The total area of the complex covers 35,000 m^2, which corresponds to the size of five soccer fields. The total workbench length in the laboratories is approx. 1.3 kilometers. 2,403 colored glass slats decorate the facade of the new building. Some of these are able to move and follow the direction of the sun. The colors of the sunshades reflect the colors of the neighboring forest as they change throughout the year.

The environment was also a major consideration during the planning, with the energy for heating coming from a geothermal system, heat recovery and a highly efficient cogeneration unit. The new building also sets great store by sustainability and energy efficiency.

PRECISE – PLATFORM FOR SINGLE-CELL GENOMICS AND EPIGENOMICS

Just like in other medical disciplines dealing with complex diseases, current findings indicate that research into the pathophysiology, diagnostics and therapies for neurodegenerative diseases will benefit greatly from systems biology approaches as the basis for systems medicine (Beyer *et al.*, 2017). The DZNE has already established first-class institutes for genetics (Tübingen) and RNA biology and epigenomics (Göttingen). However, there was a gap in the link between functional and molecular single-cell analysis and the area of genomics.

Since the University of Bonn had already invested in this field of research with the Life and Medical Sciences Institute, the partnership with the university was the best option for closing this gap. As a result, the new platform for single-cell genomics and epigenomics (PRECISE) was founded as a joint venture between the DZNE and the University of Bonn. PRECISE aims to bundle the expertise of both institutions in sample processing, automation, sequencing, data preprocessing and data analysis. It is a research body comprising scientists from the University of Bonn, the DZNE and external partners that carry out projects along the entire systems biology approach workflow on the basis of genomic data (Figure 1). Experimental processes, sequencing, computer modeling and data presentation are thus bundled into one unit.

In addition to bilateral partnerships of individual scientists at the DZNE and the University of Bonn, PRECISE also manages larger research consortia at local, national and international levels (Figure 3). Currently, PRECISE is a member of the German research association (DFG) cluster of excellence ImmunoSensation, the DFG's collaborative research center SFB704, EU consortia such as SYSCID (a systems medicine approach for chronic inflammatory diseases) and the Sparse2Big and AMPro consortia financed by the Helmholtz Association.

One current area of research for the PRECISE team is the development of a portfolio of single-cell RNA sequencing technologies (scRNA-seq). These make it possible for scientists at the University of Bonn and the DZNE to link their functional analyses of individual cells with a genome-wide assessment of single-cell transcriptomes via scRNA-seq. Single-cell-ATAC-seq also makes single-cell epigenomics possible. At PRECISE, we are sure that there is as yet no single method for all scientific questions (Beyer *et al.*, 2017). For this reason, we invest in technologies that enable, for example, a direct patch clamp analysis of individual neurons with scRNA-seq – at the same time providing approaches that enable the sequencing of tens of thousands of cells within a single experiment.

Other methods are scRNA-seq technologies that combine flow-activated cell sorting (FACS) with scRNA-seq. In cooperation with our partners, we have successfully applied these technologies in order to decode the cellular compartment of dendritic cells in peripheral blood (Heidkamp *et al.*, 2016, See *et al.*, 2017). At the same time, we were also able to explain the development of macrophages in embryogenesis (Mass *et al.*, 2016) and the role of reprogramming human monocytes during birth in order to prevent neonatal sepsis (Ulas *et al.*, 2017). One strong area of focus for PRECISE is the research of the cell population structure within the central nervous system and the human lung, with immune cell populations at the forefront of our research. In addition, we also take great interest in the role of genomics and epigenomics in chronic inflammatory disease.

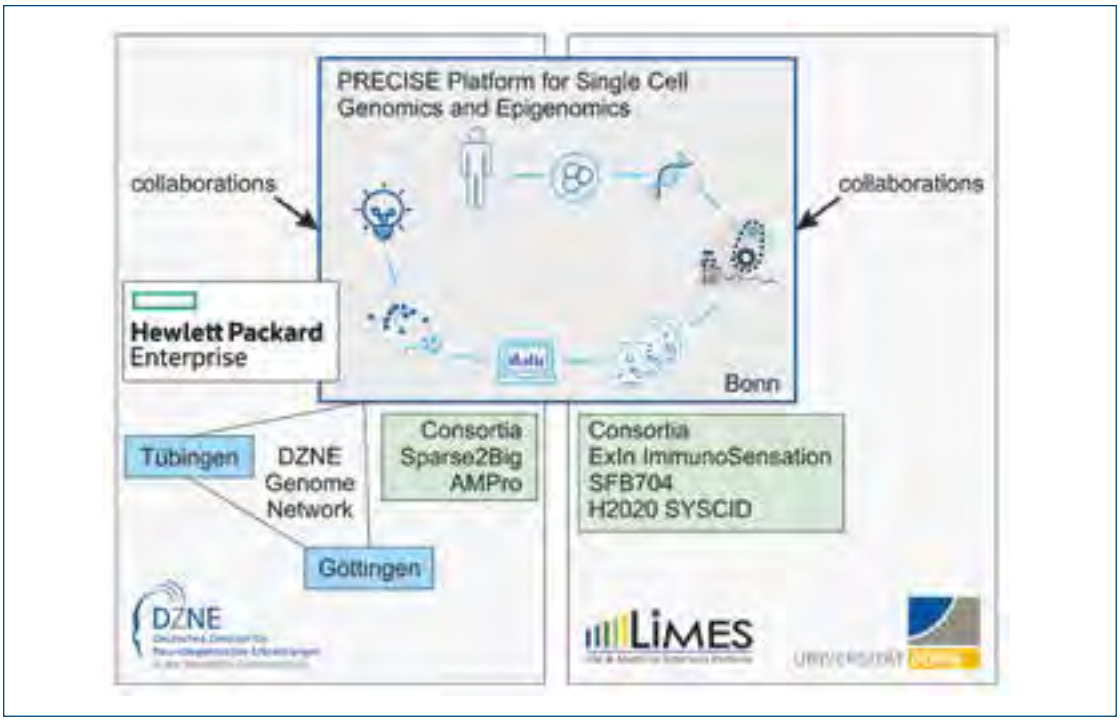

Figure 3: Structure of the PRECISE platform
Joint venture structure of PRECISE between the University of Bonn and the DZNE. PRECISE is a member of the DZNE genome network in Bonn, Tübingen and Göttingen. PRECISE is the co-chair of the Sparse2Big consortium funded by the Helmholtz Institute and a member of the AMPro consortium. PRECISE is a member of the ImmunoSensation cluster of excellence, the SFB704 and the European SYSCID consortium. PRECISE works with HPE on memory-driven computing solutions.

Source: German Center for Neurodegenerative Diseases (DZNE)

Figure 4: The new DZNE building in Bonn Source: German Center for Neurodegenerative Diseases (DZNE)

HIGH PERFORMANCE COMPUTERS ACCELERATE GENOMIC RESEARCH AT DZNE

Genomic data generation will increase dramatically with the founding of PRECISE and the genomic network within DZNE. Classic infrastructures for managing this avalanche of data include high-performance computers (HPC) with huge amounts of memory and computer clusters with data storage that is large enough to permit complex calculations. Recently, local solutions have come under competition from central cloud-based infrastructures as a result of their superior scalability and lower maintenance requirements. On the other hand, centralized models have disadvantages such as high data traffic, data reproduction and lower data security.

Regardless of the location, HPC systems currently rely on classic hardware architectures, where data has to be moved constantly between data storage and random-access memory. In order to overcome many of these obstacles, the ideal system would have a large local memory for genomic (and other) data, to which a variety of processors would have access to run various tasks (memory-driven computing). In addition, in the ideal scenario, various local systems at different locations would also be linked within a virtual network. These would mainly exchange algorithms for local computations, with data traffic reduced to much smaller metadata or calculated data (distributed mesh computing). The Gen-Z consortium, which is headed by Hewlett-Packard Enterprise, is currently transforming the processor and cloud-centric HPC infrastructure into a memory-driven, distributed mesh infrastructure. The DZNE and University of Bonn's PRECISE platform was the first external partner of HPE to test the prototype for memory-driven computing in genomic data processing. Because the initial results are so promising, the DZNE plans to expand the partnership with HPE. As such, the entire genomic data IT infrastructure is to be revised in order to switch completely to memory-driven computing.

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using algorithms to understand single cells

A Portrait of Carsten Marr, Deputy Head of the Institute of Computational Biology at the Helmholtz Zentrum München

by Kristin Hüttmann

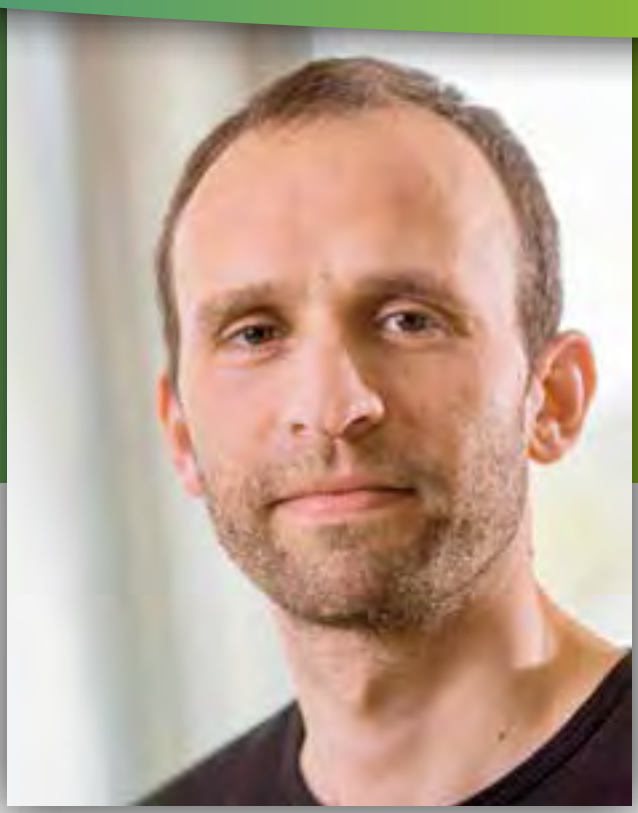
Carsten Marr has a weakness for tricky tasks – in order to understand how individual cells work, he is developing algorithms and computer-assisted simulations at the Institute of Computational Biology, Helmholtz Zentrum München.

Anyone who goes camping with four children understands the meaning of the word "challenge" by the end of the summer. Especially if the family changes campsite three times during the vacation. "We were simply looking for the perfect spot," says Carsten Marr. As a result, the family man put up and took down all their tents three times, driving down the Atlantic coast with the kids in the back – until they found the ideal place and everyone was happy.

Searching for perfection is something that is reflected in Marr's daily life at work, too. The 40-year-old is a trained physicist and loves finding the right solution to difficult problems. However, Marr has not dedicated his career to inanimate matters like many of his colleagues, but has landed in an area that he had tried to avoid at school: biology. Marr works in the north of Munich at the Helmholtz Zentrum, where he

With his team, here with doctorate student Carolin Loos, Carsten Marr works on image processing for individual cells, biomedical data analysis and mathematical modeling.

Source: HMGU



Dr. Carsten Marr, head of the Quantitative Single Cell Dynamics working group (Source: Michael Haggenemüller / HMGU).

is Deputy Head of the Institute of Computational Biology and heads the Quantitative Single Cell Dynamics working group. It might sound complicated, but it can be summarized quite easily, as Marr explains his job to his children: "I develop methods to understand how individual cells function."

Marr doesn't need a laboratory to do this. "I probably wouldn't even know how to hold a pipette," he jokes. Instead, his workstation consists of a desk and a computer. Out of the window, there is an expansive view of the green heathland to the north of Munich. This landscape is the backdrop to Marr's one-hour commute, which he makes by bicycle. He's an athletic person with short hair, dressed in black apart from a neon lanyard, and is softly spoken. Perhaps it's a trick he uses on his four children, knowing that a quieter voice makes them more likely to listen.

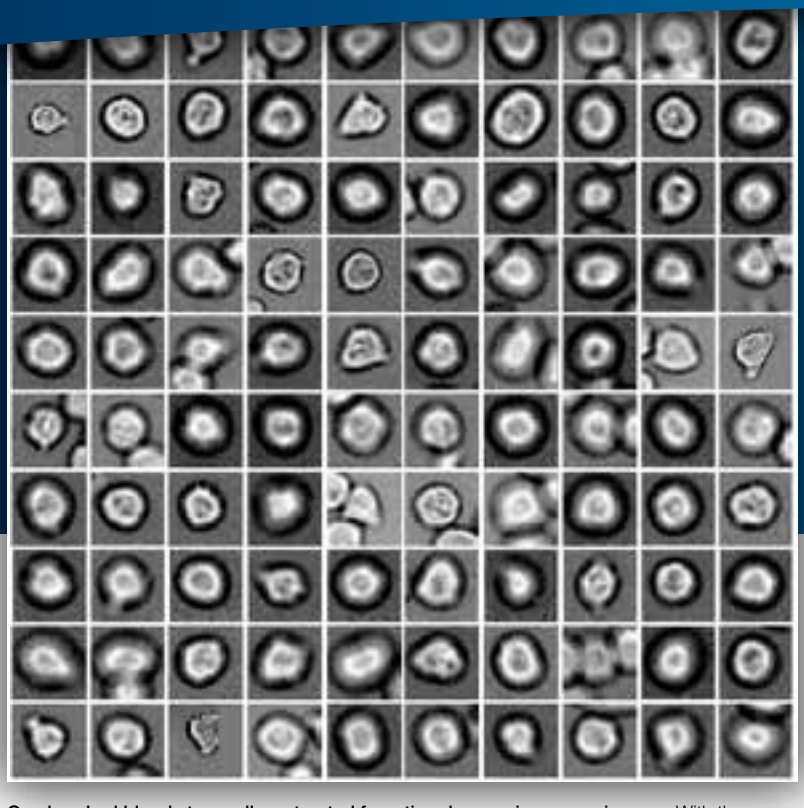
When Marr's not at his computer, he talks to his team and colleagues from other research areas at the Helmholtz Zentrum München. Around 1,000 scientists work here at over 50 departments and institutes, and Carsten Marr's institute is just one of them. "We work very closely with the institutes here," the physicist explains. "And we do the data analysis for the others." The others are biologists, stem cell researchers and clinicians both at the Helmholtz Zentrum itself and at other research institutes in Munich, elsewhere in Germany and further afield. The questions and problems arising in Marr's team find their way onto the whiteboard in his conference room, quickly followed by initial ideas for future methods.

Predicting blood stem cell decisions

Marr and his team investigate topics such as the dynamic growth of stem cells – not using a microscope and pipette, but with the aid of mathematical models and algorithms. By doing so, the scientists want to understand, for instance, how different blood cells are formed from their progenitor cells, the blood stem cells. "We are asking how and when the blood stem cells decide what kind of blood cell they are going to become," says Marr. During experiments, this process cannot be observed directly, but only be read out afterwards via cell surface markers.

In order to change this, Marr and his colleagues threw themselves into the arduous task of data collection. For four years, they sat at their monitors together with the experimentalists and tracked migrating cells in the recorded movies on the computer. It was a laborious task that finally resulted in the collection of an enormous amount of data by the scientists. "From the signals from the surface markers in sister cells, we are now able to estimate the time when a stem cell actually makes its decision," says Marr.

And that's not all. Because Marr's algorithms are not just able to calculate the time when the cells make the decision, but also predict it – using deep learning. Deep learning simulates learning processes as they occur in human neural networks – just like when a child learns how to recognize different faces or animals. In deep learning, software neurons communicate with one another within a network and work out the ideal pattern perception by computing data sets over and over. In doing so, they strengthen successful connections and eliminate less successful ones – ensuring the continual improvement of the network.



One hundred blood stem cells, extracted from time-lapse microscopy images. With the application of algorithms from machine learning, we can now very early predict the direction in which blood stem cells will develop (Source: HMGU).

Deep learning works particularly effectively when large amounts of data are available for training the network. "Our algorithm analyzes the optical microscope images and videos of individual cells and compares them with images of more mature blood cells," says Marr. In this way, the algorithm learns how certain cells behave and can even predict their development. Deep learning is not only likely to aid the analysis of blood stem cells. Research scientists want to use the method for a whole range of questions for which there are sufficiently large quantities of data. "Very similar algorithms can be used to analyze regulatory patterns in the human genome and to identify biomarkers in clinical cell screens," says Marr.

Intelligent corrective software

Marr and his team also recently developed an algorithm to deal with another problem. This algorithm helps in the analysis of images documenting the stages of development of the stem cells. Until now, the problem has been that it is often very difficult for scientists to interpret the collection of cell images in a quantitative way due to shadows in the images or different backgrounds. The new algorithm can now correct these factors. The software is called **BaSiC** and Marr's team developed it in partnership with staff from the Technical University of Munich (TUM), the University of California, Johns Hopkins University Baltimore and ETH Zürich.

BaSiC can be used with many of the image types used in bioimaging, making it possible to observe even more closely the stages of development of stem cells. "With BaSiC, we are able to make key decision-making factors visible," says Marr. Before, these factors had been obscured by measurement noise. "The long-term aim of this research is to influence the development of stem cells in a very targeted way," Marr says. The new observation options are bringing research scientists one step closer to this goal, and they hope to one day be able to help with the prediction and treatment of diseases.

This could help in the case of diseases – such as cancer – which result from changes of cellular program or when it comes to growing new heart muscle cells from stem cells for patients with a cardiac infarction. "If we know when the cells make their decision, it may be possible to influence how they make that decision," says Marr.

That's exactly what draws Marr to biology research: finding questions and answers, as well as possible applications. "It's not just finding a solution that is fun for me – I also enjoy thinking about what it could be used for." Something that, thankfully, he realized many years ago.

From quantum mechanics to systems biology

After completing his diploma in 2002 at the Technical University of Munich and at the Max Planck Institute of Quantum Optics, the Bavarian native was first relocated to London, where he

The Quantitative Single Cell Dynamics working group at the Institute of Computational Biology, Helmholtz Zentrum München in summer 2014 (Source: HMGU).

worked for a time at Imperial College London in the working group for quantum information and quantum optics. But, as he quickly realized, "Quantum mechanics was just too weird for me to think about spending the rest of my life on – it just has too little to do with reality." This realization then led Marr to TU Darmstadt, where he completed his doctorate – in biology. The title was "Dynamic processes on abstract graphs and biological networks." A few brief detours in research took him to Florida Atlantic University and Jacobs University Bremen until he returned to his homeland in 2008 and took up a post doc at Helmholtz Zentrum München, becoming head of the Quantitative Single Cell Dynamics working group in 2013 and then deputy director of the Institute of Computational Biology.

Today, Carsten Marr is known for his inventive solutions. He was recently honored with the CSB2 Prize for Systems Biology for his development of innovative computer-based simulation methods at the International Conference on Systems Biology of Human Disease (SBHD) at the German Cancer Research Center (DKFZ) in Heidelberg.

Just as he does at home in Munich, Marr also appreciates engaging in dialogue with his colleagues at conferences, such as the one in Heidelberg. At the SBHD, experts from around the world reported on how they are able to analyze the development of disease using the latest biological and mathematical models. The international conference has been taking place since 2008, hosted alternately by the DKFZ and Harvard Medical School in Boston. Marr says that systems biology recognized the importance of interdisciplinary and international dialogue early on.

From the area around Nuremberg, where Marr grew up, to Munich, London, Florida, Bremen and Darmstadt, and back to Bavaria again – Marr grins when he thinks about his resumé. "Oh, my," he reminisces. Lots of coincidences have brought him to where he lives and works now. "It's not as if it has always been my dream to do exactly this," Marr says. "But now that I am doing it, I love it."

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cells as decision-makers

Translating biopsy patterns into tumor development prediction

by Haralampos Hatzikirou, Juan Carlos Lopez Alfonso and Friedrich Feuerhake

In oncological clinical practice, clinical imaging and biopsy sampling are among the most important diagnostic and prognostic tools. Such medical images represent snapshots in time of dynamic cellular patterns. For example, a tumor is composed of dividing, migrating, or dying malignant cells, and many other non-malignant cells such as branching blood vessels with endothelial cells, stromal fibroblasts, and motile immune cells. A biopsy is like a frozen scene of these constantly changing dynamics. Combining these "snapshots in time" with knowledge on the disease, physicians attempt to predict the future of the lesion (e.g., grading malignancy) and propose an appropriate treatment. Our goal is to complement the common clinical practice and to support the temporal extrapolation of images to clinical predictions is a formidable task since imaging data are sparse in time – typically a single snapshot is available. To overcome these limitations, we propose a Systems Medicine approach, partially developed in the context of e:Med consortium SYSIMIT, that allows for the elucidation of biopsy imaging prognostic potential for tumor behavior.

To illustrate the above, we can draw an **analogy** between cells and football players. There is no way to predict the outcome of a football game from a single snapshot of the field. For instance, by watching a player ensemble (like a "pattern of image objects") close to the goalpost, it is difficult to predict if this will end up in a goal. Having more information on both teams' player quality and an understanding of the game rules would significantly increase our chances in predicting correctly. Likewise, microscopic biological "scenes" - of cellular ensembles expressing various phenotypes (corresponding to a player's capabilities and behavior) - can have true prognostic power if interpreted in the context of the underlying dynamic processes of cell phenotypic decisions and interaction with microenvironmental/stromal entities.

Figure 1: Cell decision-making

Source: Hatzikirou, HZI Braunschweig

(a) Cells live in a complex and heterogeneous microenvironment.
(b) A cell (blue) receives signals from its neighbouring cells and decides over a new phenotype (pink).
(c) In turn, the (pink) cell communicates with its immediate microenvironment inducing new cell decisions. This dynamic cellular dialogue takes place in multiple instances within tissues.

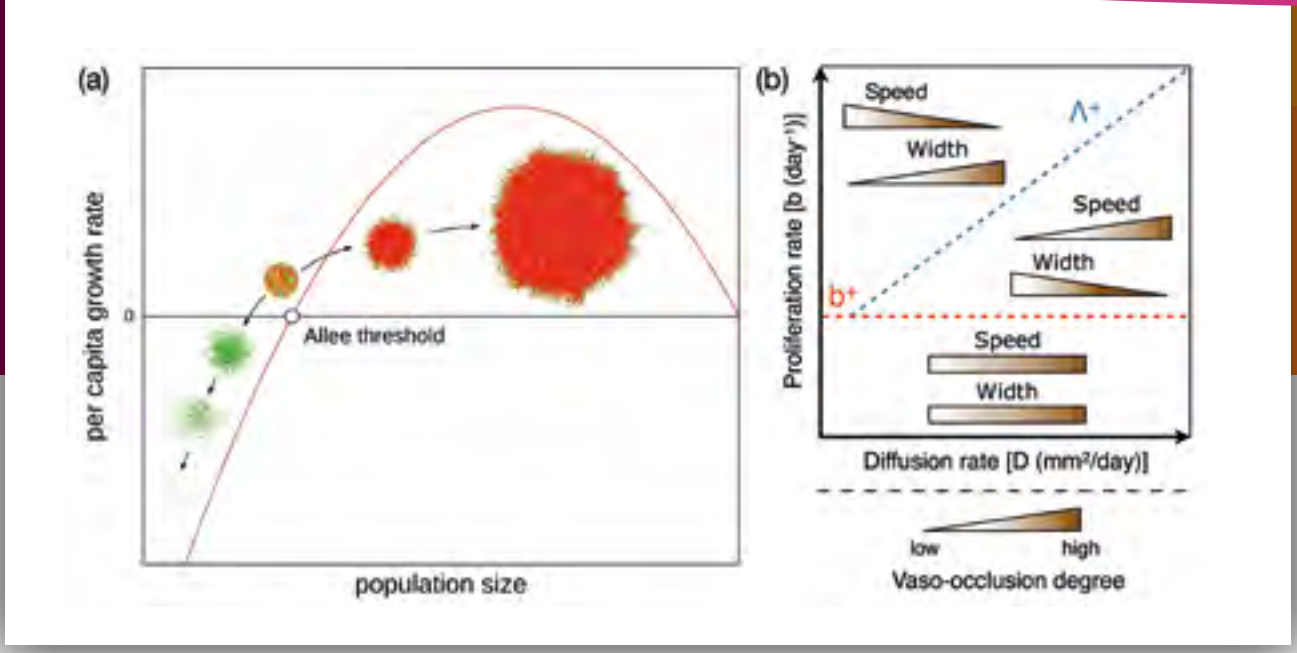

Figure 2: (a) Migration/proliferation plasticity induces an Allee effect for low grade tumors. This implies a critical tumor cell density where below tumor goes extinct and above grows uncontrollable [2]. **(b)** The effect of vasomodulatory intervention in glioblastoma tumor invasion strongly depends on the intrinsic characteristics of brain tumor cells (proliferation and diffusion rates) (Source: modified from [4]; Hatzikirou, HZI Braunschweig).

Our approach is to regard tumor cells as interacting decision-makers that dynamically select their phenotype, i. e. their behavior, according to microenvironmental cues (Figure 1). Decisions of such interacting cells define a complex multicellular system that allows for the emergence of higher order organizational structures/patterns, such as tumors composed of malignant cell formations in the context of non-malignant stroma and other tissue types. At the same time, the new emergent structures/patterns exert selective forces on the cell phenotype decision-making dynamics. With the aim of understanding the mechanistic connection between single cell decision-making and associated multicellular dynamics, we develop multiscale mathematical models. Although this rationale is very innovative, it builds on historical concepts, and we recall the infamous statement of Dr. D. W. Smithers who said in 1962 that "cancer is no more a disease of cells than a traffic jam is a disease of cars. A lifetime of study of the internal-combustion engine would not help anyone understand our traffic problems". Already 50 years ago, Dr. Smithers pointed out that the key to cancer therapy cannot be found in the biological behavior of single malignant cells, but requires also understanding of interactions between tumor cells and their microenvironment.

This new look on tumors, considering them as the emergent behavior of interacting cellular decision-makers bearing the ability to individually adapt to their microenvironmental challenges makes clear that there are at least **two crucial aspect of cell decision-making: the tumor and stromal/microenvironmental cells**. Concerning the latter, we mainly focus on the immune system, which is a pivotal part of the tumor's microenvironment. Different immune cell types exhibit a large degree of phenotypic plasticity. For instance, de/activation of effector cells or phenotypic polarization of macrophages, from anti- to pro-tumoral types, are proven to be important for the prognosis and treatment of different tumor types. Understanding the dynamic interplay of two phenotypically plastic populations, such as tumor and immune cells, is complex and requires the contribution of multiscale mathematical modeling.

Tumor cell decision-making: migration/proliferation plasticity

We have investigated a prominent tumor cell decision-making mechanism that is the so-called ***migration/proliferation phenotypic plasticity*** or *"Go-or-Grow"* behavior, particularly observed in brain tumors. This mechanism implies a mutually exclusive switching between migratory and proliferative phenotypes. The question was how this tumor cell decision-making mechanism is regulated, and what is its impact on tumor brain growth and invasion. Concerning regulation, we discovered a dependence of this cell decision mechanism on the local cell density without concluding on the exact functional form, by analyzing images of *in vitro* experiments [1].

Analyzing further the potential local cell density dependencies, we found out low-grade tumor micro-ecology potentially exhibits an emergent Allee effect (Figure 2a), i. e. a critical tumor cell density implying both tumor growth and control [2]. The precise quantification of this critical tumor cell density could be a relevant prognostic criterion for the tumor fate, since it can be easily measured in biopsies samples. Moreover, we have shown that this Go-or-Grow mechanism explains the fast tumor recurrence time of high-grade brain tumors after resection [3]. Finally based on our theoretical understanding of the Go-or-Grow mechanism, we have shown how personalized vasomodulatory glioma therapies can be optimized [4]. In particular, we showed that one-size-fits-all vasomodulatory interventions should be expected to fail, because control of glioma invasion characteristics, such as tumor front speed and infiltration width, can be very variable and may require more personalized therapeutic interventions (Figure 2b).

Microenvironmental cell decision-making: de/activation of immune effector cells

Breast cancer is characterized by different patterns of inflammatory responses, ranging from massive to sparse immune cell infiltration. Strikingly, a high number of lymphocytes is also present in healthy breast tissue, likely reflecting immunological surveillance or bystander effects during normal tissue turnover. Therefore, the density of immune cells alone does not necessarily point towards pathological conditions. On the other hand, lymphocytic lobulitis (LLO), a recurrent pattern of inflammation, characterized by lymphoid cells infiltrating lobular structures, has been associated with increased familial breast cancer risk and has been observed in increased frequency in prophylactically removed breast tissue from patients without and with cancer-predisposing germ line aberrations of the BRCA1/2 genes. As it is difficult to distinguish LLO from common variations in immune surveillance by conventional microscopy, we applied mathematical modeling to better understand the underlying dynamics and improve the evaluation of biopsies.

In particular, the e:Med scientists of the SYSIMIT consortium aimed to better understand the mechanisms leading to LLO, with the intention of using this knowledge for the development of immune cell-based prognostic markers for breast cancer, and developed a mathematical model to harness the prognostic power of immune cell infiltration in healthy and premalignant tissues [5,6]. This model integrates personal patient data (menstrual cycle length, hormone status, genetic predisposition) and advanced image analysis of breast tissue biopsies. The findings indicate that the immunological context, defined by immune cell density, functional orientation and spatial distribution, contains prognostic information previously not captured by conventional diagnostic approaches. In turn, a complementary diagnostic protocol is proposed (Figure 3). The work suggests new patient-specific parameters which help to improve predictive tools for the development of cancer and have the long-term potential to improve precision of prognosis in high-risk patient groups, e. g. women with BRCA 1/2 mutation, regarding the risk to develop malignancies [5,6].

Currently, we combine the concept of decision-making agents in tumor- and immune cells, targeting improved prognosis and new ways to guide new therapeutic designs. In particular, we intend to harness the advancements in tumor immunotherapy and chemotherapy in optimizing the timing of their applica-

Bacteria against tumor

It has been observed that intentional bacterial infections can produce efficacious anti-tumor responses in mice, rats, dogs and humans. This phenomenon has been discovered already 200 years ago (1813) by Arsène-Hippolyte Vautier, a French physician who observed that the tumors of patients shrank when they also suffered from gas gangrene. However, low overall success rates and intense side-effects prevent such approaches from being used in current clinical practice. In a recent work [9], we developed the first systematic study that combines *in vivo* experiments and *in silico* modelling towards the mechanistic understanding of the therapeutic potential of bacterial infections against solid tumors. By means of mathematical modeling, we elucidated the interplay between growing tumors, vascularization and immune recruitment dynamics in response to bacterial infections. This allowed us to suggest an optimal bacterial load based on the tumor size and the corresponding immune context. This represents a first step forward to a personalized treatment protocol using bacterial infection against tumors.

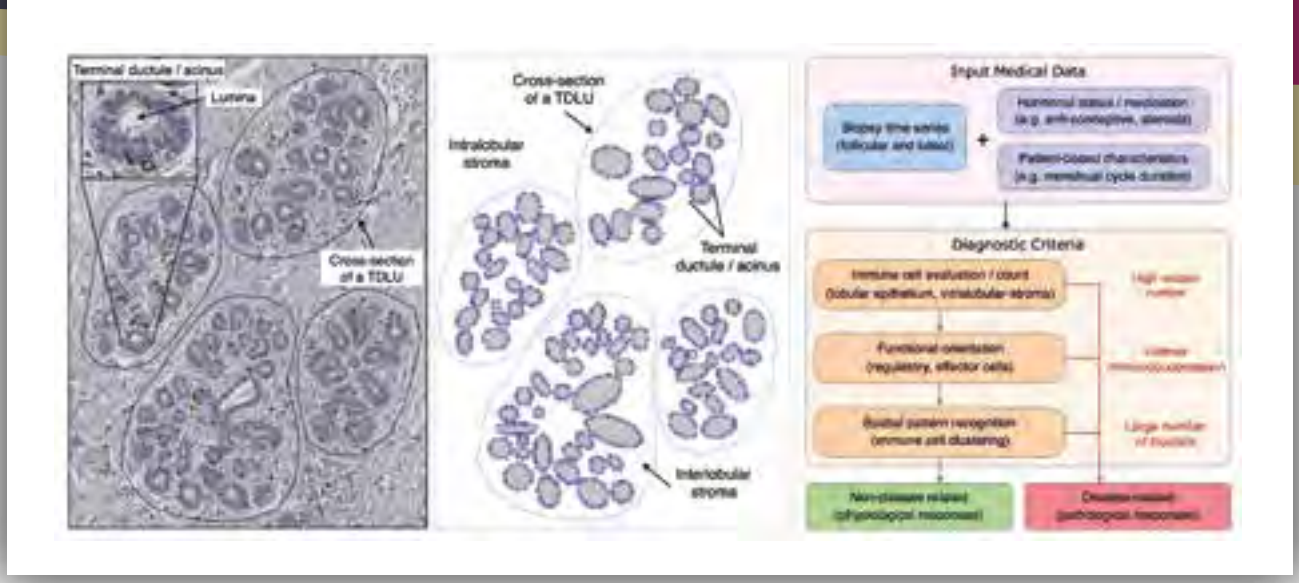

Figure 3: From biopsy patterns to novel clinical implications. The left picture shows a biopsy of a healthy breast lobule. The middle one represents the digitalized version of the biopsy sample and the corresponding simulation domain. The right figure is our proposed diagnostic protocol (Source: modified from [6]; Hatzikirou, HZI Braunschweig).

tion [7], as well as propose novel combinations with existing therapies, such as vasomodulatory modalities [8]. Finally, our *in silico* approach could be used to understand the therapeutic mechanisms of action of less conventional immunotherapies, such as challenging tumors with bacterial infections (see Box). We are confident that our systems medicine approach that starts with regarding the components of a tumor mass as decision-making agents can be translated in diagnostic utilities in clinical practice.

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BioInfra.Prot – bioinformatics for proteome research

de.NBI service center supports biomedical research on proteins

by Michael Turewicz and Martin Eisenacher

The service center "**Bioinformatics for Proteomics**" (***BioInfra.Prot***) is a service center within the "*German Network for Bioinformatics Infrastructure*" (*de.NBI*), which is focused on the processing and analysis of proteomics data from biomedical research. The main objective of BioInfra.Prot is the provision, maintenance and enhancement of a comprehensive proteomics workflow including data standardization, protein inference, expression analysis and data publication. Moreover, we offer bioinformatical and statistical consultancy, data analysis and teaching as frequently requested services with direct and intensive contact to the user. The de.NBI network is funded by the Federal Ministry of Education and Research.

The importance of proteomics in biomedical research

Proteins are the executing molecules in cells, tissues and organs of living organisms. Alterations of the abundance of proteins and their post-translational modifications (PTMs) may have a direct impact on protein functionality and cellular mechanisms while alterations of the transcription of corresponding genes may have only an indirect impact. Consequently, the investigation of proteins is crucial for the understanding of biological mechanisms. Moreover, in clinical contexts, changes of protein expression levels may be specifically associated with a disease. Some of the diseases that have a high socio-economic impact and are known scourges of mankind are protein-related disorders such as Alzheimer's disease. Thus, the discovery of disease-related proteins and the investigation of their expression profiles and PTMs may lead to the discovery of the underlying pathomechanisms, potential drug targets and biomarkers e. g. for an early diagnosis. To this end, liquid chromatography mass spectrometry-based *bottom-up proteomics* deals with the identification and quantification of all proteins from a considered proteome within the measured samples.

Technology-specific challenges

The analysis of bottom-up proteomics data results in technology-specific challenges. First, the complete information about the protein species that were in the sample is lost after the enzymatic digestion of proteins to peptides, which are the actually measured molecules. Nevertheless, medical research questions aim at the identification and quantification of proteins rather than peptides. Hence, the lost information has to be inferred from the peptide measurements. Because many peptides are shared by various proteins and are not protein-specific (unique), this task is challenging. In this context, ***protein inference*** is the task of calculating the most appropriate or probable protein composition in the sample with respect to all the identified unique and shared peptides.

Second, ***protein quantification*** aims at the determination of the amount of proteins. Therefore, in biomedical research often so called label-free quantification approaches are utilized. Here, the profile of the peptide-specific peak intensities with respect

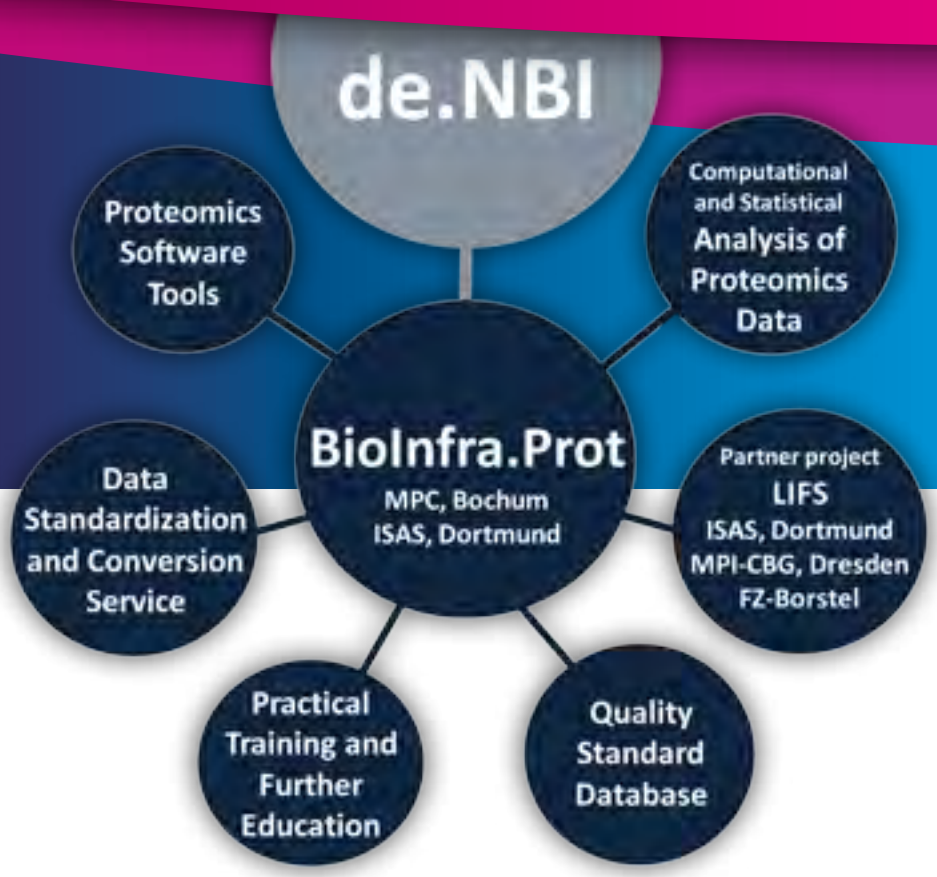

Figure 1: Overview over the de.NBI service center BioInfra.Prot and its main service categories (Source: Michael Turewicz).

to the retention times (RTs) of the liquid chromatography can be used for quantification. Because peptides have different ionization properties, only a relative quantification between the groups of interest is possible.

Third, in clinical applications protein ***expression analysis*** aims at the detection of proteins which are differentially expressed between two groups of patients (e. g., "diseased" vs. "healthy") and which may be interesting biomarker candidates. To this end, the quantified protein expression profiles are analyzed. However, the detection of correct candidate proteins is often complicated by many false positives caused by too small sample numbers, which is a common problem in biomedical research. Thus, besides usual statistical methods, sophisticated machine learning approaches are needed for reliable biomarker discovery.

Finally, ***data standardization*** is still an ongoing task in the proteomics community. Due to technological advances, proteomics data analysis pipelines usually include one or more file format conversions and there is a strong need for regularly updated data converters. Moreover, high-impact proteomics journals require the publication of data and experimental information in standard data formats in public repositories such as PRIDE (Vizcaíno *et al.*, 2016) before article publication. Since the ***data publication*** process is complex, researchers often need support to convert, annotate and upload their data.

Boosting biomedical proteome research

BioInfra.Prot provides bioinformatical services that address all above challenges (Figure 1) to facilitate reliable biomedical research of the human proteome (Turewicz *et al.*, 2017). E. g., the software tool ***PeptideShaker*** is an analysis suite for quality control and identification (Kopczynski *et al.*, 2017). For protein inference the tool ***PIA – Protein Inference Algorithms*** (Uszkoreit *et al.*, 2015) offers a user-friendly collection of protein inference methods. The software ***Protein List Comparator (ProLiC)*** provides a comparison of identification results from different experiments and databases. Besides services for LC-MS-based ***bottom-up proteomics***, BioInfra.Prot offers also software for other proteomics technologies such as a database with high-resolution reference spectra of peptides for ***targeted proteomics (QSDB)*** and the tool ***PAA – Protein Array***

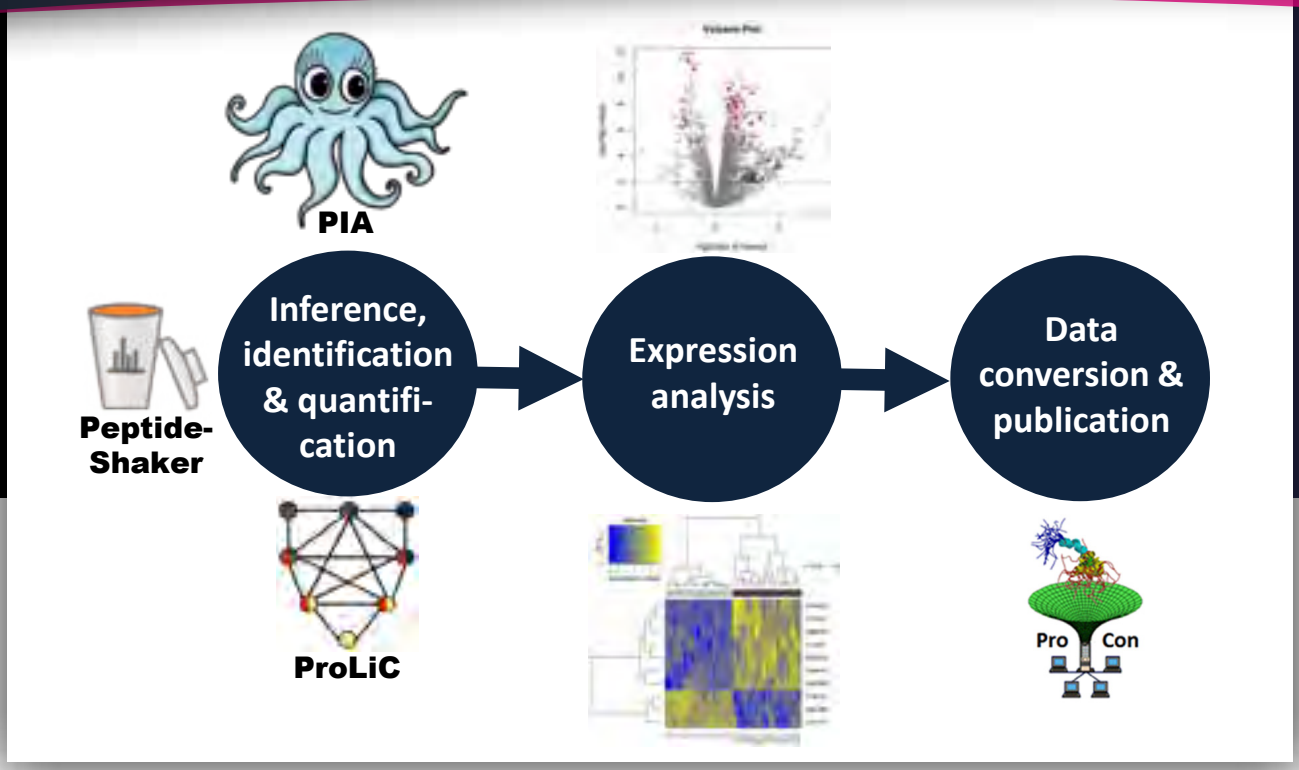

Figure 2: Data analysis of proteomics data with BioInfra.Prot-services (Source: Michael Turewicz).

Analyzer (Turewicz *et al.*, 2016) for protein microarrays. The ***CrossPlatformCommander*** facilitates the analysis of multi-omics data. Moreover, for expression analysis manifold application-specific statistical and machine learning methods are provided. Finally, the tool ***ProCon*** allows a seamless data standardization and publication.

Bioinformatics services with close user proximity

The complexity of the proteomics workflow results in a strong need for workshops and courses imparting knowledge regarding proteomics analysis and related topics. Thus, BioInfra.Prot provides regular courses with various topics regarding bioinformatics for proteomics. E. g., in the last two years courses regarding the analysis of quantitative proteomics data with R or the analysis of proteomics data in multi-omics studies have been carried out. In 2016, a de.NBI summer school on bioinformatics for proteomics has been co-organized with other de.NBI centers (CIBI and BiGi). In the next months, an R course, a workshop on our software tools and a course regarding multi-omics will be carried out.

Moreover, biomedical researchers ask us regularly to help them to analyze their data and to push their projects forward. To address these requests, we have established our frequently requested consulting services: "Computational and statistical analysis of proteomics data" and "data standardization and conversion service", which are accessible via bioinfoservice@rub.de. These communication-intensive services, where details of the data must be discussed with the users, regularly result in successfully published articles. Consequently, in the first two years of de.NBI, BioInfra.Prot members were co-authors in 28 scientific publications. Furthermore, we receive daily requests to assist users in data conversion and/or publication. Thus, the high complexity of proteomics data handling and analyses makes a deep experience and creativity for the design of customized analysis pipelines indispensable.

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Figure 3: Members of the Service Center BioInfra.Prot. From left to right: Robert Ahrends, Julian Uszkoreit, Maike Ahrens, Dominik Kopczynski, Michael Turewicz, Martin Eisenacher, Michael Kohl and Gerhard Mayer (November 2015) (Photo: Lukas Jelonek).

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Project name and main partners within the project:

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Prof. Dr. Albert Sickmann, Leibniz Institute ISAS e. V., Dortmund

Current and former members of BioInfra.Prot:

Dr. Robert Ahrends, Dr. Maike Ahrens, PD Dr. Martin Eisenacher, Gerhard Mayer, Dr. Nils Hoffmann, Dr. Michael Kohl, Dr. Dominik Kopczynski, Karin Schork, Dr. Dominik Schwudke, Dr. Andrej Shevchenko, Prof. Dr. Albert Sickmann, Dr. Michael Turewicz, Dr. Julian Uszkoreit

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RNA sequencing for finding the causes of rare diseases

Sequencing RNA, and not only DNA, boosts the diagnosis rates of Mendelian disorders

by Julien Gagneur

In about half of all patients with rare hereditary disorders, it is still unclear which exact position of the genome is responsible for their condition. We developed a new method that significantly increases the chance of a successful identification. Our new approach looks not only at DNA, but also at RNA. This article ends by discussing future perspectives towards decrypting the regulatory code: Methods that will allow predicting whether any given variation in a genome could alter the amount of protein a cell produces, in which cell type, under which condition, and by how much.

The holy grail of genetics is the perfect understanding of the relation between genetic variation and phenotype. For human geneticists, it implies being able to tell the physiological impact of any variation in the nucleic acid sequence of the human genome. This is a challenging task. Some diseases can be caused by a single nucleotide polymorphism, i. e. by the change of a single letter. Understanding the genetic text would allow predicting from a genome sequence, obtained at birth or prenatally for families at risk, which biological processes can be affected, in which organ or tissue.

Mutations: Location matters

However, most genetic variations seem to barely affect cellular processes. The remaining genetic variants are called **functional variants**. When considering the immediate impact of DNA variation at the molecular level, two classes of functional genetic variants emerge.

One group of functional genetic variants is **coding**. That means they alter the amino acid sequence of a protein, and may consequently alter how a protein can function. To date, coding variants are the best studied class of genetic variants. However, only about 1.5 % of the human genome are directly protein coding sequences. The other group of functional genetic variants is **regulatory**. Regulatory variants do not directly affect the encoding of an amino acid sequence. Instead, they affect the quantity of protein a cell produces, in which cell type, and when. Most of the genetic variants associated with genetic diseases do not lie in the genome sequence coding for proteins but in regulatory regions. However, we do not know how far we are from a comprehensive catalogue of positions with regulatory effect. In contrast to the coding regions, the regulatory regions in the genome are far from being understood.

Rare diseases: Sequence RNA, not just DNA!

So-called rare diseases are anything but rare, because they collectively affect about eight percent of the global population (Figure 1). They typically have a genetic cause. However, for most of the rare disease patients undergoing DNA sequencing, geneticists fail to pinpoint the causal genetic variants when considering only the coding variants. Without a full understanding of regulatory sequences, it is very hard to identify which non-coding variants could be the cause. Our e:Med junior research alliance **mitOmics**[1], together with the group of Dr. Holger Prokisch at the institute of human genetics in Munich, has recently pioneered an effective approach in which we directly probe the implications on gene expression by sequencing

[1] http://www.sys-med.de/de/nachwuchsforschung/juniorverbuende/mitomics/

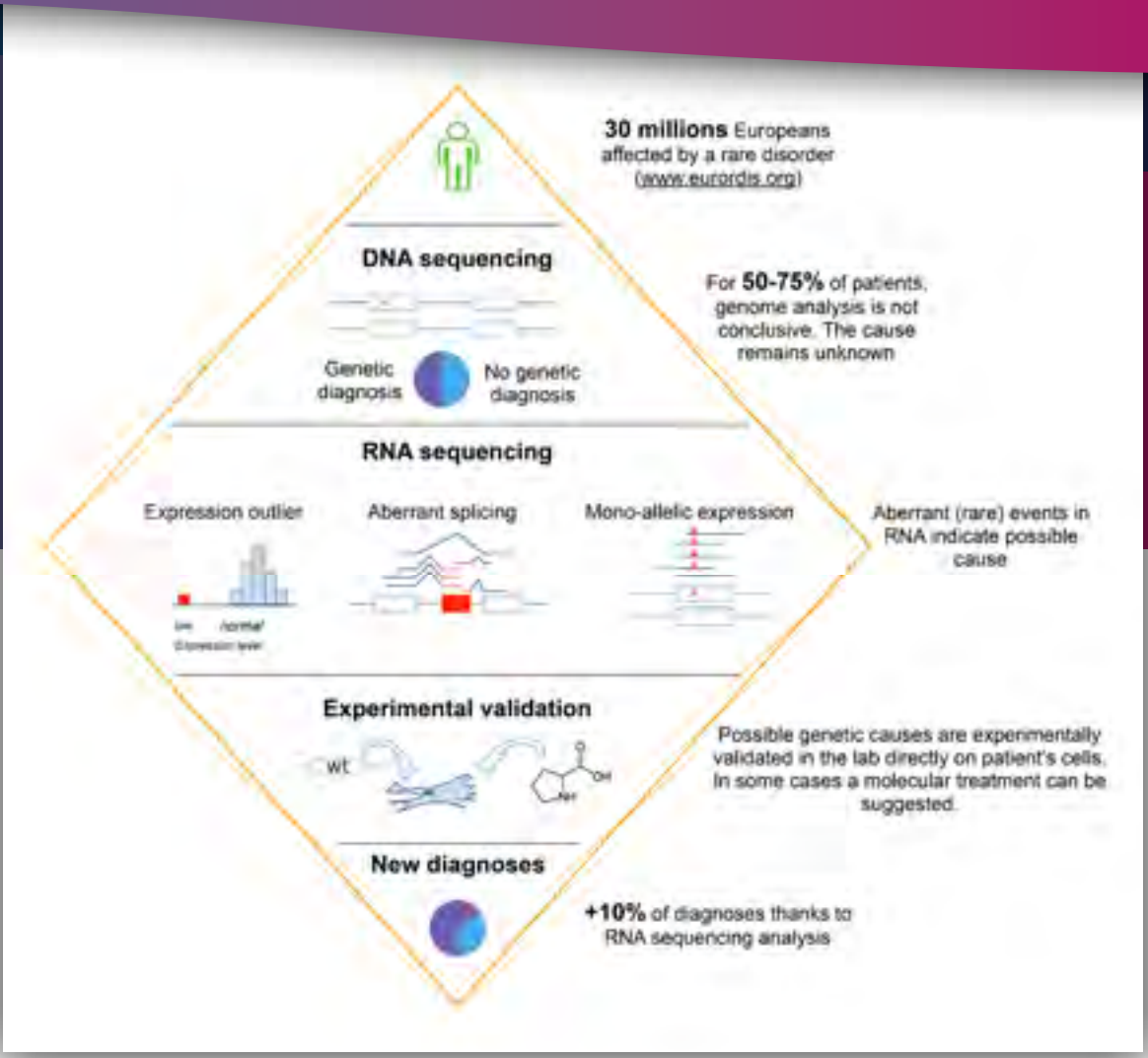

Figure 1: RNA sequencing helps providing a diagnosis to patients with a genetic disorder (Source: Julien Gagneur, adapted from Kremer *et al.*, 2017).

RNA, additional to the DNA of patients (Kremer *et al.*, 2017). We did this for patients affected by rare mitochondrial diseases, i. e. for which cellular respiration is affected.

As we knew the disease was rare, we searched for aberrant patterns in the patients' RNAs that are usually absent in controls. From a data analysis point of view, the problem can be seen as an outlier detection problem, with the specific twist that in the disease diagnosis context outliers are the signal of interest, and not artifacts to exclude from the data. We performed RNA-sequencing for more than 100 patients and developed bioinformatics methods for the prioritization of genes based on three patterns in the RNA-sequencing data (Figure 1).

Three ways to uncover disease-causing genetic defects by RNA sequencing

The first pattern is aberrant expression: genes whose overall RNA amount stood far outside its physiological range. We used a combination of a statistical significance with noise models appropriate for RNA-seq count data and a quantitative threshold on the importance of the deviation to the mean. Typically, we found genes that were barely if at all expressed in one patient, but always expressed in controls. When those genes are also known to be essential for cellular respiration, a likely molecular diagnosis can be obtained.

The second pattern is aberrant splicing. Splicing is an RNA processing mechanism, in which sections of the RNAs called introns are removed. The remaining sections called exons are spliced, i. e. joined together, to form the final or mature RNA (Figure 1).

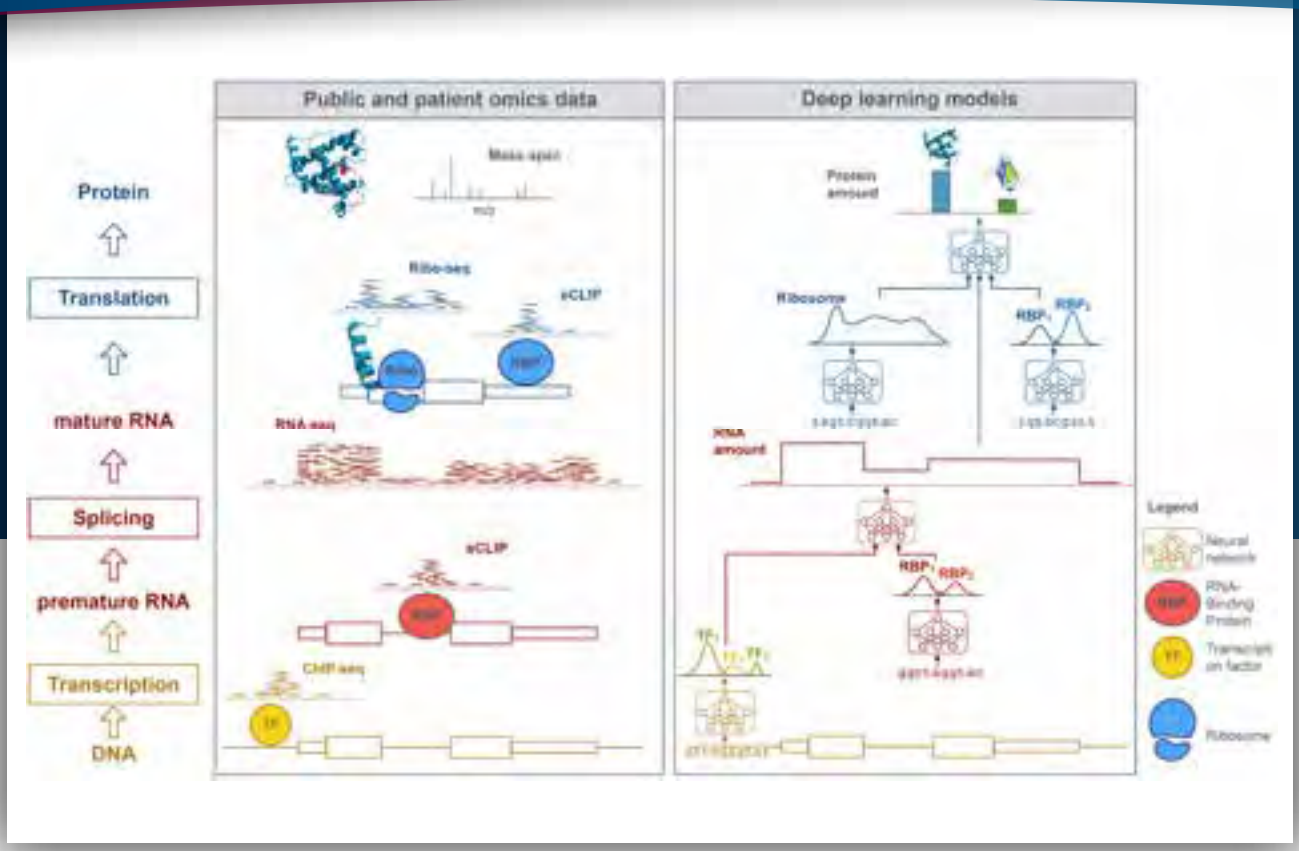

Figure 2: Outlook into omics and deep learning modelling for gene regulation. Left: Omics-technologies (ChIP-seq, eCLIP, RNA-seq, etc.) yield information-rich experimental data on every step of gene expression and are amenable to individual patients.
Right: Deep learning models of elementary molecular interactions such as the binding of Transcription Factors (TF) to DNA or of RNA-binding proteins (RBP) shall be combined to lead to predictive and interpretable models of major steps of gene expression (transcription, splicing, translation) (Source: Julien Gagneur).

A splicing defect can lead to the wrong mature RNA sequence and therefore the wrong protein sequence. Predicting that variation in DNA will alter the process of splicing is a hard task. The advantage of sequencing the RNA is that one directly observes splicing defects. We proceeded in an agnostic fashion, looking for splicing events even outside splice site annotations. This revealed a surprisingly high number of patients suffering from a splicing defect. These findings are in line with increasing evidence from many other labs that splicing plays a major role in rare and common diseases.

The third pattern is mono-allelic expression. Genes come in two copies or alleles inherited from the two parents. Having two gene copies often works well as a backup mechanism to compensate for DNA variants affecting only one allele. Hence, genetic variants found in only one allele when sequencing a patient's genome are not typically prioritized by human geneticists. It can happen that for a gene with a variant in just one allele, only RNA of the allele with the variant is expressed. Such mono-allelic expression can have various causes which are not easy to predict from a genome sequence. Sequencing the RNA is a direct way to detect those events.

Specific treatments require exact diagnosis

Altogether, we found instances of each of the three patterns. Experimental validation of which gene defects caused the disease as predicted bioinformatically could be performed directly in cultured fibroblasts of the patients; skin-derived cells that are easily accessible. This approach pinpointed the disease-causing gene for 10 % of unsolved cases in the pilot study – 15 % by now – including the discovery of a new gene-disease association with more candidates undergoing validation. In one case, a molecular treatment could even be proposed based on the function of the gene. Similar improvements could be obtained by a group at the Broad institute (Cummings *et al.*, 2017).

We are now applying the approach to larger datasets. We are developing a bioinformatics pipeline to cope with much larger datasets and to provide a secure and easy-to-use interface to

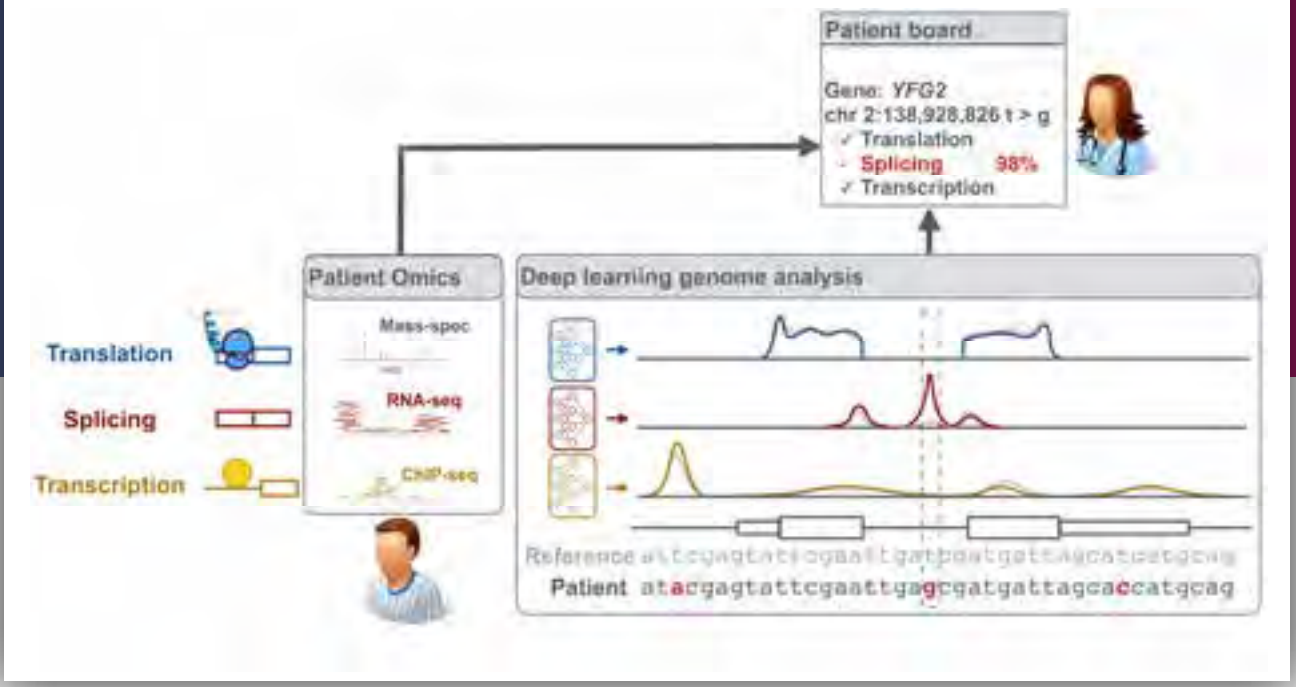

Figure 3: Outlook into omics and deep learning approaches for rare diseases. Integration of regulatory model (Figure 2) and patient omics data will help human genetics for pinpointing regulatory genetic defect. Our current pipeline already integrates personalised DNA, RNA, proteome with established variant annotations (Source: Julien Gagneur, icons for physician and patient of Ahkâm via freeiconspng.com).

clinicians. Key to such approach is the possibility to integrate multiple omics data and physiological information about the patient. We are therefore thrilled to see initiatives for patient data integration such as the BMBF-funded DIFUTURE consortium[2] starting in Munich.

Outlook: Decrypting the regulatory code with omics data and machine learning

Rapid progresses are made in the identification of gene regulatory mechanisms and how they are encoded in the genome. These progresses are driven by omics technologies measuring every step of gene expression at very high depth. Complementary to advances in measuring cell products, genome edition (CRIPSR) and assays based on very large libraries of synthesized DNA sequences (Massively parallel reporter assays) allow to probe an extremely large sequence space for millions of synthetic sequences. These data are genome-wide and quantitative. They allow to quantify the regulatory effect of a specific genetic sequence, and step by step unravel more of the regulatory code. For yeast, our latest study led to a model that can predict RNA half-life from a gene sequence alone, down to measurement accuracy (Cheng *et al.*, 2017). More work is needed to generalize these results to humans, in multiple conditions and individuals!

A very promising line of research to achieve this goal is stimulated by machine learning technologies. Over the last 5 years, machine learning has led drastic progresses in computer vision, machine translation and speech recognition. We and others are developing and adapting successful machine learning algorithms such as convolutional and recurrent neural networks to bioinformatics tasks (CONCISE[3]). Moreover, we are interested in improving the exchange of computational models in the community, by allowing code to be interoperable, leveraging on programming frameworks such as Google's Keras[4]. The ambition is that models fitted on distinct stages of gene expression with very rich data probing specific mechanisms (e. g. a model based on a RNA-binding protein assay for a given protein) can be combined with further models to model more complex phenomena (say RNA stability, which involves hundreds of RNA-

[2] http://difuture.de

[3] https://github.com/gagneurlab/concise

[4] https://keras.io

binding proteins, (Figure 2)). Ultimately the concerted effort of the community will allow decrypting the human regulatory code closing the gap between regulatory genomics research and genetic diagnostic pipelines (Figure 3).

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Profile of the e:Med junior research alliance *mitOmics*

The focus of *mitOmics* is to uncover the various molecular causes of rare mitochondrial diseases by using personalized omics-approaches. Therefore whole-genome sequencing, transcriptome analysis and genome-wide functional screens are combined. Integrative bioinformatics and statistical analyzes are used to determine causal mutations and affected signaling pathways of individual patients. This knowledge enables more precise therapies for the individual patient. The novel developed protocols and algorithms are supposed to be translated into clinical routine and used as diagnostic tools.

Research project: Junior research alliance ***mitOmics***

Partner:
Dr. Fabiana Perocchi, biologist, (Helmholtz-Zentrum / Gene Center LMU, Munich) Project Functional genomics screens
Dr. Tobias Haack, physician, (University Hospital Tübingen) Project Data generation
Prof. Dr. Julien Gagneur, applied mathematician, (TU Munich) Project Variant prioritizations

Further information:
Links see footnotes in the article and:
http://www.sys-med.de/en/young-investigators/junior-research-alliances/mitomics/
http://gagneurlab.in.tum.de

"personalized medicine is a challenge, but it is also a certainty"

Interview with the Director of the Center for Genomics and Personalized Medicine at Stanford University, Mike Snyder

Mike Snyder comes from Stanford, California, where he is the Director of the Center for Genomics and Personalized Medicine at Stanford University. It is immediately clear that the topic of personalized medicine is close to his heart. He speaks in a relaxed way, but with full concentration. Wearables are his current pet project – wearable detectors of physiological data that are slightly reminiscent of James Bond. A conversation about personalized medicine, the healthcare system and why data collection is important to him.

Systembiologie.de: *How will physicians cope with treating increasingly well-informed patients in the future?*

Professor Dr. Michael Snyder: Personalized medicine is a challenge, but it is also a certainty. Physicians have to keep up to date. I think that, in future, physicians will be the coordinators of healthcare, rather than its dictators. Patients will come along with lots of their own data and won't know how to interpret it. As it is more complicated, the physician probably won't have the comprehensive expertise and thus there will be additional need for someone who is able to interpret the genome of the patient. Information from a broad range of sources, beyond imaging and pathology data, will have to be taken into account. One good example of this is *wearables*. Initially, physicians criticized them for not being precise enough. However, they are actually able to measure heart rate more accurately than in a clinical setting because people are never quite as relaxed when they are visiting the physician. In the future physicians will have to be more open so that they do not miss out on a lot of important information. In the future, patients will have many years of patient data streaming right to the physician, who will be able to see the onset and timeline of change. As a result, we will then be able to provide much more precise treatment.

When do you think there will be software solutions available for clinics or even physician's offices in order to extract the data most important for the individual patient?

At the moment, this is mainly a subject of research. Predicting when this kind of software may be rolled out for use in a clinical setting is difficult. Let's say the first software is developed in the next year or two. In five years, it hopefully will be in full force and everyone will be wearing these little devices. Similar applications such as cancer sequencing or prenatal diagnostics have developed incredibly fast.

Because cancer is such a serious illness, everyone is worried of it. Do you see a change in the perception and translation of omics technologies for cancer treatment?

When I attended a conference on personalized medicine here in Heidelberg a few years ago, the participants said that it was too early to be using omics technologies in clinical practice. Within a short period of time here, in exactly the same place, that has completely changed. I think that the new way will be to keep people healthy by predicting illnesses before they appear. You heard in my talk at the SBHD 2017 in Heidelberg that only one of the audience members had had his genome sequenced. Even at these talks in the US, only two or three people have had it done, rarely ten people.

Source: apfelwelle - Fotolia.com

It is a wonderful aim to keep people healthy, investing in health management rather than disease management. What do you think the pharmaceutical industry makes of that?

I don't think it matters to them. The aim of the pharmaceutical industry is to sell products and medicine. The pharma industry is hesitating to invest in this area in general. It is better for the balance sheet if each patient only takes one type of drug – even if only 10 % of them respond to it. With stronger competition, almost no one is developing drugs without the companion diagnostics any more, and that's a good thing. I think the attitude is changing. In principle, it should open up more opportunities, but it also leads to a stratification of the market. For society as a whole, it is best to give the right drug to the right people at the right time.

> " Physicians will in future have to be more open so that they do not miss out on a lot of important information."

Do you think that participants in your trial are worried about the consequences of the knowledge acquired through sequencing?

No, precisely the opposite. The participants are extremely eager to get the data, even if it means bad news. Some ask specifically about disease information that is generally seen as untreatable (with drugs). We only impart such knowledge to them if it is specifically requested. By contrast, some people only want information about treatable diseases, such as a BRCA1 mutation. If they are aware of their predispositions, people can organize their lives, find a doctor and choose to do lots of things differently. It may not medically actionable, but is more a lifestyle issue.

Photo: Silke Argo / e:Med

Mike Snyder during the 2017 SBHD in Heidelberg.

Should personalized medicine to this molecular level be affordable for all?

It is sad that access is currently restricted to those who can afford it. If it turns out that it really helps, as is the case with cancer, then insurance companies will start to pay for these services. In future, people will finance to sequence their genome because they can manage their health better when they know how they really are. The prices are getting lower. Once you get your genome sequenced with interpretation for a few hundred dollars in the future, it pays off, but we're not yet there. Companies could advertise offering genomic health as a service to their employees. I think that could become a selling argument in the future.

Will insurance companies start categorizing people differently?

That's a good question. In the US, you can't be discriminated for employment and health – that's the official policy, at least. In my case, that still happened – as soon as I was diagnosed with diabetes, my premium went up. So I changed to a different insurer, who didn't have a problem with it. My own argument on life insurance is, well, it is still better to know what is wrong with you and pay a little more for life insurance than not know at all.

You are quite concerned with wearables. Besides the advantage, I wonder whether people tend to see them as a stress factor because they continuously record much personal information.

> " First of all, parents will use wearables to put on their children and elderly will wear them themselves."

I believe 40 million people wear Fitbits. They typically wear it for three months and then the device ends up in a drawer. As soon as they have found their pattern, they lose interest. But if you know that the sensors can register if something bad happens, this could be an inspiration to continue wearing activity trackers. First of all, parents will use wearables to put on their children and elderly will wear them themselves. My mother is 90 years old and lives in Pennsylvania, around 3,000 miles away from me. I wish she would wear an activity tracker so that I could keep an eye on her from California. We have 400 sensors in our cars, but we don't wear any ourselves. I would say that a human being is more important than a car.

People have physiological sensors. How would you feel taking off all your wearables?

Well, because I'm the one collecting data, that wouldn't be such a great move for me. I guess they've woven their magic on me (laughs). But I don't collect the data actively. I'm fascinated by what we can find out from them... I think that the biggest discoveries in science are not driven by hypotheses. The biggest scientific breakthroughs are always by chance. Like the *Penicillium* fungus, which kills bacteria as penicillin. And recognizing RNA as an enzyme was weird. Nobody expected to discover all these new non-coding RNAs. But it was the data that showed us the true picture. And that's exactly what I like about genomics and omics in general: It helps us to put things in context.

Do you think it makes sense to strictly separate hypothesis-driven research and discoveries?

I think there's room for both. But I believe that the biggest breakthroughs come from discovery. We analyzed cortical neurons as a cause of autism but found that the corpus callosum is actually also of great interest for morphological observations regarding autism. Many patients have a problem in the corpus callosum and it was the data that showed us this – we hadn't actually considered this area.

> "That's exactly what I like about genomics and omics in general: It helps us to put things in context."

How many wearables are you wearing at the moment? What do you think about data privacy?

I think I'm wearing eight or nine. Data privacy is a good question. I don't think that this data can be perfectly secure. If somebody wants to hack in...

Could it put carriers of certain gene variants at a disadvantage if everyone had access to this knowledge?

Nobody has the perfect genome. Every single one of us has a deleterious mutation and could be seen to be at risk of specific diseases – some are already known to us as a result of our family medical history and others will be revealed through our genomes.

Mike Snyder is the Stanford W. Ascherman Professor, Director of the Center for Genomics and Personalized Medicine and Head of the Department of Genetics at Stanford University in California, USA. He is a leading figure in the world of functional genomics and proteomics and one of the main players in the ENCODE project. His team recently carried out an integrated personal omics profiling (iPOP) of 100 healthy and pre-diabetic participants over a period of three years, combining various state-of-the-art omics technologies and wearable data in order to assess the risk of disease and monitor disease status for personalized medicine (Photo: Silke Argo / e:Med).

This interview was conducted by Silke Argo.

Further reading:

"Genomics and Personalized Medicine: What Everyone Needs to Know®" by Michael Snyder, ISBN-10: 0190234768

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predicting chronic inflammation and providing personalized treatment

Multi-omics analysis and systems medicine models optimize the treatment of chronic inflammatory diseases

by Philip Rosenstiel, Andre Franke and Stefan Schreiber

Chronic inflammatory diseases are a growing medical problem in industrialized countries. Although diagnostics continue to improve and there are now various targeted therapies available (therapeutic antibodies), the disease progression and response to a specific drug cannot yet be predicted for the individual patient. Genetic studies have identified countless overlapping and specific risk variants for the various types of disease, but this knowledge has not yet been translated into diagnostic algorithms. For this reason, an unmet need exists for an in-depth molecular taxonomy that not only includes the genetic variants but also other molecular aspects. In addition to merely identifying new markers, the systems medicine approach also promises the possibility of using these high-dimensional data sets to develop models that can accurately predict the individual course of the disease. The aim of the e:Med consortium SysINFLAME is to use innovative algorithms to improve the diagnostics and treatment of these – currently incurable – diseases.

Chronic inflammatory diseases (*CID*) are a group of immune-mediated, recurrent syndromes that can be characterized by their frequent inflammatory flare-ups in various organs. The diseases often affect barrier organs, i. e. organs that form the boundary between the human body and its environment. Typical examples are chronic inflammatory bowel disease (ulcerative colitis and Crohn's disease), skin inflammations (psoriasis and atopic dermatitis [neurodermatitis]) or joint inflammations such as rheumatoid arthritis. All of these diseases are associated with the lifestyles of Western industrialized countries. The incidence rate has been continuing to rise for most of these types of disease since the middle of the last century. Seen as a whole, the prevalence of all chronic inflammatory diseases in central Europe is around 20 %, making them a major economic burden for the healthcare system. The occurrence of cases within families is a major risk factor for developing CIDs. For people with a first-degree relative with such a condition, the risk is 40 times higher than for the rest of the population. The genetic components are also underlined by concordance (consistent occurrence) of the disease in monozygotic twins, who showed concordance of up to 50 % for Crohn's disease, for example [1].

The medical definition of the diseases is classically based on the type and location of the inflammatory reaction and the clinical symptoms associated with it. However, all CIDs have to be understood as systemic diseases that not only cause local inflammation but also significant metabolic and vascular comorbidities. The rate of occurrence of various cancer types is also significantly higher for people suffering from CIDs. With the exception of rare monogenic syndromes (e. g. specific immune deficiencies with chronic inflammation reaction [2, 3]), all types of disease occur in adulthood and each have a typical age range.

The function of the affected organs is hindered due to the chronic inflammation and, without proper treatment, can be impaired permanently. Frequently late diagnoses and a high

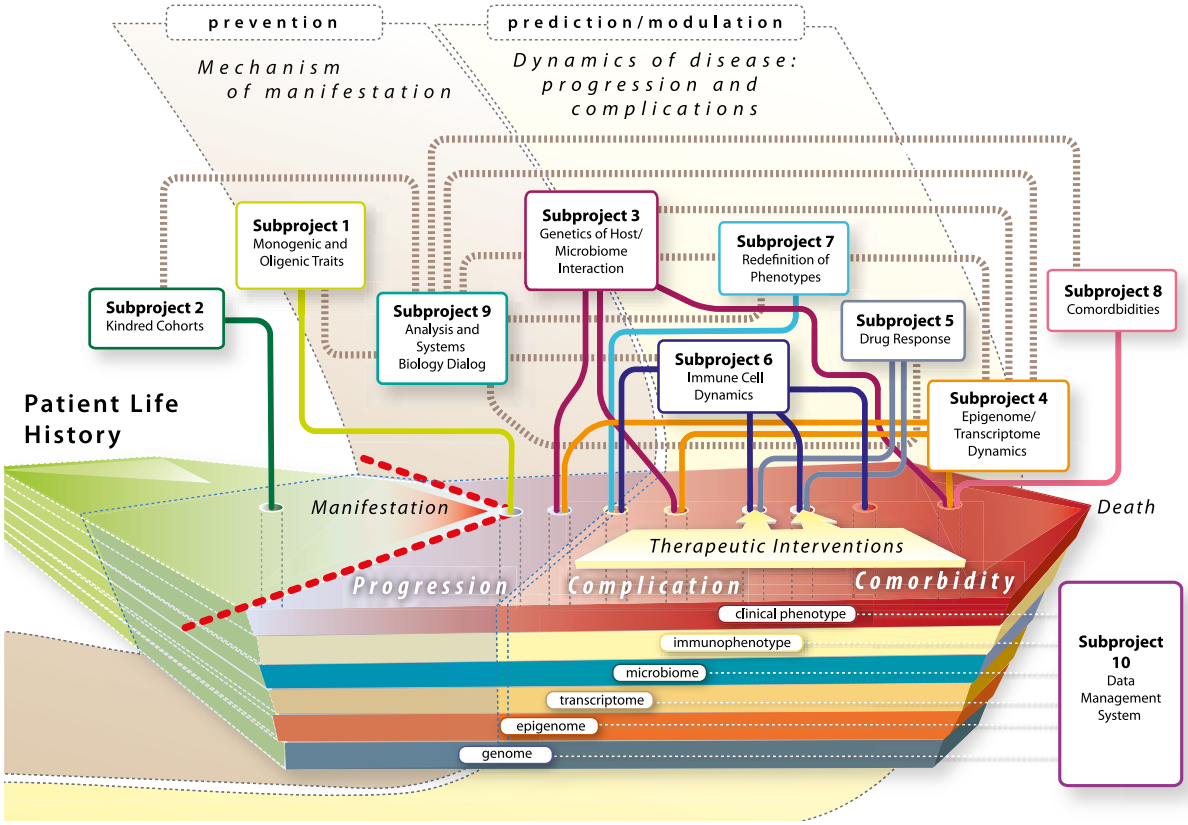

Figure 1: Schematic structure of the e:Med SysINFLAME consortium. Individual disease progression *("patient life history")* is shown as a timeline, with the horizontal layers representing various data or omics levels being analyzed. The lines refer to clinical problems being addressed by the individual sub-projects in SysINFLAME. One focus of the consortium is the definition of early molecular changes that are precursors to clinical manifestation (the occurrence of typical symptoms) (kindred cohorts in sub-project 2). Another example is the analysis of molecular patterns that accompany therapeutic interventions and distinguish response from non-response (5) (Source: IKMB).

rate of primary and secondary treatment failure all contribute to the suffering of the patients.

The SysINFLAME network has therefore tasked itself with creating a systems medicine approach to the three selected CIDs of chronic-inflammatory bowel disease, psoriasis and rheumatoid arthritis. According to the consortium's understanding, the systems medicine approach includes the integration of data from various high-dimensional "molecular spaces", made accessible via omics technologies. With the application of mechanistic and mathematical models, these data spaces will be used in ten specific sub-projects beyond correlation alone (Figure 1) in order to describe and to predict the complex phenomenon of inflammation. Earlier analyses have generally been restricted to individual time points, but longitudinal data that analyze the clinical and molecular "life history" of the patient at various time points is a major prerequisite for accurate modeling. The consortium is therefore focusing on the generation of high-dimensional genomic data sets in well-phenotyped clinical cohorts and – with the integration of longitudinal data – on modeling for the prediction of individuals' disease progression and therapeutic response.

Some of the major molecular data levels include the spectrum of genetic variants, sections of the epigenome (DNA methylation patterns in primary tissues or in isolated cells), the transcriptome, the microbiome and high-dimensional analysis of surface markers using CyTOF (flow cytometry with mass spectrometer) [4]. The consortium is restricting itself solely to experiments using human samples and data. One aspect is therefore the standardization and integration of primary clinical data and molecular data. The development of databases known as *data warehouse* architectures with secure access and opportunities for the analysis of clinical data and algorithms for fast, integrated data analysis (e.g. microbial diversity in the context of clinical parameters) are major elements of the network [5].

Figure 2: SysINFLAME consortium (Source: Christian Bauer/IKMB).

With regard to the clinical situation, the members of the e:Med SysINFLAME consortium (Figure 2) are working on three major and so far unsolved problems:

1. Definition of disease manifestation:

It is unclear what triggers chronic inflammatory disease in individual patients. Many people carry genetic risk variants and are exposed to environmental factors that facilitate inflammatory diseases. However, only some of them become ill. Manifestations must be understood by systems medicine as the threshold process of initial inflammation – clear clinical symptoms and medical diagnoses with existing methods only pertain to the late stages of the disease. In the SysINFLAME consortium, we will therefore accompany a high-risk cohort towards potential manifestation. The data from the various biomaterials will be used to derive understanding from early pathophysiological processes. In addition to identifying biomarkers, modeling aims to develop a mechanistic understanding of which molecular target structures could be used for targeted preventive measures. Finally, this systems medicine approach will require a redefinition of medicine towards a future strategy of maintaining health rather than reacting to disease.

2. Predicting disease progression and comorbidities:

One major clinical problem presented by CIDs is that neither the medium-term nor long-term progression of the disease, nor the high number of comorbidities (e. g. cancer, vascular disease) can be predicted. The early identification of patients with aggressive disease (e. g. erosion of the joints with rheumatoid arthritis or stenosis and colon cancer with chronic inflammatory bowel disease) would make a significant contribution towards the provision of individualized, precise healthcare. SysINFLAME is therefore monitoring several longitudinal cohorts in order to develop models for the prediction of complications via the integration of various clinical and molecular data levels. Initial findings indicate changes within the metabolic networks of the affected tissues, which could distinguish the mild cases from the complicated cases.

3. Prediction of therapy response:

For CIDs, various targeted therapies are approved in the form of recombinant antibodies (biologics). These neutralize various soluble neurotransmitters (cytokines such as TNF-α and IL1-β) or surface structures (e. g. the adhesion molecule integrin α4β7). These treatments are expensive (sometimes costing in excess of €100,000 per year, per patient) and have a high risk of primary or secondary treatment failure. On the individual patient level, there are currently no molecular or clinical tests that can be used to aid the choice of a specific drug for a patient. In terms of systems medicine, the elimination of individual factors with the possibilities of longitudinal follow-up is a promising scenario. Models could be developed that describe the pathophysiology of the disease in a new way via targeted perturbation but that could also predict potential therapeutic response. As a result, the SysINFLAME consortium

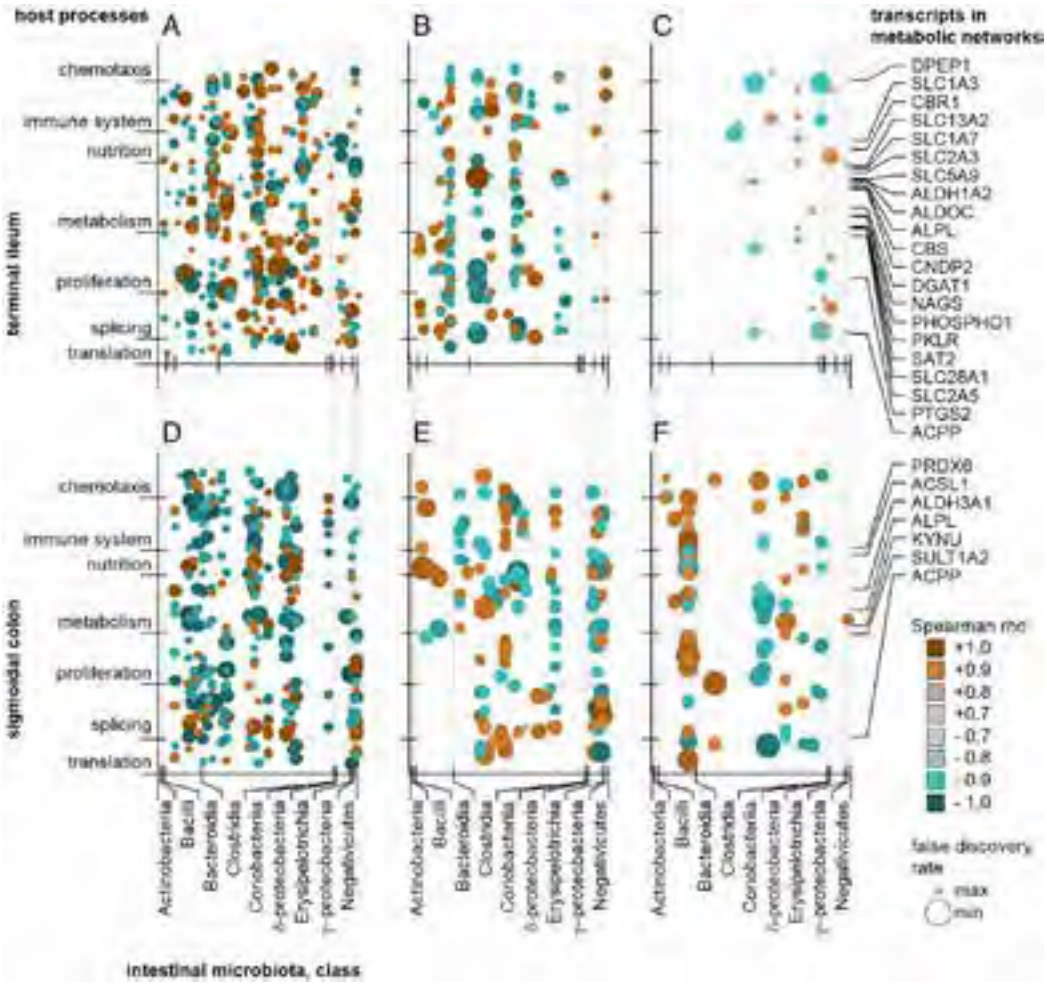

Figure 3: Uncoupling of bacterial signatures and transcriptome patterns (RNA) in the intestinal mucosa of patients with chronic inflammatory bowel disease (Source: Ref. [6] Hasler *et al.*, Gut 2016).

is establishing and investigating cohorts that monitor over time patients receiving a specific biological agent for the first time. Similar protocols will be used for the longitudinal collection of clinical data and biomaterials. With the analysis of DNA methylation, RNA and microbiome patterns over time, we will create high-resolution maps of the immunoregulatory and metabolic networks and correlate these with clinical progression [6, 7]. Using modeling, the network statuses that appear particularly promising for the neutralization of a specific factor can be identified. Initial results show that disruptions in the metabolic cooperation of intestinal bacteria could be one of these major points of interaction (Figure 3). The prediction of metabolic flux between species of bacteria could be a promising model for depicting the response to specific biologicals.

Chronic inflammatory diseases are a major problem in modern medicine and society. Combating the acute distress associated with these illnesses with precise, individualized monitoring of disease activity is therefore a major aim of translational research. The aim is not only to improve the use of existing drugs but also to redefine (earlier) pathophysiological mechanisms because, after all, all previous therapy approaches can be seen as symptomatic.

In almost all diseases of the immune system, the impaired interaction of the body with its bacteria (microbiome) plays a role in causal pathogenesis. A systems medicine approach to the cometabolic network of this interaction and the ability to integrate the disruption of various networks in predictions could be one way of developing early, causal therapies. Initial results

from the field of amino acid metabolism [6, 8] will now have to be validated in clinical trials.

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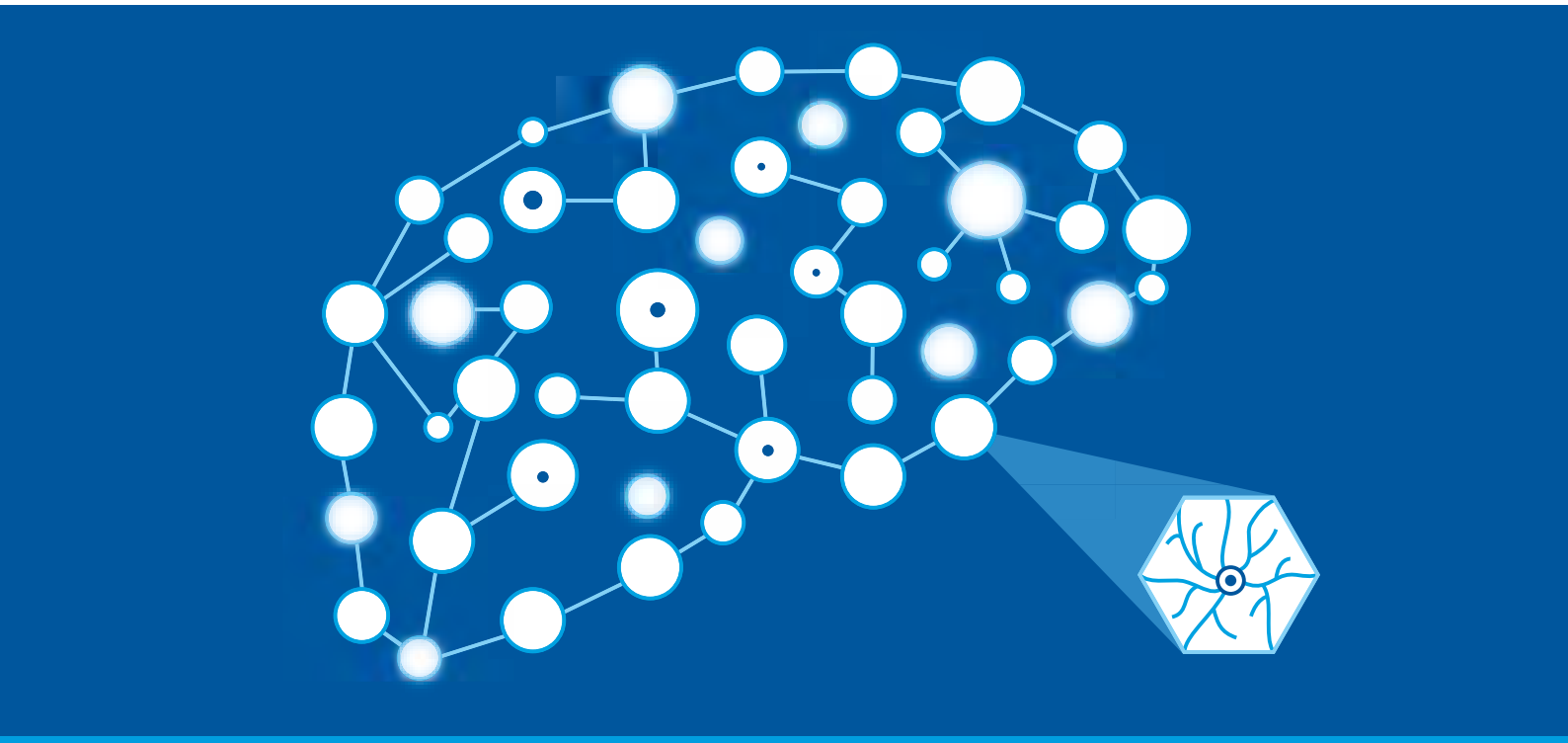

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identifying new epigenetic tumor medication

A Profile of Proteros biostructures GmbH

by Adrian Schomburg and Peter Reinemer

In recent years, modulating epigenetic mechanisms has become a promising approach for companies researching and developing new treatments for a wide variety of cancers. There are three core components to a platform for successfully identifying new active substances against epigenetic targets. (i): the identification and validation of epigenetic targets that can be of use in therapies. (ii): the best possible modeling of epigenetic activity within cells in an *in vitro* screening system for the purpose of identifying potential substances. (iii): advanced technology for optimizing leads in a target class with high technological requirements.

Over the past few years, many pharmaceutical and biotech companies have focused on epigenetic mechanisms, but it was not until recently that they were able to develop specific technologies for complex epigenetic targets. In particular, the Proteros platform for nucleosomal epigenetic assay technologies (NEAT™), combined with target identification and the technological platform for lead optimization, represents a key element in the efforts to initiate a "second wave" of drugging epigenetic targets. This enables the identification of new active substances to tackle a host of cancer types that conventional methods of medication development still cannot cope with.

Table 1: Examples of genes for which medication is already available

Gene	Medication	Tumor type
BRAF V600E	Vemurafenib	Melanoma, breast cancer, intestinal cancer
RET	Sunitinib	Lung cancer
KRAS	Cetuximab	Pancreatic cancer
PML-RARA	At-retinoic aced	Acute myeloid leukemia

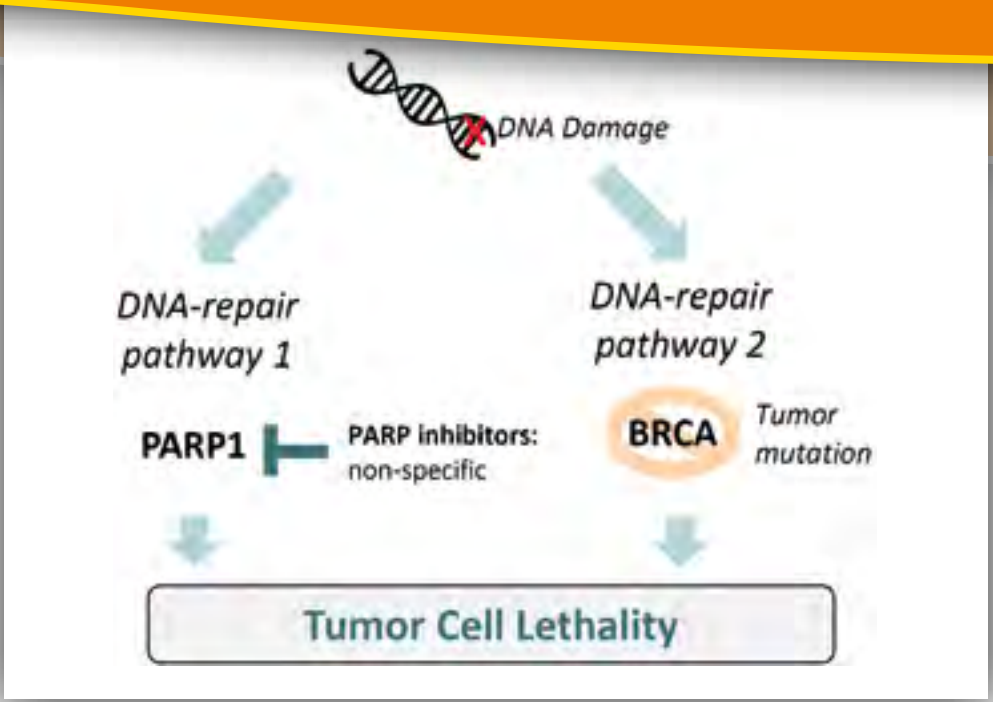


Figure 1: PARP1 is synthetically lethal in BRCA-mutated tumors (Source: Proteros biostructures GmbH).

Identifying and validating targets: a systematic search in genome databases for tumor cells' weaknesses

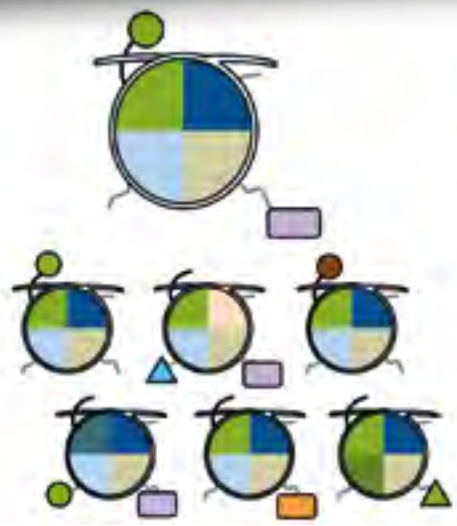
In the course of the past decade, sequencing and the molecular characterization of tumor tissue have revolutionized our understanding of how cancers form. While it is now possible to use this data to assess someone's personal risk of developing cancer, it is clear that a wide range of different cellular mechanisms can lead to cancer, even within tumor indications affecting clearly defined anatomical areas, e. g. breast cancer. This heterogeneity poses serious challenges to practitioners, as they only have recourse to a limited range of customized therapy options. Nevertheless, the molecular profiling of individual tumor biopsies already plays a major role in many clinical centers, and molecular tumor boards can indicate possible customized forms of treatment for some patients. What researchers are looking for are "clinically actionable genes," i. e. mutations for which targeted medication already exists (Table 1).

A new concept in tumor treatment is the search for synthetic lethal gene patterns that only occur in tumor cells. They are a kind of "Achilles' heel" within a tumor's biological makeup, as they only develop when a tumor is forming and are therefore absent from normal tissue. For example, mutations in the genes BRCA1 and BRCA2 are causally implicated in the development of breast and ovarian cancer. In these particular types of tumor, the BRCA1/2-associated DNA repair process is inactive, thereby favoring tumor formation resulting from changes to DNA such as mutations, translocations, and the loss of gene regions (Hanahan and Weinberg, 2011). The alternative DNA repair process, facilitated by the epigenetic protein PARP1, now becomes essential for the cell's survival, as at least one functioning repair process is always necessary. As the BRCA1/2 repair route remains unaffected in normal cells, PARP1 only becomes essential in cancer cells (Figure 1). Experts refer to this as "synthetic lethality," as lethality arises only in connection with a "synthetic" change to the tumor cells. PARP1 inhibitors are therefore approved for treating tumors of this type, and they have already proven successful when used in clinical activities (Fong *et al.*, 2010).

Can this type of synthetic lethal gene pattern be predicted? This would require new methods, along with a new take on how we understand different types of tumors. Working with the German Cancer Research Center (DKFZ), Proteros biostructures GmbH is now using a new procedure to identify targets, and this procedure makes it possible to predict synthetic lethal therapeutic targets.

Synthetic lethal tumor genes

This process starts with an analysis of the genomic data along with the associated clinical data (regardless of tumor indications), and it deploys gene pattern classifications to group tumors with similar genetic changes. Instead of looking for collective mutations the way previous approaches did, this pioneering new method searches for negative associations: for example, what gene sections only become essential in the tumor when another gene section or specific pattern is present?



Screening substrate: fully assembled, marked and modified nucleomes

Proteros toolkit: extensive library of prepared &ready-to-use nucleosomes for collaborative projects

Figure 2: Range of NEAT™ technology substrates with disease-relevant modifications (Source: Proteros biostructures GmbH).

Answering questions of this nature requires algorithms that have so far only been used in other fields, such as the automotive or finance industries. Merging technology and data resources in this manner make it possible to generate lists of target genes whose functions are essential for cancerous tissue, but not for normal tissue.

The role of epigenetic targets

As in the above-mentioned example of PARP1, many of the essential tumor genes are members of the family of epigenetic targets. These targets play a central role in tumor formation, as they are directly involved in the regulation of many other proteins that are vital to the tumors. Tumorous tissue displays a high rate of proliferation, meaning that the cells demonstrate processes similar to those during early embryogenesis. In the case of epigenetic proteins, they often involve target genes whose function is no longer important in normal, mature cells, so developing medicine against these synthetic lethal epigenetic target proteins often offers considerable potential for identifying other well-tolerated medication for customized tumor treatment.

In vitro screening systems for identifying active substances against epigenetic targets: NEAT™

Today, research into substances that act on epigenetic proteins is, along with therapeutic antibodies and immuno-oncology, the fastest-growing field in the pharmaceutical sector. The first generation of epigenetic medicines is now market-ready, and development is underway in a host of other projects. As positive as this is, the research conducted in recent years has nevertheless flagged up difficulties in connection with epigenetic medication: conventional screening methods for active substances do not address epigenetic proteins in an entirely satisfactory manner. Once again, Proteros is striking out in a new direction by establishing a technology platform for processing epigenetic targets. It uses the natural, cellular substrate of the epigenetic target as an assay substrate. This nucleosomal substrate is based on a synthetic gene section, consisting of DNA and histones, and modeled on the gene section relevant to the illness in question. Using these **NEAT™ (N**ucleosomal **E**pigenetics **A**ssay **T**echnology) substrates, it is possible to develop better active substances more quickly (Figure 2), and these medications display particularly high tolerance values thanks to their action on the specific target protein implicated in the illness. This technology also enables the development of substances for epigenetic target proteins that were previously described as "undruggable".

The strength of this approach lies in the combination of a new way of choosing targets, one which can identify synthetic lethal target genes, and the necessary technology platform that permits NEAT™ substrates and assays to be used for processing these targets.

Optimizing leads for technologically demanding epigenetic targets

One of the salient features of epigenetic mechanisms and targets is how they often consist of large protein complexes with multiple domains and a host of interaction partners. As a result, their biological functions can only be modeled *in vitro* by means of complete or virtually complete reconstructed systems (Verma *et al.*, 2012). This represents a serious hurdle for research into active substances and modern,

Figure 3: Comprehensive screening and optimization approach for epigenetic targets based on NEAT™
(Source: Proteros biostructures GmbH).

structure-supported efforts are required to optimize leads. Protein biochemistry has to be capable of producing these complexes in outstanding quality so that assay development, biophysics, and structural biology can make use of them and provide results good enough for developing and optimizing active substances. Literature on the topic contains a host of examples that use overly simplified biochemical systems for screening. This has sometimes produced results that no longer reflect the cellular functioning of the relevant complex (McGinty *et al.*, 2008) (Shi *et al.*, 2005). Proteros' platform for optimizing leads encompasses a technological advance in protein biochemistry, assay development, structural biology, and medical chemistry that satisfies the above-mentioned requirements, and it dovetails with NEAT™ technology and biology to produce a comprehensive epigenetic screening and optimization cascade (Figure 3).

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using physical methods to track biological processes

A profile of biophysicist Jeffrey Moffitt from the Harvard University in Cambridge

by Kristin Hüttmann

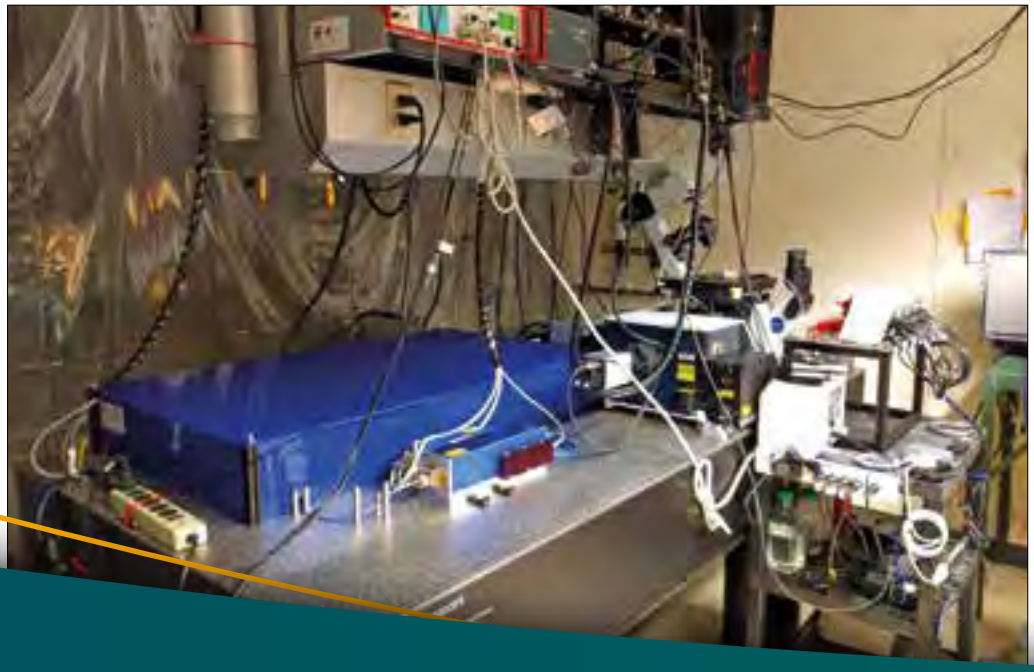
Even as a child, Jeffrey Moffitt loved to build and create things. The physicist from Harvard University has been using this passion for many years to develop innovative new measuring instruments and high-resolution microscopes. In doing so, he wants to play a part in answering fundamental biological questions.

When Jeffrey Moffitt shows you the photos on his mobile phone, you quickly see what is important to him in life. Many of the pictures show devices with lots of cables – the high-resolution microscopes that the physicist builds. The rest of his photos are of his wife and daughter. Any other hobbies? "No," laughs Moffitt. "I conduct research and have a three-year-old daughter – my life is completely full up."

There are not enough hours in a normal working day for his two passions in life. So it is no wonder that Moffitt is up by five o'clock at the latest every morning, sometimes even earlier. He then goes to the coffee shop around the corner, reads publications, writes emails and is often the first and only customer there. "I'm an early riser," he says. "I feel like my mind is fresher in the morning, and I can think better then." And it is quiet enough to do this – in contrast to the rest of the day.

Because when he goes to his laboratory at Harvard University later on, his day is defined by experiments and work on his microscopes. Originally from a little town in Ohio, his scientific career quickly took him from a small college in Wooster, Ohio, to the University of California in Berkeley and then here to Cambridge. The 37-year-old has been working for

Figure 1: The microscope constructed by Jeffrey Moffitt



In the blue box are five different lasers, violet, blue, green, red, and dark red.

Source: Jeffrey Moffitt

Figure 2: Biophysicist Jeffrey Moffitt from Harvard University in Cambridge (Sorce: Melania Strycharska).

some eight years at the renowned university as a postdoc in the Department of Chemistry and Chemical Biology. He is an uncomplicated guy with a buzz-cut hairstyle who's wearing a short-sleeve shirt and has a friendly smile. A guy you would want as a neighbour because he will collect your post and look after your plants while you are on holiday. A guy who picks his daughter up from crèche at 5.30 p.m. every day.

"I conduct research and have a three-year-old daughter – my life is completely full up."

Jeffrey Moffitt, or "Jeff" for short, is a physicist – "with all my heart," as he says. Yet he did not choose the conventional research areas such as quantum or particle physics, but instead picked a more interdisciplinary field: biophysics. His decision came during a summer internship at Cern *(Conseil Européen pour la Recherche Nucléaire)* in Geneva, where the Large Hadron Collider (LHC) is located. In this particle accelerator, scientists make particles collide in a very specific way and then observe what happens.

"It was fantastic," says Moffitt. Yet he realized in Switzerland that this was not what he was looking for. "The experiments were so enormous, and you only played a minute role in them yourself." Moffitt wanted something else. "I love to build things – I got that from my father." So he decided to pursue biophysics, a field that seemed to be made for him, since research scientists in this area use methods from physics to examine processes in biological systems and construct instruments for this. "I can build my own microscopes and do my own experiments here," he says. In this regard, Moffitt is primarily interested in questions and problems that plague biologists. And he wants to help answer and solve them.

Making minute steps visible

For his doctorate at the University of California in Berkeley, he developed for example a new type of highly sensitive optical tweezers – a sophisticated microscopic tool that can grasp and move minute particles with the help of a laser. What makes Moffitt's instrument special: it is able to observe enzymes as they move along a DNA strand in minute steps – from base pair to base pair. Distances as minute as if you placed three hydrogen atoms on top of one another, which together are only around 3.4 angstroms high, as Moffitt explains (an angstrom is a unit equal to one ten-millionth of a centimetre). His optical tweezers still hold the resolution record today.

With this newly constructed instrument, Moffitt and his colleagues were able to make a surprising observation straight away: they discovered that the variations in the movements of enzymes follow a mathematical equation. And this enabled Moffitt to fulfil a dream from his youth – the equation was

Figure 3: The flow system constructed by Jeffrey Moffitt. Each tube contains a different stain that we will apply to our sample. The system is completely computer controlled and automated, so each of these stains will be flown across the sample at the appropriate time (Source: Jeffrey Moffitt).

named after him and his colleagues: the Moffitt-Chemla-Bustamante Equation, or MCB for short. Without doubt a reason to be proud, and yet he quickly adds that it is just a small field of research and that the equation has not yet become popular.

Successful fluorescence research

Despite all his modesty, Moffitt has long been among the promising young scientists in his field. He already has a long list of publications that have appeared in numerous journals, including renowned magazines such as *Science* and *Nature*. The biophysicist has also garnered many accolades, and was recently honoured with the Anne Heidenthal Prize for Fluorescence Research at the International Conference on System Biology of Human Disease (SBHD) at the German Cancer Research Center (DKFZ) in Heidelberg.

He was awarded the prize for a method that he and colleagues developed as a postdoc at Harvard University: Its name is MERFISH, and it is hugely important for researching the transcriptome. Transcriptome is a term used by scientists to describe the sum total of all the messenger RNA molecules from which the cell produces corresponding protein molecules. By researching the transcriptome, research scientists hope to find answers to many questions about gene expression and regulation.

Up until now, it has been possible to research the transcriptome using a method that marks the RNA molecules with the help of fluorescent DNA fragments: smFISH (single-molecule fluorescent in situ hybridisation). There is a problem with this, however: up until now, biologists have only been able to examine a maximum of 10–30 different types of RNA in a cell simultaneously using smFISH, and in fact usually can only examine one to three. However, it is estimated that the entire human transcriptome contains around 100,000 different types of RNA.

It is a challenge that enticed Moffitt: he optimized smFISH to create MERFISH (multiplexed error-robust fluorescent in situ hybridisation). And in doing so, he made it possible to image thousands of RNA molecules in a cell and, in particular, identify each of these individual RNA molecules. This works by cleverly combining RNA marking and sequencing with a coding pattern that removes errors which occur the more molecules and rounds pass through.

Courage, perseverance and fearlessness

In 2015, Moffitt published the MERFISH method in the journal *Science*. It was received with great interest from other researchers all over the world. However, Moffitt was still not satisfied. MERFISH was simply too slow for him. So he and his colleagues went back to their instruments, redesigned everything and rewrote the software. Not an easy task – and not an easy time for the scientists. Because they did not know whether their rede-

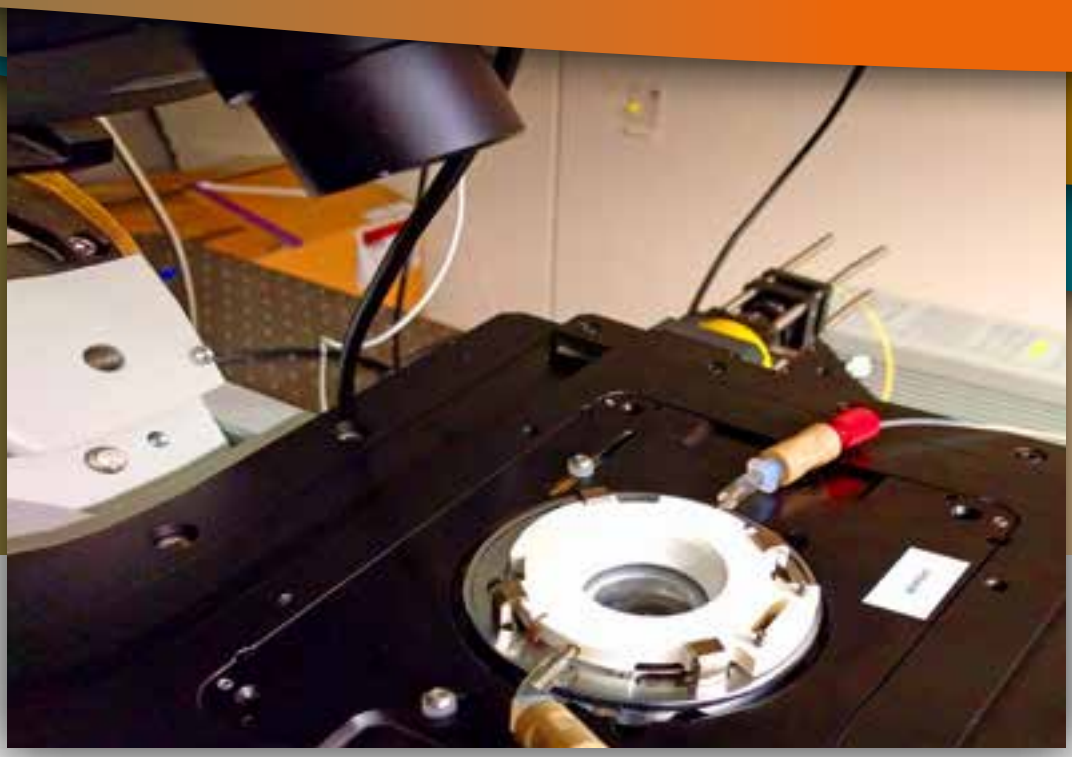


Figure 4: The sample holder in the microscope from above. This is a small chamber that holds inside of it our samples. The bottom is clear glass so that we can look from the bottom into this sample with our microscope (Source: Jeffrey Moffitt).

signs would work in the end – for two years. "It cost us quite a bit of fearlessness," recalls Moffitt today. "And we needed to remain steadfast in our belief that it would be better in the end."

> "The aim would be to fish out from the millions of cells the one that triggers a disease."

The effort and uncertainty paid off: Moffitt and his colleagues managed to significantly improve the sample throughput of MERFISH – by a factor of 400. While it took three years to measure one million cells with the original technology, this is now possible in just two weeks.

"An incredibly important step," says Moffitt. Because, after all, an organism consists of a vast number of cells, all of which the research scientists want to examine – and as quickly as possible. It should be possible to answer many fundamental biological questions with the help of MERFISH. And the method should also help to turn a great vision into reality: biologists have long wanted to create cellular atlases that contain the molecular details of tissues and organisms – from embryo to adult. And Moffitt sees opportunities for his method to be used in clinical research, too. "The aim would be to fish out from the millions of cells the one that triggers a disease," he says.

His work on MERFISH has strengthened Moffitt's self-confidence as a scientist. He was aware that the redesigns could just as easily have ended in failure. He experienced that, too, as a young research scientist. However, all of this means scientific work for him: "You always have to accept that one day you might realise that everything you have done in the previous six months was flawed," he says. "And then you go back into the laboratory the next day and start all over again."

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mapping the core units of the human body: the human cell atlas

An international initiative to map all cells in the human body

by Roland Eils, Sarah Teichmann and Aviv Regev

In October 2016 more than 150 biologists, computational scientists, technologists, and clinicians met in London to launch a new ambitious international scientific initiative – the Human Cell Atlas (HCA). The HCA aims to create an atlas of every cell in the human body as a reference map to accelerate progress in biomedical science. Similar to digital geographic maps, the HCA's maps of the human body will "zoom in" on molecular and organizational features of organs, tissues, and cells. With these maps of different cell types and their spatial organization their functions can be described and the biological networks that direct their activities can be understood. Importantly, the atlas will be open-source and collaborative, bringing together world-wide experts in networks focused on biological topics. With this, the initiative intents to ultimately accelerate the understanding of human health and improve the diagnosing, monitoring and treating of various diseases.

The Human Cell Atlas and what it is

The knowledge on human cells has progressed vastly in the past 150 years. They can be described by their shape, location, molecular constituents and function. But still information is missing of how these features are associated to each other throughout tissues, systems and organs. Furthermore, cells span substantial functional diversity, through expression of different programs, either stably as types or transiently as dynamic responses. We still do not know how many different cell types are present in the human body. To analyze the cells, scientists within the HCA will apply innovative technological approaches such as single-cell-genomics, -transcriptomics and -epigenomics that will enable to separate and sort individual cells taken from tissue and organ samples. With the analysis of the whole transcriptome, the totality of all synthesized RNA molecules within a single cell and the activity status of all genes can be identified. Also other molecules can be measured in each cell, which will then help assigning each cells identity and distinguishing it from other cell types in the body.

Additional spatial imaging or sequencing methods will provide a multi-dimensional map of how cell types work together to form tissues, will give knowledge of how all body systems are connected, and insights into how changes underlie health and disease. It would allow us to identify which genes associated with diseases are active where in our bodies and to analyze the regulatory mechanisms that govern the production of different cell types.

With these techniques, there will also be the possibility to analyze thousands of individual cells at the same time and by these produce precise comparisons of the characteristics and interaction of healthy and diseased cells and their tissue environment. Thus, the HCA will provide information about the cell types in which a given gene and its disease-associated variants are expressed, e. g. the targeted identification of specific tumor cell characteristics that prevent the patient's immune system from attacking the tumor.

The HCA will help answer fundamental questions in all aspects of biology and medicine as well as serve as a guide to unravel the secrets of human disease (Figure 1). The translational promise of the cell atlas ranges from basic biology of the human organism, to disease mechanism, diagnosis, prognosis, and treatment monitoring, to immunotherapy, drug development, and cell and organ replacement.

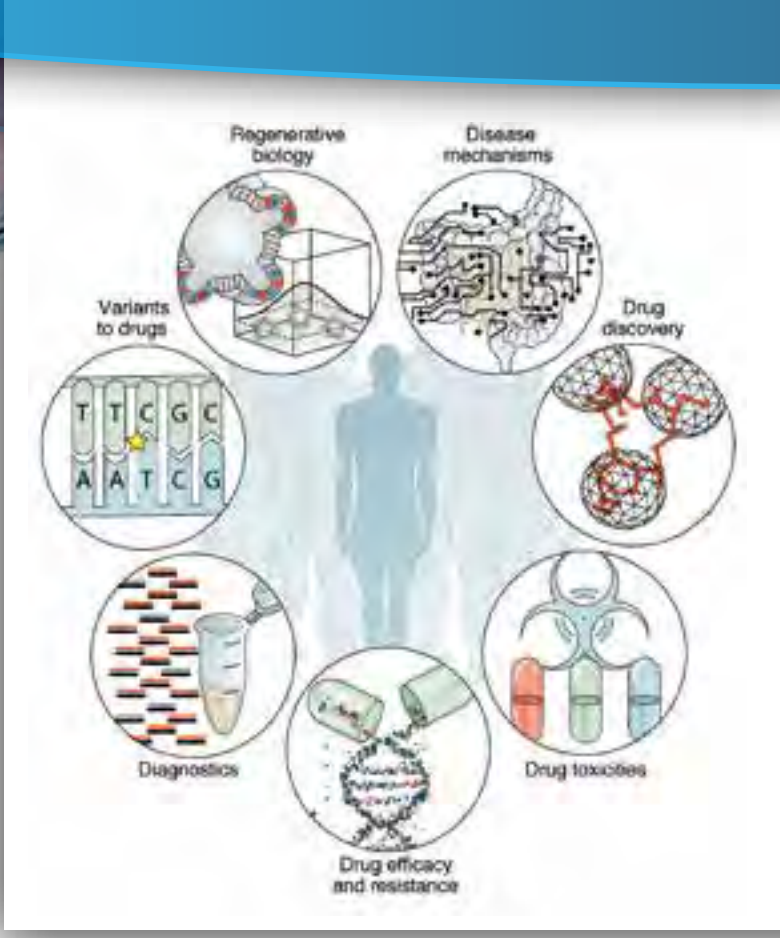

Figure 1: The Human Cell Atlas will have profound impact on biology and medicine (Source: The Human Cell Atlas White Paper. 2017 Oct 18. https://www.humancellatlas.org/files/HCA_WhitePaper_18Oct2017.pdf).

How to establish a Human Cell Atlas

In light of the enormous complexity of the human body, and the rapidly evolving technology landscape, the HCA will be built in phases, starting with cells in tissues, and eventually organs and systems, with the aim of constructing an increasingly detailed, valuable and comprehensive atlas. The HCA guidelines determine how tissues will be sampled within organs, the rarity of cells to be recovered, the resolution of spatial coordinates and the depth of molecular information that is needed. An important role for the first draft is to learn key lessons on appropriate sampling and measurement of representative tissues. Therefore, any given tissue specimen will be analyzed through a two-pronged strategy combining (1) single-cell molecular profiling of dissociated cells **(cellular branch)** with (2) highly multiplexed spatial analysis of intact tissue **(spatial branch)** (Figure 2). To relate the two, before tissue processing, physical specimens will be registered and imaged for their physical coordinates, and matching portions, such as adjacent sections, of the same specimen will be analyzed by cellular and spatial approaches.

After the HCA kick-off meeting in October 2016, several pilot projects reflecting on the identity of individual cells and their organization in tractable and medically important tissues, systems and organs were initiated. This is the first step towards the mapping of all tissues, systems and whole organs.

To realize the HCA project, experts in biology, medicine, genomics, technology development, and computation (including data analysis, software engineering, and visualization) will work together. They will need to produce data that are consistent, high-quality, and interoperable, with data collection driven by domain experts. Furthermore, the experimental and computational methods will need to be standardized. To ensure the resulting resource is truly global, samples from diverse human communities, tissue types and cell types need to be compared in consistent ways.

The results within the HCA will pile up to petabytes of data for billions of cells, across multiple modalities, generated by hundreds of labs around the world that need to be organized and standardized. Therefore, a modern, cloud-based, modular architecture for organizing and sharing data for the Human Cell Atlas will be built. As soon as possible after the first data are generated, the HCA will release them by both streaming and formal releases through an open-source, cloud-based Data Coordination Platform (DCP). The Chan Zuckerberg Initiative (CZI) supports the EMBL-EBI, the Broad Institute and the University of California Santa Cruz Genomics Institute (UCSC) to build a DCP that will incorporate all HCA data, both cellular and spatial, as well as key analytical algorithms built by the HCA community. The development team of the DCP is accompanied by a steering group led by Ehud Shapiro from the Weizmann Institute in Israel and by Roland Eils. Computational researchers will develop and share new analysis approaches and all software will be available as open source worldwide, enabling the scientific community to innovate rapidly without barriers to data access.

Source cellphotos: catalin – Fotolia.com

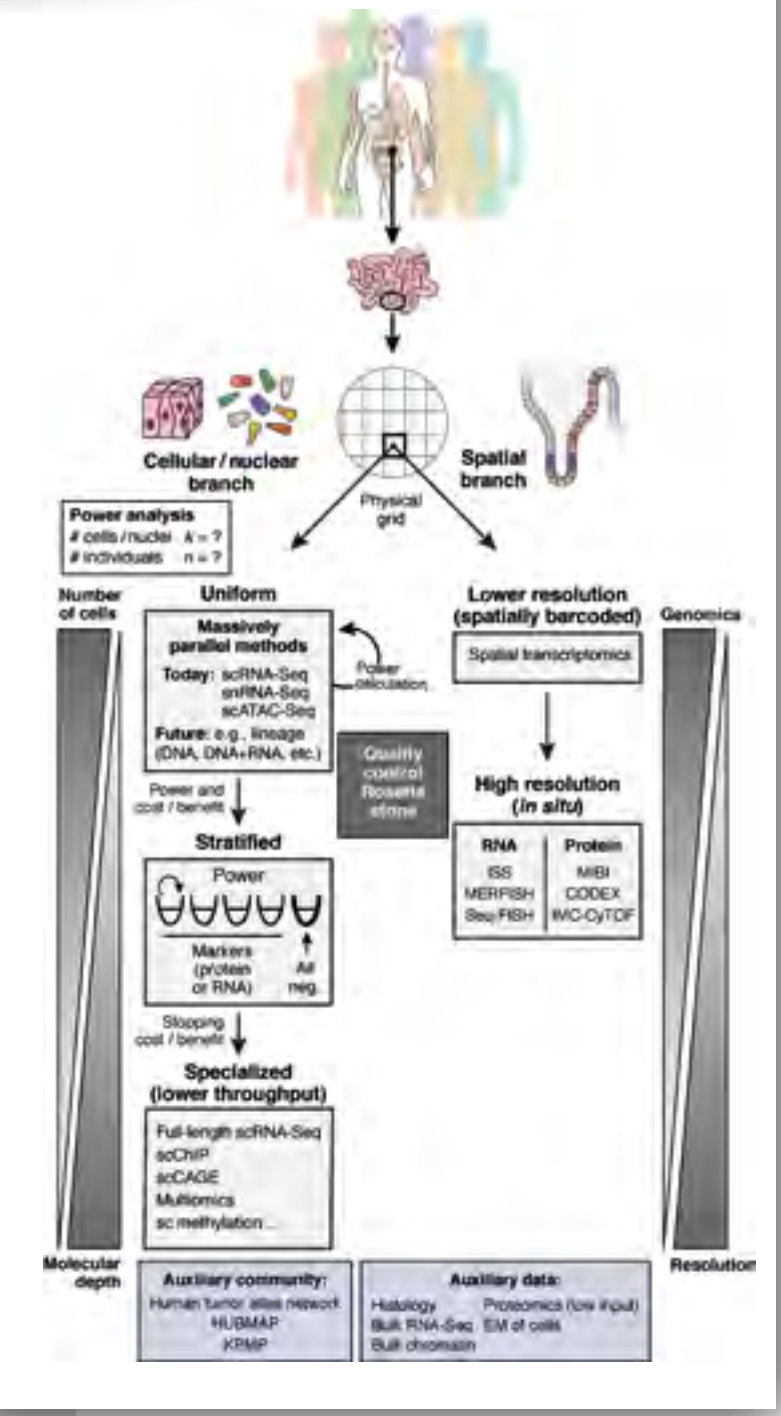

Figure 2: Framework for sampling and measurements for an initial draft. Any given tissue specimen will be analyzed through a two-pronged strategy combining [1] single-cell molecular profiling of dissociated cells (**cellular branch, left**) with [2] highly-multiplexed spatial analysis of intact tissue (**spatial branch, right**). To relate the two, before tissue processing, physical coordinates, and matching portions will be analyzed by cellular and spatial approaches (Source: The Human Cell Atlas White Paper. 2017 Oct 18. https://www.humancellatlas.org/files/HCA_WhitePaper_18Oct2017.pdf).

Coordination and Organization of the Human Cell Atlas

The Human Cell Atlas is steered and governed by an Organizing Committee, currently spanning scientists from 27 countries and diverse areas of expertise. The Organizing Committee is co-chaired by Aviv Regev and Sarah Teichmann and is the decision-making body of the HCA. The HCA is coordinated by an Executive Coordinating Office, currently located at the Sanger and Broad Institutes and also at the Karolinska Institute in Sweden, which provides administrative and scientific support to organize meetings, track progress across the many research sites and projects, help connect members of the community, triage incoming requests from scientists, funders, the public and the media, and organize overall communication within and beyond the HCA community. Further coordination offices will be established in Asia.

The HCA is open to any and all interested participants who are committed to its values, which ensure quality and rigor of the atlas. These values are:

- **Transparency and open data sharing.** We will release data openly as soon as possible after it has been collected, to maximize its immediate use and impact across the globe.
- **Data Quality.** We commit to produce high-quality data and establish rigorous standards that are shared openly and broadly and updated regularly.
- **Flexibility.** We maintain intellectual and technical flexibility to modify the design of the HCA as new data, technologies and insights emerge.
- **Community.** We encompass a global, open and collaborative community, led by a scientific steering group (Organizing Committee). The initiative is open to all interested participants who are committed to its values.
- **Diversity, inclusion, and equity.** We will maintain geographic, gender, age, and ethnic diversity in the selection of tissue samples. Similar diversity will be reflected in the distribution of participating researchers, institutions and countries.

- **Ethics and privacy.** We are committed to working at the highest ethical standards. We will obtain fully informed consent from sample donors, adhere to the terms of that consent and ensure the privacy of donors to the fullest extent required.
- **Technology development.** We develop, deploy and embrace new tools and technologies, and share these with the broader community to empower others.
- **Computational excellence.** We develop new computational methods, leveraging and driving the latest algorithmic advances, and share these through scaled, open-source software.

HCA members can engage in person and through slack channels. Further information and contact details can be found on the HCA website. In addition, mailing lists provide updates about ongoing activities and upcoming events.

Conclusion

The Human Cell Atlas will not only answer key biological questions across diverse fields but also enable the development of better drugs and the improvement of today's standard diagnostic practices. In taxonomy, it will identify cell types (including previously unknown ones) and discover unique markers and signatures for isolating them; in histology, it will relate tissue structure to spatial position of cells and molecules; in developmental biology, it will reconstruct maps of cell fate and lineage; in physiology, it will characterize dynamic states such as the cell cycle and transient responses. In addition, the cell atlas will enable research on the molecular mechanisms that form intra- and inter-cellular circuits; it will allow us to compare cell types across species to better understand our evolution, and determine how faithful models are; and will form a key foundation for disease studies and pathology, allowing the comparison of the normal reference to disease cells in their native tissue ecosystem.

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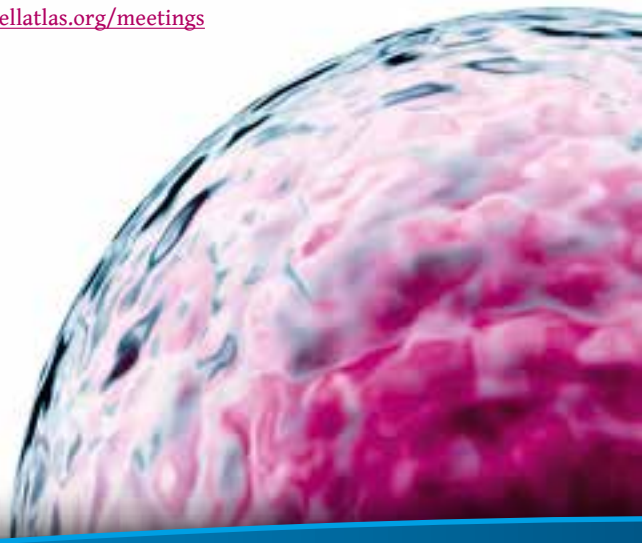
Further information about the Human Cell Atlas and forthcoming meetings:
www.humancellatlas.org
www.humancellatlas.org/meetings



Source cellphotos: catalin – Fotolia.com

events

Hackathon

Gaminfection Hackathon at the Medical Valley Center
29. September – 01. October 2017, Erlangen

MATHEMATICAL MODELS IN COMPUTER GAMES TO PROMOTE CITIZEN SCIENCE IN LUNG INFECTION

by Julio Vera and Martin Eberhardt

On average, every 20 seconds one child dies due to pneumonia in third-world countries. One of the critical issues to improve prevention and treatment of lung infections is to foster our understanding of the physiological interactions between the pathogens, bacteria and viruses, and the patient's immune system. The first 24 hours after infection are virtually impossible to investigate in the laboratory with the current technologies.

Source: Julio Vera

A participant presents one of the game prototypes developed in the Hackathon.

"Serious games" are used with a purpose beyond entertainment. Recently, some serious games have been developed to promote citizen science, that is, scientific research conducted by amateur scientists.

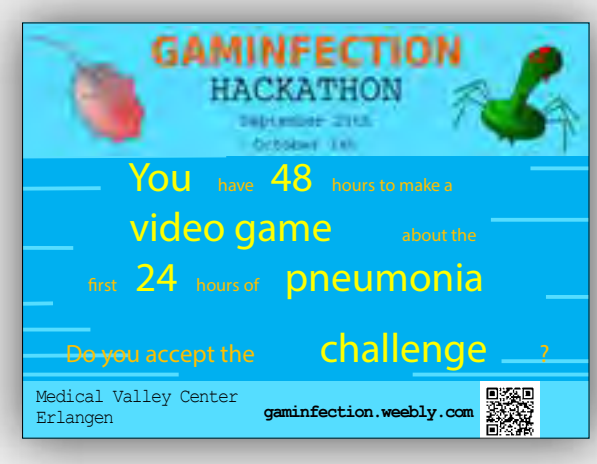
Inspired by these ideas, we organized a hackathon from 29th September to 1st October 2017 in the Medical Valley Center, Erlangen. The aim of the hackathon was to create a prototype of a serious game based on a mathematical model describing the early phase of bacterial lung infection, an undertaking of the e:Med project CAPSyS (www.capsys.imise.uni-leipzig.de).

In the hackathon, we invited interdisciplinary teams composed of computer scientists, bioinformaticians, designers, game developers and biomedical researchers. In total, forty people took part in the hackathon. After lectures on the pathophysiology of pneumonia and mathematical modelling, we conducted ideation and hacking sessions with mentors supporting the teams. A multidisciplinary committee of translational research, pharmaceutical and games industry experts selected the winners of the six game prototypes in the categories "Best Scientific Concept", "Best Game Concept" and "Best Game".

Profile of the e:Med consortium for systems medicine CAPSyS

MEDICAL SYSTEMS BIOLOGY OF PULMONARY BARRIER FAILURE IN COMMUNITY ACQUIRED PNEUMONIA

The consortium CAPSyS aims to investigate and better understand the course of pneumonia from infection to resolution with means of systems medicine. In particular, the focus lies on a severe course of disease, which may involve loss of barrier function between alveoli and blood stream and may lead to spread of infection beyond the lung. With the application of an systems medicine approach new molecular and clinical signatures predicting imminent lung barrier failure in patients with community acquired pneumonia will be identified and thus generate new insights into relevant pathomechanisms.



Source: Julio Vera and Guido Santos-Rosales

The Hackathon was organized by the Laboratory of Systems Tumor Immunology at the Medical Faculty, FAU Erlangen-Nürnberg, and the Erlangen Health Hackers association. It was sponsored by Abbvie, Bayer Grants4Apps, EIT Health, the DFG CRC 1181, and the Federal Ministry of Education and Research (BMBF) through the e:Med project CAPSyS.

More information and links to the game prototypes are available at http://gaminfectionhack.weebly.com/ and www.jveralab.net.

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e:Med consortium CAPSyS
Leader of the project "Mathematical modelling of pneumonia pathophysiology"

Links:

http://gaminfectionhack.weebly.com/
www.jveralab.net
www.healthhackers.de
www.sys-med.de/en/consortia/capsys/sp-3/

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Conference Report

10th International Conference on Systems Biology of Human Disease – SBHD 2017

July 5 – 7, 2017, Heidelberg, Germany

USING COMPUTATIONAL STRATEGIES AND SINGLE-CELL ANALYSES TO MONITOR CELLS' BIOLOGICAL PROCESSES

by Cornelia Depner

For the fourth time, the International Conference on Systems Biology of Human Disease (SBHD) was held at the German Cancer Research Center (DKFZ) in Heidelberg from July 5 – 7, 2017, during a spell of gorgeous summer weather. The first annual conference was held a few years ago on the initiative of Prof. Peter Sorger from Boston's renowned Harvard Medical School, and it has, over time, developed into a German–American event. This time around, the conference was organized by Prof. Roland Eils from Heidelberg University and DKFZ, and was supported by the Swiss systems biology initiative SystemsX, the e:Med systems medicine network (established by the German Federal Ministry of Education and Research [BMBF]), and Chroma Technology GmbH.

The program awaiting the 157 delegates from 10 European countries, Iran, Israel, Mexico, Singapore, and the USA consisted of 23 keynote speeches, 6 short talks, 12 "lightning" talks, and a poster session with 70 participants for presenting and discussing the latest systems biology research findings that are of relevance to the world of medicine. The organizing committee gave selected junior scientists and experienced researchers a chance to describe their work in a 20-minute short talk, and they were also given the opportunity to draw attention to their posters during 5-minute lighting talks.

SBHD 2017: prizes

From left to right: conference head Roland Eils (DKFZ/Heidelberg University), Georg Draude from Chroma Technology GmbH, prize winner Jeffrey Moffitt (Harvard University), prize winner Carsten Marr (Helmholtz Zentrum München), speaker Ursula Klingmüller (DKFZ), and speaker Fabian Theis (Helmholtz Zentrum München).

Photo: Yan de Andrés

Auditorium during talks (Photo: editorial team at systembiologie.de).

The overarching topic of the conference was the use of mathematical techniques and computer modeling for mapping and investigating complex biological systems on every single level, from the genome to the organism as a whole. The conference delivered a broad overview of systems biology's research applications in the medical sector, from systematic pharmacology, computational biology, and network reconstruction through to proteomics, single-cell transcript and protein analysis, and the mathematical modeling of therapy resistance mechanisms.

With 12 talks to this topic, the focus of the event was single-cell analyses, a new technology with a wide range of uses in biomedical research. Developing even more effective therapies hinges on being able to identify rare cell types that are different from the bulk of the cell population, such as in the case of resistant tumor cells, or identifying cells at a certain stage of the cell cycle. Despite possessing identical DNA, cells can diverge considerably in terms of behavior in seemingly homogeneous populations. Using bioinformatic assessments and modeling for individual cells, it is possible to investigate their basic biological processes and pinpoint cell types. Improving the process for developing single-cell analysis procedures can produce targeted systemic therapy options that promise greater efficacy than what is currently available.

Single-cell analysis generates large quantities of data (big data) that can be evaluated using computational methods and mathematical modeling, and these analytical methods were presented and discussed at the conference. Knowledge gleaned about cellular processes can, ultimately, be harnessed for treating diseases such as cancer and neurodegenerative conditions.

Physicist **Dr. Carsten Marr** is also working on individual cells. At the Institute of Computational Biology at the Helmholtz Zentrum München, he heads up a group for quantitative single-cell dynamics and is using innovative, computer-aided simulations and intelligent image recognition in an attempt to understand how individual cells develop. In the future, this could play a key role in grasping the complex processes involved in the development of diseases such as skin cancer and leukemia, and even help to predict them. At SBHD 2017, Dr. Marr received the **CSB2 Systems Biology Prize**, sponsored by Merrimack Pharmaceuticals, for his work on developing innovative computer-based simulation techniques.

Dr. Jeffrey Moffitt from Harvard University also works with single cells. He received the **Anne Heidenthal Prize for Fluorescence Research**, sponsored by Chroma Technology GmbH, at SBHD 2017 for his research into the MERFISH method, which he developed during his postdoctorate at Harvard. This technique makes it possible to simultaneously identify hundreds of thousands of different RNA types in an individual cell. Researchers hope that their work on the transcriptome, the sum total of all the messenger RNA molecules, will yield answers to a host of questions concerning gene expression and regulation, including issues relating to a large number of illnesses.

The wide-ranging and extremely interesting scientific program was rounded out by a boat trip on the Neckar river, with a barbecue and music until late at night for everyone to enjoy. The positive feedback from participants and lively scientific discussions of a host of topics with so much potential meant that everyone involved is already looking forward to SBHD 2018.

The 11th International Conference on Systems Biology of Human Disease (SBHD) will take place at UCLA in Los Angeles, USA, from June 4 – 6, 2018.
Information about the conference is available at www.sbhd-conference.org/2018

On the move: Digital Integration in the Healthcare Sector

Research Program for Promoting Medical Research and Improving Patient Care

by Roland Eils and Isabel Göhring

Covering everything from blood results and X-rays to medication lists, and doctors' letters, the quantity of health-related data available in electronic form for everyday clinical activities is constantly growing. High-throughput technology used in clinical research and patient care also generates huge amounts of data that contain an abundance of information. For example, the use of high-throughput next-generation sequencing means that the entire human genome could be sequenced in just a few hours. By analyzing the genome and comparing the findings against a database, medics can provide for patients using the latest in molecular diagnostic methodologies.

Despite the fact that large quantities of medically important data are available, researchers and doctors are often unable to access them. Prof. Benedikt Brors from the German Cancer Research Center (DKFZ) in Heidelberg explains, "For example, if a cancer patient at clinic A is undergoing treatment, an oncologist with a similar patient profile at clinic B will not hear about the therapeutic process and success at A, because there is no systematic sharing of therapy data between different clinics. If the treatment data were stored in a centralized manner, this could help the attending physician make decisions or perhaps consider another form of therapy that is more effective."

Being able to exchange electronically stored biological and medical data would be a tremendous boost for cooperation between researchers and doctors, as it would provide a faster way of obtaining information that could then be transformed into applicable medical knowledge.

Even when data are available, they frequently lack uniformity in terms of quality, type, and format. The IT systems used at different clinics are often incompatible as well, which makes it difficult to merge existing data drawn from a variety of sources. As a result, making medical data from clinical research activities and patient care available to a range of institutes is often a very challenging undertaking.

To overcome the problems outlined here and to make the best possible use of the potential offered by having large quantities of medical data, the German Federal Ministry of Education and Research (BMBF) is providing funding worth EUR 150 million to medical informatics. This funding is intended as a means of promoting the exchange and use of data regarding healthcare at different sites.

Speaking at a press conference on July 10, 2017, Germany's research minister Johanna Wanka said, "Our vision is for doctors, whether they work at clinics, as family doctors, or as specialists, to be in a position to draw on all available experience and research results at the touch of a button when making their therapy-related decisions. This will deliver even better treatment and advice for patients in the future."

Putting these words into action began in January 2018, when four medical informatics consortia (HiGHmed, MIRACUM, DIFUTURE, and SMITH), comprising university clinics and other academic and private-sector partners, started the work of creating what are known as data integration centers. These centers will use innovative IT solutions to establish the technological parameters for exchanging data between sites. The functionality and benefits of the IT solutions are demonstrated using specific medical use cases. Headed by bioinformatics specialist Prof. Roland Eils, the HiGHmed consortium is made up of three leading and complementary medical faculties and university hospitals from Heidelberg, Göttingen, and Hanover plus the DKFZ in Heidelberg. In the field of oncology, the consortium focuses on the challenge of harnessing the huge quantities of data from genome sequencing and radiology for customized therapy

Germany's research minister in 2017, Johanna Wanka, and Alexander Hörbst with representatives from the four medical informatics consortia DIFUTURE, SMITH, HiGHmed, and MIRACUM. From left to right: Alexander Hörbst (chair of the reviewers' group), Klaus A. Kuhn (DIFUTURE), Markus Löffler (SMITH), Roland Eils (HiGHmed), Johanna Wanka, Hans-Ulrich Prokosch (MIRACUM) (Source: BMBF/Hans-Joachim Rickel).

options. Serving as a platform for exchanging information, a virtual oncology center will play a key role in identifying other patients, including those suffering from rare types of cancer, thereby promoting more personal and patient-centered treatment.

The medical informatics consortia now have four years to demonstrate that data can be shared between different institutions in a manner that benefits those involved in research and patient care. Creating an electronic information exchange system between medical researchers and practitioners will be no easy task, but medical informatics experts, academics, and doctors are coordinating their efforts closely so they can tackle the problems and ultimately deliver better care quality for patients.

Professor Eils says, "We are addressing the challenges of digitalization in medicine so that we can make advances in collaboration between university hospitals and research institutions in addition to designing more efficient processes for exchanging existing information between clinics and researchers. Together, we will develop new technological solutions that improve how different medical institutions can share information gathered during the course of a patient's illness or therapy. This will help doctors to make faster and more effective decisions about a patient's specific therapy needs in the future."

Further information:
Information about the medical informatics initiative:
http://www.medizininformatik-initiative.de/en/start
Information about the HiGHmed consortium:
http://www.highmed.org
Background information about the consortia from BMBF:
https://www.bmbf.de/de/medizininformatik-3342.html

MTZ®-Award for Medical Systems Biology 2018

The MTZ-Award for Medical Systems Biology honors future-oriented innovative doctoral theses in the field of medical-oriented systems biology.

The prize will be awarded for the sixth time by the MTZ foundation and is supposed to give promising young scientists special attention and public recognition. For this purpose, the MTZ foundation is cooperating with the Federal Ministry of Education and Research (BMBF) and the Project Management Jülich (PtJ).

MTZ stiftung – for a better future –

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Federal Ministry of Education and Research

The award promotes young scientists who have made an outstanding contribution to the field of systems biology and disease research.

The prize money is divisible and will be awarded for the **three best doctoral theses**. The award ceremony will take place during the 7th International Conference SBMC "Systems Biology of Mammalian Cells" from **July 4th to July 6th in Bremen**.

Further information can be found on the following websites:
www.mtzstiftung.de, www.systembiologie.de
...or on the website of Project Management Jülich
https://www.ptj.de/mtzaward
Source: Project Management Jülich

news

German Network for Bioinformatics Infrastructure – de.NBI

A Successful Start for Germany's Network for Bioinformatics

by Yvonne Pfeiffenschneider

March 2015 marked the starting date of Germany's Network for Bioinformatics, or de.NBI. Today, this successful bioinformatics network offers a wide range of bioinformatic software packages for evaluating data in life science and medical research projects.

de.NBI
GERMAN NETWORK FOR BIOINFORMATICS INFRASTRUCTURE

The participating centers also provide training courses and answer questions from users.

In September 2017, an international committee of experts attested the outstanding quality of this bioinformatics infrastructure and provided recommendations about the network's continued expansion. As of March 2018, the German Federal Ministry of Education and Research (BMBF) has started a second round of funding to support the network.

One element of this support will take the form of creating an innovative IT infrastructure for the network ("de.NBI cloud") at the sites in Giessen, Bielefeld, Freiburg, Tübingen, and Heidelberg. This cloud will help resolve issues regarding storage and computing capacities in the life sciences.

The latest information about de.NBI is available on the network's website:
www.denbi.de

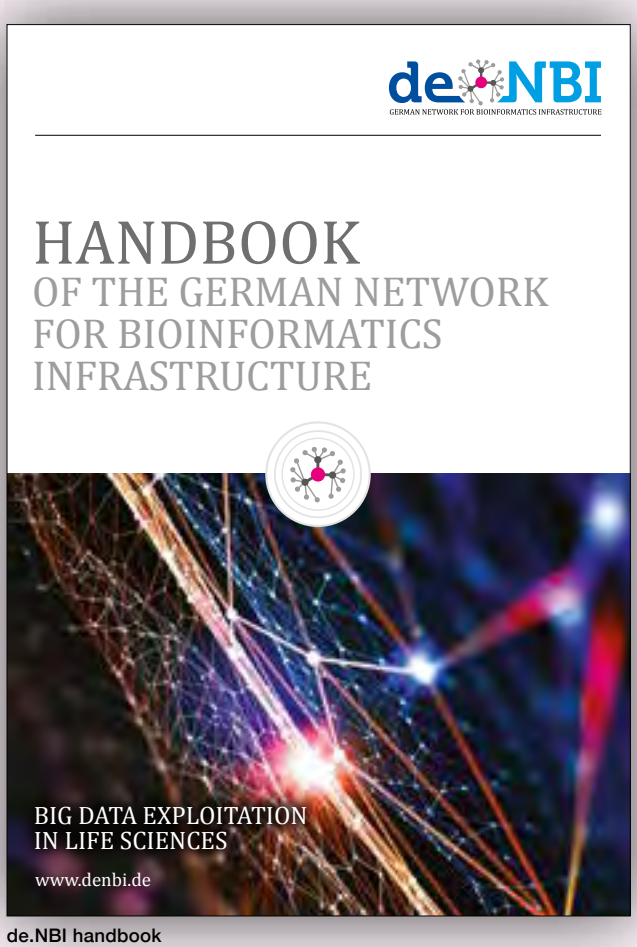

Image: Sergey Nivens – Fotolia.com

de.NBI handbook

INCOME
INtegrative COllaborative Modelling in Systems MEdicine

CONFERENCE & HACKATHON

Integrative pathway modeling in systems biology and systems medicine

October, 15-19, 2018

Bernried, Lake Starnberg, Germany

KEYNOTE SPEAKERS

Edda Klipp, HU Berlin
Clemens Kreutz, University of Freiburg
Julio Saez-Rodriguez, RWTH Aachen
and others

IMPORTANT DATES

Abstract submission closes: Aug, 6
Registration deadline: Sep, 3

Conference: Oct, 15-17
Hackathon: Oct, 18-19
(separate registration possible)

Conference and hackathon will gather experts in pathway modeling to discuss the state of the art in reuse, integration, visualization and parameterization of models. The hackathon will provide the participants with time and space to work on problems and improve existing tools in a collaborative way.

http://www.integrative-pathway-models.de/meeting-2018/

ORGANIZERS

Jan Hasenauer, Helmholtz Zentrum München
Wolfgang Müller, HITS
Olaf Wolkenhauer, University of Rostock

INCOME project funded by
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September 24-26, 2018
Urania, **Berlin**

e:Med Meeting **2018** on Systems Medicine

Main topics

Modeling in Systems Medicine

Systems Medicine of **Diseases**

Translational Approaches in Systems Medicine

Technologies in Systems Medicine

+++

International Systems Medicine

Poster FlashTalks & Poster Exhibition

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Company Exhibition

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

Abstract Deadline: **June 30**, 2018

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- Presentations
- Hands-on experiments and virtual reality
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www.sys-med.de/de/TagPerMed

SEPTEMBER 24, 2018 | URANIA, BERLIN | 9:30 - 4:00 pm

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imprint

systembiologie.de – International Edition

The magazine for Systems Biology Research in Germany – International Edition Issue 12, May 2018

systembiologie.de publishes information on German systems biology research. It is published alternately in German and in English.

ISSN 2191-2505

Publisher:

systembiologie.de is published by the Helmholtz Association, Cross Program Topic Systems Biology and Synthetic Biology, the Liver Systems Medicine (LiSyM) program and the Project Management Jülich (PtJ).

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Design and layout:
LANGEundPFLANZ Werbeagentur GmbH, Speyer (www.LPsp.de)
Translations:
EnglishBusiness AG, Germany
Printed by:
Ottweiler Druckerei und Verlag GmbH, Ottweiler, Germany

Subscriptions:
The magazine is funded by the Helmholtz Association and the German Federal Ministry of Education and Research (BMBF). It is published as part of the public relations work of the initiatives listed as "Publisher". It is supplied free of charge and must not be sold.

For subscription please visit www.systembiologie.de or contact:

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apops - Fotolia

Please visit our homepage if you would like to find out more about systems biology.

What you can expect:

- Exciting stories from everyday research – find out more about ongoing projects
- Profiles of systems biologists – get to know the faces behind the research
- Extensive overview of events in systems biology – never miss another important date
- Information about current funding – stay up to date
- Get involved – suggest your own topic

We look forward to your visit on our homepage!

about us

Presenting the systembiologie.de editorial team

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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Photo: Tobias Schwerdt / DKFZ

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EMBL Events 2018

MAY

14 – 17 MAY	EMBO | EMBL Symposium	Cellular Mechanisms Driven by Liquid Phase Separation
23 – 25 MAY	EMBL Conference	BioMalPar XIV: Biology and Pathology of the Malaria Parasite
27 – 30 MAY	EMBO | EMBL Symposium	Microtubules: From Atoms to Complex Systems

JUNE

3 – 5 JUN	EMBO | EMBL Symposium	Biological Oscillators: Design, Mechanism, Function
7 – 9 JUN	EMBL Conference	Hematopoietic Stem Cells: From the Embryo to the Aging Organism
11 – 15 JUN	EMBL Course	Fundamentals of Widefield and Confocal Microscopy and Imaging
24 – 27 JUN	EMBO | EMBL Symposium	Innate Immunity in Host-Pathogen Interactions
24 – 29 JUN	EMBL Course	Advanced Fluorescence Imaging Techniques

JULY

2 – 5 JUL	EMBL Course	Shift Your DNA and RNA Sequencing Library Preparation into Hyper-Drive
9 – 14 JUL	EMBL Course	Super-Resolution Microscopy
10 – 11 JUL	EMBL Course	Exploring Human Genetic Variation
15 – 17 JUL	EMBL Conference	Microfluidics 2018: New Technologies and Applications in Biology, Biochemistry and Single-Cell Analysis
17 – 20 JUL	EMBL Course	Metagenomics Bioinformatics
24 – 27 JUL	EMBO Workshop	Imaging Mouse Development

AUGUST

25 – 28 AUG	EMBL Conference	Transcription and Chromatin
26 – 31 AUG	EMBO Practical Course	Molecular Geobiology
29 AUG 1 SEP	EMBO Workshop	Chemical Biology 2018

SEPTEMBER

2 – 10 SEP	EMBO Practical Course	Cryo-Electron Microscopy and 3D Image Processing
3 – 7 SEP	EMBL Course	Structural Bioinformatics
5 – 8 SEP	EMBO | EMBL Symposium	Principles of Chromosome Structure and Function
10 – 13 SEP	EMBO | EMBL Symposium	Organoids: Modelling Organ Development and Disease in 3D Culture
10 – 14 SEP	EMBL Course	Attacking Open Chromatin with ATAC Sequencing
10 – 18 SEP	EMBO Practical Course	Membrane Protein Expression, Purification and Characterisation (mPEPC1)
16 – 19 SEP	EMBO | EMBL Symposium	The Human Microbiome
16 – 21 SEP	EMBL Course	Genome Engineering: CRISPR/Cas

OCTOBER

2 – 5 OCT	EMBL Course	Introduction to Next Generation Sequencing
3 – 6 OCT	EMBO | EMBL Symposium	The Complex Life of RNA
7 – 11 OCT	EMBL Course	Whole Transcriptome Data Analysis
15 – 18 OCT	EMBL Course	Software Carpentry
15 – 20 OCT	EMBL Course	Liquid Biopsies

OCTOBER

17 – 20 OCT	EMBO Workshop	Experimental Approaches to Evolution and Ecology Using Yeast and Other Model Systems
22 – 26 OCT	EMBL Course	Analysis of High-Throughput Sequencing Data
23 – 25 OCT	EMBL Course	Assay Development for Drug Discovery and Characterisation

NOVEMBER

5 – 9 NOV	EMBL Course	Bioinformatics for Plant Biology
5 – 9 NOV	EMBL Course	Deciphering DNA Methylation
10 – 13 NOV	EMBL Conference	From Functional Genomics to Systems Biology
27 – 29 NOV	EMBL Course	Exploring Biological Sequences
14 – 15 NOV	EMBL Course	Microinjection into Adherent Cells
15 – 16 NOV	EMBL Science and Society Conference	Infectious Diseases: Past, Present and Future
19 – 26 NOV	EMBO Practical Course	Solution Scattering from Biological Macromolecules
20 – 22 NOV	EMBL Course	Microinjection in Zebrafish and Medaka: From Transgenesis to CRISPR
22 – 24 NOV	EMBL PhD Symposium	Game Changers - Taking Life Sciences to the Next Level
27 – 29 NOV	EMBL Course	Digital PCR

DECEMBER

3 – 7 DEC	EMBL Course	Microbial Communities: Modelling Meets Experiments
6 – 8 DEC	EMBL Conference	From Images to Knowledge with ImageJ & Friends

For further details visit our website:
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