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

Cover photo: Wounded area of an organotypic *in vitro* model of human skin 3 days post wounding. Epidermal cells which were stained 24 h post wounding with the red cell tracker CMTPX have been distributed on top of the extending epidermal tongues which have fused in the middle of the wound area to build a neoepidermis. The green occludin staining marks the tight junctions of the keratinocytes which are being shed (purple cells) after occludin peptide perturbation.
Source: Hamamatsu TIGA-Center (Bioquant, Heidelberg University)

welcome note

Esteemed Reader,

Health research in Germany is facing major challenges. Age-related and complex chronic diseases are occurring more frequently and have become commonplace in everyday medical care. This is why research for healthy, active and independent living is a major priority in the Federal Government's new High-Tech Strategy.

A great number of different factors can trigger diseases, and it is not unusual for older people in particular to suffer from multiple illnesses. We must pay individualized attention to patients' complex disease processes if suitable treatments are to be found. Systems biology can deliver some important answers because it links knowledge in the field of molecular biology with methods applied in mathematics and engineering.

The Federal Ministry of Education and Research (BMBF) supports systems biology among others through funding programmes in research on ageing and cancer (GerontoSys and CancerSys). This is making it possible to characterize tumours at the molecular biological level and develop custom tailored treatments for patients. The BMBF's new "e:Med – Paving the Way for Systems Medicine" funding measure is aimed at enabling the systematic study of diseases and preventative measures and accelerating the process of translating basic research into medical practice.

The successful projects featured in this issue of systembiologie.de showcase the great potential of systems biology for medical research. I hope you enjoy reading about them.

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

INTERNATIONAL CONFERENCE ON

SYSTEMS BIOLOGY OF HUMAN DISEASE

JULY 6-8, 2015

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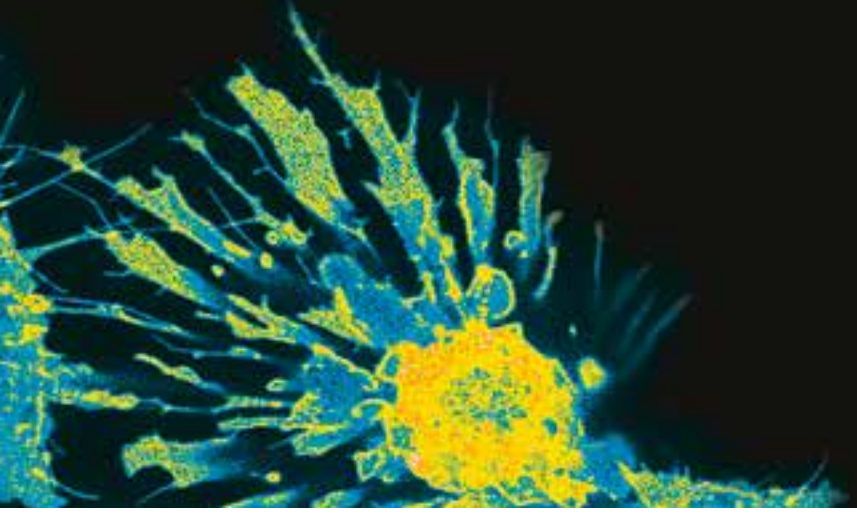

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foreword

We did everything right...

That's what my friends at Phenex Pharmaceuticals AG (see company profile, p. 44) probably thought when we all raised our glasses to a second incredibly satisfying deal within a short period of time. The gesture was not without irony – we were toasting the fact that the nuclear receptor that was bringing in almost $0.5 billion for Phenex was effective at treating fatty-liver disease. As a result, the third cocktail at the party tasted twice as good in view of the fact that fatty liver would soon be caused more often by obesity, diabetes and other metabolic disorders than by excessive alcohol consumption. So, everything done right? This I had to ask myself as well. Didn't I join forces with one of the Phenex founders a few years ago to create a company, one that we just about managed to sell off successfully in the difficult time following the collapse of the dot-com bubble? After that, I returned to the safer shores of public research, while my colleague continued as an entrepreneur and launched Phenex. A good ten years later, I can say, just like my friend at Phenex, that yes, we did everything right!

Fruit sellers have also been doing everything right for centuries. At farmer's markets, you can often marvel at amazing architectural creations: apples or oranges that have been piled high into enormous pyramids. Astronomer and mathematician Johannes Kepler worked out 400 years ago that this was the most efficient way of stacking round objects of about the same size in order to save space. Typical for ingenious conjectures, generations of scholars had a tough time to prove this simple claim. After countless failed attempts, Thomas Hales, then at the University of Michigan, stunned his faculty colleagues by proving the Kepler conjecture. As is standard in the mathematics community, a good dozen of his colleagues then set about trying to find a mistake in his reasoning or to see the conjecture as having been proven. As a result of the unusual, computer-based reasoning, the review process was difficult. After four years of hard work, the process was abandoned partly as a result of exhaustion with the result that people were only 99% sure that proof had been established. This proof was, however, still published in the renowned journal Annals of Mathematics, albeit with a kind of disclaimer that people were not completely sure that the proof would stand up to future scrutiny.

Hales didn't want to settle for this unsatisfactory result. If his colleagues had given up through exhaustion, an inexhaustible computer should take over. Following an independent research project, which lasted over 12 years, the person who originally posited the theory recently announced that the computer found no mistakes in the reasoning. As such, the proof is now to be accepted, he maintained. Whether the mathematics community accepts the argumentation that humans can be replaced by computers as a final testing authority remains to be seen.

Computers have also found a permanent role within systems biology – however, more in the role of a model-based ideas generator for experimental life scientists. There's still a long way to go before a mathematical model on a computer can be accepted as a final scientific testing authority (see also "systems biology modelling: what's next?" on p. 61). What impressed me about this story was also the unshakable determination to reach a goal and the perseverance involved in looking for mistakes in your own work for over a decade. This is a rare virtue in the discipline of the life sciences, which all too frequently hurries from one sensational discovery to the next. Sometimes I long for the good, old days of slow mathematics and I ask myself: did we do everything right?

Well, if you are dedicated to reading about the astonishing reports from the exciting world of systems biology, you can certainly say yes to that! Enjoy reading this issue!

Yours, Roland Eils

Editor in Chief

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how wounds heal

After 40 years, systems biology clarifies wounds' healing mechanism

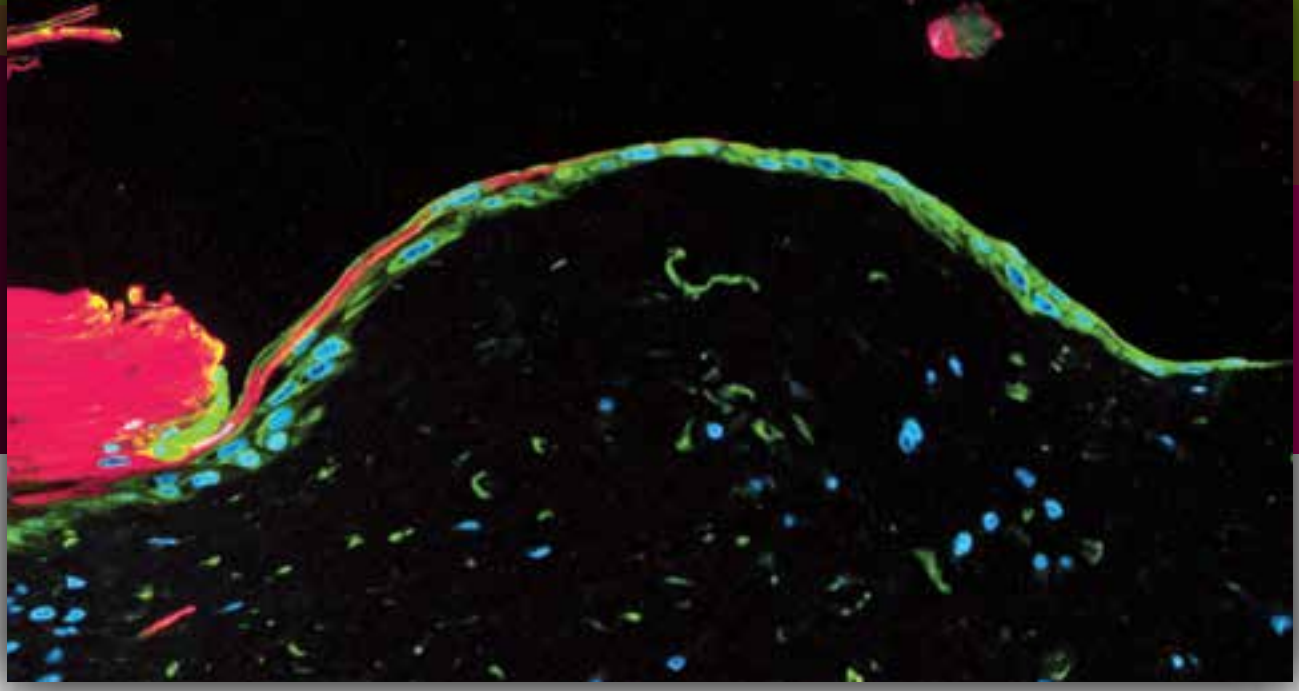
by Kai Safferling, Thomas Sütterlin and Niels Grabe

The skin, humankind's largest organ, surrounds us like a protective shield, protecting us from harmful environmental influences and dangerous microorganisms while also preventing loss of vital water. Without the skin's manifold functions, we would be entirely at the mercy of our environment. Yet for most of the time, we are unaware of all the processes that take place in our skin. That changes, however, and changes painfully, when we are injured and the skin's barrier function is damaged. As part of the MedSys Chronic Wounds project funded by the Federal Ministry of Education and Research (BMBF), a group of research scientists at the Tissue Imaging and Analysis Center (TIGA) in Heidelberg applied systems biology to investigate wound healing and decode fundamental wound closure mechanisms.

Human skin consists of several cell layers that are replenished by a steady stream of newly forming cells. In the process, the individual skin cells, the keratinocytes, migrate from the basal layer to the surface of the epidermis. As they do so, they change their structure and harden, providing essential protection in the uppermost layer of the skin. This protective layer is penetrated if an injury occurs. To close the wound and restore the organism's integrity, cellular processes such as proliferation, migration and differentiation interact closely. The wound healing process can be divided into four phases. In phase 1, fresh blood that emerges from the wound creates a scab on contact with the air. The scab for one prevents the loss of further bodily fluids and for another serves as a reservoir for blood platelets, or thrombocytes. The thrombocytes attract immune cells by secreting messenger substances (e.g. platelet derived growth factor, PDGF). In phase 2, the inflammatory phase, these cells in turn eliminate dangerous microorganisms that have found their way into the wound. They also secrete growth factors that lead to a proliferative burst in the surrounding tissue and to a mobilisation of the keratinocytes. In phase 3, the reepithelialisation phase, these activated cells migrate beneath the scab into the wound and seek to repair the damaged tissue. The immigrant cells form a triangular structure, the extending epidermal tongue: starting from the edge of the wound, it grows steadily thinner and consists of a single cell layer at its tip. In the final wound healing phase, the remodelling phase, the connective tissue that surrounds the wound is remodelled, and after this phase the healing process is complete and a scar is all that remains of the wound.

How exactly is the wound closed?

In spite of the division of wound healing into different phases, many of the cellular reactions and interactions in this complex mechanism have yet to be clarified. A central question that has been unanswered for 40 years is how the keratinocytes in the reepithelialisation phase organise themselves within the extending epidermal tongue to close the wound. In scientific literature, there have hitherto been two fundamental models that sought to describe the reepithelialisation mechanism. One, the tractor tread model, states that the epithelium pushes itself into the wound as a single block to close it, which implies unchanged rigid positions for the keratinocytes in the migration tongue. The other, the leapfrog model, postulates a migration of suprabasal keratinocytes, i.e. the upper layer of skin, via the basal cells of the lower layer of skin to close the wound. To clarify whether one of these two fundamental models is correct, the migration mechanism of the epithelium was investigated by means of systems biology at the TIGA Center as part of the BMBF-funded MedSys Chronic Wounds project. The entire regeneration process of the skin was broken down systematically into the cellular processes of proliferation, migration and differentiation, each of which was measured quantitatively. On the basis of this data, a new multicellular systems biology model to explain the wound healing mechanism was drawn up (Safferling



Cell migration after wounding
According to the organotypic wound healing model, the skin cells (basal cells green, suprabasal cells red) migrate into the wound area to close the wound (Source: Tissue Imaging and Analysis Center).

et al., 2013). This model first shows that the previous wound closure theories are wrong. Instead, the research disclosed a new 3D structure to cellular movement, the extending shield mechanism (ESM), and at the same time revealed the key role played by the intact skin surrounding the wound.

Deep insights into the complex process of wound healing

To quantify cellular mechanisms during wound healing, an *in vitro* wound healing model was developed at the TIGA Center. This model is based on human skin that has the same cellular layering *in vitro* as ordinary human skin. In this model, a circular wound was created and the division of the epithelial cells was quantified by means of the proliferation marker Ki-67. The data shows that after wounding occurs, the model responds with an initial proliferation impulse that activates the basal cells of the entire model. While cell division activity subsides over time in the areas of the model that are far from the wound, it stays at a constant high level in the wound. Due to this proliferation behaviour, the tissue that surrounds the wound generates a sufficient number of new cells that migrate to the wound area and take part in wound closure. Although the proliferation data provides information on the systemic tissue reaction after a wound, many other issues relating to the wound healing mechanism remained unresolved after this initial investigation. How are the cells organised in the extending epidermal tongue? Do they stay in contact with each other during migration or do they migrate into the wound in a loose formation? Are all cells the same or do they perform different tasks during wound healing?

Basal cells as key drivers in wound healing

To clarify these questions about the mechanisms involved, a new kind of fluorescence double staining based on the principle of applying a green and a red dye to the wound in succession was developed. Directly after the wound is inflicted, a green dye (CMFDA) is applied to the wound and collects in the cell membranes of the keratinocytes. This green dye marks the skin cells that immediately surround the wound. As a result of the wound, the cells stained green begin to migrate into the wound. The red dye (CMTPX) is applied on the second day after wounding (Fig. 1). In addition to staining the "old" cells already stained green on the edge of the wound, it stains the "newcomers" from further afield. These staining patterns enable analysis of spatial cell distribution in the extending epidermal tongue. Analysis of the staining patterns revealed an accumulation of both dyes in the upper cell layers, whereas basal cells on the edge and in the middle of the emerging cell tongue showed no staining. These basal cells must therefore have migrated to the wound area from unwounded tissue that was not affected by either of the two dyes. The fact that basal cells migrate actively into the wound while the suprabasal keratinocytes remain stationary in the upper part of the cell tongue disproves the two previously postulated migration models.

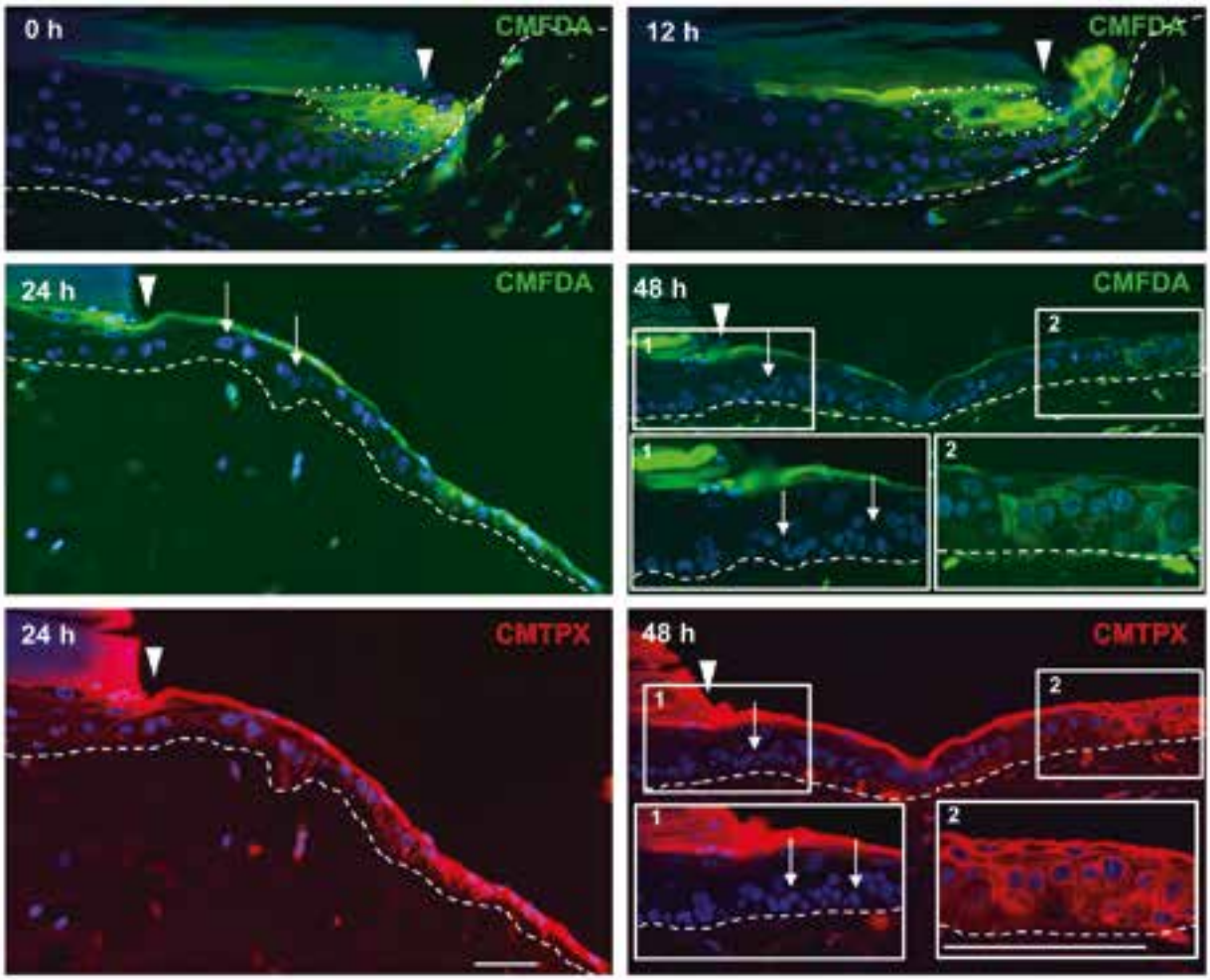


Figure 1: Basal cells migrate into the wound beneath a protective shield of suprabasal cells
To investigate the dynamic spatial distribution of keratinocytes in the extending epidermal tongue, a two-stage fluorescence experiment was undertaken in which, at an interval of 24 hours, first a green (CMFDA) and then a red dye (CMTPX) was applied to the wound. After 12 or 24 hours, unstained basal keratinocytes (white arrows) migrate into the wound while the cells stained green create a protective layer over the migrating cells. The cells stained red after 24 hours also accumulate after 48 hours in the upper layer or on the migration front (the extending epidermal tongue that is taking shape). The experiment thus demonstrates graphically the active migration behaviour of basal cells and disproves the migration mechanisms postulated for 40 years. The white arrows indicate the edge of the wound and the dotted line the basal membrane. Scale bars 100 µm (Source: Tissue Imaging and Analysis Center).

But why do two different cell behaviours exist in the extending epidermal tongue? Analysis of the cellular contacts provided an answer, as the migration tongue was divided into two distinct compartments. While the upper compartment was characterised by the formation of strong and rigid cell connections, the lower compartment was characterised by very flexible, easily degradable cell-cell proteins. These rigid connections give the upper compartment mechanical stability and form a protective shield over the cells beneath them. The basal cells of the lower compartment are extremely mobile as a result of their flexible cell contacts and migrate beneath this protective shield into the wound area in order to close the wound.

The combination of results supplied data for a new kind of model to account for the wound healing process: the extending shield mechanism (Fig. 2). The extending epidermal tongue must be visualised as a dynamic structure that is constantly on the move. While cells at the forefront of this migration tongue migrate into the wound area in order to smooth the way for their successors by restructuring the wound, basal cells press forward from the rear, from unwounded areas. These basal cells coming up from the rear stack on top of each other by means of targeted control of cell-cell connections at the "lifting point" and form a multilayer epithelium. Looking at the triangular structure of the extending epidermal tongue, the lifting point logically marks the point at which the individual cell layer changes into a multilayer epithelium. The extending shield mechanism owes its name to the basal cells that migrate beneath the protective shield of the upper compartment and extend it successively by moving into the upper compartment at the lifting point.

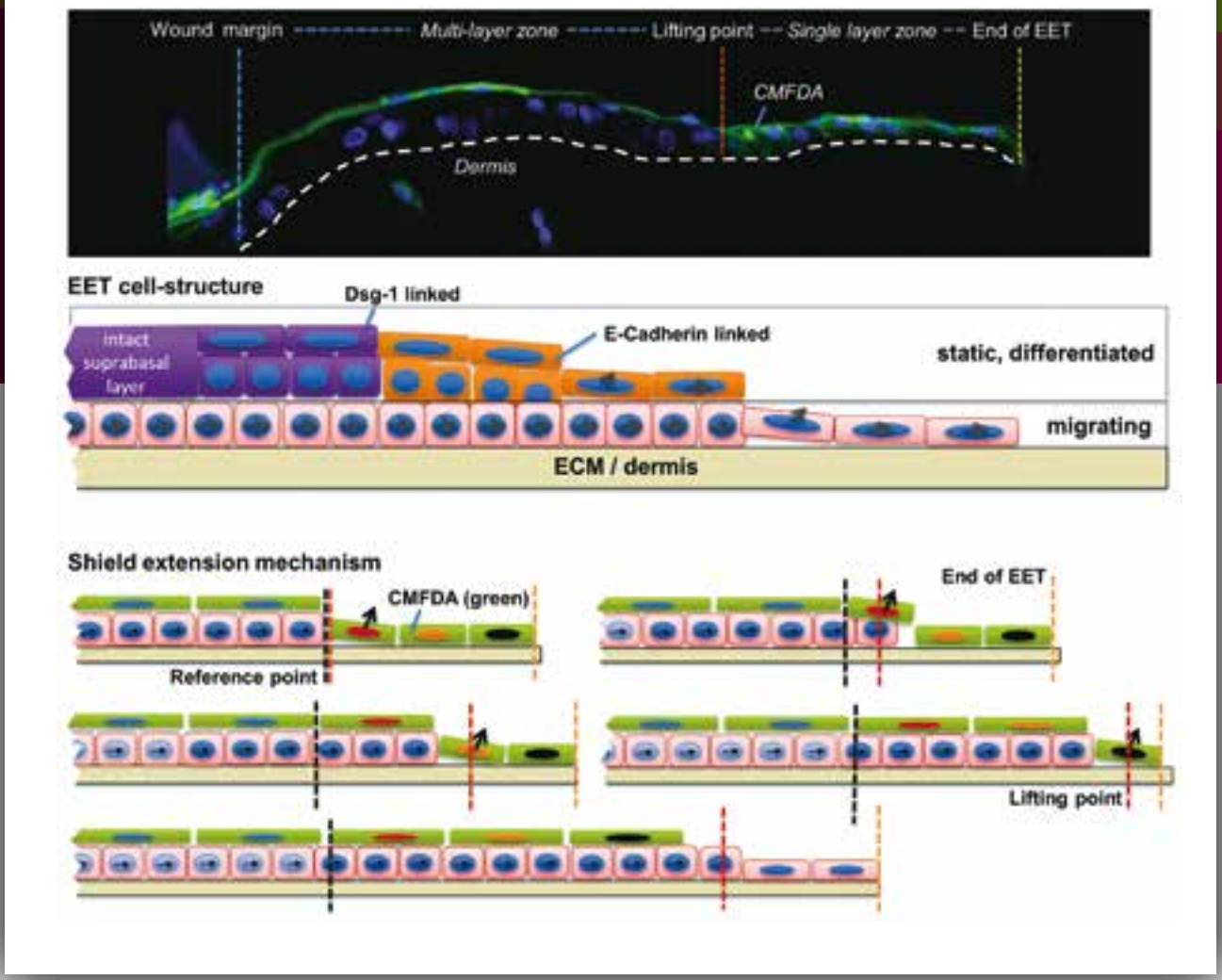


Figure 2: Schematic representation of the extending shield migration mechanism
After the wound is created, two compartments take shape in the extending epidermal tongue: I) a protective, mechanically stable suprabasal compartment characterised by rigid cell-cell connections and II) a dynamic basal compartment that is actively migrating. After the wound has been created, the basal cells migrate into the wound area beneath the protective shield of the suprabasal cells. The cells that migrate into the wound are pushed by the cells behind them into the suprabasal compartment at the "lifting point" so as to extend the protective shield (Source: Tissue Imaging and Analysis Center).

In silico wound healing: modelling the migration mechanism

The extending shield mechanism developed in this manner is based on histological data. This data provides snapshots of the wound healing process that permit statements about possible cellular distribution patterns in the migration tongue but that do not provide dynamic data of any kind. To gain a direct insight into the dynamic cellular migration behaviour during the wound healing process, the *in vitro* model was reproduced *in silico*, based on the experimental data in respect of cellular contacts, proliferation, differentiation and migration integrated in the EPISIM multicellular modelling platform that was also developed at the TIGA Center (Sütterlin *et al.*, 2009; Sütterlin *et al.*, 2013). On the basis of these results, the final *in silico* model contained four specific cell populations with distinct properties (Fig. 3). Every cell in these populations influences itself and the cells that surround it due to the effects of adhesive and intercellular compressive forces. The resulting dynamic cellular behaviour patterns permit statements about the biological migration mechanism. In the *in silico* model, the basal cells migrated beneath the protecting suprabasal cells and were pushed at a certain point by the basal cells that followed them into the suprabasal compartment, thereby extending the protective shield. *In silico* modelling thus facilitated dynamic insights that would not have been possible with purely experimental methods and supplied the last piece of the puzzle for the extending shield mechanism.

Outlook

A wide range of medical interventions can be derived from an understanding of the epithelial migration mechanism during wound healing. By integrating growth factors into plasters and wound dressings, for example, reactions of the surrounding tissue can be controlled and accelerated, leading to a swifter wound closure and a reduced risk of infection and chronic wounds.

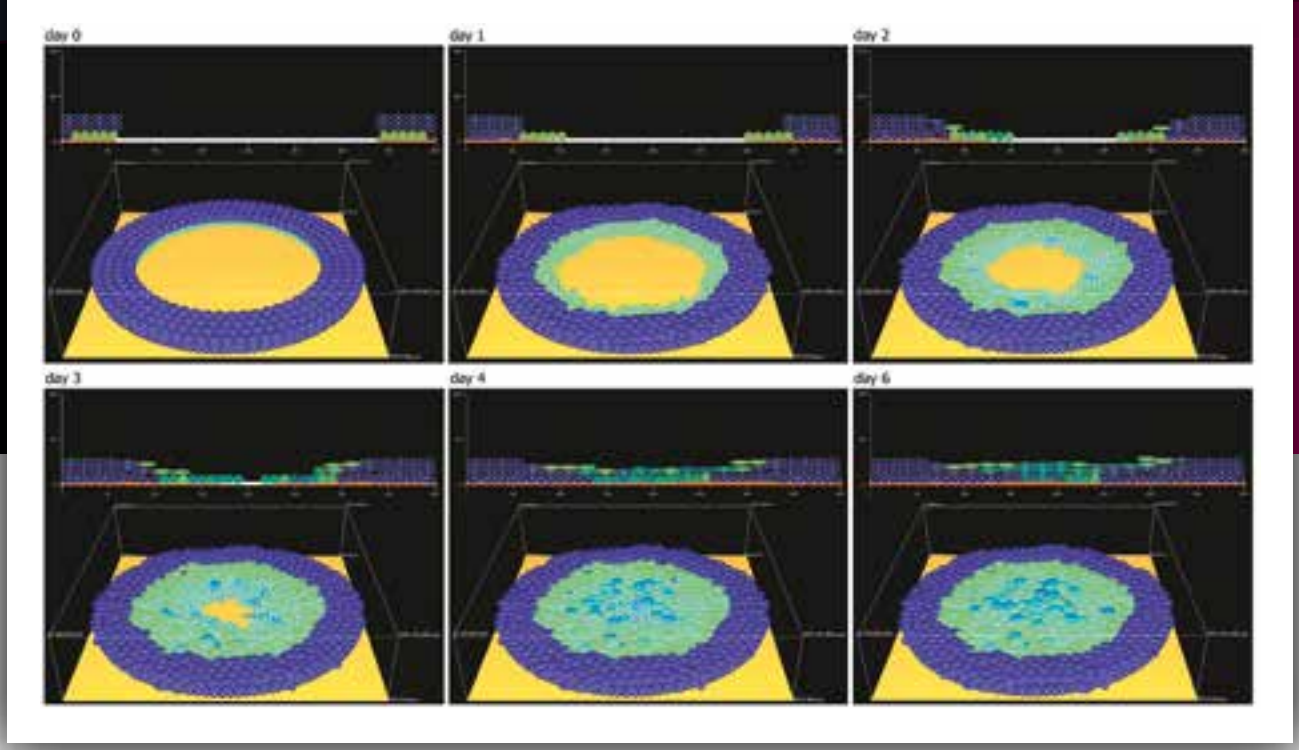


Figure 3: Wound healing *in silico*
The wound healing process can be modelled *in silico* on the basis of the experimental data. The computer model makes a decisive contribution toward dynamic analysis of the reepithelialisation mechanism (Source: Tissue Imaging and Analysis Center).

The research project in brief:

Project name:
MedSys-Chronic Wounds (BMBF consortium)
Participating partners:
Coordinator: Prof. Dr. Peter Angel, DKFZ Heidelberg
Prof. Dr. Petra Boukamp, DKFZ Heidelberg
Prof. Dr. Peter Schirmacher / Dr. Kai Breuhahn, Institute of Pathology, Heidelberg University Hospital
Prof. Dr. Günter Germann, Ethianum, Heidelberg University Hospital
Prof. Dr. Roland Eils, DKFZ Heidelberg
Dr. Jürgen Eils / Dr. Chris Lawerenz, DKFZ Heidelberg
Dr. Hauke Busch, Institute of Molecular Medicine and Cell Research, Center for Biochemistry and Molecular Cell Research, Freiburg

References:

Safferling *et al.* (2013). Wound healing revised: A novel reepithelialization mechanism revealed by *in vitro* and *in silico* models. Journal of Cell Biology *203*(4), 691-709.
Sütterlin *et al.* (2013). Bridging the scales: semantic integration of quantitative SBML in graphical multi-cellular models and simulations with EPISIM and COPASI. Bioinformatics, *29*(2), 223–229.
Sütterlin *et al.* (2009). Modeling multi-cellular behavior in epidermal tissue homeostasis via finite state machines in multi-agent systems. Bioinformatics, *25*(16), 2057–2063.

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controlling cells with light

Perturbing cellular processes with the aid of optogenetics

by Julia Ritzerfeld, Dominik Niopek, Roland Eils and Barbara Di Ventura

The function of many proteins is controlled by dynamic changes in their subcellular localisation. Being able to control protein dynamics in living cells would allow understanding how they trigger specific cellular responses. To this aim, scientists at the University of Heidelberg and the German Cancer Research Center (DKFZ) have developed a new method to translocate proteins of interest in the nucleus of living cells using light as trigger. The new system is called LINuS, just like Snoopy's cartoon pal. LINuS stands for "light-inducible nuclear localisation signal", because the signal is active only when cells are stimulated with light. This system facilitates new studies on intracellular protein movement and is therefore of interest for both basic and applied research. The scientists have recently published their findings in Nature Communications (Niopek *et al.*, 2014).

Many important transcription factors dynamically translocate in and out of the nucleus upon activation by an external stimulus. This pulsatile response can trigger different gene expression patterns depending on the number and amplitude of the pulses (Purvis and Lahav, 2013). "Probing protein dynamics is essential to understand cellular processes" says Dominik Niopek, first author of the study and PhD student at the DKFZ. "Simply switching a protein on or off in a cancer cell is not enough. The time-dependent subcellular localization of cancer-relevant proteins such as the tumour suppressor p53 is just as important and can now be studied using LINuS".

Optogenetics: Controlling cellular processes with light

"Optogenetics, the use of genetically encoded photosensors to steer cellular proteins with light, is becoming an essential tool for cell biologists, because light is the ideal trigger for controlling proteins in individual cells," says Barbara Di Ventura, group leader for Synthetic Biology in Roland Eils' division. In contrast to chemical triggers, light can be applied with enormous spatiotemporal precision, can be easily removed and re-applied. The field has been originally developed to help understand how individual neurons work in the brain. In the seminal paper that kicked optogenetics off, Edward Boyden, Karl Deisseroth and colleagues were able to control neuronal spiking with short pulses of blue light (photostimulation) in well-defined neuronal populations simply by expressing in those neurons the algal light-sensitive ion channel channelrhodopsin 2 (ChR2) (Boyden *et al.*, 2005). Currently, optogenetics relies on natural photosensors, proteins that change their conformation when exposed to light of a specific wavelength converting an external light stimulus into intracellular signals. By extracting the light-sensing domains of photosensors and engineering their connection to functional protein domains, it has been possible to control cellular functions in a highly targeted manner and with almost no side-effects. A prominent example is the construction of photoactivable Rac1, which allows controlling cytoskeleton remodelling and cell movement with light (Wu *et al.*, 2009). A variety of optogenetic approaches have already been used to accumulate proteins within the cell nucleus. However, these previous systems were either relatively slow, irreversible or required exogenous chromophores and were thus not ideally suitable for simulating complex protein localisation changes in individual cells (Crefcoeur *et al.*, 2013; Yang *et al.*, 2013).

Conversion of a plant photoreceptor into an optogenetic protein shuttle service

LINuS, however, enables swift, reversible and tunable control of nuclear protein localisation. As the system is completely genetically encoded it allows for targeting individual cells within a cell population. The fact that the light-absorbing moiety (the chromophore) of the LOV domain does not require external supplementation further facilitates the applicability of LINuS in model organisms, such as zebrafish. LINuS is based on the LOV2 domain from the light-sensitive protein phototropin 1, which in wild oats (Avena sativa) is involved in movement towards the sunlight (phototropism). This plant protein was converted

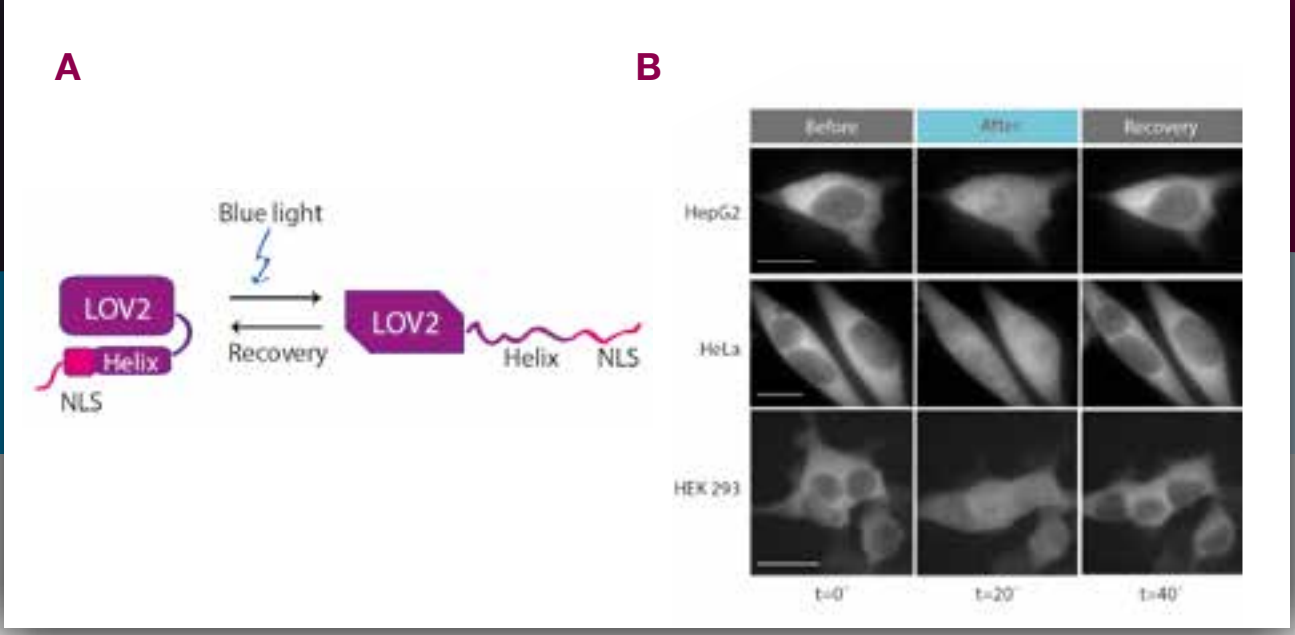

Figure 1:

A) Schematic representation of LINuS function. In the dark, the hybrid Jα helix is folded and interacts with the central LOV2 domain. Blue light leads to unfolding of the Jα helix and rendering the nuclear import signal (NLS) accessible to the cellular import machinery.

B) Localisation of the mCherry-LINuS protein in human cells prior to activation, after activation with blue light and after an additional recovery phase in the dark. (Source: D. Niopek, B. Di Ventura, DKFZ and University of Heidelberg)

into a light-sensitive protein shuttle service that functions in different cell types. A nuclear localisation signal (NLS) that mediates the transport of proteins into the cell nucleus is caged within the modified LOV2 domain in the dark and therefore inactive. In the presence of blue light (450-495nm), the LOV2 domain's C-terminal Jα helix is unfolded. The exposed NLS can now be recognised by the nuclear import machinery, resulting in the transport of the LINuS-tagged protein from the cytoplasm to the cell nucleus (Fig. 1A). Characterisation of LINuS using the fluorescent reporter protein mCherry revealed that LINuS can be used both in yeast and in various mammalian cell lines to transfer the fluorescent signal into the cell nucleus with light. Since in the absence of blue light the LOV domain switches back to the conformation characterized by a docked Jα helix, the NLS is concealed again when cells are kept in the dark. The presence of a nuclear export sequence (NES) ensures that the LINuS-tagged protein accumulates in the cytoplasm in the dark recovery phase (Fig. 1B). Given the reversibility of the system, LINuS-tagged proteins can be accumulated in the nucleus in a pulsatile fashion. Furthermore, the strength of the signal can be varied according to the light intensity and duration, and different versions of LINuS can be "customised" to suit the needs of the protein that is under investigation. This opens up a large number of possible application areas and facilitates research on complex spatiotemporal signals.

An artificial light switch for cell division

With the aid of LINuS, the Heidelberg research scientists can now directly perturb very basic cellular functions, such as cell division (mitosis). In cancer cells, mitosis is usually accelerated and uncontrolled, leading to genetic defects that can support tumour growth or promote resistance to certain drugs. Genetic repair mechanisms are also often incorrectly programmed or even switched off in cancer cells. "In healthy cells, all these processes rely on a complex and well coordinated movement of the corresponding signaling proteins that we can now understand much better," says Roland Eils, who is actively engaged in cancer genome research at DKFZ. To take a closer look at these processes, Niopek, Dräbing and Di Ventura designed cell cycle protein variants with fluorescent tags that can be activated with LINuS. Low concentrations of a complex consisting of cyclin B1 and CDK1 (cyclin-dependent kinase 1) in the nucleus are sufficient to induce early mitotic events such as chromatin condensation, cell rounding and nuclear envelope breakdown, which are usually preceding the division of a parental cell into two daughter cells. Upon irradiation with blue light, translocation of cyclin B1-mCherry-LINuS and CDK1-mCherry-LINuS into the nucleus triggered the cellular transition into mitosis (Fig. 2). Interestingly, the researchers were able to control this process not only with a high temporal but also a high spatial resolution. Only irradiated cells showed early mitotic features, whereas surrounding cells in which the fusion proteins were not activated by light were not affected (Fig. 2). Further disease-relevant signal proteins are already under investigation in the Di Ventura research group.

Synthetic biology as a research toolkit

Optogenetics is only one focus of the Di Ventura research group in Roland Eils' division. For years, the two scientists have been engaged in intensive research in the field of syn-

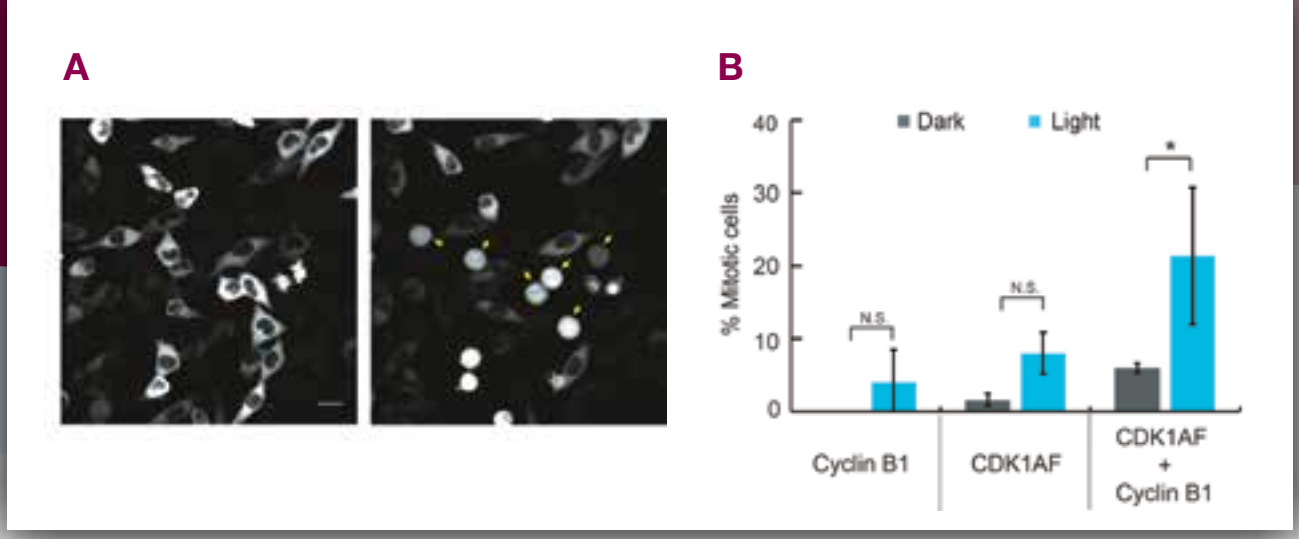

Figure 2:

A) Representative microscopy images of human HeLa cells that express the cyclinB1-mCherry-LINuS and CDK1-mCherry LINuS fusion proteins before (left) and after (right) illumination. Yellow arrows depict illuminated cells showing early mitotic features, including cell rounding and nuclear envelope breakdown.

B) Quantification of light-dependent mitosis induction in cells expressing the cyclinB1-mCherry-LINuS and CDK1-mCherry-LINuS fusion protein before and after illumination with blue light.

(Source: D. Niopek, B. Di Ventura, DKFZ and University of Heidelberg)

thetic biology. This emerging research area uses engineering principles for developing genetic tools to equip cells with completely new properties that do not occur in nature. Using standardised building blocks ("BioBricks"), complex genetic circuits ("devices") are constructed, which can be transferred into organisms with a basic genetic configuration (or "chassis"). Drew Endy, a synthetic biology pioneer at Stanford University, calls this approach "making biology easy to engineer" (www.openwetware.org/wiki/Endy:Research). Promising application areas include biomedicine, biotechnology and environmental engineering. In 2013 and 2014, Eils, Di Ventura, Niopek and their students were the first German team ever to win the world championship in the prestigious iGEM (international genetically engineered machine) competition in Boston – and the first to win the synthetic biology's world championship title twice in succession.

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is punctuality really a virtue?

Temporal variation in the activation of endogenous and synthetic gene expression

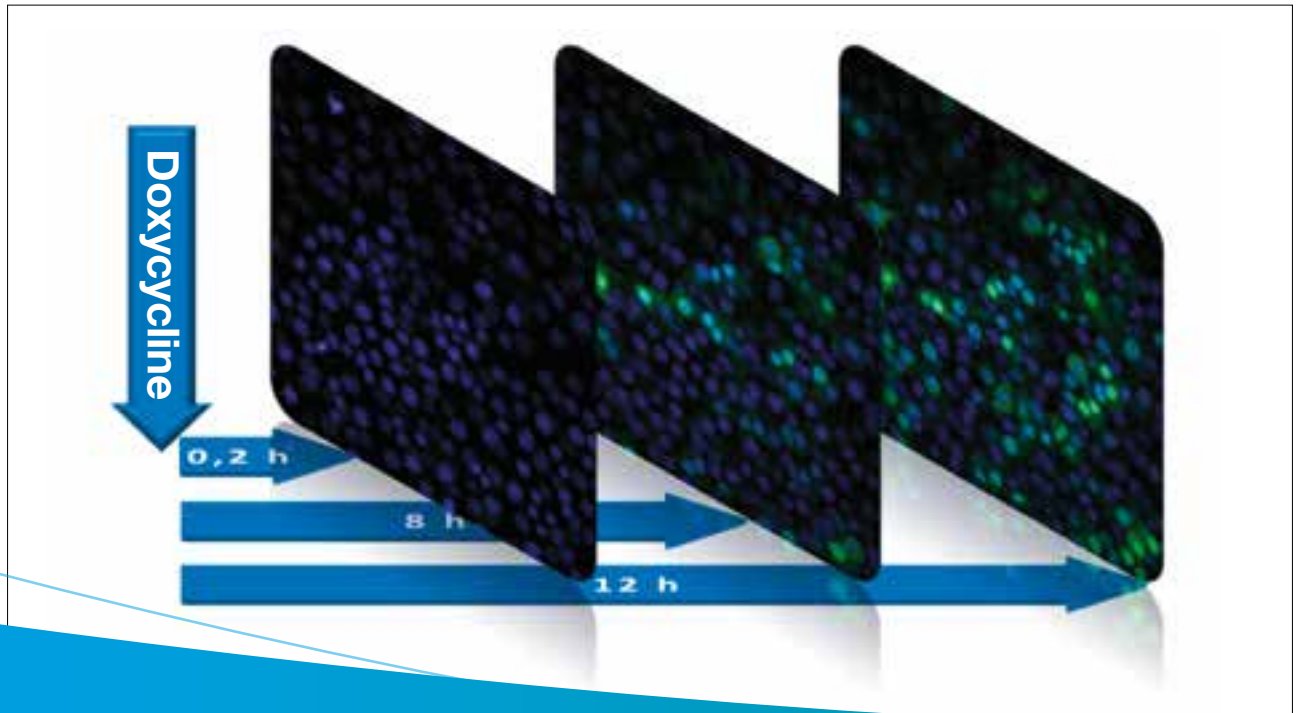
by Ulfert Rand, Hansjörg Hauser and Dagmar Wirth

Activation of cellular genes is controlled by external and internal stimuli. Cells often do not react in the same way, however. Experiments with clonal, i. e. genetically identical, mammalian cells show that some signals lead to gene activation in some cells, whereas other cells fail to react in spite of the same conditions. Even among the cells that react, there is a large heterogeneity in respect of, say, the moment of activation. We will be describing phenomena of this kind in the activation of interferon β (IFN-β) after viral infection and the establishment of antiviral protection by the secreted IFN. The use of synthetic gene expression modules makes it possible to intervene in cellular processes and thereby answer important questions.

Heterogeneity in cell populations by means of stochastic gene activation

Two fundamentally different gene activation patterns are observed in cell population. In gradual activation, the gene activity of individual cells in a clonal population increases with the signal concentration and all cells react uniformly. What is known as bimodal activation, in contrast, is characterised by a part of the cell population showing total activation while the other part does not react – even though all of the cells in the population are genetically identical. With this bimodal reaction, the concentration of the signal molecule determines not the extent but the likelihood that gene expression will be activated (stochastic activation).

Temporal variation in activating a synthetic gene expression module.
Shown here: cells (cell nuclei are marked blue) in which the doxycycline-dependent TetOn module controls the expression of green fluorescent protein (GFP).



Source: Ulfert Rand, HZI

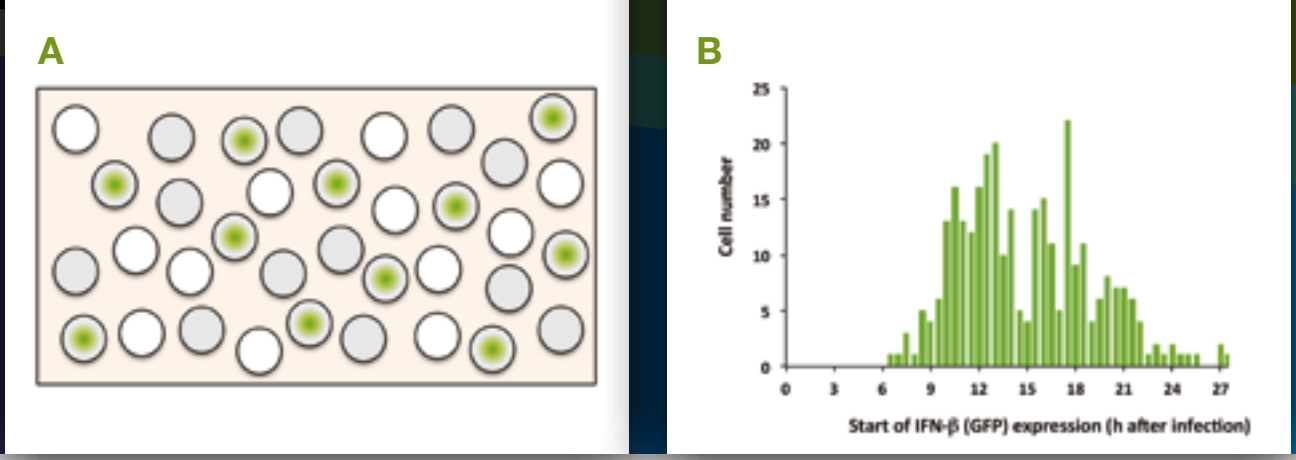


Figure 1: Heterogeneity of IFN-β formation after virus infection

A) Schematic representation of the stochastic reaction of cells to infection by an RNA virus (Newcastle Disease Virus, NDV). If, in the case of an infection, 70 % of the cells of a clonal population are infected (grey), only half of them are able to create IFN (green). Uninfected cells are shown in white.

B) Temporal heterogeneity of the IFN-β response. In the A cells that can be activated, the start of IFN-β promoter activation (the start of IFN-β production) is spread over a period of 6 to 30 hours after infection.

(Source: Dagmar Wirth, HZI; experimental data from Rand *et al.*, 2012. Molecular Systems Biology)

An estimated 15 % to 20 % of genes follow this stochastic activation mechanism. Examples of cellular genes that are activated stochastically are to be found in, for example, the IFN signal cascade. The cascade is activated as soon as a virus infects cells and constitutes a swift and very efficient immune response to viruses. The IFN cascade is activated by viral RNA or DNA and triggers a number of different antiviral immune responses that prevent the spread of a wide range of viruses. After a viral infection, Type I IFN (such as IFN-β) is released. This signal activates an antiviral protection programme that is both autocrine (in the secreting cell itself) and in neighbouring cells and, after distribution via the bloodstream, systemic too.

If the kinetics of activating the IFN-β gene is followed via time-lapse microscopy with the aid of authentic fluorescent reporter cells, it is clear, surprisingly, that the viral induction of IFN-β is bimodal, in other words stochastic (Rand *et al.*, 2012). This means that only some of the infected cells in the tissue activate the antiviral protection programme and secrete IFN-β (Fig. 1A). Furthermore, not only *whether* a cell is activated but also *when* it is activated is not merely random. If gene activation is followed over time, it becomes clear that induction of expression can vary over 30 hours (Fig. 1B and Rand *et al.*, 2012).

What biological meaning might there be in a mechanism of this kind that allows many cells to build up antiviral protection either not at all or only after a delay? That has yet to be fully clarified. It could be that the variability of the population due to heterogeneity is beneficial because the virally induced effects are activated over a longer period. Preventing overproduction of IFN and with it the cytokine's various toxic side effects may also be important. Furthmore, a system biology model based on biological data shows that just a few IFN-β producing cells can be enough to provide antiviral protection for a large population of surrounding cells (Rand *et al.*, 2012). Heterogeneity was also observed in the response to IFN-β: the expression of antiviral genes. This heterogeneity plays a part in determining whether certain herpes viruses infect cells latently or lytically, either remaining inactive in the cell or proliferating in it and thereby damaging it (Dag *et al.*, 2014).

Investing the dynamics of cellular antiviral protection by means of synthetic expression cassettes

How can the methods of synthetic biology help us to understand a) the dynamics of virus suppression by the cellular protection programme and b) the blockade of this programme by viral antagonistic proteins? In the course of evolution, viruses have developed mechanisms by which they block or undermine the antiviral activity of cells. A large number of viral antagonistic proteins intervene in the IFN cascade at different points and interrupt it. We were, however, long unaware of what the dynamics of this race between infected cell and virus looks like. To investigate it, we chose to intervene by means of manipulation, bringing about controlled perturbation of the endogenous IFN signal cascade by introducing modules such as synthetic promoters to control transcription (cf. Botezatu *et al.*, 2012) that can be regulated externally. One of the best-investigated orthogonal (and thus independently activatable) modules is the tetracycline bacterial module with which gene expression can be strictly regulated by an exogenous addition of tetracycline or derivatives such as doxycycline (Dox). By combining synthetic expression modules of this kind with model viruses, fluorescent reports and time-lapse microscopy, we have succeeded in decoupling activation of the IFN cascade from the expression of antiviral proteins and in following the repercussions in individual living cells 'live' (Rand *et al.*, 2014).

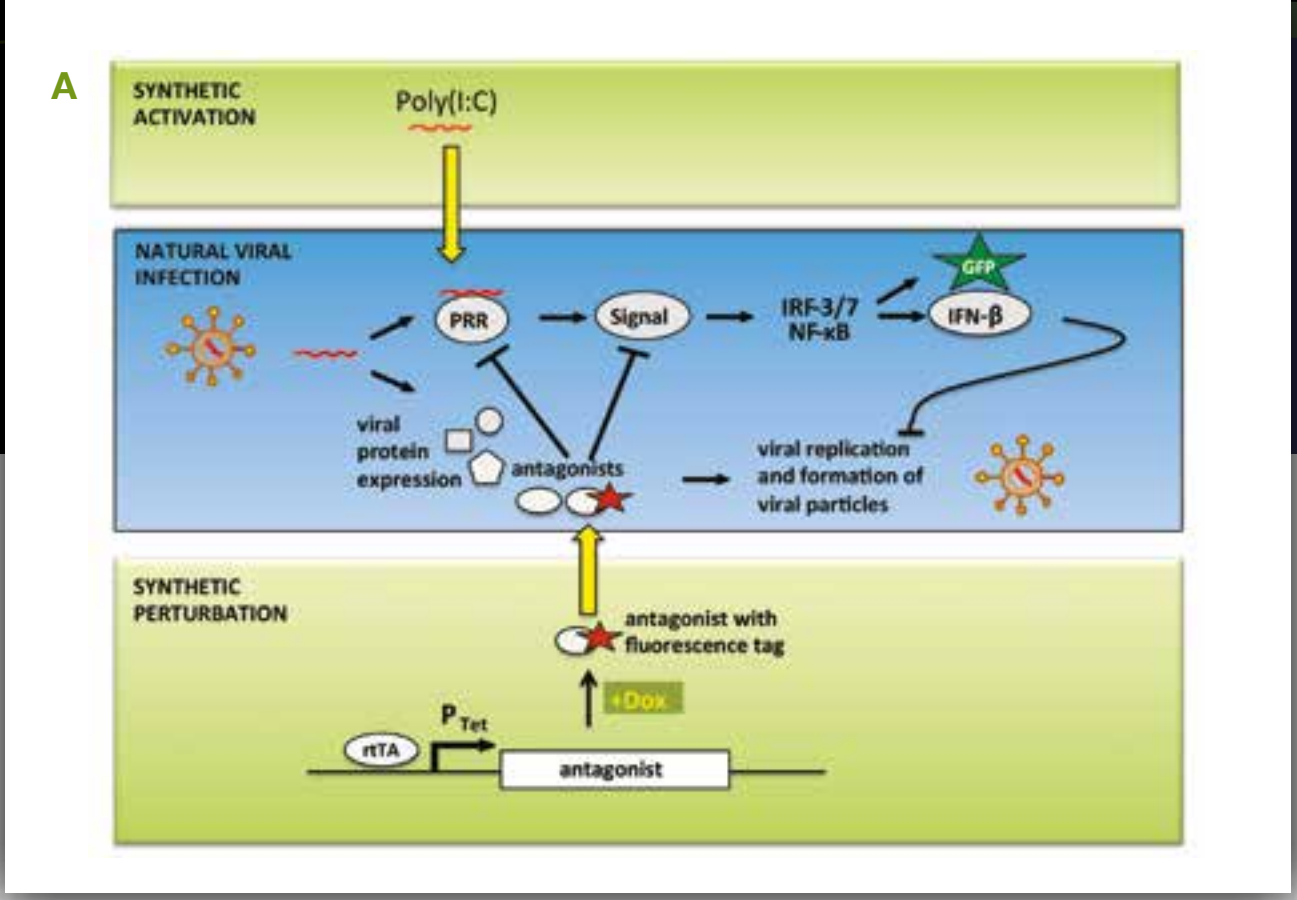


Figure 2A: Synthetic intervention in the interferon system

Blue box: Schematic representation of the virus induction of IFN-β and its autocrine inhibition of virus proliferation. Viral antagonists in turn have an inhibiting effect on the IFN-β signal cascade. The IFN-β reporter GFP leads to green fluorescence of the production cells.
Green box (above): The IFN-β signal cascade can be activated by adding synthetic dsRNA at the PRR level.
Green box (below): Expression of IFN antagonists via a synthetic Dox-dependent module. The antagonists are marked by a red fluorescent protein in order to follow their temporary effect.
(Source: Dagmar Wirth, Ulfert Rand, Hansjörg Hauser, HZI)

To investigate temporal regulation of the IFN cascade, cells were constructed in which both activation of the IFN-β promoters and expression of the viral antagonistic proteins can be followed via fluorescent reporters at the level of individual cells. To do so, we introduced Dox-dependent synthetic modules into IFN-β reporter cells to bring about a controlled expression of antagonistic fluorescent marked proteins (influenza virus NS1 or hepatitis C virus NS3/4A). That enabled us to decouple temporally the activation of the IFN promoter (by means of synthetic dsRNA such as poly(I: C)) and the creation of antiviral protection from counter-regulation by antagonistic proteins (Fig. 2A).

In the process, we observed cells that had both activated the IFN-β promoter and showed expression of the antiviral antagonist. Correlation of the start of expression by the synthetic antagonistic cassette and deactivation of the previously activated endogenous IFN-β promoter led to an important observation: activity of the IFN-β promoter previously stimulated by a virus infection can even be brought to a halt retroactively by antagonistic proteins. This would suggest that IFN-β activation does not occur along "hit-and-run" lines. The promoter needs permanent stimulation to maintain expression. If this stimulus is interrupted by, for example, the expression of viral antagonistic proteins, IFN production is brought to a halt once more. That would seem to indicate that viruses are able to block the IFN cascade with the aid of their antagonistic proteins even when the virus has already been identified by the cell and IFN genes have been activated (Fig. 2B and Rand *et al.*, 2014).

Heterogeneity in activation of synthetic expression cassettes too

Interestingly, we observed in this study that synthetic, Dox-dependent expression cassettes also show a clear stochasticity in respect of the time of gene activation. In a population of genetically identical cells, for example, Dox-activated transcription does not start synchronically but varies over a period of more than 20 hours. Further investigation revealed that this is the case not only with gradually regulated synthetic modules but also applies in principle to auto-regulated modules (Rand *et al.*, 2015). Synthetic modules thus also show temporal heterogeneity in activation.

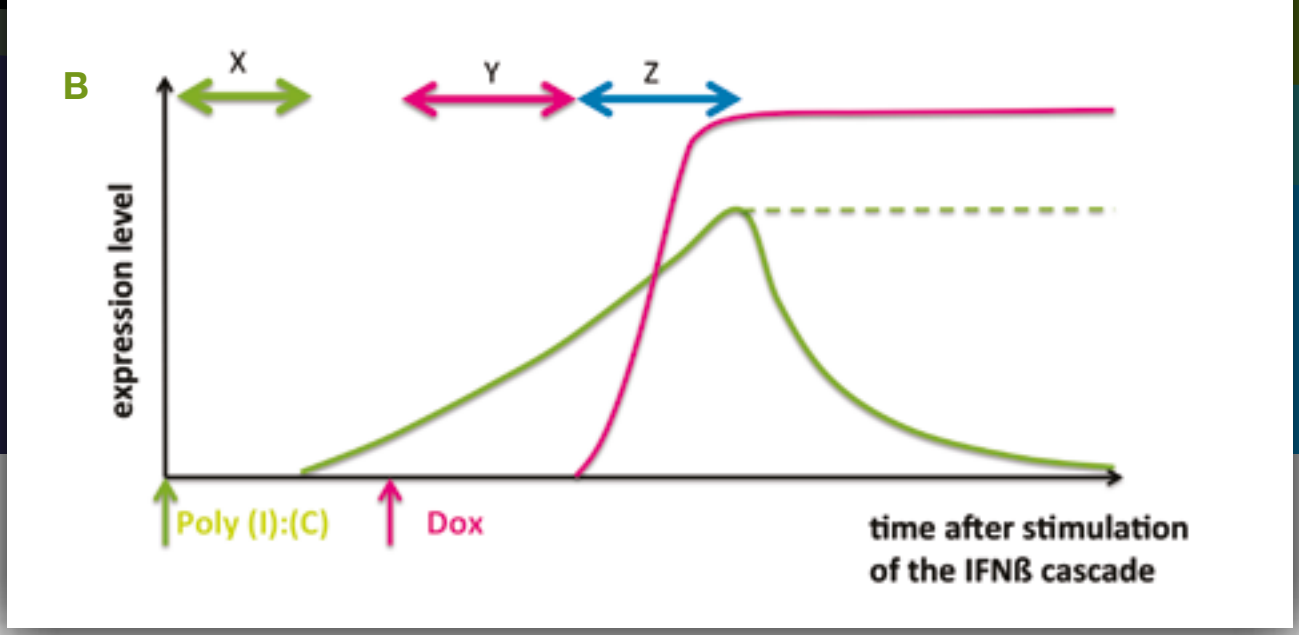


Figure 2B: Synthetic intervention in the interferon system

Schematic representation of IFN-β expression after induction by dsRNA (green line) and Dox-induced expression of an antagonist (red line) in the same cell by means of time-lapse microscopy (live-cell imaging). The time lapse between poly(I: C) stimulation and the start of interferon expression (X, green) and the time lapse between doxycycline administration and the start of antagonist expression (Y, red) are indicated by horizontal double arrows.
The continuous green line shows the course of expression after perturbation by the synthetic cassette, while the dotted line indicates expression without perturbation. The blue double arrow (Z) indicates the time between the onset of antagonist expression and the inhibition of IFN expression.
(Source: Dagmar Wirth, HZI)

The consequence of the heterogeneity of endogenous and synthetic control loops is that a large number of cells must be followed at single-cell level and statistically evaluated in order to reach meaningful conclusions (Rand *et al.*, 2014; Rand *et al.*, 2012).

The random variation in responses by different cells to the same stimulus contributes toward generating an appropriate response at the tissue level. Temporal variation between cells is an important part of this regulation and may, with precise observation, temporally resolved and at the level of single living cells, reveal new and interesting mechanisms.

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supporting young systems biologist

Three young research scientists look back

Never wanting to be boxed in, some people start looking beyond the horizons of their own disciplines almost from the outset of their careers. Interest alone is not enough, however, and interdisciplinary projects take time and money. To help young systems biologists take this route, the Federal Ministry of Education and Research (BMBF) has funded 22 groups of young research scientists for up to five years with its "FORSYS – Forschungseinheiten der Systembiologie" (FORSYS – Research Units in Systems Biology) initiative. Support for young research scientists continues to be an integral part of systems biology funding, for example in the form of the current programme, "e:Bio – Innovationswettbewerb Systembiologie" (e:Bio – Systems Biology Innovation Competition). Three young scientists of the FORSYS programme take a look back to their beginnings in systems biology and explain what fascinates them about this area of research.

The medical doctor:

"Modelling subjects our hypotheses to merciless scrutiny"

systembiologie.de: *What can systems biology accomplish in your research?*

Prof. Dr. Bernd Schmeck: My area of specialisation is pulmonary diseases. These include allergies such as asthma, infectious diseases such as pneumonia and environmental diseases such as COPD, known colloquially as "smoker's lung". All of them are triggered by an inflammatory reaction in the lungs. We developed experimental models for these conditions and looked into regulatory mechanisms. It is important to understand the point or points at which an inflammatory reaction gets out of hand after having previously been normal and beneficial. That is where we want to take a systems biology approach to developing new therapies.

What fascinates you about systems biology?

The fact that it gives us totally new ways of working that we can use to sound out our patients' complex clinical pictures. On the computer, we aim to follow developments within the body exactly as they occur in nature. In the process, we frequently find that things don't work in the way we had previously imagined. Computer modelling is a rigorous – even merciless – way of checking our hypotheses. It shows us where we were too uncritical with our suppositions and where we need to adopt a new approach.

Were you able to acquire a taste for interdisciplinary work right away?

I always linked my medical training with experimental research. In the process, I increasingly came across areas that I could no longer understand using intuition and simply methods alone. I first came into contact with systems biology as a young postdoc: starting out, I had to learn how a mathematician tackles problems, for example. The experimenter must understand what drives the modeller and the modeller must develop an understanding of biological problems. This basic principle is a constant challenge.

The FORSYS initiative was also intended to promote the ability of scientists in different fields to networking with one another. What role has this funding played in your career?

For me personally, FORSYS was a crucial source of support. As a young head of a working group, I could never have taken on such a risky and complex project without the funding and the network to support me. Interdisciplinary collaboration does not, after all, lead to publishable results in a matter of months. Systems biology projects require a long-term perspective and wide-ranging cooperation. Furthermore, the group of young scientists gave me an enormous impetus and, in the final analysis, enable me to work on my projects at an institute of my own.

Bernd Schmeck (Photo: 5D fotografie, Thorsten Doerk)

What advice would you give to young research scientists who want to move into systems biology?

In my view, there is no ideal way to go about it. Speaking personally, my passion is transferring the findings from systems biology research to medical practice. That is why it is hugely important to get doctors and medical students enthusiastic about the discipline. But medical training is subject to enormous economic pressures: the main focus is on training general practitioners who are to treat the 100 most frequent illnesses in the least expensive way possible. That is not exactly an environment that invites you to deal with innovative approaches that involve financial risks. I believe that systems medicine will in future be able to achieve much more, including a cost-effective diagnosis and therapy, but for that we need to think in terms of a ten-year timescale.

Where do you see systems biology in ten years' time?

Systems biology will become increasingly commonplace, much like molecular biology, which is now no longer a separate subject but an integral part of nearly all lines of medical and biological research. Both further technological development and the creation of a community will lead to more and more projects that feature systems biology components. In many areas, systems biology is already a sure-fire success. In systems medicine it will take longer.

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The biologist:

"Many biology students are afraid of maths"

systembiologie.de: *How did you find your way to systems biology?*

Prof. Dr. Anke Becker: Looking at the interplay between individual cell processes as a whole was what attracted me to genome research. To then go into systems biology was, for me, the natural way to progress. When you look at genome transcription, for example, you will find that it in no way proceeds in as orderly a manner as you may have assumed. A stochastic view of the overall system is necessary for understanding the processes, and to reach this, we biologists need theoreticians who take our data and study the issues in the right way.

You are referring there to interdisciplinary cooperation in systems biology. As a biologist, how did you feel about it?

I had previously worked with bioinformaticians. We had to approach each other and learn to understand each other's languages. Years later, we were able to jointly develop ideas that one discipline alone could not have produced. I was unable to transfer experience from bioinformatics to systems biology, however. The familiarisation process took as long as beforehand, but this time it was with mathematicians and physicists. After three years of FORSYS funding, it slowly transpired that we could achieve something together. Today I have a successful DFG project with the same mathematicians from Freiburg.

What role has FORSYS funding in general played in your research career?

A major one. During the funding period, I switched from genome research in Bielefeld to systems biology in Freiburg.

Anke Becker (Photo: FRIAS, University of Freiburg)

There, I was able to expand my interdisciplinary environment and make important progress in cooperation with modellers. I don't think that I would have been offered my position at the LOEWE Center for Synthetic Microbiology without that background. The combination of a basic training in microbiology with expertise in bioinformatics and cooperation with modellers was crucial for my career. I can't imagine synthetic biology without systems biology. If I have to understand a system that I want to change or to integrate into another organism, I can only do that by collaborating with modellers.

There are now even special degree courses in systems biology. Do you see them as the ideal way to get into this area of research?

The problem is that systems biology is highly interdisciplinary. Many biologists are afraid of maths. A student studying for a first degree in biology has usually opted for the subject because they wanted as little as possible to do with mathematics, but any biologist who wants to go in for systems biology needs at least some affinity for maths. Interdisciplinary study programmes should not make the mistake of wanting to train students to be experts in experimental biosciences and modelling at the same time. In most cases, this produces students who are not really capable of either. An interdisciplinary degree course would do better to promote individual strengths and, by teaching the basics in both areas, promote interdisciplinary communication skills.

Where do you see systems biology in ten years' time?

I hope that systems biology will by then be a fixed part of research into biological systems at the cellular level. That will only be possible if we biologists are able to supply appropriate data, and that continues to be a major problem. We frequently lack the techniques, or else generating data over a reasonable period is far too expensive. That is why projects can often only start if a great deal of experimental data is already available, and that can sometimes take several years.

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A brief outline of FORSYS funding

The BMBF initiative "FORSYS – Forschungseinheiten der Systembiologie" focussed on two main objectives. For one, the systems biology infrastructure was to be developed as a way of uniting teams of interdisciplinary research scientists under one roof, as it were. For another, young research scientists were to be funded with a view to permanently strengthening the growing systems biology community in Germany. From 2007 to 2011, the BMBF financed the creation of four FORSYS centres in Potsdam, Freiburg, Heidelberg and Magdeburg. Funding totalled €45 million, and it also included grants for ten groups of junior scientists.

In addition, as part of the supplementary FORSYS Partner programme, a further twelve teams of young research scientists received around €14 million in funding. This support was important in helping many of the young scientists to find their way into systems biology and continue research in the field. Following the success of this, the scheme was expanded to include groups of young research scientists in the current research programmes "Systembiologie für die Gesundheit im Alter – GerontoSys" and "e:Bio - Innovationswettbewerb Systembiologie".

Photo: Julio Vera-González (left) in a discussion with colleagues (Photo: Julio Vera-González).

The physicist:

"In systems biology, you become a discoverer"

systembiologie.de: *Do you feel there is an ideal way of entering systems biology? And if so, what is it?*

Prof. Dr. Julio Vera-González: In my view, it would be good if young systems biologists gained a basic knowledge of modelling or molecular biology during their master's course or in the first year of their PhD studies. Sadly, I have frequently found that young experimental scientists have very limited prior knowledge of mathematics. Among the theoreticians, in turn, many know too little about molecular biology, but they can make much more headway with their models and may even understand how the experiments are conducted. In Germany, there are now a number of outstanding master's degree courses in systems biology. I feel that the new generation of systems biologists will therefore be able to avoid many difficulties that we had in our early days.

What fascinates you about systems biology?

A great deal of research has been undertaken in most scientific fields, so the basics are well known and the methods are established in these areas. The likelihood of contributing something toward progress in them is therefore very slight. In systems biology, in contrast, you can still conduct research in so many new areas. You become an explorer, a discoverer!

Has BMBF funding played an important part in your career?

FORSYS funding was very important for me. It enabled me to embark on systems biology cancer research and to find partners. I have established permanent cooperation with a number of young scientists from the programme. I also thought it was very good that there was a prospect of long-term funding for a period of five years: if you are setting up a group and would like to make genuine progress, you need time. Three years would definitely not have been enough.

Systems biology has now put its teething troubles behind it. Is targeted funding, even of groups of young scientists, still necessary?

I think that funding groups of young scientists should continue. For a young research scientist, it is difficult to gain access to conventional project funding because you often lack the references that are required. Promoting young scientists also always attracts new, young people with a wealth of fresh ideas. That keeps the field vibrant and alive.

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Interviews by Melanie Bergs and Gesa Terstiege.

e:Med – establishing systems medicine in Germany

Systems medics meet in Heidelberg to found a new network

by Silke Argo

e:Med has set its sights on no less a goal than establishing a systems medicine network in Germany. e:Med is a new "child" of the Federal Ministry of Education and Research (BMBF) and involves a host of participants: clinicians, biologists, mathematicians and IT specialists. Gathering for the kick-off meeting in November 2014, they formed a shifting pattern of people in the "glasshouse" foyer of the DKFZ Communication Center in Heidelberg. Partners of many years' standing congregated in clusters and closely-knit groups, scientists in new research partnerships introduced their teams and their projects, while researchers moved between groups, posters and the buffet. These people are part of a highly motivated community that is setting out to open up a new scientific field.

But what is systems medicine exactly? What is its objective? And why is systems medicine so important that an entire funding concept is dedicated to it?

Systems medicine – Putting Big Data to work for the patient

The swift pace of technical progress and increasingly precise methods of conducting analyses with digital data processing are helping to generate larger and larger amounts of medical data from genetic material, proteins or metabolic products. In current high-throughput approaches, sophisticated technical methods are used to investigate enormous numbers of samples simultaneously and at very high speeds. The amount of resulting data is gigantic, and it is increasing day by day.

To ensure that this inundation of Big Data can actually benefit the patient, medics and biologists are joining forces with experts in computer science and mathematics. Their aim is to quantitatively and chronologically record the complex molecular processes that determine bodily functions and the development of diseases. The systems biology approach – using a combination of laboratory experiments and computer models to analyse data – plays a central role in this. That is not all, however: increasingly, scientists are also aiming to gain an understanding of pathological processes that encompass a whole range of different diseases. With this approach, systems medicine is a chance for offering improved treatment and prevention to the patient.

e:Med – Modular, flexible, future-oriented

The hall was full as Andreas Weller of the DLR project management agency that is supporting the BMBF project funding presented the structure and objectives of e:Med at the official opening. Emphasising the importance of systems medicine for understanding many diseases, he voiced his belief that e:Med would provide a crucial stimulus for a systems medicine network throughout all of Germany. Markus Nöthen of the University of Bonn, one of the speakers of the e:Med Project Committee, stressed that e:Med, as a modular funding concept, had come at the right time and that further funding was urgently needed to ensure that Germany developed an internationally competitive systems medicine network.

The e:Med community at the Kick-off Meeting in November 2014, DKFZ Heidelberg (Photo: e:Med).

e:Med consortia get off a good start

The more than 230 participants had a welcome opportunity to gain an overview of projects at this internal e:Med meeting. Fourteen consortia and the first of nine junior research alliances were presented and described by their coordinators. Only a few aspects can be taken up here...

Using an integrated approach, the e:Med consortium **PANC-STRAT** aims to personalise treatment of pancreatic cancer. Computer-assisted modelling is being combined with patient-based tumour models. Roland Eils of the DKFZ and the University of Heidelberg explained the Omics-based approach to investigating pancreatic tumours and their liver metastases by means of parallel research into individual patients' tumour-initiating cells.

Transplantation and cancer medicine are the focal points of **SYSIMIT**. Friedrich Feuerhake of the Hannover Medical School (MHH) said that they had in common an immune response visible under the microscope. Until now, however, these responses have been insufficiently recorded. Now, the consortium is using the latest methods in automated image processing and mathematical modelling for dynamic processes to include temporal and spatial factors in the assessment of microscopic findings – and make use of them for early detection (see SYSIMIT article on page 28).

CAPSyS scientists deal with serious cases of pneumonia. Markus Löffler of the University of Leipzig explained how systems medicine will be used to analyse data and patient

e:Med – A new research and funding concept

e:Med is a new research and funding concept established by the Federal Ministry of Education and Research (BMBF). e:Med stands for the electronic processing, mathematical modelling, and integration of medically relevant data from various knowledge levels in systems medicine. The concept consists of five modules, and the ministry is providing €200 million in funding for an initial eight years. In module I, fourteen systems medicine research consortia are working on specific issues at 42 research facilities in 28 German cities and three universities in other countries. In Module II eight "demonstrators for an individualised medicine" started their work in 2015. These pilot projects of systems medicine are investigating different diseases and preventive measures by close interaction of life sciences and information sciences. The demonstrator projects aim to show how data from high-throughput research can directly enhance individualised prevention, diagnosis and therapy. In nine "junior research alliances" of Module III, three to five young scientists interdisciplinary work on medical questions of different diseases. e:Med module IV, Future-oriented and Cross-cutting measures, will facilitate a flexible response to innovative requirements and currently constitutes an interface with other BMBF initiatives such as de.NBI and i:DSem. Module V, Internationalisation, deals with participation in important international projects such as ICGC, IHEC, ERA networks and CASyM. At the same time, the BMBF is funding projects on ethical, legal and social aspects of systems medicine.

material from three German study groups to identify new signatures that indicate an impending failure of the barrier between the lung and the blood vessels.

More than 50 new genetic loci and lifestyle factors that are associated with heart attacks or strokes have been recently identified. Jeanette Erdmann of the University of Lübeck and spokesperson for the e:Med Project Committee reported on how **e:AtheroSysMed** is analysing genetic and lifestyle data by means of various Omics technologies. The objectives of this consortium are to discover therapeutic target structures, develop better forecasts of personal risk and put newly developed algorithms and tools into clinical use.

Hepatocellular carcinoma (HCC) patients are the focus of **Multiscale HCC**, said Bernd Pichler of Tübingen University Hospital. This interdisciplinary consortium combines the results of multiparametric imaging and Omics methods with the findings of clinical examinations, and it develops or refines mathematical models of tumour development. They are used to examine and optimise dosage regimens for combination therapies.

Systems medicine – An innovative field for lateral thinkers

Networking is of tremendous importance. Full of enthusiasm and dedication, e:Med members attend inaugural meetings of project groups to discuss methods, technologies and scientific content with colleagues and to launch initiatives. The poster session, followed by the evening get-together, is an event that encourages scientists to take part in relaxed, lively discussions with one new group after another. While some ideas are shelved, others are born. More and more, this community of lateral thinkers does interlink, and we can expect a lot from it in the years ahead.

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workshop on

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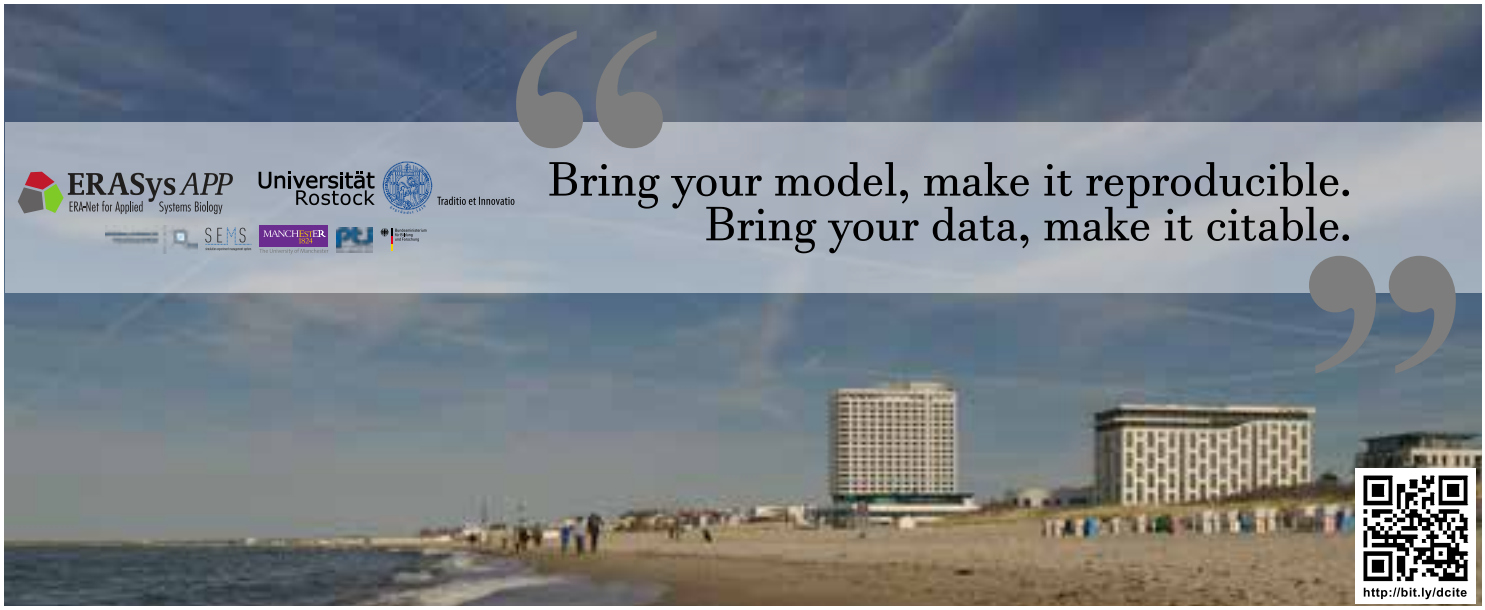


NEW DATE AND LOCATION

The 16th International Conference on Systems Biology

ICSB-2015 Singapore

23 - 24 November @ Biopolis

25 - 26 November, Workshops/Tutorials @ Fusionopolis

For details: http://www.2015icsb.com/

Jointly organized by

Agency for Science, Technology and Research (A*STAR), Singapore
Japan Science and Technology Agency (JST)

lymphatic tissue where it doesn't belong

Mathematical models for the development of tertiary lymphoid structures

by Michael Meyer-Hermann and Friedrich Feuerhake

The human immune system is highly complex. An enormous number of different cells and proteins make up our immune defences against bacteria, viruses and other intruders. Lymphatic organs are specialised in generating cells for our immune system, as well as in training them and mobilising them in the event of an emergency. Systems medicine's research approaches can contribute toward a better understanding of the role that lymphatic organs play in the development of diseases. This article provides an insight into the mathematical modelling of immunological processes.

Immune system cells are present around the entire body. Originating in the bone marrow, they create organs at various points in the body where they mature and specialise. A finely tuned system of reciprocal activation and genetic processes results in highly specialised cells that are ready for action. They produce antibodies, kill cells that are a threat for the body, or trigger a tightly regulated immune reaction.

The body can form new lymphoid tissue

There are primary, secondary and tertiary lymphoid organs. Immune cells are created in the primary lymphoid organs. Lymphocytes are formed in the bone marrow and then proliferate and mature. Selection of effector and regulatory T cells takes place in the thymus. Secondary lymphoid organs (SLOs) are the interface between potentially pathogenic molecules and the immune system. Immune cells come into contact with pathogens in the lymph nodes and lymphoid tissue of the mucous membranes and the spleen, and they are prepared for organised defence responses. Tertiary lymphoid organs (TLOs) are similar to SLOs in structure but originate at places in the body that are not really part of the lymphatic system. TLOs are frequently associated with autoimmune disorders and originate in the synovial tissue (joint lining) in the case of rheumatoid arthritis, and in the meninges in the case of multiple sclerosis (Pitzalis *et al.*, 2014). Funded by the Federal Ministry of Education and Research, the SYSIMIT consortium is studying a range of issues, including whether TLOs play a role in organ rejection after a kidney transplant.

Secondary lymphoid organs: A dynamic equilibrium of cells

In SLOs, the cells are not arbitrarily distributed: instead, they are grouped. T zones are dominated by dendritic and T cells. Follicles are oval structures dominated by B cells with T zones adjacent to their "south poles". In histological sections this may appear like a static structure; however, in reality it is a highly dynamic system of constantly immigrating and emigrating cells. On average, a cell spends ten hours in the lymph node. The "snapshots" of SLOs as provided by a microscope may look like a stable structure, but represent in reality the result of a flow-equilibrium of motile cells.

Self-organisation of stable structures

There has been speculation as to whether the structures in the SLOs are predetermined by the extracellular structure in the lymph nodes. The *Delaunay Object Dynamics* mathematical model demonstrated that motile cells on an amorphous background are able to create these stable structures (Beyer *et al.*, 2007). That indicates that the lymph node does not predetermine any fixed structures. The structures are actually self-organised by cellular interactions. If you think a step further, this means that similar structures must be able to self-organise elsewhere.

What are the minimal preconditions for lymphoid organs?

Researchers have identified signals that are needed for the development of SLOs. It is clear that these interactions must include threshold values and feedback loops to make self-organisation possible. Using the mathematical SLO model,

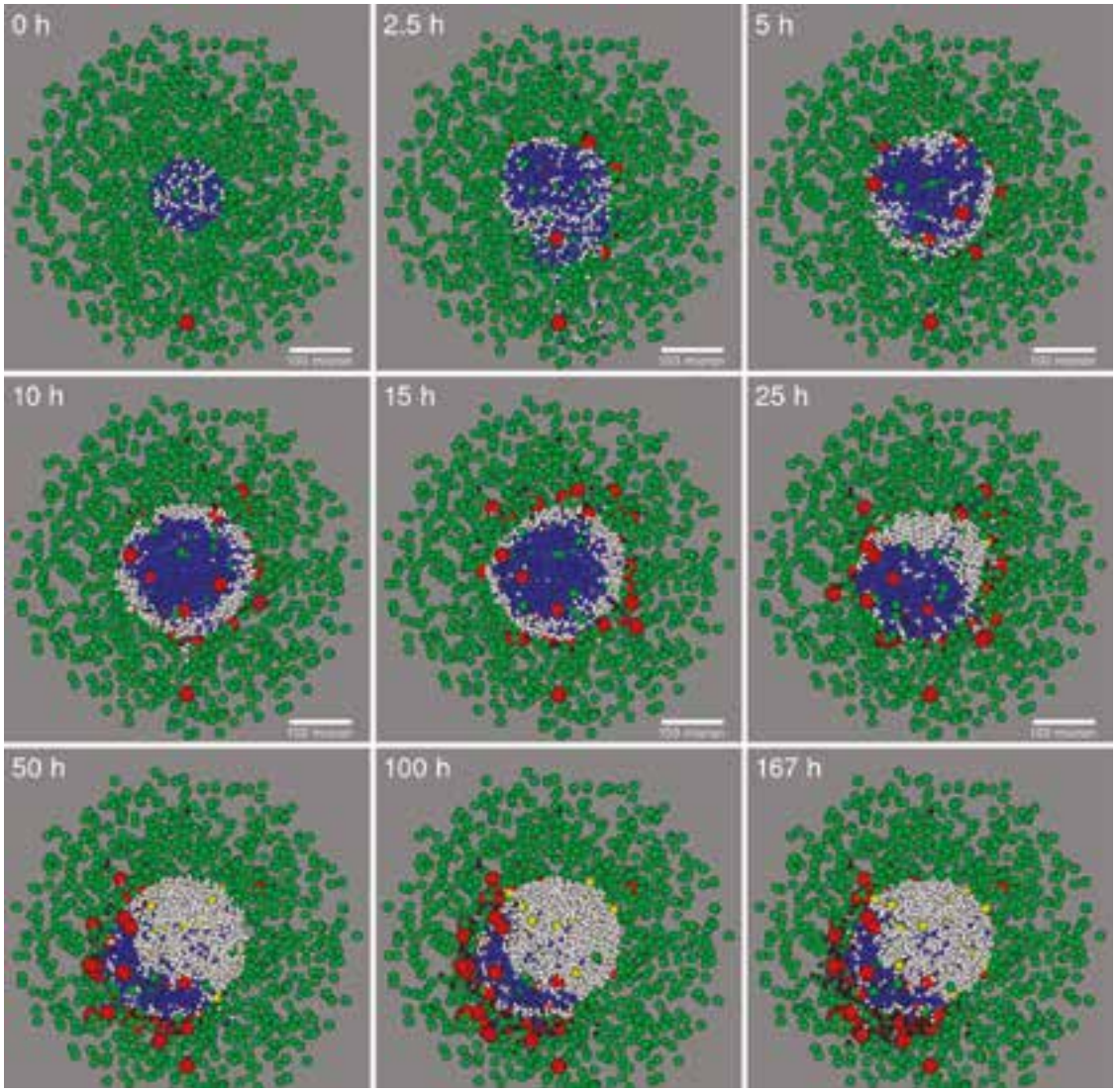


Figure 1: Cross-sections of a three-dimensional simulation of the development of lymphoid structures using Delaunay Object Dynamics
Initially, B cells (white) and T cells (blue) are intermixed on a background of stromal cells (green). T and B cells immigrate via high endothelium venules (red) and follow a dynamically generated distribution of chemokines (not shown) that attract the B and T cells. The cells leave this area via lymphatic vessels (dark grey). Stromal cells differentiate themselves via interaction with B cells into chemokine-producing cells (yellow). A transient shell structure takes shape, and it then leads to a stable flow-equilibrium with a realistic separation of T zone and B cell follicles. Times are shown in hours (h). The scale corresponds to 100 micrometres. Parts were previously published in: Meyer-Hermann, M. (2008). Delaunay-Object-Dynamics: Cell mechanics with a 3D kinetic and dynamic weighted Delaunay-triangulation. Curr. Top. Dev. Biol. 81, 373-399.

a set of minimal preconditions for their development was formulated (Fig. 1, Beyer *et al.*, 2008a). One aspect of this is the interaction of migrating cells and the static stromal background in the lymph node. The migrating cells exchange signals with the stromal cells and prompt them to produce chemokines that attract further migrants. This self-reinforcing process attracts more and more cells. Analogously, negative feedback regulates the size of the follicle that takes shape.

Structures on the borderline of instability

The simulated SLOs have a stable form and display the flow-equilibrium of immigrating and emigrating cells that is known to exist in genuine lymph nodes. Can the stability of the dynamic structure be disturbed? Starting with a single chemokine source that is an attractor of the simulated cells, a computer experiment calculated the diffusion of the chemokines. Furthermore, the chemokines are bound and internalised by the cells' receptors. That acts back onto the chemokine distribution and changes the sensitivity of the cell to the chemokine. The astounding result of this experiment is that the cells do not arrange themselves spherically and symmetrically around the point source but are in a state of constant fluctuation (Fig. 2, Beyer *et al.*, 2008b). This is an example of a multiscale effect because the internalisation of receptors has an effect on the organisation of cells into a tissue structure. Its shape changes constantly. To suppress these

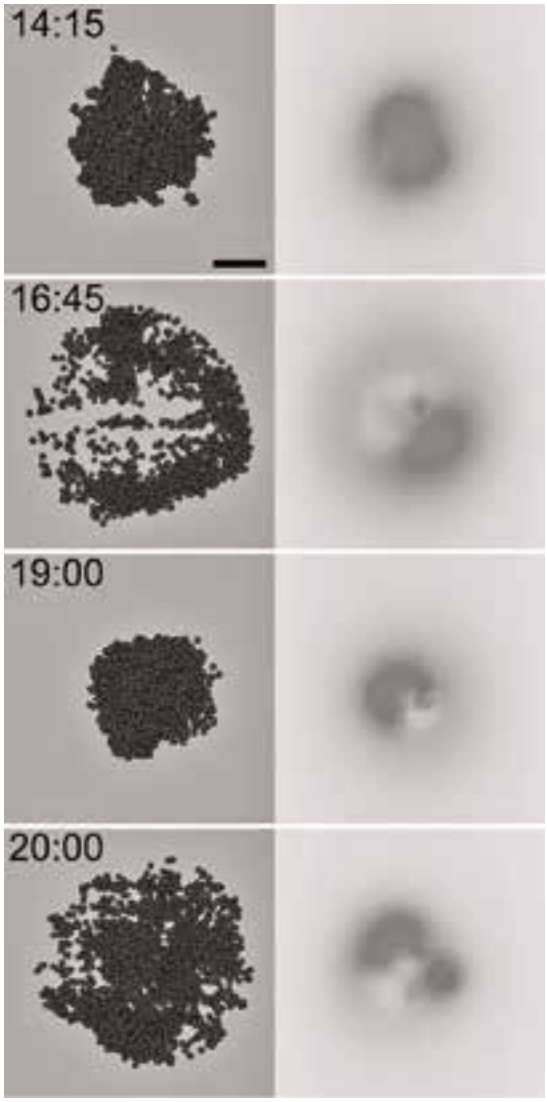


Figure 2: Simulation of a single cell type (left) that is sensitive to an attracting signal (right) generated in the centre of the area shown. Each cell contains a set of coupled differential equations that describes the internalisation of the signal receptor. The dynamics of the internalisation and the retroactive effect on the diffusive signal (right) destabilise the basically spherical structure. Original simulations from Beyer *et al.*, 2008b.

fluctuations SLOs need stabilising factors. It is obvious that these factors might be missing in an environment not primarily intended for generation of lymphoid tisue, and TLOs in synovial tissue are indeed referred to as dysmorphic follicles (Krenn *et al.*, 1996). From the viewpoint of the computer model, these follicles are not stable. The structures identified on the histological cross-sections are in reality a snapshot of a highly dynamic compound of cells unstable in shape.

How do tertiary lymphoid structures originate?

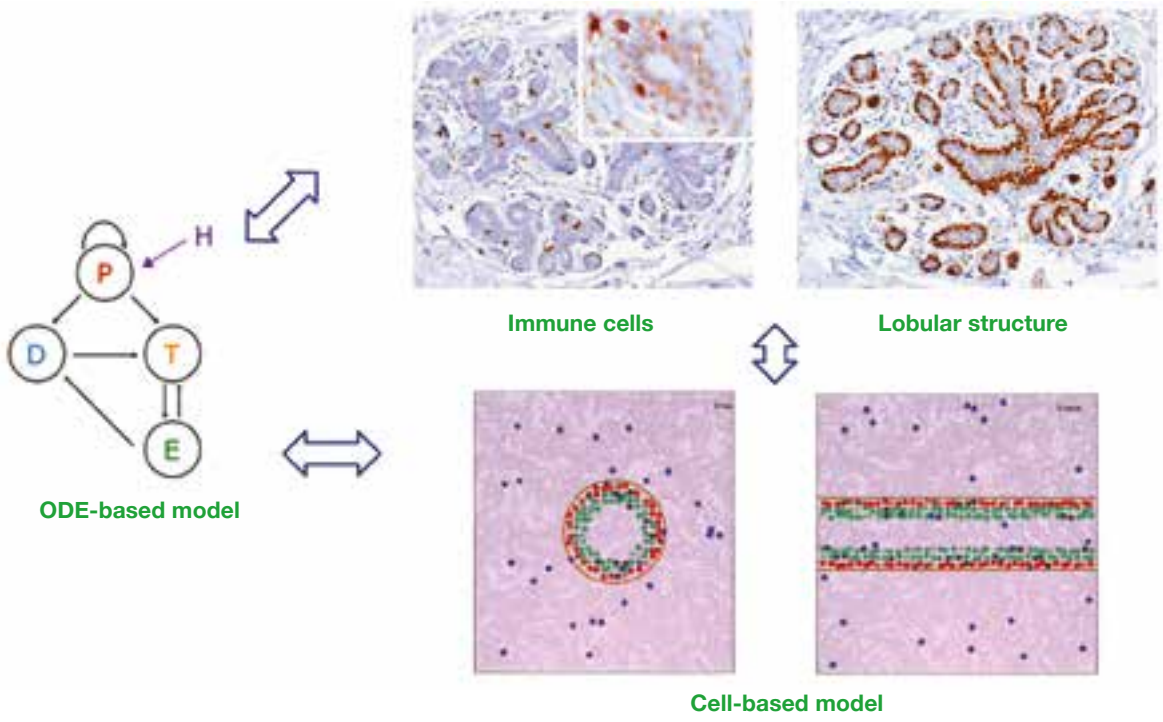
We do not know how TLOs develop. An intuitive hypothesis is that the fundamental mechanisms are similar to those in the development of SLOs. The stromal background and the anatomy of non-lymphoid organs are fundamentally different. As a consequence, one should expect that the exchange of signals should change, too. On the other hand, the feedback loop described above between migrating cells and the production of molecules that attract further migrating cells will nonetheless probably be an important precondition. Just as in the SLO, the loop must require a threshold value to prevent a single cell from activating this process. Where does this threshold value lie? How many cells must come together to trigger TLO development? Can the density of lymphocytes in biopsies be an indicator of the risk of TLO development? Finding answers to these questions is a central point of the SYSIMIT consortium's research. The results will facilitate a new understanding of the role of TLOs in organ rejection and may lead to improved treatment methods.

The research project in brief:

Project name:

SYSIMIT: Discover all the relevant information hidden in the "snapshot" of a biopsy.

Microscope images of tissue biopsies have proved to be reliable markers for the classification and prognosis of many diseases. But do we really make use of all the information that is hidden in tissue samples? That is doubtful, especially in the case of inflammatory diseases. The human eye may be able to recognise certain patterns, but a comprehensive evaluation of the density, spatial relationships and interactions between immune cells exceeds the limits of our visual perception. Furthermore, immune cells are mobile and a microscope image reflects many dynamic processes as a "snapshot". Systems medicine provides a new approach toward eliciting this previously hidden information from microscope images. Complex immunological interactions are mapped in mathematical models. Predictions on the behaviour of dynamic biological systems are specifically compared to corresponding laboratory experiments. The **SYSIMIT** consortium (**SYS**tem immunology, **I**mage **MI**ning and complex image analysis in **T**ranslational transplantation and tumour research) follows this systems medicine approach to improve diagnosis and treatment of hereditary breast cancer and after kidney transplants. Experts in mathematical modelling, image analysis and tumour and transplantation research collaborate closely. The main focus is on the role of tertiary lymphoid tissue in immune reactions after kidney transplants and on the inflammatory reaction to breast cancer cells.



A systems medicine approach to a better understanding of lymphocytic lobulitis, an inflammation of the mammary gland often associated with hereditary breast cancer. Modern methods of image analysis enable classification of every single immune cell as an image object and relating their coordinates to surrounding structures and anatomical knowledge. Mathematical models map the spatial and temporal factors in a clear visual format. Other aspects, such as hormonal influences on the glandular epithelium, are incorporated by means of differential equations. The circle closes when predictions from mathematic models are compared with experimental data. *ODE = ordinary differential equations.* Images and graphics by Dr. J.C. Lopez (TU Dresden) and Dr. N. Schaadt (Hannover Medical School).

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Federal Ministry
of Education
and Research

News from the BMBF

The new High-Tech Strategy – quicker translation of ideas into innovations

The aim of the new High-Tech Strategy (HTS) is to move Germany forward on its way to becoming a worldwide innovation leader. Scientific findings must be incorporated quickly in the development of innovative products and services because innovative solutions are the key to more growth and prosperity in our country. The Federal Government made €11 billion available for this purpose in 2014 alone.

"In the face of the great international competitive pressure we must take care to hold onto our leadership position in science and industry", explains Federal Minister of Research Johanna Wanka. "Germany must now also become the world champion in innovation. This is why the new HTS aims to turn creative ideas into real innovations."

THE NEW HIGH-TECH STRATEGY
Innovations for Germany

The HTS focusses on research areas which promise to deliver creative answers to the urgent challenges of our time while also increasing our level of prosperity. The core elements of the Strategy are the digital economy and society, sustainable economy and energy, the innovative workplace, healthy living, intelligent mobility and civil security.

New instruments will be applied to accelerate the transfer to practical application. The Strategy will give greater support to universities of applied sciences, leading-edge clusters and comparable networks will become more internationally focussed. Industry and science will cooperate in a great number of projects with the support of the Federal Government. One special focus of support is on small and medium-sized enterprises (SMEs).

Since 2006 the High-Tech Strategy has encouraged government and industry to invest as much in research and development as ever before. Germany is a leading exporter of high-tech products. A great many innovations have emerged from research in this time – from energy-saving LED lights to the autologous heart valve. "This is proof that research matters to all of us", said Minister Wanka, "which is why the dialogue with the public will play a major role in the new HTS."

www.hightech-strategie.de/de/The-new-High-Tech-Strategy-390.php

Enhanced cooperation between the Federal Government and *Länder* in science

Germany's higher education system is about to undergo major changes. The Bundesrat also recently granted approval of an amendment to the Basic Law paving the way to greatly increase the opportunities for the Federal Government and the *Länder* to cooperate in science. Students, teaching staff and the research community all stand to benefit.

Federal Research Minister Johanna Wanka has called it "a milestone for our science system" and a "confident step towards the future." The amendment to the Basic Law opens up the brightest of prospects for higher education institutions in Germany. "By enabling the Federal Government and the *Länder* to cooperate and engage in strategic planning, it is a win-win situation for them, for universities and for students", said Minister Wanka.

Up to now the Federal Government and the Länder could only provide joint funding to non-university research institutions, whereas universities merely received federal funding for specific thematic projects for a limited amount of time. The amendment to the Basic Law makes it possible to grant long-term funding to universities, individual institutes or collaborations involving institutes. It will also be easier than in the past for the Federal Government and the *Länder* to grant joint support for the networking of universities and non-university institutions.

www.bmbf.de/en/17975.php

BAföG amendment saves the *Länder* billions

The Federal Government is responsible for funding all disbursements under the Federal Training Assistance Act (BAföG) starting 2015. This will permanently reduce the financial burden on the *Länder* by around 1.2 billion euros each year. In the past the *Länder* covered 35% of the costs, with the other 65% funded by the Federal Government. The *Länder* will now have greater freedom to make additional investments in universities, for example to create permanent positions.

Furthermore, the Federal Government is raising BAföG grant rates by 7% as of the winter semester 2016/2017; housing and child care benefits will also be greatly increased. As of 2017, more than €500 million per year in new funding will be allocated from the federal budget for this purpose.

“The Federal Government is investing in equity in education and educational opportunities”, said Federal Minister of Education Johanna Wanka. “The higher BaFöG grants will give more schoolchildren and students access to financial support. The increased housing benefits also take real living conditions into account.”

The rise in allowable income deductions will increase the number of pupils and students benefiting from BaFöG grants by an annual average of about 110,000. The number of people benefiting from this support is expected to reach the highest level in over 30 years in 2017.

www.bmbf.de/en/892.php

Three pacts for science

The Federal Chancellor and heads of the *Länder* governments have agreed to extend the three major pacts for science. By extending the Higher Education Pact the Federal Government and the *Länder* are responding to the consistently high number of incoming first-year students. The Pact will continue to make higher education accessible to everyone interested in studying and create places for 760,000 additional first-year students by 2020. This represents an average of nearly 37% more new entrants at higher education institutions per year between 2016 and 2020. The Federal Government and the *Länder* have been providing €26,000 per additional new entrant since 2007 – in other words about €19 billion in the new framework.

“The tendency of school leavers to pursue higher education continues on a high level. The Higher Education Pact will provide good study conditions at higher education institutions for everyone who chooses this path”, explains Federal Education Minister Johanna Wanka. "The Higher Education Pact will continue to be one of the major instruments for coping with demographic change. It is already supporting training for the skilled staff that we will so urgently need in the coming decades."

The Pact for Research and Innovation will ensure financial planning certainty for organizations which are jointly funded by the Federal Government and the *Länder* and for the German Research Association. The grants they receive will increase by 5% per year between 2011 and 2015. In return, they commit to science policy goals.

The Excellence Initiative sets out to strengthen the role of institutions of higher education as places for training talented young scientists. The higher education institutions will thus become more attractive for students and researchers from Germany and abroad. The decision of principle of the new Federal Government/*Länder* initiative states that the volume of joint funding for the Excellence Initiative must be at least as high after 2017 and must be made available for supporting excellent top-class research at higher education institutions.

www.bmbf.de/en/1321.php
and
www.bmbf.de/en/6142.php

More equity in education and educational opportunities: BAföG grants to increase by 7% in 2016.

Image: WavebreakMediaMicro – Fotolia

New hotline for qualified international personnel

The new "Working and Living in Germany" hotline was launched in December 2014. The hotline is the Federal Government's first multilingual, one-stop counselling point for migration and integration matters. Migrants and qualified personnel, students and vocational trainees interested in moving to Germany can phone +49 (0)30-1815-1111 for personalized advice on issues such as entry and residence, German language classes, job seeking and recognition of foreign vocational qualifications.

"With the new hotline we are expanding the range of advisory services for international skilled staff and including all types of issues, from entry into Germany and learning German to recognition of qualifications", said Federal Education Minister Johanna Wanka. "We are offering people a central point of contact to get their bearings in Germany more quickly and easily."

Good counselling is key to attracting international professionals to the German job market. The hotline is a sign of welcome and aims to make Germany more attractive as an immigration country.

www.anerkennung-in-deutschland.de/html/en/index.php

Green prospects for industry, work and environment

Federal Research Minister Johanna Wanka and Federal Environment Minister Barbara Hendricks introduced the new research agenda at the international Green Economy conference in Berlin. The agenda is the result of a two-year process which involved representatives of government, industry and research as well as trade unions and business associations. The process was triggered by the question of which innovations – technological and societal – are necessary to drive the full-scale societal transformation to a green economy. "The research agenda unites research and industry to develop solutions which are environmentally friendly and competitive at the same time", explains Research Minister Wanka.

The topics on the research agenda cover everything from the use of biomass as the basis for new plastics, the networking of energy supply systems (electricity, heat, gas), the use of CO_2 in chemical products and the recycling of rare raw materials, through to the determination of the impact of new, energy-efficient technologies on consumer behaviour. The priority action fields of the agenda are production and resources, sustainability and financial services, sustainable consumption, sustainable supply and use of energy, and work and qualification.

The Federal Ministry of Education and Research is making a total of €350 million available for the Strategic Research Agenda until 2018.

www.fona.de/en/index.php and www.fona.de/mediathek/pdf/Green_Economy_Agenda_bf.pdf

Federal Minister of Research Johanna Wanka (r.) and Federal Minister for Environment Barbara Hendricks present the new Green Economy Research Agenda.

Image: Photothek / FONA – Forschung für Nachhaltige Entwicklungen

Service telephone for continuing education counselling

People in the workforce who would like to make a career change or who are looking for skills development can get advice by phone with effect from 1 January 2015. Anyone who needs counselling can call this first nationwide, free service with any questions about continuing education on work days from 10:00 to 17:00.

“Continuing education is a very important way tool for creating individual life and work opportunities. The new service telephone will help people navigate the varied and sometimes confusing opportunities for continuing education”, says Federal Education Minister Johanna Wanka.

The phone service provides easy access to coordinated and provider-neutral advisory services. The phone counsellors determine the caller's individual aims in continuing education and personal needs to find an ideal training format .

The other partners of the jointly operated service telephone are the Federal Employment Agency (BA) and the Federal Office for Migration and Refugees (BAMF).

www.bmbf.de/en/lebenslangeslernen.php

BMBF strengthens health care research in Germany

The pressure of high costs in the health care system is increasing but every patient is nevertheless entitled to the best possible and safest treatment. The BMBF is supporting the establishment of high-powered health care research in Germany through its “Action Plan Health Care Research – Research for a patient-oriented health care system” in order to continuously improve this area of research.

“Germany already has a very good health care delivery system. We want to ensure that it continues to do so”, explains Federal Minister of Research Johanna Wanka. “We must therefore determine which measures are actually effective and which are not and where resources may possibly not be being put to good use.”

Any questions about continuing education? Call 030 – 2014 90 90 for personal advice. The service is free of charge.

Image: Monkey Business - Fotolia

Analyses on the overuse, underuse or misuse of resources are just as necessary as the trialling of new health care concepts or studies on the economics of the health care system. The Ministry is making a total of 50 billion euros available for the action plan in the period between 2015 and 2018. The action plan is part of the Federal Government's Health Research Framework Programme.

Qualified and recognized health economics research is helping in the health policy decision-making process and creates the necessary conditions for financing the health care system in the long term. This is why the BMBF has already been providing funding for health care research projects and for the establishment of four interdisciplinary centres of health research since 2012.

www.bmbf.de/en/16170.php

Contact:

For more information about this and other interesting topics concerning the new High-Tech Strategy click on:
www.hightech-strategie.de/de/The-new-High-Tech-Strategy-390.php

QUANTITATIVE BIOTECHNOLOGY AT FORSCHUNGSZENTRUM JÜLICH:

One-stop shopping for strain and process development

The cycle of data generation and model design is generally seen as an essential part of systems biology research projects. In industrial biotechnology, and particularly in metabolic engineering, this cycle has played a central role since the 1990s. The development of microbial high-performance production organisms based on systems biology methods and the subsequent development of bioprocesses are the mission of the IBG-1 biotechnology research institute at Forschungszentrum Jülich. In Jülich, insights based on systems biology are used to genetically modify industrial platform organisms such as *Corynebacterium glutamicum* and *Escherichia coli* in order to reach industry-relevant producers of basic and fine chemicals, pharmaceuticals, natural substances and proteins.

FAST PHENOTYPING OF STRAIN LIBRARIES

Industrial biotechnology is a quantitative discipline. What counts is, ultimately, the profitability of a production process when compared with established chemical or biotechnological processes. The swift identification of suitable production strains and the preparation of optimally composed media for their cultivation are among the first important building blocks for the development of new biotechnological production processes. Conventional cultivation systems such as shake flasks quickly reach the limits of throughput and process control. By using microtitre plate-based technologies like the BioLector (m2p-labs, Aachen), which among other things permit non-invasive measurement of process parameters such as pH, pO_2 and biomass, these limitations can be partly remedied. That said,

Figure 1: The Mini Pilot Plant consists of a liquid handling station (JANUS, Perkin Elmer), a microtitre plate cultivation system (BioLector, m2p-labs), a centrifuge for cell separation (IXION, Sias AG) and a plate reader (EnSpire, Perkin Elmer). The entire system is housed in a laminar flow enclosure (Cleanair) for sterile work.

Source: © IBG-1, FZ Jülich

Staff of the IBG-1 biotechnology research institute at Forschungszentrum Jülich.

Source: © IBG-1, FZ Jülich

standalone approaches of this kind still involve a greater deal of manual input to prepare media compositions, inoculate individual cultures, and induce production formation should that be required.

With a view to achieving fully automated phenotyping of microorganisms under controlled growth conditions, a new plant, the Mini Pilot Plant (MPP), was developed at the IBG-1 (Fig. 1). Connected to a BioLector, the liquid handling robot facilitates automated media preparation and inoculation of cultivation experiments in microtitre plate format along with the timed or signal-triggered addition of substances, such as inductors, during cultivation. In addition, cultivation samples can be taken, centrifuged and then analysed automatically with the aid of quantitative photometric assays.

Using the MPP, we recently succeeded for the first time in comprehensively phenotyping a library of 17 new L-lysine-producing *Corynebacterium glutamicum* strains in a few days (Unthan *et al.*, 2015). Each strain was cultivated on different media and evaluated for industrially relevant process parameters such as specific growth rate, substrate uptake rate, product titre and productivity. As a result, a new production strain was identified with a 20 % higher product titre compared with the best model producers previously available. The MPP approach is therefore perfectly suited for determining new genetic targets for metabolic engineering of existing production strains.

TARGETED DEBOTTLENECKING USING QUANTITATIVE OMICS METHODS

When it comes to optimising existing high-performance producers, metabolic engineering also constitutes a sophisticated testing ground for systems biology because further improvements can only be accomplished on the basis of a detailed understanding of the function of metabolic networks. For the omics “toolkit”, this means quantitative measuring methods are required to measure intracellular resources and processes on an absolute scale. This approach, hereafter referred to as quantitative biology, sets the highest demands for the as-yet undeveloped measurement protocols, and it can in no way at present be considered established. Most methods currently in use are at best semi-quantitative or function only in relation to a suitable reference.

At Jülich, researchers have developed quantitative omics methods in recent years that make possible detailed measurement of the expression pipeline of individual genes from transcription, translation and folding of the active enzyme to metabolite concentrations and the resulting metabolic fluxes. Only this detailed information permits effective inferences to be made about the cellular organisational level, where the bottlenecks that impede an increase in productivity are located. With the aid of genome-wide metabolism models such as the metabolic network of *C. glutamicum* (further developed at the Institute), the intracellular material flow can be channelled in the direction that is desired.

The development of quantitative omics methods is made a little easier by the fact that industrial biotechnology mostly focuses on the central metabolism of a microorganism and on a few synthesis routes. Quantitative measurements must not necessarily be genome-wide: indeed, they can be developed for specific metabolic sections of the cell (Fig. 2). This is an important difference from the fingerprinting methods aimed at genome-wide integrity that are used at the institute to complement the undirected examination.

The IBG-1 in Jülich is constantly developing the following omics methods and using them for systems biology research:

- Since the 1990s, the institute has been one of the pioneers in metabolic flux analysis using isotope labelling experiments (Wiechert and Nöh, 2013). This is a model-based method that determines metabolic fluxes from measured intracellular labelling enrichment. It is also an outstanding example of the aforementioned interplay of experiment, modelling and prediction in metabolic engineering.
- The Institute enjoys a no less international reputation for developing methods for quantitative metabolome analysis. Recent work has clearly shown that established measurement protocols often show systematic errors that can no longer be neglected because the data cannot be used for quantitative modelling purposes (Noack and Wiechert, 2014).
- The first quantitative proteomics method for a prokaryotic organism based on characteristic peptide sequences was also recently established in Jülich (Voges *et al.*, 2015).

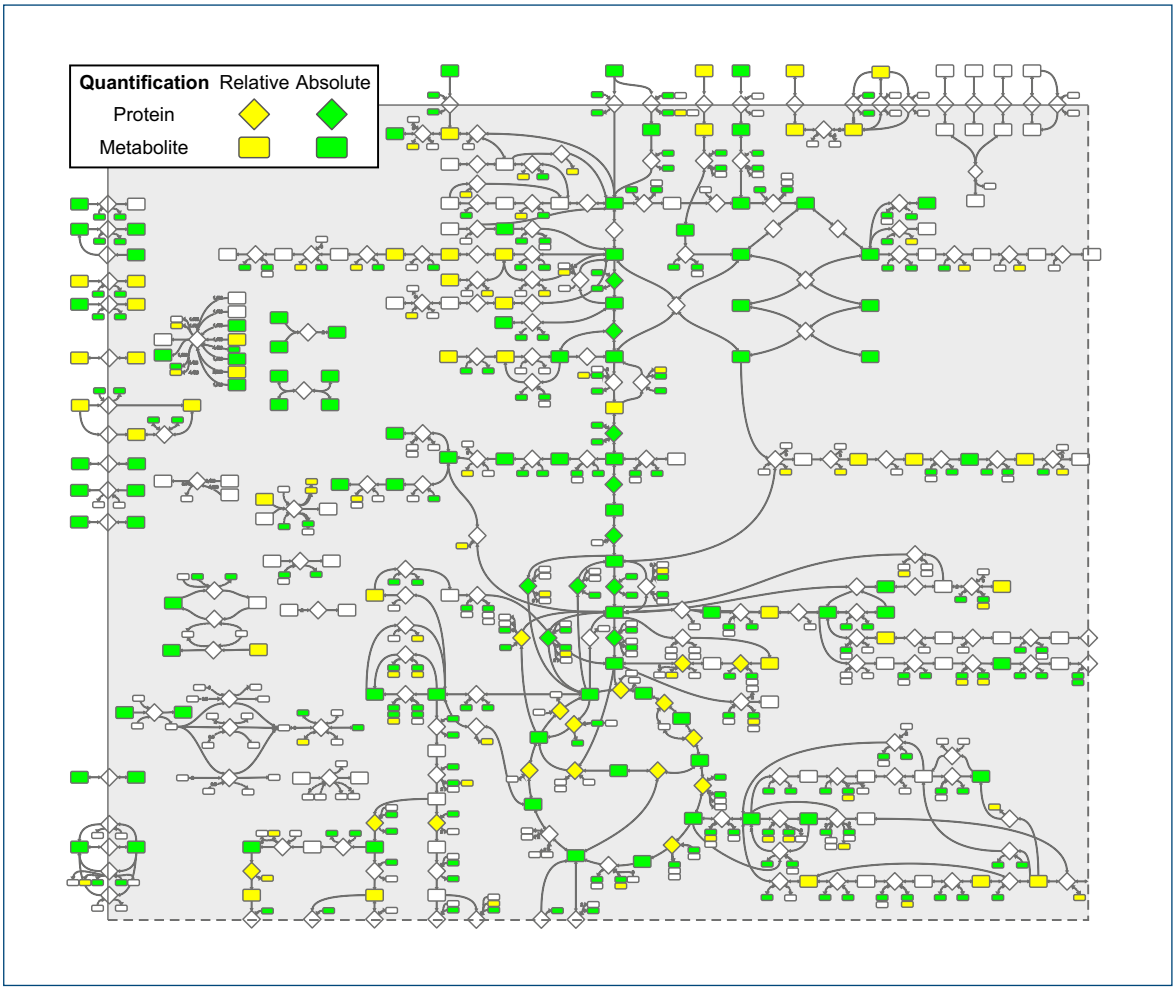
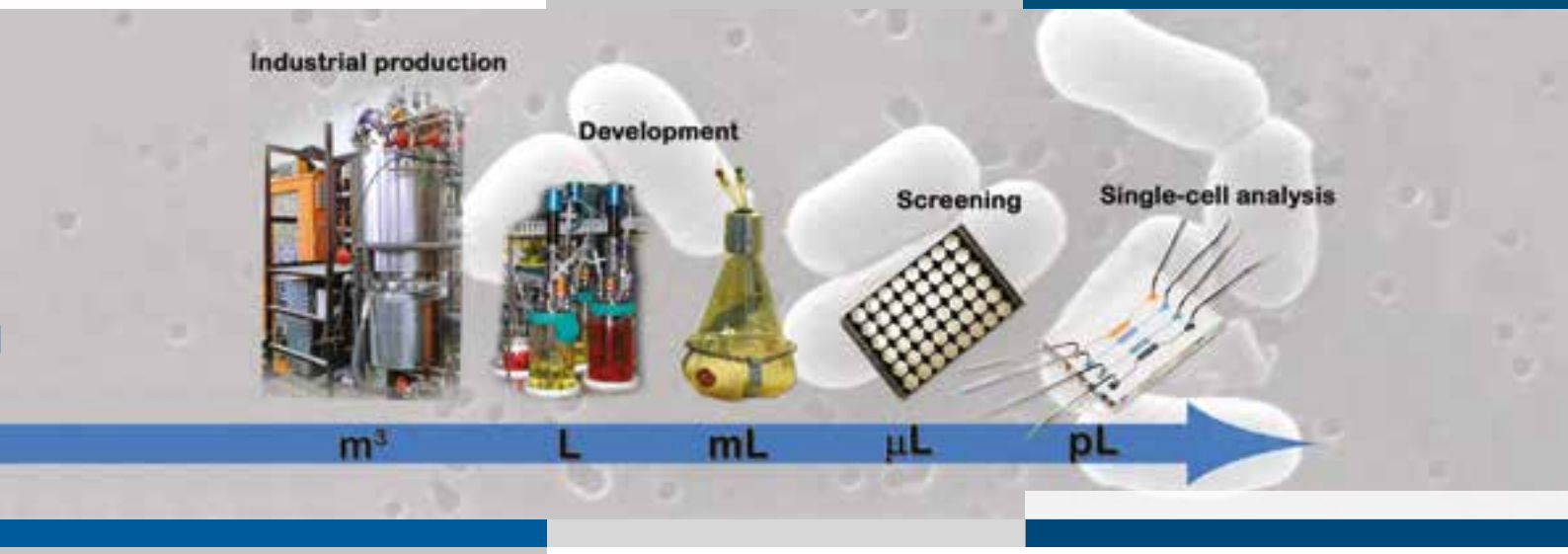


Figure 2: Part of the metabolic network of *Corynebacterium glutamicum*. Proteins and metabolites quantifiable at the Jülich institute by means of mass spectrometric methods are shown in colour. Absolute quantification (green symbols) presupposes the existence of corresponding standards with a known substance concentration.

Source: © IBG-1, FZ Jülich



Cultivation platforms at the JMPC for analysing microorganisms at different scales.

Source: © IBG-1, FZ Jülich

The interplay of different omics methods in optimising production strains can be illustrated very well by means of a recently completed research project aimed at the improvement of L-lysine production with *C. glutamicum* (van Ooyen *et al.*, 2012). All over the world, around two million tonnes of L-lysine are now produced every year using biotechnology. To a great extent, this process has been industrially optimised and so represents an outstanding test case for the development of new methods. As published, L-lysine production strains already account for 30 % of the glucose-enriched carbon atoms in L-lysine, and production must strike a balance between cellular growth (i. e. biomass synthesis) and product synthesis.

This balance can be influenced most effectively by regulating the metabolic flux in the citric acid cycle. Most surprisingly, a significant effect only occurred after a tenfold reduction in the promoter strength for the citrate synthase (the enzyme of the first reaction step in the citric acid cycle). Detailed measurement of the individual steps in the expression pipeline of citrate synthase and neighbouring enzymes clarified how this effect comes about. The desired reduction in gene expression proved to be correctly implemented from translation and transcription to enzyme activity. It took metabolome and flux analysis to reveal that the citrate synthase operating point had shifted to higher substrate concentrations, as a result of which the same metabolic flux was still possible despite a reduction in enzyme quantity.

JMPC – QUANTITATIVE BIOTECHNOLOGY MADE IN JÜLICH

The MPP, developed in Jülich, and the quantitative omics platform constitute the core of bioprocess technologies and bioanalytics at the Jülich Microbial Phenotyping Center (JMPC). It also includes extensive experimental facilities for cultivating microorganisms at different scales from the picolitre scale (microfluidics) and microliter scale (microcultivation) to the litre scale (laboratory bioreactor) and pilot scale (300 L bioreactor). All told, the JMPC provides a one-stop shopping solution for the optimisation of industrial production strains.

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models and methods for systems biology and systems medicine

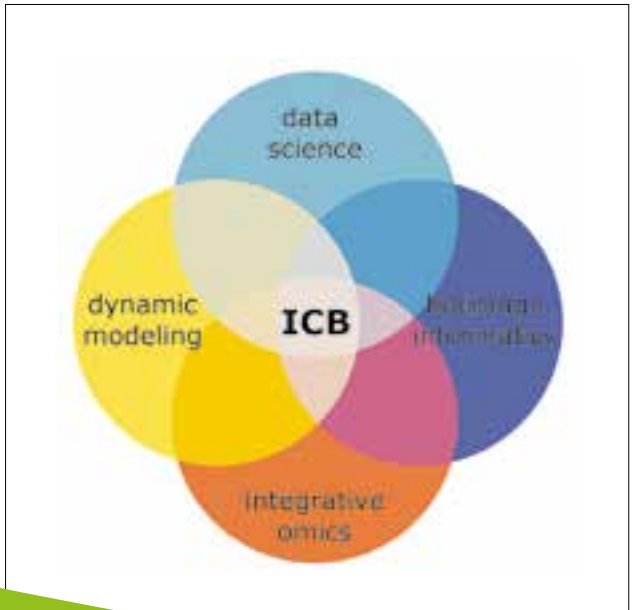
The Institute of Computational Biology at the Helmholtz Zentrum München

by Carsten Marr, Jan Hasenauer and Fabian J. Theis

The number of people with chronic illnesses continues to increase dramatically around the world. The key to understanding many such illnesses lies in the interaction of genetics, environmental factors and lifestyle. Innovations in biotechnology and the continual development of analytical methods permit us to obtain increasingly accurate measurements at the molecular, cellular and organismal level. This is associated with a rapid increase in the volume of data, which enables us to analyse a biological system from many different viewpoints. Today, for example, cells may be analysed using their genome, transcriptome, proteome or metabolome.

As a result, modern biological research requires mathematical and statistical methods to allow for efficient analyses of large amounts of data and the integration of various viewpoints. In addition, there is a growing need for statistical and mechanistic models to properly interpret the data obtained. In close collaboration with our experimental partners, our institute aims to establish analytical tools to enhance our understanding of diseases and their treatment options.

Research areas of the Institute of Computational Biology

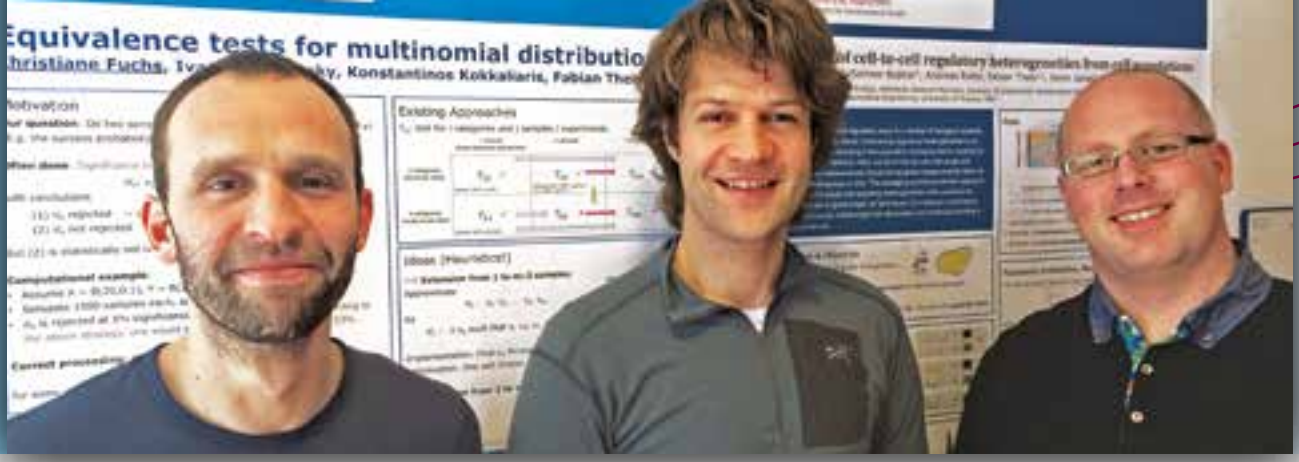


Graphic: ICB

The Institute of Computational Biology (ICB) resulted from the amalgamation of the Institute for Biometry and Biomathematics and the Research Group for Computational Modelling in Biology. The expertise of both groups was pooled in order to create new possibilities for the data-driven analyses of biological systems. Founded in 2013, the ICB is staffed by around 50 scientists and postgraduates. In addition to scientific work, our employees also lecture at Technische Universität München and supervise Master's and Bachelor's dissertations in the fields of mathematics, statistics, information systems and bioinformatics. The ICB works together with theoretical, experimental and clinical research groups at a national and international level. In addition, it is also part of several national industrial partnerships.

Science at the ICB

The ICB develops models and methods for analysing data in systems biology and systems medicine. We analyse information on a variety of scales – from time series of individual cells to Omics data from large patient cohorts. In our ten research groups, we are developing new methods for biostatistics, bioinformatics, image processing and mechanistic modelling, as well as integrative Omics analyses and data science. We apply these to the modelling of cellular decisions and the quantification of gene-environment interactions in disease pathologies. This article describes three of these research projects in greater detail.



From left to right: Carsten Marr, Fabian Theis and Jan Hasenauer (Photo: ICB).

Analysing cell-to-cell variability using statistical methods

Biological systems are highly adaptive and therefore very variable. Individual cells of the same type may react in very different ways to the same stimulus. Thanks to technological advances in imaging and the miniaturisation of reaction volumes with microfluidics, the description and analysis of this cell-to-cell variability is a new and exciting field of research. The ICB works to describe heterogeneity in the cellular context, e. g. gene expression variations in a mixture of differentiated and undifferentiated cells, using both statistical and mechanistic models.

Cellular heterogeneity is an essential factor in a range of projects in developmental and stem cell biology, but also in oncology. For example, we are working on acquiring a better understanding of the initial stages of murine embryonic development. After three divisions, a mouse embryo consists of eight cells, which start to differentiate into different types of cells. Experiments provided data on gene expression in individual cells after each cell division, which in turn provided us with expression analyses for different cell types. In order to detect differences between the cell types, we projected the 48-dimensional space of the gene expressions from single-cell qPCR onto a two-dimensional subspace. Each cell profiled thus corresponds to a point in the plane. With the aid of this projection, we were able to analyse which cells are very close together and which genes are responsible for transitions between cell types. Previously, it was only possible to differentiate cells after six divisions using standard projections. However, using the non-linear expansion developed and adapted by us, which also allows for group affiliations in the projection, it is possible to see that the cells can be categorised as one of two sub-groups already after four divisions (Buettner *et al.*, 2012). In practice, we ascertained that the resolution of transcriptomic data at the single-cell level resulted in new artefacts that were "averaged out" in the relevant data at the population level. For example, similar cells in different phases of the cell cycle could have significantly different levels of expression. In partnership with our colleagues at EBI, we recently recommended a method based on variance analysis in order to compensate for relevant confounders, such as the cell cycle (Buettner *et al.*, 2015). Thanks to the combination of single-cell analyses with statistical models, cells could be grouped into sub-populations that would otherwise have remained undiscovered.

From the cell to the patient

Interestingly, the methods developed for single-cell data can also be used for completely different types of data, such as individual measurements in large patient collectives. One such example comes from the field of diabetes research, in partnership with experts at Helmholtz Zentrum München.

Diabetes mellitus has been classified as an international threat and epidemic by the United Nations and is thus one of the biggest challenges faced by western industrialised nations. The mechanisms causing the disease are largely unknown. Until now, the best way of predicting the risk of type 1 diabetes was by examining family medical history and HLA genotypes. As part of a collaborative project, we were recently able to identify weighted gene combinations using statistical analyses that enable us to better predict the risk of type 1 diabetes (Winkler *et al.*, 2014). Our risk model with ten selected genetic positions enables improved risk prediction and therefore better screening of children in observational and intervention studies.

In addition to lists of known genetic risk markers, the institute also works with large Omics data sets. For example,

The academic staff of ICB at the retreat 2014 (Photo: ICB).

we recently created metabolomics networks that are able to depict the interactions between metabolic molecules specific to a type of tissue or organism. These networks were then expanded using genome-wide associations with genetic polymorphisms in order to create large, integrated metabolic maps showing metabolic and genetic correlations (Shin *et al.*, 2014). We then used these for a variety of purposes, such as analysing phenotype associations of the metabolome in order to simplify the biological interpretation of large results lists.

From measuring heterogeneities to understanding mechanisms

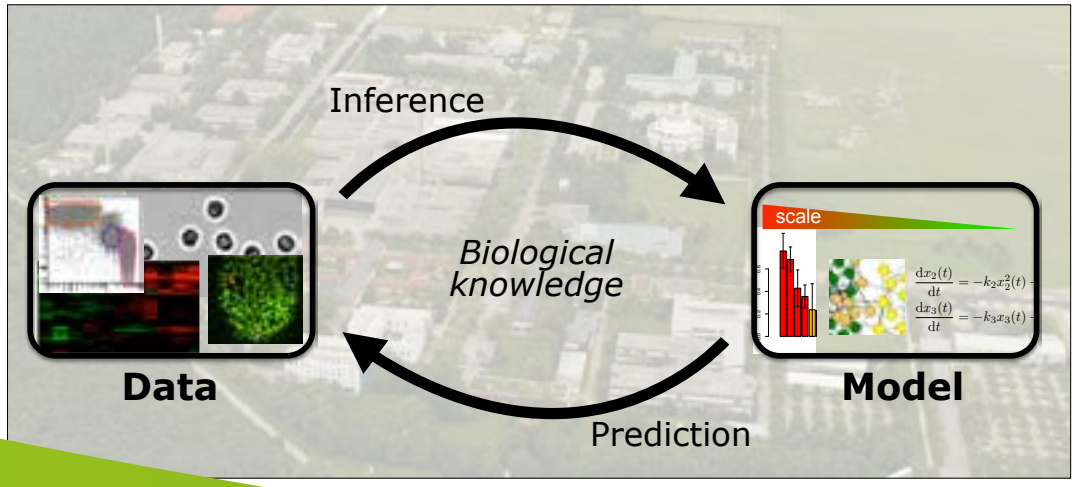
In order to better understand cause-and-effect chain, we use mechanistic dynamic models to analyse the *in vivo* characteristics of, for example, leukaemia, thereby promoting the mechanism-based stratification of carcinomas, or investigating cellular signal transduction. The development of deterministic and stochastic models is complemented here by tailored statistical evaluation methods. Together with other groups, we have developed algorithms that can be used to optimise models with several hundred parameters within hours. This allows the analysis of more complex data sets from a number of experiments.

We recently used such methods to identify various subgroups of neurones involved in transmitting and modulating pain (Hasenauer *et al.*, 2014). Through the combination of statistical and mechanistic models, we were able to determine the cause of differences between the subgroups, despite the fact that the cause had not been directly observed (Fig. 1). In similar projects, we worked with others to determine a potential target for the treatment of chronic pain, which is a major socio-economic issue.

Outlook

Innovative statistical methods and mechanistic modelling approaches are required in order to push ahead with establishing systems biology and systems medicine in the long term, both at our facilities and within Germany. Complex, high-dimensional, potentially longitudinal data sets are more and more available – partly within the specific project and partly via public databases – although clarification is still required on the questions of how to work with them and their integrative analysis in a wide range of projects. As a result, we want to develop tailored methods for complete analysis – from the cell to the patient – one step at a time, and push ahead with the development of multi-stage data-integration processes and genome-scale mechanistic models.

Data-based modelling at the Institute of Computational Biology



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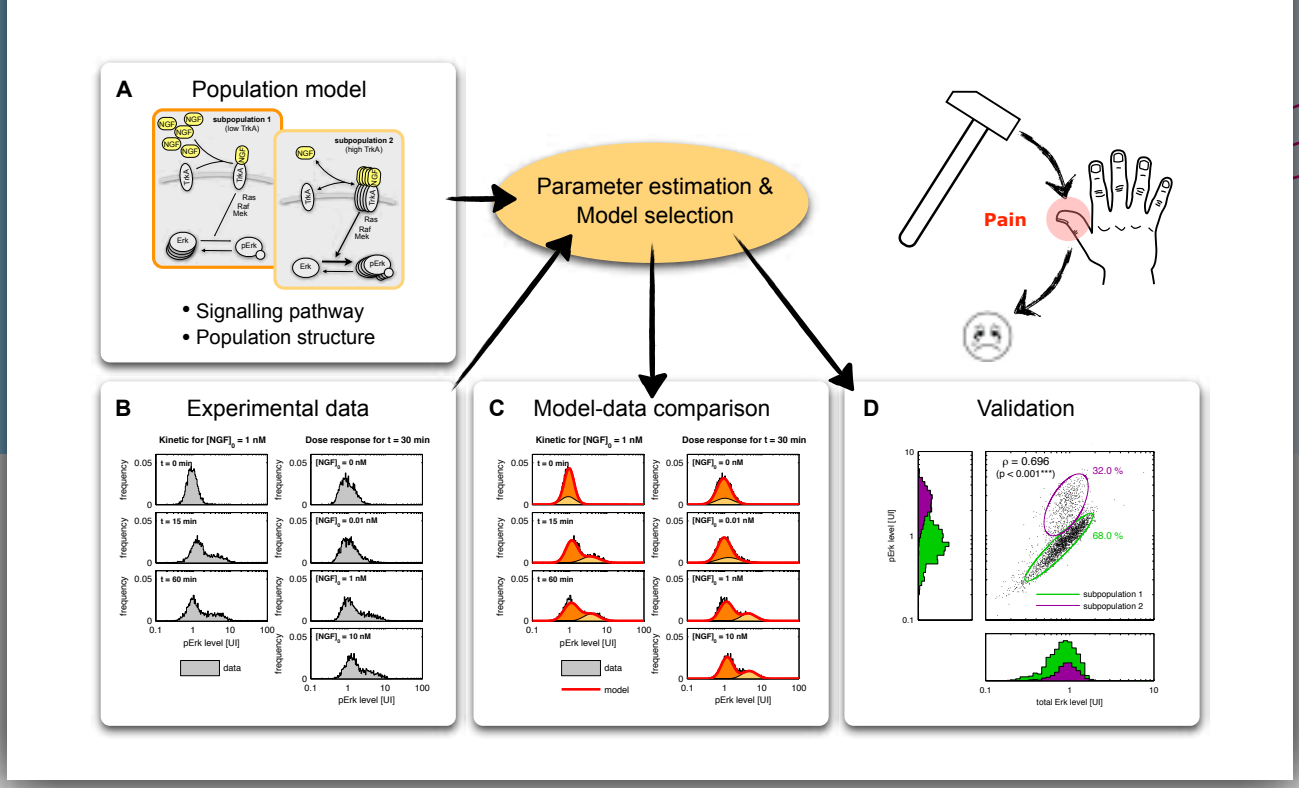

Figure 1:
Illustration of ODE-MM (Hasenauer *et al.*, 2014), a new modelling approach that draws on the advantages of synergies between mechanistic and statistical models. The intracellular dynamics of individual sub-populations can be described using mechanistic, ordinary differential equations. Cell-to-cell variability is depicted using mixture models. Using parameter estimation and model selection, these models (A) were adapted to experimental data (B), e. g. microscopy data. The resultant models (C) are reliable, with predictions of differences between cellular sub-populations, for example, having already been validated in a pain context (D).

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nuclear receptors signalling the way to success

Company profile: Phenex Pharmaceuticals AG

by Thomas Hoffmann

Biotech and glacier crevassesn

Research into innovative drugs is dominated by the big pharmaceutical companies. There is often a gap, if not something of a crevasse like in a glacier, between the end product of academic research, usually a publication, and the point where the research catches the interest of the pharmaceutical companies, ideally in order to find a concrete new drug candidate. Biotech companies such as Phenex Pharmaceuticals AG help to bridge this enormous gap.

Phenex
Pharmaceuticals AG

Our proximity to academic research, the fact that we can act quickly and flexibly, and our deep understanding of biology are what distinguish us from big pharmaceutical companies. We have facilities and expertise in the field of applied research and development, market knowledge and business acumen, and we also possess the necessary capital – these set us apart from academic study groups.

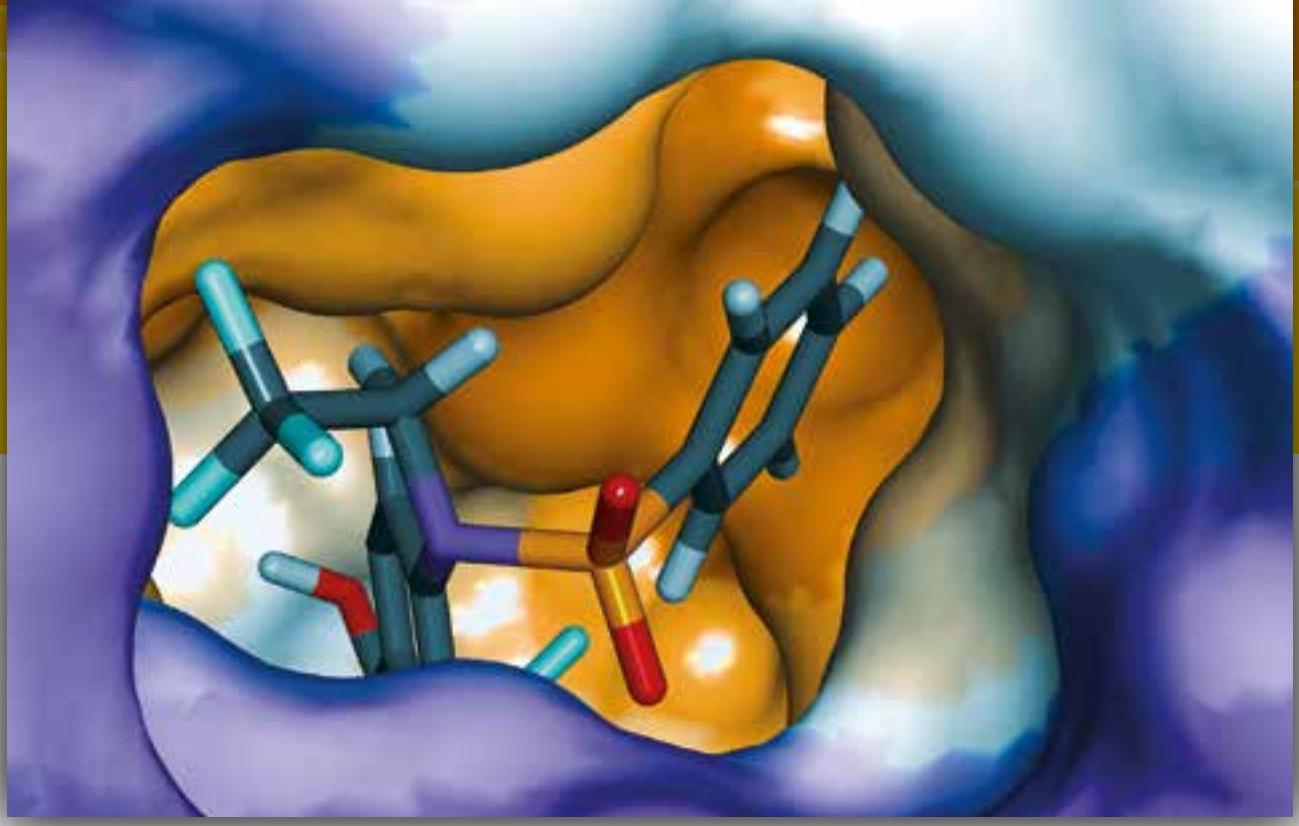
Admittedly, working as a German biotech company is not always without its risks – at least if you want to develop new drugs, that is. Research and development is expensive, and there is much less venture capital available in Germany than in the US, for example. Balancing the intrinsic business risks with intelligently selected projects, commitment and passion is the aim of a biotech company. With a little luck, it is possible to be successful in the field in Germany too.

Foundation and breakthrough

The history of Phenex Pharmaceuticals AG started at the end of 2002 in Heidelberg. Using a comprehensive collection of clones and assays for the nuclear receptors target class, as well as chemical substance libraries, the company wanted to develop new drugs for liver and metabolic disease. Right from the start, our focus was on the FXR receptor, which is discussed in more detail during the course of the article.

However, capital had to be found before any investments could be made, and that at a time when securing financing for new companies was a very bleak prospect. After the NEMAX technology stock exchange crash in 2000/2001, venture capital practically evaporated in Germany, forcing the company to recruit foreign venture capital.

Photo: Phenex Pharmaceuticals AG



Inverse agonist T1317 in the ROR-gamma ligand-binding domain (PDB:4NB6) Illustration of the protein surfaces by hydrophobicity (Graphic: Phenex Pharmaceuticals AG).

At first, we had little success, and this was unfortunately followed by a spate of more bad luck.

Venture capitalists are by nature risk-averse and feel more at home in the company of other venture capitalists than on their own. It was our bad luck that another new company was being founded at the same time in Strasbourg – also with a focus on nuclear receptors – which, due to existing French venture capital commitments, sucked in further European capital in the manner of a black hole. After negotiations with around 60 investors, the final result was EUR 30 million for the French competition and nothing for us. Not exactly the perfect start!

Initial use of the platform as a cash cow

What we had to do was stand our ground. We were helped by two major service projects for a pharmaceutical company that we were, luckily, able to acquire via the fantastic contacts of one of our co-founders. These projects were the first time we used our technological assays and tools to generate molecular receptor ligand profiles for a customer in order to characterise its drug candidates.

The biology of nuclear receptors, which interfere with various signalling pathways such as transcription factors, including regulating the transcription of target genes, is complex. They are of major interest to pharmaceutical companies as pharmacological targets because their modulation is generally associated with extremely high therapeutic efficacy: they are some of the most important target structures for the whole of the pharmaceuticals industry. Every year, turnover from drugs that target nuclear receptors is in the region of double-digit billions. Many well-known and successful drugs, such as cortisone, the contraceptive pill and various oncology drugs, target nuclear receptors.

On the other hand, the pharmacological targeting of nuclear receptors as a result of their central biological function and their often pleiotropic mode of action (acting on various target structures and thus producing different effects) also has its price. A well-known example of this is the glucocorticoid receptor with its ligand, cortisol. Everyone is aware of the exceptional anti-inflammatory properties of cortisone, but unfortunately, also of its potential side effects. To put it simply: in order to develop successful drugs in the nuclear receptors target class, it is necessary to take a close look at their biology. The biology of nuclear receptors enables us to develop selective modulators that offer a much better therapeutic window in terms of the ratio between therapeutic effects and side effects. Or, to put it in non-scientific terms, receptors not only have an “on” or “off” mode, but it is also “dimmable” and can sometimes even be regulated gene-selectively or tissue-selectively by ligands.

With our technologies, we are in a position to classify ligands more precisely with regard to their molecular-biological fingerprint in order to move towards the selective profiles we want. This is the central focus of Phenex as a company, and it was also the touchstone for our service business in the form of dif-

ferently scaled partnership projects on the basis of our nuclear receptor assays – services that we still offer pharmaceutical companies to this day.

From the time it was founded until mid-2005, the company, financed by cashflow, continued to generate a contribution margin for internal research activities and provided essential contacts, feedback and credibility for the future. In retrospect, we were anything but idle in the years when we enviously viewed all well-financed biotech companies and had to earn our money through services.

First injection of venture capital and investments in internal projects

More than two years after the company was founded, Phenex received its first venture capital in mid-2005, in conjunction with significant funding from the German Federal Ministry of Education and Research for the development of the FXR project. Receiving these financial resources meant we could finally start researching our own programmes.

Between 2005 and 2008, the money was primarily invested in our FXR project. The FXR, or farnesoid X receptor, is a nuclear receptor expressed in the liver and gastrointestinal area that binds bile acids. It is like a kind of "bile acid overload switch", physiologically regulating a complex process in order to protect the liver and gastrointestinal system from excessive, toxic concentrations of bile acid, but it also protects the liver in other ways, such as from metabolic stress. On the basis of the first pharmaceutical leads available at the time the company was founded, we improved the molecules' pharmacology to such a degree that early 2008 saw us present our first candidate molecule suitable for further pre-clinical development.

Following negotiations with various investors, we were able to conclude a further round of financing in mid-2008, and this was supplemented once again in 2010 by the same group of investors. In retrospect, we were very lucky that we decided against the major foreign investment offered and placed our trust instead in a mixture of smaller German venture capital funds and private investors, who have remained loyal to us all this time.

The company was certainly able to use the additional funding from 2008 to develop a second R&D programme for the RORg receptor, with a focus on autoimmune disease. This meant we had two R&D projects in our portfolio, which we developed in parallel and which, by 2012, had reached such a level that their sale to a pharmaceutical company seemed practical.

Reaping the benefits

All of us know the feeling of cycling with and against the wind, and this is one way of describing the sale of R&D projects. It's easier to cycle with the wind behind you, and this was also the case with the licensing of the RORg project. Without much effort on our part, the receptor became a hot topic after 2010 (that is to say, in the books of business development departments in the pharmaceuticals industry) as a result of high-profile scientific publications and clinical data on therapeutic antibodies that at least validated the signalling pathway, if not the target itself. Since we had already published the first substance patents by this time, Phenex became a highly sought-after partner and we were able to enjoy the fruits of our labours after a few months of negotiations. The RORg programme was licensed to Janssen Pharmaceuticals (the healthcare branch of Johnson&Johnson) for funded research payments of up to USD 135 million and potential additional commission.

Fortune continued to favour the brave. Although the FXR project never attained the same level of prestige as RORg in the pharmaceuticals industry during the ten years in which Phenex worked on the programme, this was to change very suddenly. Intercept Pharmaceuticals, our sole R&D competitor in the field, published the results of its phase II trial in patients with fatty liver disease, and this sent its company value skyrocketing on the stock exchange to an incredible USD 9 billion during the days that followed.

Biotech company Phenex Pharmaceuticals AG in Heidelberg

Photo: Phenex Pharmaceuticals AG

(Photo: Phenex Pharmaceuticals AG)

Why the hype? Obesity, diabetes, high blood pressure and high blood lipids are widely known symptoms of metabolic syndrome. What is not as well known is that the liver can also play a part in metabolic syndrome. High blood lipids lead to fatty deposits and inflammation of the liver in some patients (also known as NASH – non-alcoholic steatohepatitis). If left untreated, this inflammation can lead to liver cirrhosis and liver cancer with the concomitant mortality rates. According to estimates, the rate of metabolic-induced cirrhosis will soon outstrip that of alcohol-induced cirrhosis. Because the few clinical trials of substances in this indication had failed miserably, Intercept was able to terminate its trial after the enrolment of half of the patients as a result of spectacular efficacy. Overnight, FXR became a target for the pharmaceuticals industry looking for a slice of what would become a very attractive market.

We used this unexpected boost provided by Intercept to our advantage. Following competitive negotiations with interested parties, we concluded a sales contract and cooperation agreement in December 2014 with Gilead, the global market leader in the treatment of liver disease, for a figure of up to USD 470 million.

Unlike other successful biotech companies, this does not mean that our journey is at an end – we will still invest some of the revenues from this endeavour in new, interesting projects so we can forge ahead with German-made success stories.

Phenex Pharmaceuticals AG profile:

Phenex Pharmaceuticals AG is a privately financed biotech company with offices and laboratories in Heidelberg and its headquarters in Ludwigshafen am Rhein. We work on researching and developing drug candidates for the treatment of liver, gastrointestinal and autoimmune disease. Our team of 20 experts designs and tests new small molecules, and we work together with a global network of partner companies on the chemical synthesis of active substances and conducting pharmacological investigations of substances.

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CyanoGrowth – the architecture of phototrophic growth

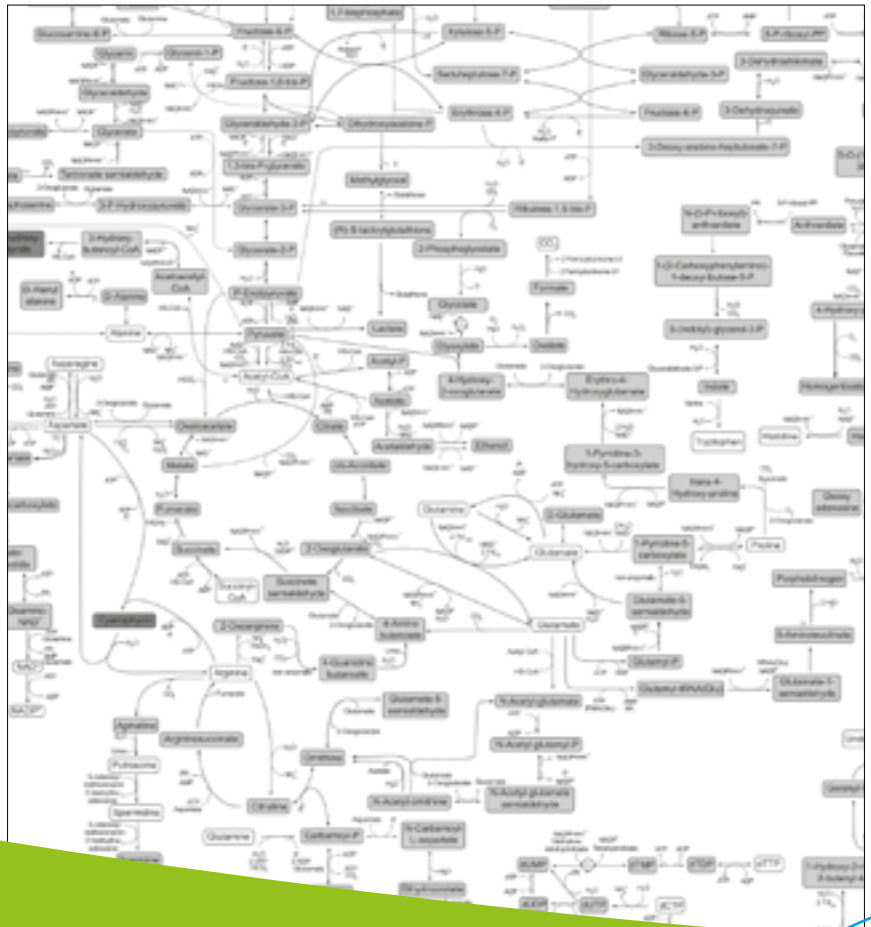
From systems biology to biotechnological applications

by Ralf Steuer

Cyanobacteria are fascinating organisms. As evolutionary inventors of oxygenic photosynthesis and as precursors of modern chloroplasts, cyanobacteria have influenced the Earth's biochemistry like no other organism. In addition to their glorious past, cyanobacteria also hold great promise for the future. The potential offered by phototrophic microorganisms will play a major role in mastering the challenges of the 21st century – from securing global food supply to the synthesis of renewable raw materials. The project CyanoGrowth, funded as part of the "e:Bio – Innovationswettbewerb Systembiologie" [e:Bio – systems biology innovation competition] initiative, aims to better understand the mechanisms of phototrophic growth and thus establish systems biology of phototrophic growth as a key technology for a sustainable bioeconomy.

Our planet is green! Oxygenic photosynthesis is perhaps the most important biological process within our entire biosphere. Plants and cyanobacteria supply almost all organic carbon compounds that form the building blocks of life. The by-product of oxygenic photosynthesis, molecular oxygen, serves as the electron acceptor for aerobic respiration and is thus the basis of

Figure 1: Visualisation of a large-scale reconstruction of the metabolic network of the cyanobacterium *Synechocystis* sp. PCC 6803.



Metabolic reconstructions are like complex route maps of cellular metabolism and enable us to systematically analyse the biochemical repertoire of a cell. The reconstruction of the cyanobacterium *Synechocystis* sp. PCC 6803 comprises approximately 700 metabolic reactions and describes the biochemical pathways from carbon fixation to the synthesis of the building blocks that are required for growth (Graphic: from Knoop *et al.*, 2013).

Cultivation of cyanobacteria under controlled conditions (Photo: Cyano Biotech GmbH Berlin).

almost all multicellular life on our planet. Without the evolution of oxygenic photosynthesis, there would be almost no free oxygen in the atmosphere, there would be no protective ozone layer and most likely no complex life on Earth as we know it today. The evolution of oxygenic photosynthesis in the ancestors of modern cyanobacteria just over three billion years ago changed our planet forever.

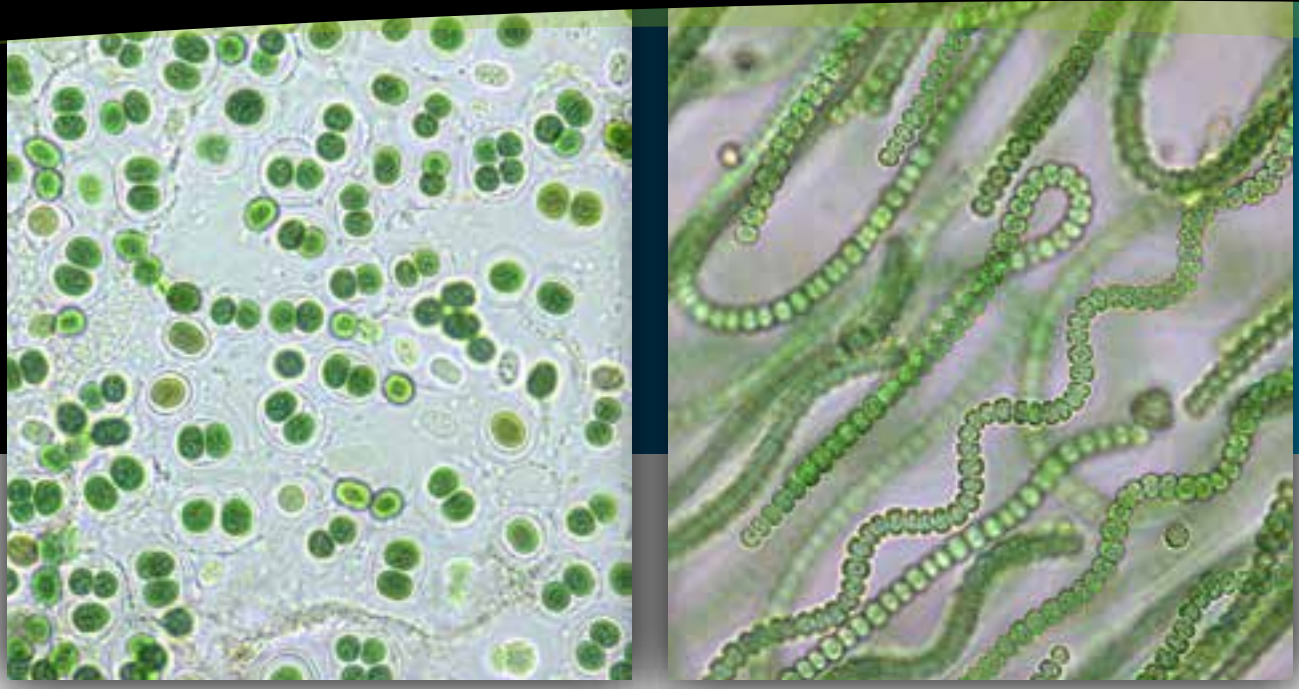
To this day, cyanobacteria still play an important role in global geochemical cycles. Cyanobacteria live in almost all environments – in rivers and lakes, in low-nutrient regions of the oceans, in the fur of animals, and also often under adverse or extreme conditions, such as in salt marshes and brackish water, deserts and in Antarctica. This diversity of cyanobacterial life forms, in conjunction with their ability to achieve high phototrophic growth rates even under challenging conditions, makes them of particular interest for biotechnological applications. Cyanobacteria are particularly suited for generating renewable raw materials and biofuels, as well as proteins, natural products, animal feed and comestible goods. Their cultivation does require neither traditional farmland nor freshwater.

Reconstruction of the cyanobacterial metabolism

The aim of our research is to describe cyanobacterial phototrophic growth with the aid of mathematical models in order to facilitate the use of cyanobacteria as a renewable resource. Our starting point is a computational characterisation of cyanobacterial metabolism. Metabolism is the focal point of growth and translates the genetic information held in the DNA into biochemical reactions. Many metabolites, notably the co-factors ATP and NADPH, are also global regulators and indicate the intracellular state of the organism.

In recent years, flux balance analysis (FBA) has become an established method for the computational analysis of metabolism. Flux balance analysis draws on the principle of conservation of mass in biochemical reactions and allows us to predict the biochemical fluxes using evolutionary optimality principles. An advantage of flux balance analysis is that it only requires very few kinetic parameters, thus making it suitable for a quantitative description of large metabolic networks. The application of FBA requires a reconstruction of cellular metabolism – a comprehensive compendium of all biochemical reactions that can take place within a cell (or cellular compartment) (Figure 1). Once such a compendium exists, we can analyse important questions with regard to potential biochemical pathways and their characteristics. In particular, a metabolic reconstruction enables us to systematically analyse the biochemical repertoire of a cell and to identify any missing or wrongly annotated reaction steps.

Metabolic reconstructions are based on the annotated genome of an organism and resemble a complex route map of cellular metabolism. A metabolic reconstruction is the result of an iterative process requiring literature research, sequence comparisons, the integration of high-throughput data and targeted biochemical tests, all of which requires a close interaction of different disciplines in biology. A major touchstone is growth experiments in photobioreactors. Under controlled conditions, specific parameters of cyanobacterial growth, primarily CO_2 assimilation, pH of the medium and release of O_2, can be compared with predictions from the model.



Cyanobacterial metabolism is highly diverse and has evolved to survive and prosper even in challenging ecosystems and environments. Left: *Gloeothece*, a single-cell cyanobacterium. Right: Filaments of the cyanobacterium *Nostoc* sp. Heterocysts, specialised cells for fixing atmospheric nitrogen, form inside the filaments (Photo: Cyano Biotech GmbH Berlin).

Systems biology of phototrophic growth: from light to biomass

Phototrophic growth is an organismic process. It is therefore a specific challenge for systems biology to connect the various cellular levels and time scales of phototrophic growth. It is not so much one single process that gives rise to cellular growth but rather the interplay of various different processes (Figure 2). Many of these building blocks of cellular growth have been very well researched. Entire scientific communities are often concerned with specific processes, providing excellent work to establish knowledge on the functioning of individual building blocks. The challenge of systems biology is now to collate this knowledge into predictive models of cyanobacterial growth.

While individual cellular processes are often reasonably well understood, the mathematical modelling of their interactions is no straightforward task. The various sub-processes and time scales involved in cellular growth often require very different mathematical and methodical approaches, which cannot always be easily reconciled with one another. Phototrophic growth starts with the absorption of light, water splitting, and the photosynthetic electron transport chain – complex biophysical processes that have been very well researched but are still far from being fully understood. Since the discovery of light-dependent reactions by the biochemist Robin Hill and others, many details of the electron transport chain are known. A variety of mathematical models of photosynthetic electron transport are available, often with a focus on photosystem II. These models are usually not based on differential equations but use other methods to describe the very fast time scales and transitions between a large number of states.

The chemical energy and the regenerated NADPH harvested through the electron transfer chain are then used to assimilate carbon dioxide. The biochemical steps involved in carbon assimilation, including the upstream CO_2 concentration mechanism, have been well researched but are still insufficiently understood from a quantitative point of view. The relevant time scales are much slower than in the electron transfer chain and the relevant computational models are often based on ordinary differential equations.

The carbon assimilated by the central enzyme in the Calvin-Benson cycle, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), is used to build up storage substances and to synthesise new cell components. This process can be described by flux balance analysis using large-scale metabolic networks reconstructions. Other levels of cellular regulation include the cyanobacterial circadian clock, whose precise interaction with metabolism is not yet properly understood, as well as global transcriptional regulation, including diurnal changes in DNA topology. Based on these individual processes, we want to understand how phototrophic growth functions. How is it regulated? How does the coordination of metabolism work in order to synthesise the right macromolecules at the right time? How do environmental conditions or different day lengths affect phototrophic growth? What factors limit phototrophic growth? What maximum growth rates can phototrophic cyanobacteria achieve under ideal conditions? The project CyanoGrowth does not specialise in a small area of cyanobacterial molecular biology but is instead more generalist in nature, with the aim of uniting the relevant processes and aiming to understand their interactions.

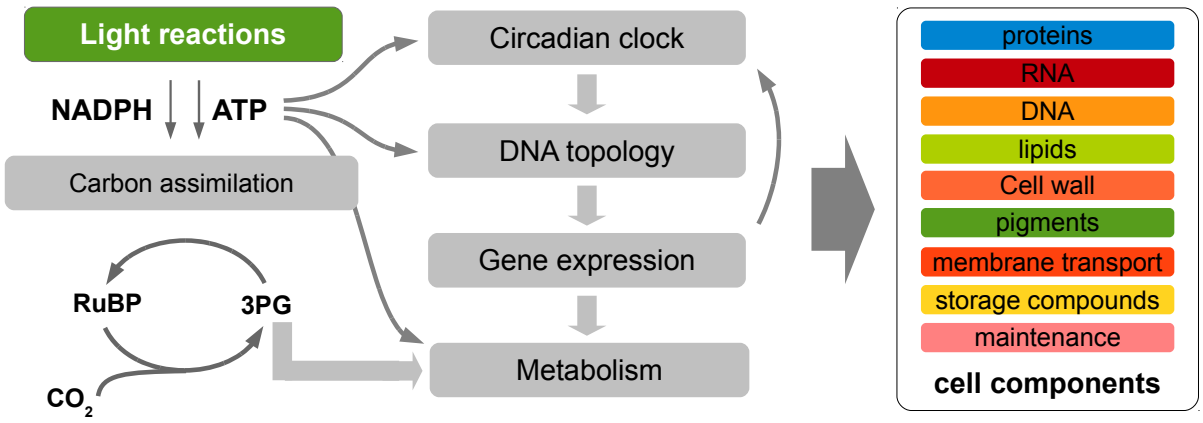


Figure 2: Phototrophic growth is an organismic process. Our research objective is to understand phototrophic growth as the interaction of cellular processes and to describe these interactions using mathematical models. Phototrophic growth involves the fast time scales of the light-dependent reactions, carbon assimilation with an upstream CO_2 concentration mechanism, the circadian rhythm and the synthesis of new cell components (Graphic: Ralf Steuer).

New challenges in green systems biology

In addition to the aim of reconciling cellular processes, systems biology of phototrophic growth is faced with further challenges to successfully establish cyanobacteria as a green resource. An important aspect is the diversity of cyanobacterial metabolism. Until now, experiments and mathematical models have been mostly restricted to a small number of laboratory strains. However, based on reference organisms such as *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942, newly sequenced genomes offer the opportunity to better understand phototrophic growth of other strains – specifically, their adaptations to diverse ecosystems and environments.

Understanding metabolic diversity is closely linked with the evolution of systems biology towards an ecosystems biology. Like all organisms, cyanobacteria live in complex environments with which they interact and which influence them. The list of cellular processes listed above therefore does not end at the cell wall but has to be expanded to describe the interactions between organisms, as well as complex ecosystems. Cyanobacteria are particularly well-suited for the analysis of simple forms of multicellular life and cooperation: many cyanobacteria form specialised cells (cell differentiation), biofilms and microbial communities (microbial mats) with a complex division of labour. A quantitative understanding of the interactions in such microbial communities is only in its nascent form – giving rise to new challenges for mathematical modelling.

The impact of computational modelling in green biotechnology is still modest. Our aim, therefore, is to work with international partners to contribute to a systemic understanding of cyanobacteria and to bridge the gap between knowledge-based research and applications in green bioeconomy making use of predictive models. Models of cyanobacterial growth have direct applications in green biotechnology, including gauging the potential yield of commercial cultivation, optimising growth conditions, identifying suitable genetic intervention strategies and increasing the yield of specific products.

The research project in brief:

The project CyanoGrowth is funded by the German Federal Ministry of Education and Research as part of the “e:Bio – Innovationswettbewerb Systembiologie” [e:Bio – systems biology innovation competition] initiative (reference: FKZ 0316192), and it is part of the junior research group on Metabolic Network Analysis. The research group is based at the Institute of Theoretical Biology (ITB) at the Humboldt University of Berlin, an innovative unit within the Humboldt University of Berlin and the Charité – Universitätsmedizin Berlin medical school, currently housing six professors and four junior research groups.

The focus of the research group is the mathematical description of cyanobacterial phototrophic growth. The research focuses on the integration of cellular models, dynamic flux balance analysis, problems of resource allocation in dynamic metabolic networks and numerical methods in green biotechnology.

Models of cyanobacterial growth have direct applications in green biotechnology. The extraction of biofuels using cyanobacteria is being researched in the research project CYANOSYS II (reference: FKZ 0316183), funded as part of the "e:Bio – Innovationswettbewerb Systembiologie" [e:Bio – systems biology innovation competition] initiative. Above, the first pilot facility run by our cooperation partner Algenol for the production of ethanol (Photo: Algenol Biofuels).

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JACOBS UNIVERSITY

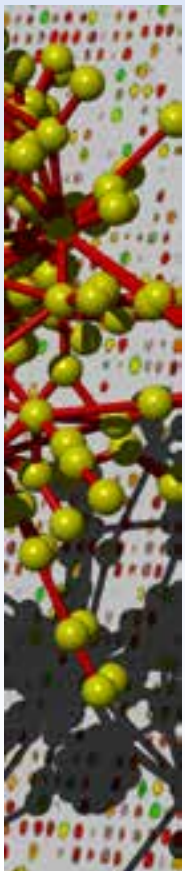
WE Heraeus Physics School

The Physics Behind Systems Biology

This WE Heraeus Physics School is open to graduate students, PhD students and post-doctoral researchers. We want to explore the Physics foundations of Systems Biology and show how this novel discipline stands on a basis paved by physical principles. Core topics are complex networks, robustness of biological processes, methods of mathematical modeling, synchronization and cellular rhythms.

Confirmed Speakers

Reka Albert, Pennsylvania State University, Pennsylvania, USA
Stefan Bornholdt, Universität Bremen, Germany
Thilo Gross, University of Bristol, UK
Shlomo Havlin, Bar-Ilan University, Tel Aviv, Israel
Hanspeter Herzel, Humboldt Universität Berlin, Germany
Thomas Höfer, DKFZ, Heidelberg, Germany
Heinz Koeppl, TU Darmstadt, Germany
Michael Lässig, Universität Köln, Germany
Annick Lesne, Université Pierre et Marie Curie, Paris, France
Karsten Kruse, Universität des Saarlandes, Saarbrücken, Germany
Kim Sneppen, Niels Bohr Institute, Copenhagen, Denmark



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a closer look at codes within the cell

Interview with Alexander Hoffmann

At the University of Los Angeles (UCLA), systems biologist Alexander Hoffmann is researching how immune responses are regulated. His team is developing multi-scale models that describe the dynamic process of pathogen recognition and concerted immune response across various scales – from the network of interacting proteins within the individual cell to tissue and organ function – and is thus taking a closer look at the codes that regulate immunity.

After completing his undergraduate degree in physics, Hoffmann switched to biology, fascinated by the detailed insights about molecular processes in organisms. At that time, he had no idea how important his knowledge of physics would be to him. For him, systems biology leverages all technological, conceptual and algorithmic advances of modern biology.

Systembiologie.de: *Mr Hoffmann, how did you become a systems biologist?*

Prof. Dr. Alexander Hoffmann: During my postdoc at Caltech (California Institute of Technology), I worked on the biochemical characterisation of a transcription factor and also wanted to understand its physiological function. Like most people, I assumed that I would be able to use a knockout mouse to understand the phenotype on the basis of the biochemistry: how the transcription factor binds, which genes it regulates, etc. But I quickly discovered a major discrepancy between the biochemistry and the actual biology. The phenotypes did not correspond to my expectations in any way, which, in retrospect, was what other people, studying other genes, were finding as well. Now we know that the relationship between the phenotype and genotype is very complex and that it is a dynamic system. At that time, I realised that in order to understand such a dynamic system, the tools that I had learnt about in physics would be relevant. It was really unexpected revelation that I had not wasted my time studying physics, but that that way of thinking and skillsets would end up being very useful. It really boosted my enthusiasm. We didn't use the term systems biology, however, because at that time in the US it was generally associated with genome-wide in other words "system-wide" or "systematic" studies and not so much with the emergent characteristics of biological systems.

You have been committed for some time to strengthen and boost the profile of systems biology. What have you already achieved, and what remains still to be done?

I have tried to show that the type of systems biology that uses mathematical models to understand biological systems can provide important physiological and clinically relevant findings. Mathematical models have long been an established method of investigating biological hypotheses and most of these models are very simple and abstract, yet still very informative. In general, this discipline remains the preserve of physicists and was, in the US at least, often relegated to the background. One of my aims has been to show biologists that mathematical models are useful, and another was to link systems biology with high-throughput technologies. Quantitative analysis and bioinformatics methods form the basis for creating good mathematical models. The data obtained is then used for the parameterization of the models. I work towards linking all aspects of systems biology, from genomics to physics.

What do your mathematical models tell us about the immune system?

We have found that immune response and associated regulatory processes are very dynamic and that the dynamics of signal processing and transcription represents a kind of code that determines the behaviour of the cell. Similar to the Morse code, this enables a lot of messages to be sent via a sequence of different events or activities – dynamic aspects. The cell uses a similar strategy to transmit a lot of different signals to the various compartments of the cell. This means there is a kind of cellular

Alexander Hoffmann in his office at the newly founded Institute for Quantitative and Computational Biosciences at UCLA (Photo: Reed Hutchinson).

language that is not a genetic code but a code used for communication. We are trying to understand how this language works, what vocabulary it uses and what the words mean. That's keeping us pretty busy just now.

What is particularly challenging about it?

In the past few years, we've learnt that cells have a very precise level of regulation but that they also behave differently – even if they're genetically identical – and that the response of a cell to a pathogen seems to be unreliable when individual cells are compared. This raises a lot of questions. After all, cells in our body don't function on an individual basis but in a coordinated way. In order to understand the molecular regulatory mechanisms, we need models that describe the biochemical responses in each cell. But we also want to understand how cells – despite their heterogeneity - work together to produce reliable biological function. A straightforward approach would be to establish a mathematical model for each individual cell, although all these models – and there could be up to a thousand of them – would have to run parallel at the same time. This would be an agent-based model, where each individual cell is treated as an agent, or unit. However, this approach quickly outstrips computational resources. Therefore we need other modelling approaches that allow a scale-independent simulation, ranging from tissue with millions of cells right down to the molecular details of protein interactions, without the need for a supercomputer or having to wait several days for the results.

How are you aiming to solve the problem?

We need to find an elegant way of abstracting the model to larger scales without losing the relevant molecular details. Only by doing this can we achieve the ultimate goal of simulating clinical trials. We want to test new drugs on the computer before they are tested in humans. Of course, we need a certain level of detail in the models, i. e. precise information about the molecular interactions of the drug and its metabolism – for all cells in the body and for a large number of patients. It's a huge challenge to be sure, but one that we want to, and have to, address.

How far have you come with that?

We're currently working on a model that enables us to predict B cell and antibody response as a function within the molecular network in each B cell. When exposed to pathogens, B cells start to divide very quickly until they decide to differentiate at a certain point, when they release antibodies and then die off. These decisions are made at a molecular level. We have developed models that describe these molecular events and if we unite them, we will be in a position to be able to predict how the B cell population will develop. The result is a model for population dynamics as a function of the molecular networks in each individual cell. This is one example of this type of multi-scale modelling.

So this model will enable you to predict immune response?

Exactly. Well, for now, one aspect of the immune response. This work shows that the seemingly random decisions of cells actually result in a very predictable overall response. If we imagine the B cell population as a lymphoid organ, for example, there is a predictable immune response at the organ level. We have identified the origins of cell heterogeneity and are thus one step closer to achieving our goal of being able to predict immune response.

As well as to your goal of being able to conduct clinical trials on a computer?

At the moment, we are only able to simulate the dynamics of a cell population in a Petri dish. In the future, we hope to be able to do this for cells in the body. We want models that enable us to predict the course of a viral infection based on the patient's genetic profile, and predict whether the patient is in a position to be able to survive a disease such as ebola. On the basis of these models, it would be possible to manufacture tailored vaccines that are safer and more effective. We want to enable improved treatment for patients with autoimmune diseases and improved diagnostics, which would lead to earlier detection and perhaps even the prevention of a disease.

So your biggest challenge is creating a multi-scale model for the patient?

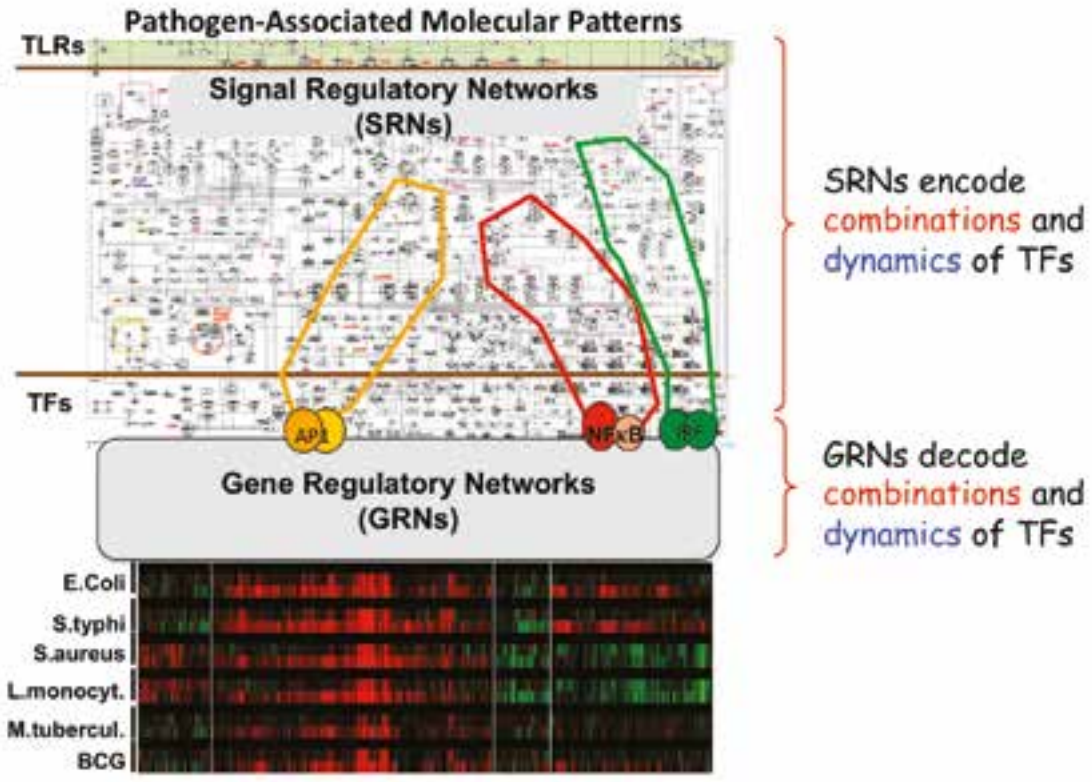
Yes, that's one of many immodest challenges that I am passionate about. I recently moved away from San Diego, where I lived for ten years. One reason for switching to UCLA was the exceptional links between the hospital, the medical faculty and the basic sciences. The institutes here work together very closely. I hope that I will be able to contribute with our research interests in taking the next steps: the clinical application of our knowledge of the molecular mechanisms of gene regulation and the regulation of the immune system. This means lots of huge mathematical models for lots of interacting cells on the way towards the largest scale – the patient.

And UCLA provides you with the ideal conditions for your work?

Yes – I've got all the essential ingredients here: a fantastic hospital and a similarly fantastic medical faculty with strong basic sciences. Right next to us, we have the physics, math and the engineering departments with great bioengineering department and computer sciences faculties. The partnerships are already in place, but we are still missing a central platform to strengthen them even further – a common institute. UCLA leadership recognised this and decided to form a new initiative. I was lucky enough to get this job and am now entrusted with creating the new institute, which is called the Institute for Quantitative and Computational Biosciences.

What kind of researcher is interested in systems biology, do you think?

Anyone can be! I think that it is increasingly seen as the contemporary form of biology. There are definitely several variations, but generally people agree that the primary task of identifying molecules is now complete. We have the human genome, we can determine all RNAs and identify almost all proteins in the cell very quickly. The question now is how they all interact. I think that people agree that this is the biggest challenge in biology today. As a result, I don't view systems biology as a sub-discipline of biology but as a way of providing biology with the tools and approaches it needs to address current scientific questions.



Schematic representation of the molecular network that regulates the innate immune system and the inflammatory immune response of a cell to pathogens. Many molecular interactions have already been documented in medical literature, but it is still unknown how they interact to encode and decode the information of a particular pathogen in intracellular signals, and how these signals regulate the complex process of gene expression. Alexander Hoffmann's research laboratory primarily deals with the three major transcription factors AP1, NF B and IRF, shown in colour (Graphic: A. Hoffmann).

In your opinion, which country has the better research conditions – Germany or the US?

I think the conditions in Germany and Europe are very good. However, I would say that the fixed-term contracts offered to young postdocs in Europe are less attractive than comparable contracts in the US, which offer much better opportunities for long-term employment when successful in the field. However, the research conditions for senior scientists are just as good in Germany as they are in the US. The competition in the US is huge, just as it is in Germany, and this means that the job is an attractive one.

Mr Hoffmann, you are German and have been living in the US since your doctoral studies. Have you ever thought about coming back to Germany?

Yes, I have. Living in different countries and places is exciting, but it's also a challenge and a balancing act. Two years ago, my family and I spent a four-month sabbatical in Berlin. It was a fantastic experience. But after the sabbatical, we decided that, for personal reasons, the next phase of our lives would be spent in California, where we had already integrated and where we feel at home. We'll have to see what happens next...

Interview conducted by Miriam Colindres.

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towards fulfilling the promises of systems biology

Joint Research Center for Computational Biomedicine (JRC) Aachen – a newly established partnership of computational biomedicine

by Andreas A. Schuppert

In 2003 the estimated cost for research and development for a new drug was 1bn US $. Ten years later, these costs rose to 2.6bn US according to the same source[1]. Systems biology has been proposed as a new paradigm to improve drug discovery, but it seems that it could not turn this trend so far. Even worse, the new drugs, designed to cure complex diseases, tend to be ineffective in a significant, yet unpredictable, set of patients. Has Systems Biology provided any benefit so far? Do we see at least light at the end of the tunnel?

Today, modeling and simulation already play a key role in understanding complex biological mechanisms, including some of clinical relevance such as drug resistance, and have contributed to new therapeutic concepts, in particular:

- **Detailed physiology-based models for the pharmacokinetics of new drug candidates allow optimization of dosing strategies in clinical trials, even in non-standard populations.**
- **Network models of molecular mechanisms in cells can be used to simulate of the effects of combinatorial drug therapies and of the evolution of resistance as well as of potentially unexpected side effects (Gammon, 2012). Such models enable the setup of new therapeutic strategies (Lee *et al.*, 2012).**

However, the primary goal, namely a significant reduction of the overall costs of drug R&D, has not been achieved so far. To improve the situation, models are required to predict clinical toxicity and efficacy based on pre-clinical data. Such models will have to cover all factors affecting the diseased cells arising from the biological heterogeneity in patients – i. e., not only the genetics but for example the individual history of disease, lifestyle or co-medication, as well as their interaction. Such models need to include a vast amount of biological heterogeneity that would be impossible in a classically designed and data-poor experimental setting. However, we are now beginning to live in a data-rich world, where a deluge of data from very different experimental settings is starting to become available. Extracting the relevant information and integrating it into pragmatic predictive models requires new concepts in modeling and data analysis that are adapted to the new challenges:

- **Modeling of the therapeutic effects of molecular interventions is basically a multi-scale challenge in space and time. Processes have to be integrated from the molecular level across cell populations up to the patient level.**
- **Biological systems can adapt their functional structures to the drug-induced stress. This "biological plasticity" plays a crucial role in emergence of resistance and corresponding failure of a drug, but it can also be used for the design of new therapeutic concepts that can overcome resistance.**

Modeling of therapies for complex diseases is therefore very much like a jigsaw puzzle, where the shape of the jigsaw pieces are known, but the overall final picture is very unclear. Even worse, the picture can change in response to therapy, owing to biological plasticity. The good news, however, is that it is not necessary to put together the full picture in order to arrive at predictive models for specific diseases and therapies. It is sufficient to identify the relevant parts and focus on assembling only those. This is what we are doing by hybrid modeling integrating data into mechanistic understanding of biomedical processes.

To realize this concept, Bayer Technology Services GmbH, together with RWTH Aachen University and University Clinics

Figure 1: Opening Ceremony of the Joint Research Center for Computational Biomedicine, October 9, 2013
(from left to right: Prof. A. Schuppert, Head, Dr. D. VanMeirvenne, CEO Bayer Technology Services GmbH, Prof. W. Plischke, Member of the Board of Bayer AG, Prof. S. Uhlig, Dean of Medical Faculty of RWTH Aachen, Prof. E. Schmachtenberg, Rektor of RWTH Aachen) (Foto: Bayer AG).

Aachen established the Joint Research Center for **Com**putational **Bi**omedici**ne** (www.combine.rwth-aachen.de). Two research groups, focused on mechanistic and data-driven modeling respectively, will develop novel approaches to establish hybrid models integrating machine learning, mechanistic modeling and "Big Data" analysis for pragmatic solution of pressing needs from industry and clinics. This research is fully pre-competitive and we would be happy to welcome further partners, on the industrial as well as the academic side. The tight embedding into a strong mathematics and computational sciences community allows us to find helpful analogies from complex systems modeling in other areas of science and technology.

Our activities are supported by a scientific advisory board, as of 2015 consisting of Douglas Lauffenburger (MIT), Peter Kohl (Imperial College) and Philip Maini (Oxford University).

Our goals are...

- **to identify the molecular mechanisms that drive therapies, from public data repositories and dedicated experiments,**
- **to translate information from lab-gained drug activity profiles into efficacy of drugs in patients**
- **to characterize patients' individual disease status for optimal therapy**

An example of our activities is the development of stable pattern recognition algorithms in large genome-wide –omics datasets covering a wide range of biological heterogeneity. They allow monitoring and interpretation of lab data on the background of clinically relevant physiology as well as providing tools for cross-species analysis of biological mechanisms. Such algorithms have primarily been developed for the quality assessment of induced pluripotent stem cells and their physiological differentiation products (Lenz *et al.*, 2013), but now they are applied to other problems such as tumor characterization.

Another research area of the Joint Research Center for **Com**putational **Bi**omedici**ne** is the unsupervised identification of biological mechanisms and their mutual interactions from data. The algorithms use intrinsic correlations of large heterogeneous data sets providing multiple-input multiple-output data structures. They are based on the mathematics of functional networks, which had been developed for modeling chemical processes and have already been successfully applied to the mode-of-action analysis of tyrosine kinase inhibitors in resistant and wild type leukemia cells (Balabanov *et al.*, 2013). As they allow the unsupervised identification of mechanisms, these algorithms might be beneficial for the analysis of poorly understood biological systems, such as plants, or for the understanding of toxic action.

A third, newly established area of activities is focused on leveraging of methods from non-linear systems theory for monitoring and prediction of critical states along disease progression. Here our focus is the early identification of transitions from a chronic to a malign disease state.

Figure 2: Speakers of the scientific symposium "Computational Biomedicine for Translational Research"
f.l.t.r: Adriano Henney (Virtual Liver Network); Douglas Lauffenburger (MIT); Andreas Schuppert (RWTH Aachen); Philip Maini (Oxford University); Franz-Josef Müller (CAU Kiel); Tim Brümmendorf (UK Aachen), Peter Kohl (Imperial College London); Joerg Lippert (Bayer Health Care AG); Rune Linding (University Copenhagen); Jacob de Vlieg (Bayer Crop Sciences AG) (Foto: Bayer AG).

Why invest in computational modeling to support translational medicine along the drug R&D workflow? Is there a silver line being readily detectable?

The examples outlined above illustrate how our integrated modeling strategy can deliver valuable results out of huge data sets already today. To discuss the options and risks of computational biomedicine, we hosted an international symposium „Computational Biomedicine for Translational Research" (http://www.combine.rwth-aachen.de/index.php/cbtr2014.html, October 2014) bringing together scientists from industry, academia and clinics, as well as from modeling, wet lab biology and clinical research, to discuss state of the art, the challenges and the unmet needs.

Even if a generic framework for predictive modeling along the drug R&D pipeline is not available yet, the presentations demonstrated clearly that application-specific combinations of experimental design, data analyses and multi-scale modeling technologies provide the tools to assemble more and more jigsaw pieces. There is definitely light at the end of the tunnel!

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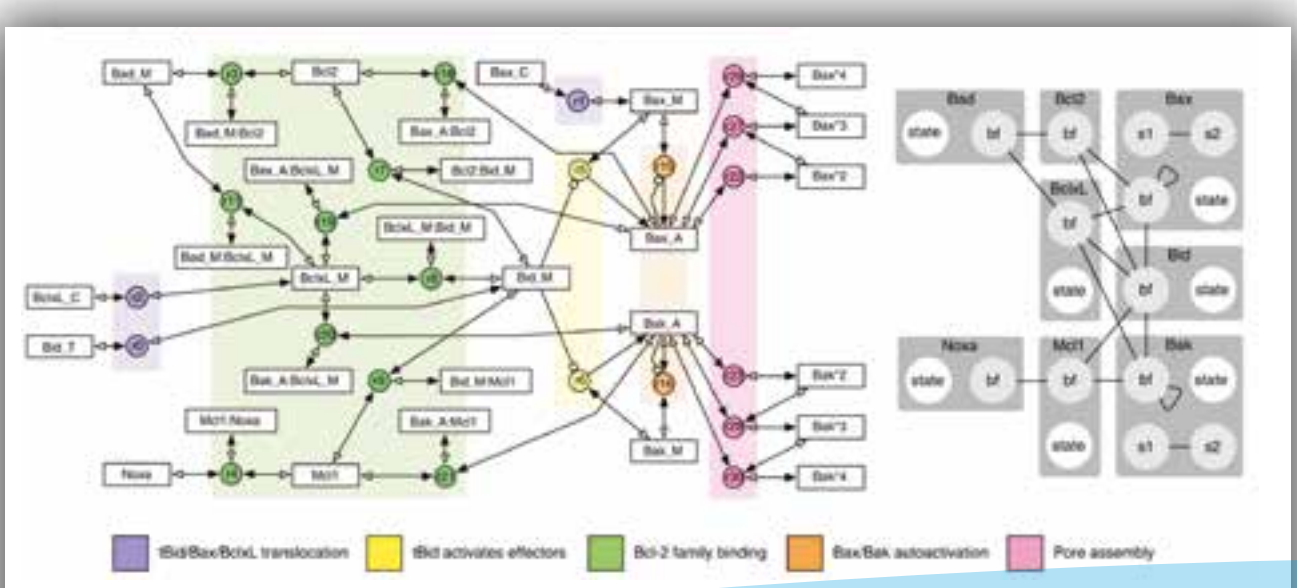
systems biology modelling: what's next?

by Thomas Lemberger

Ten years ago, *Molecular Systems Biology* was launched as a journal covering all flavors of systems biology, from quantitative to genome-scale biology.

It was clear from the very beginning that complementary approaches would be necessary to study (at any scale) the collective properties of the components that form a biological system and to answer key questions in systems biology: how can we understand the organization of the tremendous variety of biological components that make up living organisms; how can we understand the time-dependent behavior of biological processes whose dynamics are essential to biological functions? To interpret the very diverse types of data that were being generated, a variety of methodologies have been developed by computational systems biologists, ranging from classification methods, graph analysis to statistical and kinetic modelling. At the period when MSB was launched, the use of systems of differential equations for modelling the dynamics of biochemical reaction networks considerably gained in popularity. The Systems Biology Markup Language (SBML) format had just been specified to facilitate the exchange of mathematical models and the BioModels database was launched. Even though kinetic modelling had its roots in prior tradition in the fields of metabolism, biochemistry and biophysics, it gained momentum at that time, likely due to the convergence of several factors. First, more efficient and flexible experimental approaches became available and allowed obtaining quantitative biological measurements at the molecular and cellular level. Second, it coincided with the possibility to systematically map the organization of biological systems with omics platforms and the resulting realization that biological processes are executed by interconnected circuits rather than linear cascades. The possibility to combine quantitative data with dynamical systems theory and computational simulations allowed building explanatory and predictive kinetic models of biological networks. This approach was so attractive that it almost became synonymous to 'systems biology modelling'. A decade later, it is instructive to reflect on how this particular form of systems biology modelling has evolved.

Partial representation of an apoptosis model (programmed cell death)



Graphic from: Lopez *et al.*, Mol Syst Biol 2014

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Computer code for the simulation of a kinetic model (Graphic from: Lopez *et al.*, Mol Syst Biol 2014).

The notion of 'molecular mechanism' in classical molecular biology had traditionally been focusing on the description of a sequence of molecular interactions and biochemical reactions. However, an entire new range of fundamental questions became amenable to a deeper mechanistic understanding with the help of kinetic models based on experimental observations: how are steep thresholds or precise boundaries created; how do biological systems maintain their function in spite of random fluctuations in the number and activity of their constituents; how are oscillatory behaviors sustained and what determines their directionality; how can the same system adopt several different steady states under the same conditions and how does this depend on its past history; how is specificity achieved in signaling pathways that share components. A number of biological phenomena and pathways became 'classical model systems' for successive generations of kinetic modelling, including bacterial chemotaxis, yeast pheromone and stress signaling, *Drosophila* morphogen gradients, mammalian signaling pathways such as the NF-κB, EGFR and apoptosis-controlling signaling pathways, circadian clock oscillators and the cell cycle. With each iteration, models were extended by the inclusion of additional components and reactions, accounting for more experimental observations and incorporating further mechanistic details. Progress was further made to analyse more systematically ensembles of models and their complex parameter landscape. However, further increasing model size and complexity brings about the considerable challenge of obtaining sufficient data to constrain them. Furthermore, now that, at least in some model systems, several key dynamical phenomena have been deciphered, it seems more and more challenging to use new models that reveal novel concepts. What will be the new frontiers, beyond adapting what has already been done to perhaps less investigated processes? Is this the end of modelling?

In fact, quantitative studies have only scratched the surface of the complexity of living organisms and the field has considerably matured. An increasing number of research groups successfully navigate from small-scale mechanistic investigations to large-scale 'omics' studies. This entails a perhaps more flexible and pragmatic modelling approach, which adopts solutions adapted to the question posed, using a suitable blend of kinetic bottom-up, statistical top-down or 'middle-out' phenomenological modelling. Ten years ago, mechanistic modelling and omics were clearly separated. Today, it is possible to generate time-resolved phosphoproteomics data with sub-minute resolution, to map protein physical interactions at a large-scale in a condition-dependent manner, and to perform large-scale multi-dimensional perturbation experiments. Consequently, new methods are being developed to integrate omics data into genome-scale metabolic stoichiometric models. These technological advances, amongst many others, are now progressively blurring the distinction between the large-scale and quantitative 'dynamical' branches of systems biology. An additional avenue that may contribute to the junction between large-scale and small-scale quantitative biology is the use of

Thomas Lemberger, Chief Editor of the scientific journal *Molecular Systems Biology* (Photo: EMBO)

coarse-grained models whereby the dynamics of a biological 'module' are described in a phenomenological manner rather than in all molecular details. This approach greatly benefits from the past efforts in systematical mapping such 'modules' both functionally and mechanistically. Finally, the use of well-tested models as 'molecular engines' in multi-agent simulations will open the door to the multi-scale modelling of cell populations based on the properties of molecular networks. Even if it is still in its infancy, the importance of a multi-scale approach to biology has been concretely illustrated in the recent years by the advances in single cell level molecular and phenotypic profiling methods that revealed the extent of cell-to-cell heterogeneity and its impact at the population level.

The advances made in systems biology and modelling in the last ten to fifteen years have irreversibly changed our perception of living organisms. With the knowledge gained from genome-scale analyses, it is no longer possible to ignore the complexity of living organisms, even when focusing on the analysis of a particular biological process. With the availability of time-resolved and single-cell level measurements techniques, the notion of molecular mechanisms has evolved to include the dynamics and variability of biological processes. As such, quantitative thinking and computational modelling are more pervasive and necessary than ever in biology. So, what's next? While it is not possible to predict which of the modelling formalisms and simulation methods will be successful for the interpretation of these new data into novel concepts, it is clear that we have not reached the end of modelling. To quote Winston Churchill, even though the circumstances are not quite as bellicose:

„This is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning".

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focussed on solutions: gene myers makes tools for cell biologists

A profile of the Max Planck director

Among other things, Gene Myers is known for developing BLAST, the world's most widely used program for analysing biological sequence data. His work also contributed to the early conclusion of the human genome project. A mathematician and computer scientist, Myers develops computerised methods and technologies that facilitate the identification of solutions to biological problems. As the director of a research group for image analysis and microscopy at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPI-CBG) and the founding director of the new Centre for Systems Biology Dresden (CSBD), he pursues one single aim: to create effective, interdisciplinary teams that support cell biologists in solving current issues facing cell biology using a systems biology approach.

Myers found his way into sequence biology when, a few years after earning his doctorate, the biologist David Mount was looking for a computer scientist with whom to establish a centre for bioinformatics. Myers stepped up and became increasingly involved in biology from that point on. "I enjoyed the dialogue with biologists, scientists from another culture, and I enjoyed the openness and creativity," he says. The practical approach to scientific dialogue at MPI-CBG in Dresden is evident as soon as you enter the modern research facility: a large, open cafeteria with plenty of seating invites people to discuss issues with their colleagues while they have their first cup of coffee in the morning. "That's why we don't have any coffee machines in the working groups," Myers reveals. "Here, people are encouraged to leave their office to get a cup of coffee and to get talking to colleagues in the process." Regular discussions are a pillar of interdisciplinary work. It is particularly important in developmental and cell biology because these deal with such complex systems, says Myers. "People studying cells, tissues and organisms, like those here at MPI-CBG, also deal with material touching on physics," he explains. He is particularly interested in the topic of molecular self-organisation. "The physics is very complex and the

The Max Planck Institute of Molecular Cell Biology and Genetics in Dresden

Photo: MPI-CBG

Gene Myers in his office at the MPI-CBG in Dresden (Photo: M. Colindres).

scales required are so large that it's impossible to deal with them without the use of modelling and informatics."

The MPI-CBG in Dresden pursues its mission "from cells to tissues". It's not by chance that Myers switched to cell biology following his career in DNA sequencing. "After sequencing the genome in 2002, almost everyone was pretty busy with genomics and expression analyses," Myers says. "To be honest, even if I am partially responsible for the sequencing of the genome, I thought that it wasn't possible to understand it all by continuing to sequence genomes or simply by looking at overall expression." Myers wanted to understand what the units that were created through the genome do in the cells. Although it is sometimes possible to draw major conclusions about the functioning of a system by looking at graphs and partial lists, it is often the case that these conclusions may be wrong and simply not enough to be able to explain life itself. Although Myers was already an expert in genomics, he decided to switch to imaging following the conclusion of the human genome project and dedicate himself to a new class of computer algorithms and methods. His opportunity came in the form of Janelia Farm, the Howard Hughes research campus in Virginia. "There, I was completely free to be a postdoc again," Myers explains. He studied microscopy and image analysis in neurobiology until he was given the opportunity to switch back to cell and developmental biology in Dresden in 2012. At the MPI-CBG, he works on understanding how cells coordinate within tissue and how various molecular components make up entire cells. For Myers, this means considering time and space, thinking about physical forces and taking phase transitions into account, for example. It's a challenge that, in his opinion, can only be mastered by efficient teams of physicists, computer scientists and biologists.

Myers creates tools that make biologists' work easier and, sometimes, are even what make it possible. He emphasises that he doesn't develop biology hypotheses himself as he is a technology specialist who creates platforms. His areas of research include optical engineering, bioimaging, informatics and, to a certain extent, modelling. Myers and his team of information technology specialists and physicists develop software for data capture in microscopy and other imaging procedures, and they build customised optical microscopes. "Virtually no one is investigating a gene in a transgenic animal in quantitative terms, for example, and creating a model of what they can see in the microscope," Myers says. "Our technologies make this possible." He is particularly interested in following long development axes – for example, the development of a nematode, embryo development and the development of wings on fruit flies, or embryo development in the zebrafish. "If we take *Drosophila* as an example, we see that, within 24 hours, the fertilised ovum turns into a fully developed larva comprising around 100,000 cells. I would love to be in a position to be able to watch the genome as it is expressed on a cell-to-cell basis on its journey from one to 100,000 cells," says Myers. His vision is of cellular atlases of tissues and organisms that are annotated with molecular information. *C. elegans*, the nematode, is a particularly well-suited model organism because the development of the individual cells is determined very early on. It would seem obvious to create a cellular atlas here. With such a resource, transgenic constructs could be observed under the microscope, for example, and molecular annotations, such as the time of gene activation, could be carried out in real time.

The aim Myers has is to digitalise and quantify cellular developmental biology. His work benefits collaboration partners who need algorithms and software in order to extract and interpret images. In a current project, he is working on creating a model for the development of the wings on *Drosophila*. Each individual cell is tracked over a period of 18 hours – a total of 20,000 marked cells. At the start of the project, it took a month to process the data using the software available at the time. This was a very limited timespan given that perturbations were to be carried out on the organism and each experiment took a month. Myers is working on a solution that can achieve the same thing but within one day, and with very high performance. "We have the necessary expertise within my group. It's easy to achieve an 80 % solution, but if you want a 99 % solution – well, that's when you give me a call."

Explaining 99 % of the data in a reproducible, automated way is a real challenge. However, things are looking good with regard to the wings on *Drosophila*. The data is clean enough that the software is able to produce a complete and sufficiently accurate model. However, things look a little different for a complete organism, such as *C. elegans*. Currently, neither the microscopes nor the available software is good enough. "We can recognise patterns and get an impression of cell migrations," Myers says. "With a preliminary, approximate model, we are already able to answer some questions at least qualitatively." The requirements for the long-term observation of a living organism are high. Myers is restricted, for example, by the natural resolution limits in optical microscopy. How is it possible to achieve higher resolutions with consistently low light levels so as not to negatively influence the sample in its development? Distorted images are also a problem, caused, for example, by tissue as a result of refraction on lipid membranes. Myers achieved a significant improvement in image quality by automatically controlling and adjusting the beam focus and other microscope parameters in real time via computer. This enabled him to optimise the resolution and reduce visual artefacts. "We are gauging the limits of technology here and pushing the potential of optical microscopy forward with our customised microscopes," Myers explains. As a mathematician who is new to the area of physics, he is enthusiastic yet bewildered by the fact that there is so much uncharted territory in this field and still so much to do, despite the fact that optical microscopes have been around for a long time.

Myers's microscopes are designed for very specific applications. The microscope for the embryo development of *Drosophila* is specially adapted to the particular shape of the embryo. In addition to microscopes for developmental processes in living organisms, Myers also constructs microscopes for intracellular imaging in order to depict organelles, for example objects that are smaller than 5 µm. This has a whole host of different requirements, including high temporal resolution because interesting processes are only observable within very short time spans. Here, image processing uses FPGAs and GPUs. The integrated robotics enable us to observe and adjust the subject in 3D on the computer, and all in real time. "We are able to do that as information scientists. We are great engineers. Our microscopes can do things that standard microscopes can't and so permit scientific discoveries that would otherwise not be possible," Myers says with pride.

Myers was the founding director of the new Centre for Systems Biology in Dresden, the CSBD. Its mission it is to unite all aspects of systems biology in one building – analytical biology, bioinformatics and systems biology. "We want to combine the best of physics for modelling, the best of informatics, i.e. computer algorithms, methods and techniques, and the best of systems biology in terms of work on the cells and tissues," Myers emphasises. In principle, the CSBD is the logical combination of the MPI-CBG and the Max Planck Institute for the Physics of Complex Systems. "It arose from the well-established cooperation between biologists and physicists in Dresden." The new office building will house physicists, mathematicians and information scientists who will actively work with the biologists next door at the MPI-CBG and further afield. It will serve as a data centre for a large computer cluster. A high-speed link to the Dresden University of Technology has already been constructed and it will offer a wide range of resources in terms of optical technologies, interpretation and modelling. Although the building is not yet finished, the centre has already begun operations. In addition to teaching students, the CSBD also offers a postdoc programme and grants for PhD students.

For Myers, it is very important to assemble teams of a very high calibre. To do this, it is not enough to simply encourage dialogue between postdocs and doctoral students in research groups, as is usually the case. In line with his model, it is the heads of the working groups or "experts" who primarily work together and meet regularly for scientific discussions. Only by doing this can the centre function at the highest level, with the aim of driving basic science forwards.

The X-Wing is a microscope that is used to record images of and track all cells in a developing Drosophila embryo. The microscope gets its nickname from its four arms, which project laser beams into the embryo, and make the microscope look like an X-wing spaceship from Star Wars. The two other arms at the front and back each contain a very high-resolution lens and a specially controlled camera. In total, six lenses and a lot of control software developed in-house are used in order to achieve the highest possible image quality without affecting the development of the organism being observed (Photo: M. Colindres).

According to Myers, his biggest challenge is to actually create the things that he believes he can make. Always keeping the solution to a problem in mind, he is very busy with what he is currently working on and still has plenty to do in the future. There are some unsolved problems that he is interested in. "A mechanism for measuring physical forces at the cellular level would be great. I would like to understand hydrostatic pressure and how membranes interact with one another in terms of forces. It would also be great to be able to conduct individual cell sequencing. Then we would be able to investigate the expression status of individual cells." Myers has everything he needs to foster his creativity at the MPI-CBG: a positive environment, motivated colleagues, a healthy pressure to perform and good coffee. "This institute isn't just unusual in terms of its science departments, but also in terms of its sociology." He is also extremely happy with the research conditions in Germany. He appreciates the excellent access to resources and praises the ambition and intellect of doctoral and postdoc students: "The German university system produces exceptional scientists!" Not least, he also feels at home in Germany for personal reasons: he and his wife love the city of Dresden, its lifestyle and German culture.

Interview conducted by Miriam Colindres.

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BioComp – complex data analysis in life sciences and biotechnology

A new research initiative at the University of Kaiserslautern

by Dorothea Hemme, Christina Surulescu, Holger M. Becker, Joachim W. Deitmer, Timo Mühlhaus, Christoph Garth and Michael Schroda for BioComp research

The University of Kaiserslautern – an excellent location for research in systems biology

The University of Kaiserslautern specialises in technology and engineering in the fields of architecture, civil engineering, biology, chemistry, electrical and computer engineering, computer sciences, mechanical and process engineering, mathematics, physics, regional and environmental planning, social sciences and business studies.

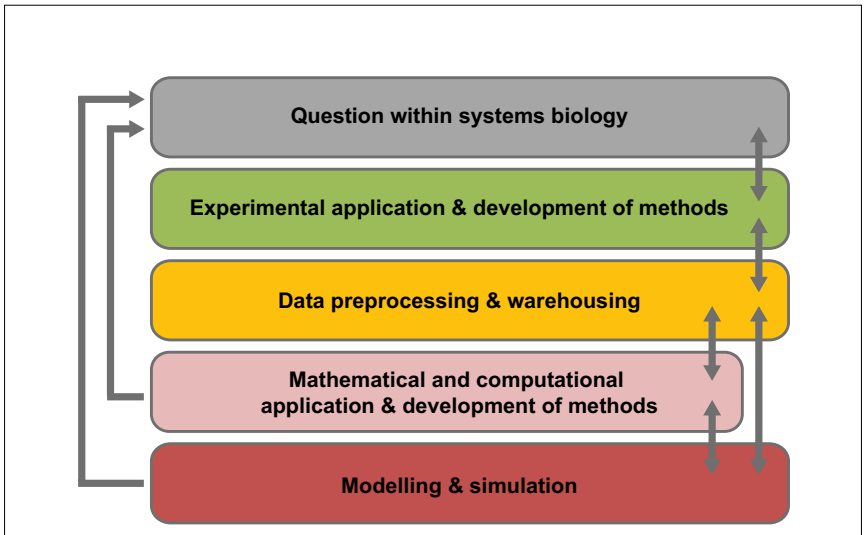
The scientific landscape of the University of Kaiserslautern is also defined by highly respected research institutes, such as the Fraunhofer Institute for Industrial Mathematics (ITWM) and the Fraunhofer Institute for Experimental Software Engineering (IESE), the German Research Centre for Artificial Intelligence (DFKI), the Institute for Composite Materials (IVW) and the Max Planck Institute for Software Systems (MPI-SWS). The close proximity of the faculties and research institutes outside the university significantly facilitates partnerships.

The life sciences at the University of Kaiserslautern have a very good analytical and resource infrastructure. For example, three high-throughput platforms have been developed that are able to conduct mass spectrometry of proteins and metabolites, the automated quantification of protein structures via CD spectroscopy and the localisation of molecules in living cells via fluorescence microscopy. Using this infrastructure and interdisciplinary partnerships as a foundation, the research area "BioComp – Complex Data Analysis in Life Sciences and Biotechnology" was established in 2014 in order to address hypotheses in systems biology as part of the state of Rhineland-Palatinate's research initiatives.

The consistent structure of all BioComp projects creates synergies

BioComp comprises 23 principal investigators from the fields of biology, physics, mechanical and process engineering, mathematics, computer sciences and the Fraunhofer ITWM in 14 sub-projects. In order to foster an interdisciplinary approach, all BioComp projects are built up of five basic elements (Figure 1).

Figure 1: All BioComp projects are built up of five basic elements



Within BioComp, all projects share a common structure for addressing biological questions based on bottom-up and top-down systems biology approaches (Graphic: Dorothea Hemme).

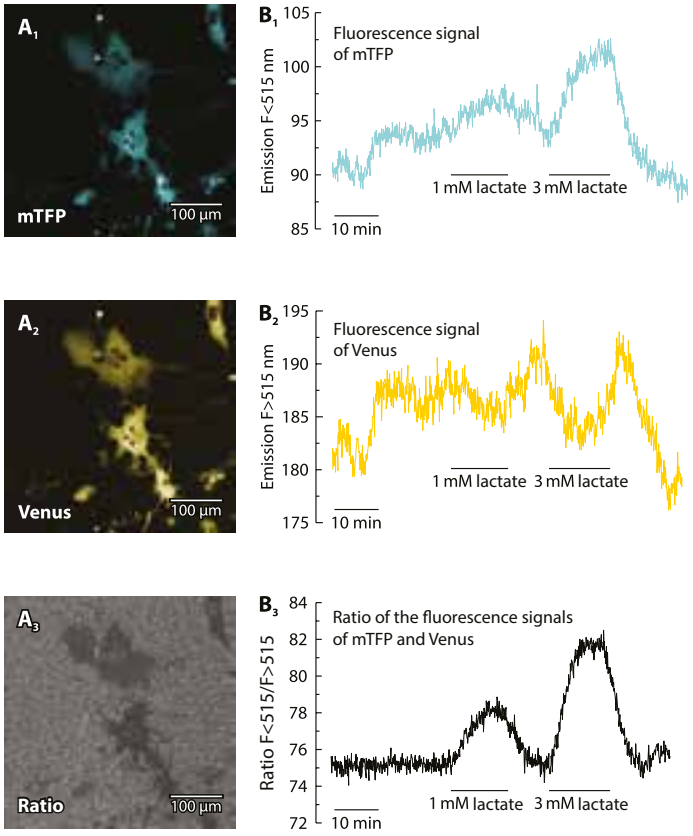


Figure 2: Real-time measurement of the relative intracellular lactate concentrations in human MDA-MB-231 breast cancer cells using the lactate-sensitive FRET nanosensor, Laconic.
In order to measure the intracellular lactate concentration, the lactate-sensitive, FRET-based nanosensor Laconic (San Martín, A. *et al.*, 2013, PLoS One) was introduced to human MDA-MB-231 breast cancer cells via adenoviral transduction and the fluorescence signals of the FRET donor mTFP and the FRET acceptor Venus recorded using a confocal laser scanning microscope.
A_{1-3}) Fluorescence signal from mTFP (**A_1**), Venus (**A_2**) and ratio of both signals (**A_3**) in MDA-MB-231 cells expressing Laconic.
B_{1-3}) Changes in the fluorescence of mTFP (**B_1**), Venus (**B_2**) and the ratio of both signals (**B_3**) during application of 1 and 3 mM lactate. The increase or decrease in the ratio during application or withdrawal of lactate shows an increase or decrease in the intracellular lactate concentration, which indicates that lactate ions are transported via the cell membrane.
(Data: Samantha Ames, Graphic: Holger M Becker)

Depending on the biological question, the members of a BioComp project cover four to five of these elements. This consistent structure behind the individual projects leads to a high level of communication between the researchers involved in the respective BioComp project.

The close cooperation between life scientists, mathematicians and computer scientists enables the processing and interpretation of complex data on the one hand, and on the other, the data generated and structured for long-term use offer a comprehensive basis for pursuing mathematical and informatics-based hypotheses. On the one hand, experimental data obtained by scientists from life sciences are used for providing explanations and predictions about various biological processes. On the other hand, innovative mathematical models raising new biological questions are to be tested experimentally. Iterating between these two approaches is expected to deepen the knowledge about the phenomena of interest.

The hypotheses and experimental approaches developed in BioComp cover a wide spectrum within the life sciences.

Two BioComp projects from the bottom-up and top-down categories are presented below:

Modelling pH regulation in tumour cells and surrounding tissue to determine their influence on the migration and invasion of cancer cells

Within BioComp, C. Surulescu (mathematics), J. W. Deitmer (biology) and H. M. Becker (biology) work together to investigate the influence of the intracellular and extracellular pH on cancer cell migration and their invasion of healthy tissue. In recent years, there have been growing indications that the tumor microenvironment can determine the phenotype of its cells (Gatenby, R.A., and Gillies, R.J., 2007, Int. J. Biochem. Cell Biol.; Hanahan, D., and Weinberg, R.A., 2011, Cell). For example, insufficient oxygen supply (hypoxia) and acidosis in the tumour tissue can trigger the transition from benign to malignant cell growth (Webb, B.A. *et al.*, 2011, Nat. Rev. Cancer). In order to survive in their environment, tumour cells upregulate specific proton extrusion mechanisms. The extrusion of protons from the cell results in an acidification of the extracellular space, which causes the death of the surrounding, healthy cells, allowing the tumour tissue to expand in the space they left behind. Acidosis in the tumour restricts blood

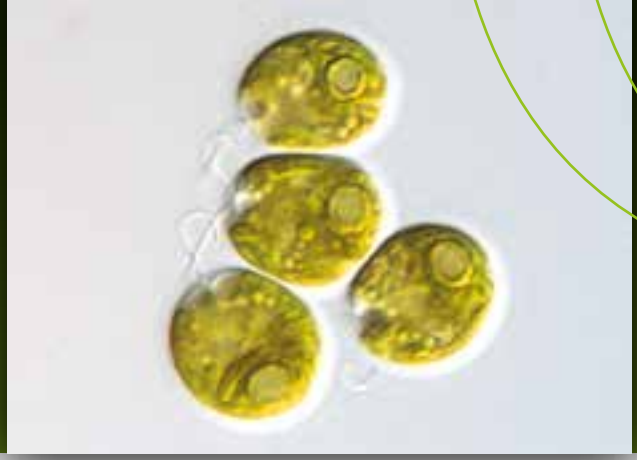


Figure 3: Microscopic image of the single-cell green alga *Chlamydomonas reinhardtii* (Photo: Michael Schroda).

supply and alters the metabolism of the cancer cells. Moreover, the pH also affects the metastatic potential of tumour cells (Martinez-Zaguilan, R. *et al.*, 1996, Clin. Exp. Metastasis; Stock, C., and Schwab, A., 2009, Pflugers Arch).

Multiscale mathematical models have been used to investigate the effect of the intracellular and extracellular pH on cancer cell migration and invasion (Stinner, C. *et al.*, 2014, IMA J. Appl. Math.; Hiremath, S., and Surulescu, C., 2015, Nonlin. Analysis B: Real World Appl., Hiremath, S., and Surulescu, C., 2015, preprint, TU Kaiserslautern). The modelling scales extend from the microscopic level, at which the intracellular proton dynamics is described via ordinary or stochastic differential equations, up to the macroscopic level of cancer cell populations and tissue. On the latter scale, the development of tumour cells is characterised by accounting for their interaction with healthy tissue and extracellular protons and modelled by reaction/diffusion taxis equations. Particular attention has been paid to the regulation of both intra- and extracellular protons.

The underlying experimental data are collected by the biologists on the team. Physiological experiments on human tumour cell lines and multicellular tumour spheroids enable the generation of quantitative data, including absolute changes in the intracellular and extracellular pH or the identification of intracellular concentrations of metabolic products. This is done using modern imaging processes, such as ratiometric measurement based on pH-sensitive fluorescent dyes and single-cell metabolite imaging using FRET-based nanosensors for glucose, lactate and ATP under a confocal fluorescence laser scanning microscope. The validity of the results gained from the mathematical models is then experimentally tested (Figure 2).

One major long-time objective of this project is the development of possible therapy strategies in oncology. To aim this, the theoretical sensitivity of a tumour to various treatment regimens is investigated by using numerical simulations and qualitative analysis for the developed mathematical models.

Analysis of the cellular response of *Chlamydomonas reinhardtii* to environmental changes

Another BioComp project sees the cooperation of T. Mühlhaus (bioinformatics), C. Garth (informatics), D. Hemme (biology) and M. Schroda (biology), who are investigating cellular response of the eukaryotic single-cell green alga *Chlamydomonas reinhardtii* to changes in environmental conditions (Figure 3).

Regardless of their genetic composition, all living organisms are able to adapt to changes in their environment. This ability is essential to their survival in a constantly changing environment. A comprehensive understanding of the molecular principles of this adaptation strategy is necessary in order to be able to specifically manipulate crops so that they can survive more effectively in extreme environmental conditions, such as heat waves, which are increasingly frequent as a result of global climate change.

Cellular adaptation to environmental conditions is based on dynamic changes in the expression of genes and proteins, as well as the metabolism. These consist of a chronological sequence of defined response elements. In heat-stressed cells, for example, CO_2 fixation decreases in order to divert ATP and reducing equivalents from the light reactions of photosynthesis to the synthesis of saturated fatty acids. The latter is required immediately after exposure to heat in order to reduce the increased fluidity of biomembranes. As soon as this has been achieved, CO_2 fixation is reactivated in order to dispose of ATP and reducing equivalents, and thus counteract the accumulation of electrons from the light reactions (Hemme, D. *et al.*, 2014, Plant Cell). Identifying such elements and their occurrence in time during a response to changing environmental conditions requires time-resolved experiments that collect data on physiological (e. g. photosynthetic and respiratory activity), cytological (e. g. cell size, number and

Members of the BioComp research area at a meeting with colleagues from the University of the Greater Region (www.uni-gr.eu) in Kaiserslautern in March 2015 (Photo: Dorothea Hemme).

morphology) and molecular parameters (e. g. transcriptome, proteome, metabolome and lipidome profiles).

The challenges posed by these top-down systems biology approaches are two-fold. Firstly, experimental platforms need to be established in order to generate high-quality, high-throughput data on molecular parameters. Such a platform for the time-resolved analysis of relative changes of (now) ~2,000 proteins was described in issue 02 of systembiologie.de (Hemme, D. *et al.*, 2010, systembiologie.de) and was used in studies that investigated the response of *Chlamydomonas* to heat stress (Mühlhaus, T. *et al.*, 2011, Mol. Cell. Proteomics; Hemme, D. *et al.*, 2014, Plant Cell), an increase in light intensity (Mettler, T. *et al.*, 2014, Plant Cell) and nitrogen deprivation (Schmollinger, S. *et al.*, 2014, Plant Cell). Secondly, the essential relevant information has to be extracted from the large, highly complex data sets. This data is often fragmented (not all proteins, metabolites and lipids are recorded) and influenced by technical and biological background noise. An analysis that is too fine-grained can lead to an overinterpretation of individual processes and thus the misidentification of an element in the adaptation response. This problem is well known from statistics and machine learning and is referred to in these fields as model overfitting. However, an overly coarse-grained analysis may overlook certain elements of the response. For this reason, it is important to develop an algorithm that conducts the data analysis in the correct granularity in order to identify components of the adaptation response robustly. This algorithm is based on an intelligent combination of the depiction of the responses at the levels of functional ontologies and individual molecules.

The research project in brief:

The "BioComp – Complex Data Analysis in Life Sciences and Biotechnology" research area was established in January 2014 as part of the state of Rhineland-Palatinate's research initiatives and comprises 14 sub-projects. Team members cooperate on an interdisciplinary level and develop processes up to the point of application in order to understand biological systems in their entirety.

www.uni-kl.de/biocomp

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ImmunoQuant: the race between viral infection and innate immune response

An interdisciplinary research association of virologists and systems biologists

by Marco Binder, Lars Kaderali, Melanie Rinas, Diana Claußnitzer and Thomas Höfer

Viruses cause a large number of infectious diseases. Hundreds of millions of people around the world suffer from serious viral infections every year, which cause both pain and suffering as well as incurring high treatment costs for the healthcare system. In order to ward off viruses, we need a healthy innate immune system. One of the key mechanisms in the antiviral immune system is the interferon response: cells infected with a virus form cytokines from the interferon family that warn uninfected cells and trigger their antiviral protection mechanisms. Human viral pathogens inhibit the interferon response and cause severe acute infections or chronic disease, which can lead to progressive damage to the organs affected.

The dengue virus and hepatitis C virus (HCV) are two related viruses that cause acute or chronic disease. As yet, there is no specific therapy or vaccine for dengue fever. There is also no effective vaccine against HCV, although an efficient antiviral therapy that has to be adapted specifically to the lifecycle of the virus has recently become available.

The ImmunoQuant joint research project, funded by the German Federal Ministry of Education and Research, pursues an integrative, systems biology approach in order to express in quantitative terms the race between the spread of a virus within an infected organism and the protective interferon response. The resultant systemic understanding of interferon response should aid in the development of more efficient therapies. In order to achieve this aim, the ImmunoQuant project involves scientists from a broad range of disciplines: virologists, systems biologists, biophysicists, chemists and information scientists. While some partners are based in Dresden, Magdeburg, Braunschweig and Frankfurt, the majority of the team conducts its research in Heidelberg. This close geographical proximity facilitates scientific partnership.

Together, the scientists investigate the innate immune response at the molecular, cellular and organismal level. One particular focus for ImmunoQuant is on the use of imaging processes. With the aid of fluorescence microscopy and related techniques, the scientists can observe the replication of viruses, the production of interferons, the protective response that they induce and the cell death caused by viruses in both living cells and laboratory mice. This data is then used to develop mathematical models that simulate the "race" between viral infection and innate immune response. These theoretical analyses provide data about the molecular processes in the host cell and virus that determine whether the immune response or the virus "is first over the line", i. e. either the infection is warded off or it proliferates within the organism. The findings from this then trigger further experiments.

Many interdisciplinary partnerships within ImmunoQuant developed from the preceding project in Heidelberg, ViroQuant, which was also funded by the German Federal Ministry of Education and Research. The findings from ViroQuant indicated that the reactions of the host cells to the virus could vary dramatically and comprised an element of chance (Rand/Rinas *et al.*, 2012). A central task of the current research, therefore, is to understand the extent to which the

Members of the ImmunoQuant research association at the status meeting in April 2015 at the BioQuant Center in Heidelberg (Photo: Ulrike Conrad).

very heterogeneous individual cell responses contribute to a coherent view of the dynamics of infection within the organism. In addition to microscopy, this involves the use of quantitative methods from biochemistry to analyse the molecular networks responsible within the cells. This varied data poses an exceptional challenge in terms of mathematical modelling: multiple-scale models integrate the experimental data about the molecular, cellular and organismal levels.

In order to determine mechanistic principles, models highly suited to experiments in laboratory mice (e. g. infections with the Newcastle disease virus) and human viral pathogens (dengue virus, hepatitis C virus) are being investigated. All these viruses induce an interferon response and inhibit this in various ways in order to successfully infect the host. This research work will be expanded with investigations into human immunodeficiency virus-1 (HIV-1), whose mechanisms in terms of innate immune response have yet to be thoroughly researched. The ImmunoQuant research work on the mechanisms of innate immune response to viruses are part of a long-term strategy that aims to understand the dynamics of viral infections and immune response in quantitative terms on the organismal level. While ImmunoQuant partnership projects cover a whole range of topics, we will introduce two in more detail below.

Antiviral signal cascades in hepatitis C infection

In order to better understand the race between an infectious virus and the innate immune response within the cell and potentially enable a more precise therapeutic response, ImmunoQuant adopts an interdisciplinary approach: biological findings from the relevant literature and data obtained through experiments performed specifically for this purpose are collated in a mathematical model that enables the simulation and quantitative prediction of highly complex processes during a viral infection. In order to be able to create this model to start with, the entire system (the viral infection and cell's own innate immune response) first needs to be broken down into more manageable signalling pathways and processes. There are two basic sub-systems: the replication of the virus in the host cell itself on the one hand, and the RIG-I/IRF-3 pathway on the other. The latter functions similar to an

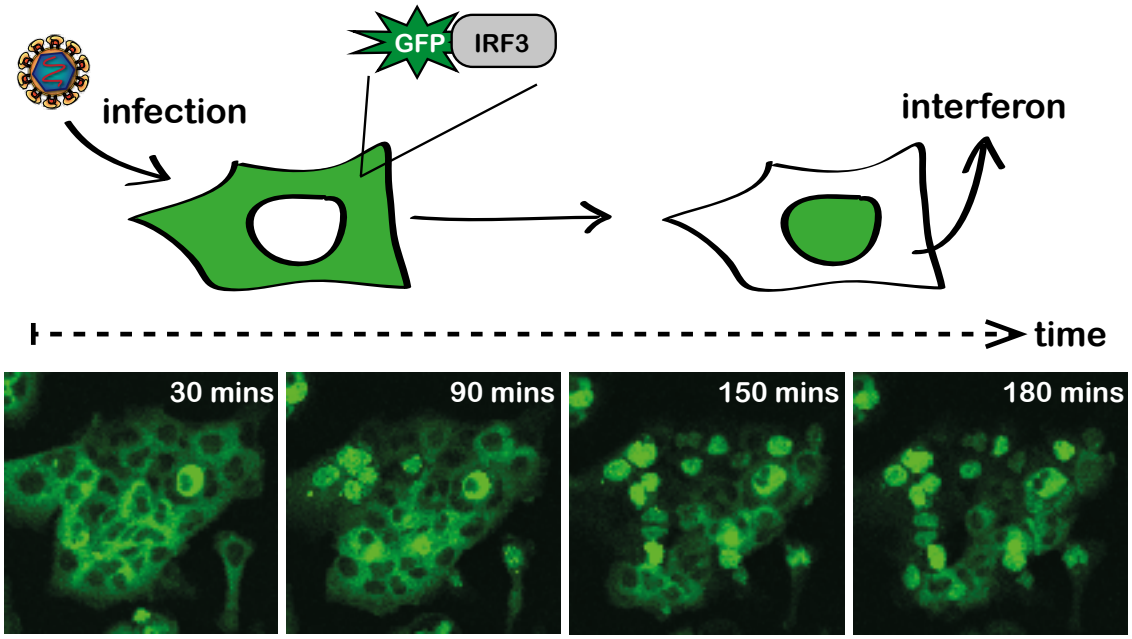

Figure 1: In order to analyse the dynamics of antiviral response in infected cells, the scientists use a genetically modified cell system. The central transcription factor of this intrinsic immune response, IRF3, has been marked here with green fluorescent protein (GFP). Only once a cell has been infected with a virus does the RIG-I signalling cascade lead to IRF3 being phosphorylated and allowing it to migrate from the cytoplasm to the cell nucleus, where it leads to the production of the antiviral cytokine interferon. This migration can be observed and quantitatively evaluated using live-cell microscopy (Graphic: Marco Binder).

early-warning system in the cell, recognising tell-tale characteristics of the virus and triggering the antiviral alarm in the cell and its neighbouring cells. These two systems have been investigated within ImmunoQuant by the groups headed by Lars Kaderali (Dresden University of Technology) and Marco Binder (German Cancer Research Center, Heidelberg), who have been successfully collaborating in the field for many years.

As part of the preceding EU-funded project called SysPatho, Kaderali and Binder were able to develop and test a mathematical model for the clinically highly important hepatitis C virus (Binder *et al.*, 2013). This model is now being developed further by ImmunoQuant: above all, it is being augmented by including those interfaces where the virus depends on its host cell and where the antiviral defence mechanisms of the cell are potentially triggered. To do this, Binder treats HCV-replicating cell cultures with defined amounts of interferon – the substance that is released by virus-infected cells and that triggers the antiviral response. With high sensitivity and time resolution, data is then collected on how the activation of the antiviral response affects HCV replication over time. The mathematical model enables conclusions to be drawn about the individual stages in virus proliferation, which is inhibited by the interferon system.

The model of the viral lifecycle is complemented by a mathematical description of interferon production. The signalling cascade of the innate immune system that triggers the production and secretion of interferon is the RIG-I/IRF-3 signalling pathway, which starts when the RNA virus genome is identified. The issue of how the sensor, the RIG-I molecule, is able to distinguish between the cell's own and foreign RNA was already the subject of earlier research within Binder's working group (Binder *et al.*, 2011). Identifying RNA is now also an important first step in the mathematical model of the signalling pathway. A simplified signal chain is then used as the basis for the model, enabling the quantitative and dynamic prediction of how the cellular antiviral defence mechanisms are activated, including the production and secretion of interferon (Figure 1). Throughout the project, other important stages that are key to the regulation of the signalling cascade are integrated into the model via the time-resolved, experimental characterisation of highly specific individual protein/protein interactions along the signalling pathway.

As one aim of the ImmunoQuant project, both modules – the viral lifecycle and innate immune response – are to be linked and collated as part of a large, comprehensive model. What will be critical is making sure that the interdependencies are correctly represented: the output of the virus model, i. e. the amount of synthesised virus RNA over time, will be used as the input for the immune model. This in turn predicts the dynamics of the production of antiviral cytokines, primarily interferon, whose concentration must then be integrated as a negative factor in the virus replication model. In addition, the virus also has mechanisms with which it can actively counter immune response: an enzyme coded into the virus genome, the protease NS3/4A, can break down and thus destroy a central signalling molecule in the RIG-I pathway (Cardif / MAVS) within the cell (Meylan *et al.*, 2005). This active protective mechanism for the virus can also be implemented in the models because both the amount of viral proteins (including NS3/4A) and the dependence of the RIG-I/IRF3 pathway on the available amount of MAVS can be predicted.

Finally, this combined model will help to better understand the complex interdependencies between the virus and cellular immune response and therefore give us an insight into the mechanisms behind deciding which side – the virus or the immune system – wins the race. With this research, Binder and Kaderali hope to advance our understanding of why most viral infections are over within a week and why some viruses (such as HCV) manage to circumvent the body's immune system and cause chronic infections lasting years or even decades.

An early window for stopping the spread of dengue viruses

Around half of the world's population lives in predominantly tropical or sub-tropical regions, where mosquitoes transmit dengue virus to humans. Every year, around 390 million people are infected with the dengue virus, which can by asymptomatic or can produce flu-like dengue fever, which is life-threatening in around 500,000 cases per year. Because there is neither a tested vaccine nor an antiviral therapy, dengue fever is a global health problem.

Following the infection of a host cell, the dengue virus activates the production of interferons but tries to block the reaction of the cells to interferons at the same time. A team of scientists led by Ralf Bartenschlager at the University of Heidelberg and the German Cancer Research Center have been able to show that dengue virus cannot reproduce in cells that contain interferon signals. As a result, it is a mystery as to how the virus manages to infect people with an intact interferon system.

In order to understand the dynamics of the race between the proliferating dengue virus and the antiviral interferon response, the Bartenschlager working group is cooperating with the working group led by Thomas Höfer at the German Cancer Research Center. As part of this partnership, the researchers developed the first live-cell microscopy system that allows them to simultaneously observe the replication and spread of a fluorescent-marked dengue virus and the induction of interferon response using fluorescent reporter proteins (Figure 2A).

This real-time analysis shows that individual cells can react extremely randomly to the interferon secreted and that the absence of an immune response promotes the spread of the

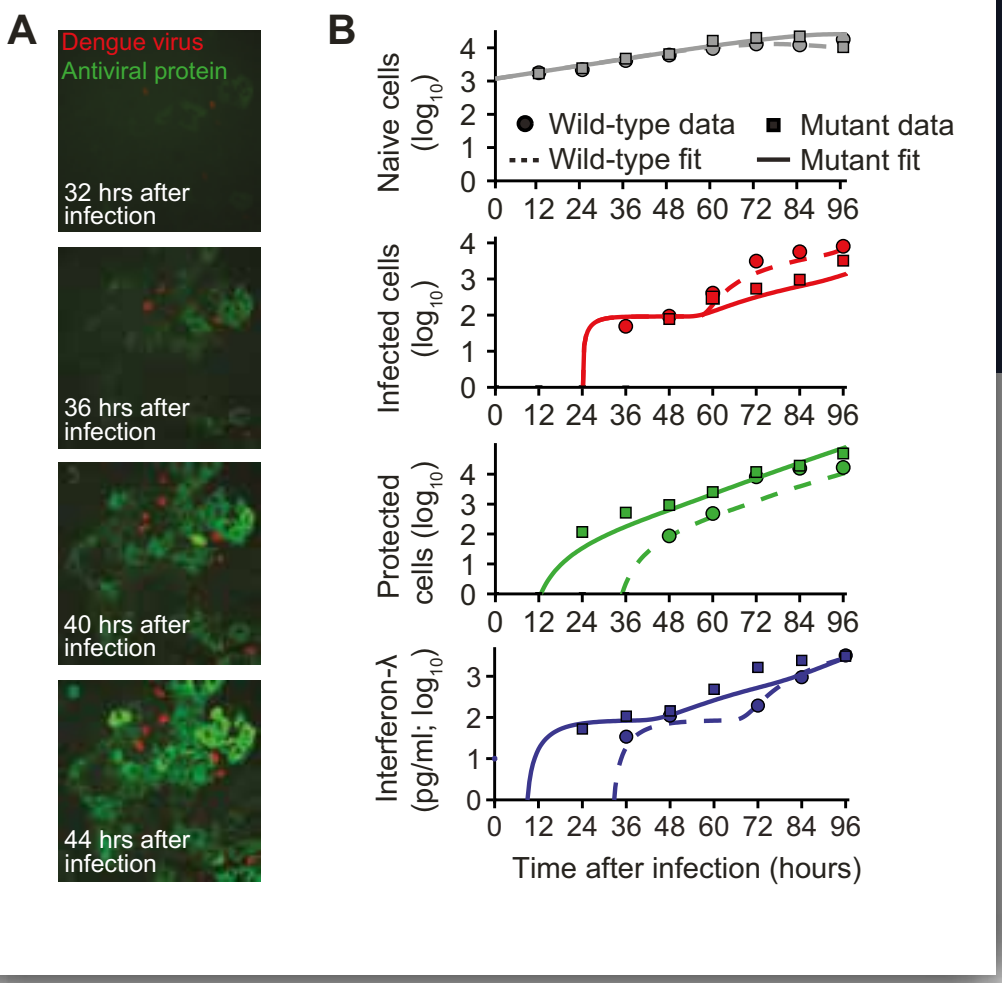

Figure 2: Single-cell analysis and data-driven modelling to research the race between the spread of dengue viruses and the antiviral interferon response (Graphic: Bianca Schmid, Melanie Rinas).

virus in unprotected cells. In order to find out which elements of the interferon system have a decisive influence on the proliferation of the virus, the researchers compared the infection dynamic of the dengue virus wild type with that of an investigational vaccine, namely, a mutated dengue virus. Quantitative data on the comparison between the infection kinetics of the wild type and the mutants show that the mutated virus stimulates a much stronger innate immune response and hardly proliferates (Figure 2B).

Based on this kinetic data, the scientists developed a mathematical model in order to analyse in greater detail the temporal correlation between virus replication, virus production and the release of interferon. The adaptation of the model parameters to the data (Figure 2B) showed that the formation of interferon following infection with the wild-type virus occurred almost simultaneously with the release of new viruses by infected cells – an important indicator that the immune response was too late to prevent the spread of the virus. By contrast, cells that were infected with the mutant virus secreted interferon much earlier. This result confirms that the mutants may be suitable for a vaccine. It triggers a strong immune response that may check the infection in the early stages.

How exactly does the early release of interferon prevent the spread of the virus? To investigate this, the scientists first conducted simulations of their mathematical model. Surprisingly, this showed that the protective function of interferon on yet to be infected cells actually had a very low impact on the spread of the dengue virus. This prediction by the model was then confirmed through validation tests. The protective function of interferon in terms of the spread of the dengue infection primarily affects cells that have already been infected. The scientists discovered that host cells are still receptive to the antiviral effect of interferon in the early phase of infection, while this effect is later inhibited by the dengue virus. In order to determine this antiviral interval with more precision, the researchers now want to look in more detail at the stages of the replication cycle of the dengue virus.

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ImmunoQuant

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events

10th International Conference on Genomics (ICG-10)

23–25 October 2015, Shenzhen, China

The ICG conference is one of the most important annual events in Omics research. ICG-10 will be celebrating its tenth anniversary in Shenzhen, China, with talks by outstanding, international scientists from all fields of Omics research, including single-molecule analysis, genome editing, synthetic genomics, phenotyping, bioinformatics and dealing with the comprehensive analyses associated with ever-expanding big data sets. This will also result in discussions on bioethics and social implications, which continue to grow in importance. The event is intended as a way of marking the start of a new era, where Omics research could aid efforts to improve the treatment of diseases and to preserve health in the coming decade.

For more information and registration, please visit:
www.icg-10.org

3rd International Systems Biomedicine Symposium Big Data in Health Care – Challenges, Innovations and Implementation

28–29 October 2015, Luxembourg

The Luxembourg Centre for Systems Biomedicine (LCSB) and the EuroBioForum Foundation are joining forces to host the third international Systems Biomedicine Symposium in Luxembourg. The symposium wants to bring together experts from the worlds of science, industry, clinical practice, politics and patient organisations all working in the field of big data in healthcare in order to promote dialogue about the latest technologies and scientific discoveries.

The event will take place on 28 and 29 October at the Hôtel Légère in Luxembourg-Munsbach.

For more information on the programme and to register, please visit:
http://bigdata2015.uni.lu/eng

LCSB

EURO BIO FORUM

3rd International Systems Biomedicine Symposium

"Big Data in Health Care"

28-29 October 2015

Luxembourg

Save the date

Conference report

7th International Conference on Systems Biology of Human Disease – SBHD 2014
June 17–19, 2014, Boston, USA

BRINGING SYSTEMS BIOLOGY TO CANCER, IMMUNOLOGY AND INFECTIOUS DISEASE

by Kelvin A. Janes* and Chun-Chao Wang

Over 250 scientists converged upon Harvard Medical School for the seventh annual International Conference on Systems Biology of Human Disease (SBHD). Originally conceived by the systems-biology working groups of Boston and Heidelberg, the SBHD has grown to become an important venue for disease-relevant research at the systems level. The modest size of SBHD hits that conferencesweet spot, where you can still make five or six new friends while touching base with 15 or so old ones.

SBHD dedicates itself to systems biology, a field that is in its teenage years as a discipline. Like most teenagers, systems biology no longer yearns for the approval of its parents (molecular biology and mathematics), even though it cannot possibly succeed on its own without them. We get caught up as a group in the latest fashions (cellular heterogeneity), whereas others fall out of style (interaction hairballs). Fortunately, the upbringing of the systems-biology community has been healthy so far – cliquishness is at a minimum, and good science is at the forefront. During the meeting, we heard from cell biologists, engineers, geneticists, theoreticians, technologists and bio-informaticians, who all connected with systems biology in various ways. There were also multiple invited talks on microbiology, an area that has been somewhat slower to adopt systems approaches outside of model organisms.

Mining cell-to-cell heterogeneity

Heterogeneity pervaded the meeting so much that SBHD could have stood for 'single-cell biology and human disease'. Appreciating cell-to-cell heterogeneity is pretty straightforward – just look at cells with a microscope and you will see that no two are identical. But, do we know how different they truly are? Chris Bakal (Institute of Cancer Research, London, UK) asked this question with respect to cell shape and its impact on cell signaling. Focusing on signaling through nuclear factor-κB (NF-κB), Bakal found that the strongest responders in a population exhibited unique nuclear-shape characteristics compared with those of the average. Cell density played an important role, as cells at the leading front of a collectively migrating sheet showed preferential NF-κB activation in response to tumor necrosis factor. A more complete inventory of shape-sensitive pathways would be valuable for interpreting high-content imaging screens with such information already embedded.

Equally striking were the live-cell examples of cell-to-cell heterogeneity. Using modern Förster resonance energy transfer (FRET) reporters of extracellular signal-regulated kinase (ERK) and 5'-AMP-activated protein kinase (AMPK) activity, John Albeck (University of California Davis, USA) showed time-lapse videos of how the single-cell response to environmental stimuli can differ qualitatively from the population average. Pulses of activity were observed over many hours, with time-dependent characteristics that depended on the sensor and the perturbation. Understanding how cells interpret these pulses will require computational models that combine data on signaling, gene expression and post-translational modifications of immediate-early gene products.

FRET-based indicators of kinase activity can be problematic for multi-color applications and for kinases with rapid deactivation kinetics. To address these limitations, Sabrina Spencer (University of Colorado Boulder, USA) and Markus Covert (Stanford University, USA) presented independent designs of one-color sensors that report kinase activity by localization. The trick is to engineer substrates within a tandem nuclear localization and export sequence, such that phosphorylation disrupts the more-potent sequence and causes relocalization of the reporter. Spencer designed a reporter for cyclin-dependent

Poster Session of SBHD 2014 in the spacious hall of the Joseph B. Martin Conference Center at Harvard Medical School in Boston (Photo: C. Bird).

kinase 2 in order to investigate the cellular decision to proliferate or quiesce, revealing a new restriction point in G_2 phase that had eluded the 'starve-and-refeed' experiments of 40 years ago. Covert expanded the premise of 'kinase translocation reporters' (KTRs) more broadly to the mitogen-activated protein kinases (MAPKs). Covert showed proof-of-concept multiplexing of one-color KTRs by tracking ERK, c-jun N-terminal kinase (JNK) and p38 activities concurrently in single cells. KTRs showed better reversibility than standard FRET reporters, suggesting that they could become the sensor of choice for pathways that are rapidly deactivated.

Bernd Bodenmiller (University of Zürich, Switzerland) took deep analysis of static images to the next level with imaging mass cytometry. In Bodenmiller's setup, tissue sections are immunostained with heavy metals and then raster ablated with a UV laser before detection of the released metals by mass spectrometry. Work in progress seeks to identify intermediate states during the epithelialto-mesenchymal transition of mammary cancer cells. The sensitivity of imaging mass cytometry should improve substantially as the technology matures.

Immune cells and cytokine signaling

Another recurring theme at SBHD 2014 was systems analysis of the immune system and circulating cytokines. We crossed over from epithelial to immunological heterogeneity with talks from Kathryn Miller-Jensen (Yale University, USA) and Grégoire Altan-Bonnet (Memorial Sloan-Kettering Cancer Center, USA) about single-cell responses of myeloid and lymphoid effectors. Miller-Jensen reported on the cascade of paracrine factors triggered by lipopolysaccharides in monocytes and macrophages. Using nanowell chips to capture and profile cytokines released from single cells, Miller-Jensen compared the population-level secretion patterns with those obtained from individual cells isolated from the population. Several late-phase cytokines – including interleukin-6, interleukin-10 and granulocyte-macrophage colony-stimulating factor – appeared to be emergent properties of the population that could not be recapitulated by the aggregate response of individual cells. Although Miller-Jensen focused on a bacterial stimulus, these results could be especially relevant to solid tumors and atherosclerosis, where macrophage accumulation is recognized.

Altan-Bonnet embraced the intersection of cancer biology and immunology by investigating the receptor-proximal behavior of transformed B cells in chronic lymphocytic leukemia (B-CLL). Comparing B-CLL tyrosine kinase signaling with that of B cells from healthy donors, he reported bimodal and hysteretic responses of B-CLL cells to inhibition of tyrosine phosphatases. Altan-Bonnet tied these dynamical-systems properties of B-CLL cells to aberrant B-cell receptor clustering, which gives rise to cooperativity and a saddle-node bifurcation of the network. This mechanism provides a potential explanation for why B-CLL cells escape negative selection, and the bimodality itself could be exploited as a sensitive diagnostic for staging B-CLL patients.

Of course, good systems biology is still taking place at the population level for blood cells and their signaling pathways. Ursula Klingmüller (German Cancer Research Center, Hei-

delberg, Germany) examined the potential dangers of erythropoietin (Epo) therapy for treating anemia in lung cancer patients undergoing chemotherapy. By combining modeling with assay development, Klingmüller showed that not all Epo variants were equivalently bioactive towards non-small cell lung cancers (NSCLC) and erythroid progenitors. This suggests that some variants might be better suited for the treatment of chemotherapy-induced anemia than others.

Cytokine crosstalk was an important motivation for the systems work on endometriosis presented by Douglas Lauffenburger (Massachusetts Institute of Technology, USA). By monitoring the cytokine profiles of aspirates of peritoneal fluid from women stricken with the disease, Lauffenburger showed how one could infer the secreting and receiving cell types that were most consistent with the observed profiles. This analysis suggested the existence of macrophage hyperactivity and JNK signaling in endometriosis patients with cytokine signatures that correlated to pain and emphasized the practical constraints that must be considered when combining systems biology with clinical material.

Infectious disease

The latest entries into the systems-biology arena at SBHD 2014 involved the bacteriology of infectious disease. Pathway and network models are a long way off because most genes lack detailed functional characterization, and it is not even clear which ones are essential under different conditions. Christopher Sassetti (University of Massachusetts Medical School, USA) and Tim van Opijnen (Boston College, USA) tackled this problem for *Mycobacterium tuberculosis* and *Streptococcus pneumoniae* at the genomic level. Using different insertional-mutagenesis and sequencing-based approaches, Sassetti and van Opijnen showed how 'conditional essentiality', dictated by nutrient availability and other stresses in the host microenvironment, could be the norm for these infectious agents. Versatile pathogens have not one network but many that reconfigure according to the growth conditions. A systems-level dissection of the constraints on these networks might investigate whether bacteria could be 'trapped' in certain configurations that eradicate infection.

Concluding remarks

Amidst all the great things brought to SBHD 2014, it was striking to note what was missing. For example, aside from our talks and that of Luis Serrano (Center for Genomic Regulation, Barcelona, Spain), no presentation showed results with quantitative immunoblots, as if systems biology had disowned one of its parents. Indeed, Serrano showed that immunoblots were more robust for absolute protein quantification than multiple reaction monitoring, a mass-spectrometry-based approach that is currently in vogue. Diverse perspectives imply a diversity of methods that intermingle experimental and computational techniques, both old and new. Like teenagers, we want nothing but to race around in a fast sports car, forgetting that we must still use two legs to walk to the driver-side door.

Abbreviations:

AMPK: 5'-AMP-activated protein kinase; B-CLL: B-cell chronic lymphocytic leukemia; Epo: Erythropoietin; ERK: Extracellular signal-regulated kinase;
FRET: Förster resonance energy transfer; JNK: c-jun N-terminal kinase;
KTR: Kinase translocation reporter; NF-κB: Nuclear factor-κB;
NSCLC: Non-small cell lung cancer; SBHD: Systems Biology of Human Disease.

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news

20 “Add-on Fellowships for Interdisciplinary Science” to support graduate students and post-doctoral researchers in systems biology

The Joachim Herz Stiftung has launched a new fellowship program to foster interdisciplinary skills and support the careers of young scientists from various disciplines who conduct research in systems biology and neighboring fields.

The “Add-on Fellowships for Interdisciplinary Science” do not cover living expenses. Instead, they allow PhD candidates and postdocs who are interested in further training in neighboring disciplines to attend conferences, finance research stays or undertake professional training needed in order to gain new insights into relevant research methods. Up to 20 young researchers will receive a maximum amount of € 12,000 per person to be spent over a period of two years. For fellows with children, additional funds are available to cover specific costs, e. g. related to childcare.

Selection committee 2015: Rudi Balling, Patrick Cramer, Roland Eils, Andreas Kremling, Gene Myers, Nicole Radde, Nikolaus Rajewsky, Fred Schaper, Petra Schwille, Jens Timmer, Albrecht Wagner, Matthias Wilmanns, Olaf Wolkenhauer, An-Ping Zeng.

The Joachim Herz Stiftung promotes education, science and research in the natural sciences, economics and business administration, and in the field of personal development. Educating and empowering youth and young adults at all stages of education are the common goals of all operational projects and grant-making activities. Programs for talented and driven individuals create academic opportunities and offer access to different cultures and environments. The Joachim Herz Stiftung specifically promotes excellence in science and research and supports promising junior scientists working at the cutting edges of their respective fields.

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The “Add-on Fellowship” program is part of the Foundation’s initiative to support junior scientists looking to develop interdisciplinary skills within the context of systems biology. In addition to the “Add-on Fellowships”, the Joachim Herz Stiftung also provides support for research and events at the newly established Centre for Structural Systems Biology (CSSB) in Hamburg as well as for extracurricular project courses aimed at high school students.

Apply now! Deadline: September 1, 2015

Please see **www.joachim-herz-stiftung.de/add-on** for further information regarding the application procedure.

ERACoSysMed – European research and development funding to promote the implementation of systems biology approaches in clinical research and medical practice

The first ERA-NET focussing on systems medicine – ERACoSysMed – started in January 2015 under the auspices of the European Commission's Horizon2020 research programme. The aim of the ERACoSysMed consortium, which comprises 14 European funding agencies, is to develop a common agenda for the targeted promotion of research and development in systems medicine.

Systems medicine is tightly linked with systems biology and utilizes systems-based approach to conquer the current challenges in medical research and practice. By integrating modern -omics technologies with mathematical-modelling and -simulations, new, more effective and tailored treatment concepts can be designed efficiently. With the active integration of the patient, these concepts can then be used to enable early detection, specific prevention as well as a rational drug development. One medium- to long-term aim of implementing approaches from systems biology in classical medicine is to achieve a paradigm shift towards a personalised, preventive, predictive and participatory medicine (P4 medicine).

Based on the strategic roadmap for implementing systems medicine in Europe, that the CASyM consortium (www.casym.eu) published in 2014, ERACoSysMed has set itself the following aims: (I) The development of a strong European systems medicine community, (II) the formation of a network of European funding agencies pursuing a common RTD agenda, and (III) the publication of transnational calls for systems medicine. Three joint transnational calls (JTCs) are planned within the five-year duration of ERACoSysMed. The first call (JTC1) will be supported by additional funds from the European Commission as part of the ERA-NET co-fund model.

JTC1, which was published simultaneously in all partner countries in mid-February 2015, aims at proving the socio-economic benefits of the systems medicine approach for a concise clinical question. Such demonstrator projects shall develop novel concepts for P4 medicine and pay specific attention to the integration of biomedical data and mathematical models from systems biology. Successful projects with a duration of 3 years will be selected during a two-step reviewing process (pre- and full proposal phase), whereas the first projects will be launched at the beginning of 2016.

ERACoSysMed profile:

- **Title:** ERACoSysMed – Collaboration on systems medicine funding to promote the implementation of systems biology approaches in clinical research and medical practice
- **Term:** January 2015 – December 2019
- **Consortium:** 14 funding agencies from 13 European countries
- **Coordination:** Forschungszentrum Jülich GmbH, Project Management Jülich (PtJ)
- **Budget:** approx. €12.5 million (first cofund call)
- **Homepage:** www.eracosysmed.eu

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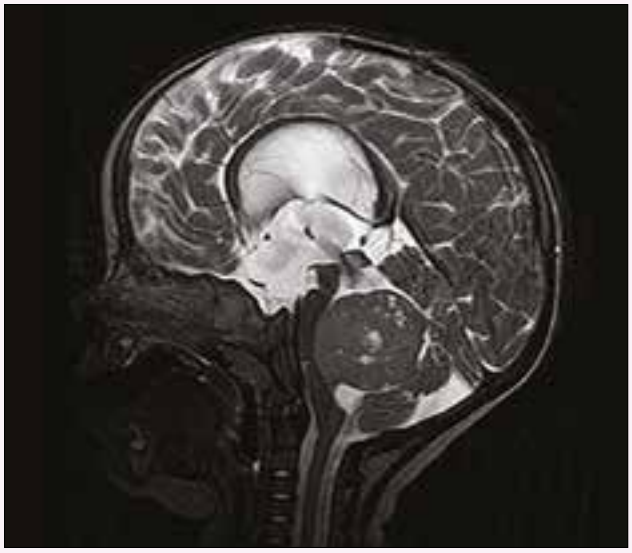
Oncogenes hijack foreign enhancers

Medulloblastoma is the most common type of malignant brain tumour in children. Until now, it was unclear as to why frequently metastatic group 3 medulloblastoma showed particularly aggressive behaviour despite few mutations in genes that promote growth. Together with an international team of colleagues, scientists at the German Cancer Research Center recently discovered that, for this particularly malignant group of medulloblastomas, the oncogenes are usually not altered in terms of their genetic code, but their transcription is facilitated instead. Previously unknown control mechanisms are responsible, with the oncogenes commandeering foreign enhancers.

As part of the International Cancer Genome Consortium ICGC (www.icgc.org), researchers in the PedBrain Tumor Research Project are systematically analysing all changes in the genome of paediatric brain tumours in order to identify target structures for new treatments. According to coordinator Prof. Dr. Peter Lichter (German Cancer Research Center), in the case of a particularly aggressive and therapy-resistant group of medulloblastomas, apart from amplification of the MYC oncogene in a fraction of cases practically no changes to the genome are known to drive tumour growth and serve as suitable target structures for the development of therapeutic drugs.



MRT scan of a medulloblastoma
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Dr. Paul Northcott and his colleagues, however, were able to track down a phenomenon that was completely unknown in solid tumours within this particularly aggressive type of medulloblastoma. A variety of structural changes to the DNA in the tumour genome of various tumours in this subgroup underwent an identical experience, despite their heterogeneity: the transcription of one of the two oncogenes GFI1 or GFI1B, which are not active in healthy brain tissue, occurs in these tumours and thus results in oncogenesis.

The PedBrain researchers also discovered the cause of the strange phenomenon at the same time: the heterogeneous structural changes “push” the oncogene from its hereditary, inactive environment by hijacking active enhancers, which leads to the awakening of these oncogenes. Such “hijacked” gene enhancers may also play a major role in the activation mechanism of many other types of cancer. “However, they can only be discovered through the extremely precise analysis of genetic material and are therefore easy to overlook,” says Prof. Dr. Stefan Pfister, molecular geneticist and member of the PedBrain team at the German Cancer Research Center and paediatrician at Heidelberg University Hospital. Substances that block the mechanism of the oncogenes GFI1 and GFI1B have already been subject to preclinical trials and may prevent the growth of particularly aggressive medulloblastomas.

For the first time, scientists have discoverd a molecular ”Achilles heel” in group 3 medulloblastoma that cooperates with MYC and can now be used to develop targeted drug therapies.

Original publication:

Paul A Northcott, Catherine Lee, Thomas Zichner, ..., Peter Lichter, Jan O Korbel, Robert J Wechsler-Reya und Stefan M Pfister im Auftrag des ICGC PedBrain Tumor Project:Enhancer hijacking activates GFI1 family oncogenes in medulloblastoma. Nature 2014, DOI:10.1038/nature13379

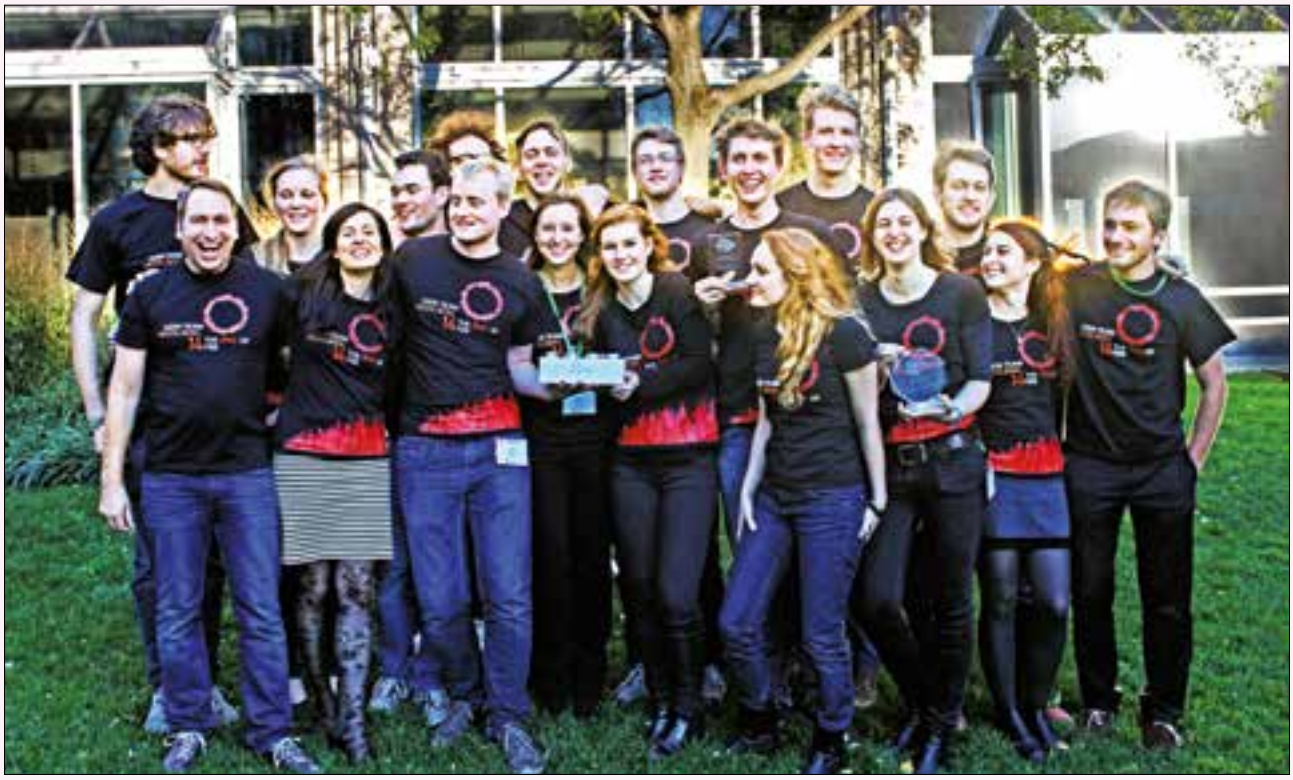
Source: Press release German Cancer Research Center, Heidelberg

The Ring of Fire project wins world championship in synthetic biology – student team from Heidelberg once again impresses the judges in Boston

For the second time, the student team from the Heidelberg University and the German Cancer Research Center has won the grand prize and several special prizes in the international iGEM competition. The team from Heidelberg defeated teams from internationally renowned universities such as Harvard, Yale and Stanford to take the top spot in November 2014 in Boston. The Heidelberg team's success once again shows that Germany is a world-class location for research and teaching in synthetic biology.

With their "Ring of Fire" project, the students from Heidelberg solved a common problem in the application of biological molecules: proteins are often not very stable and therefore cannot be used in many applications in research and biotechnology. The solution of the students from Heidelberg: the two ends of the protein strand, which looks like a twisted yarn, are joined by "linkers", which function like another piece of yarn. Using this new system, the proteins join up to form a ring, which significantly increases their stability. The ring-like structure protects the delicate ends of the proteins and increases their potential for use in new technologies.

Supervised once again by Prof. Roland Eils (German Cancer Research Center and the University of Heidelberg) and Dr. Barbara Di Ventura (Heidelberg University), the team of twelve Bachelor's and Master's students entered their project in the International Genetically Engineered Machine (iGEM) competition in Boston. During the competition, student teams from around the world search for solutions, often for very common problems, and draw on the potential of syn-



The Heidelberg team with the iGEM World Cup trophy – the silver biobrick. The team was supervised by Prof. Roland Eils and Dr. Barbara Di Ventura (front left).

thetic biology to do so. In this up-and-coming research field, scientists follow engineering principles so they can equip microorganisms with new properties for innovative applications in biomedicine, biotechnology or environmental research.

The team from Heidelberg won the undergraduate category, beating other internationally famous universities, in the Giant Jamboree of the tenth iGEM competition in 2014, which included over 200 teams from 32 countries in four continents. In addition to the grand prize, the Heidelberg team also won several special prizes, such as "Best Technological Advance" and the "Best Software", and they were also voted the Audience's Favourite, winning the "iGEM'ers price". The second prize went to Imperial College London (UK) and the third prize to NCTU Formosa (Taiwan). Following their major success in 2013, which saw the first German team win the international iGEM competition, the Heidelberg team is now the first team ever in the history of the competition to win the Grand Prize twice, and even in two consecutive years.

An example of how a ring-shaped protein leads to significant improvements in research applications has already been tested by the Heidelberg iGEM team. In biomedical laboratories, DNA is often amplified using the polymerase chain reaction (PCR), which requires very high temperatures. During the amplification process, the epigenetic imprints on the DNA are lost because the enzyme methyltransferase (DNMT1), which copies these marks, cannot withstand the heat. A ring-shaped, heat-resistant methyltransferase can help: not only are the four letters of the genetic code copied, but so are the epigenetic DNA modifications that are essential for the transcription of the code and for controlling the activation and deactivation of entire genes. The students assume that closing the ring could also be used to protect therapeutic proteins from degradation in body cells, or that it could stabilise enzymes that are used in food technology.

The team from Heidelberg provided the scientific community with a universally applicable standard "kit" for closing protein rings, winning them the special prize for the "Best Technological Advance" in addition to the main prize. In addition, the students developed two new software applications, enabling the precise measurement of the linker length required to join both ends of the protein strand without affecting its structure and function. Because these applications are computationally very demanding, they also developed the iGEM@Home platform, which is able to use the capacity of unused computers around the world for processing data. Their achievements in software development were honoured with a further special prize.

The Heidelberg iGEM team was supported by the Klaus-Tschira-Stiftung (Klaus Tschira Foundation), the Dietmar-Hopp-Stiftung (Dietmar Hopp Foundation), the Helmholtz Initiative on Synthetic Biology and the CellNetworks Cluster of Excellence at Heidelberg University, among others.

Source: Press release German Cancer Research Center and the University of Heidelberg

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Human promoters operate directionally

Enzymes that are responsible for gene transcription attach to promoters (recognition sequences) on the DNA. After high-throughput data to precisely investigate the activation of genes had become available, scientists assumed that most of these promoters were not assigned to one direction and that the DNA was transcribed on both strands in the helix. Prof. Uwe Ohler from the Max Delbrück Center for Molecular Medicine (MDC) and Prof. James T. Kadonaga from the University of California in San Diego (UCSD) determined that the core promoter only permits transcription in one direction in human cells. If copies of the opposite DNA strand are generated, they therefore depend on their own core promoter.

Our genetic material, DNA, is generally wrapped around nucleosomes within each cell in the tiny cell nucleus, but some DNA segments are unoccupied and accessible. These DNA segments are where the promoters are found, and where enzymes that transcribe the genetic material bind in order to make a copy of the blueprint for the production of proteins. This process is known as transcription.

A promoter is made up of several parts, and the core promoter right in front of the gene to be transcribed is directly responsible for initiating transcription. Uwe Ohler and his colleagues were able to show that this core promoter is unidirectional in human cells. The transcription mechanism starts here and only travels in one direction, which means that it does not transcribe the opposite DNA strand.

If the second strand is copied as well, it is due to its own core promoter. This second core promoter is found in the same area as the first one, which is why researchers previously assumed that the direction of the gene was not determined in the promoter sequence.

With a combination of high-throughput data to track the transcription machinery and computational analyses, the scientists established that more than 50% of the genes have two core promoters opposite one another at various

imprint

distances. Researchers assume that these divergent core promoters are involved in the transcription regulation of adjacent genes.

Original publication:

Duttke SHC, Lacadie SA, Ibrahim MM, Glass CK, Corcoran DL, Benner C, Heinz S, Kadonaga JT, Ohler U (2015) Human Promoters Are Intrinsically Directional. Molecular Cell 57, 674–684.

Source: Press release, Max Delbrück Center for Molecular Medicine

The Systems Medicine Web Hub

www.systemsmedicine.net is the Internet's hub for information related to Systems Medicine, supporting scientists, projects and initiatives with the communication of their efforts. The benefits for scientists are an increased visibility and improved dissemination of their research and the more effective search for resources, results and partners.

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www.systemsmedicine.net

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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 twice per year in German and once in English, is produced jointly by the Helmholtz Association, Cross Program Topic Systems Biology and Synthetic Biology, Virtual Liver Network, and Projektträger Jülich. It is financed by the Helmholtz Association and by the German Federal Ministry of Education and Research (BMBF).

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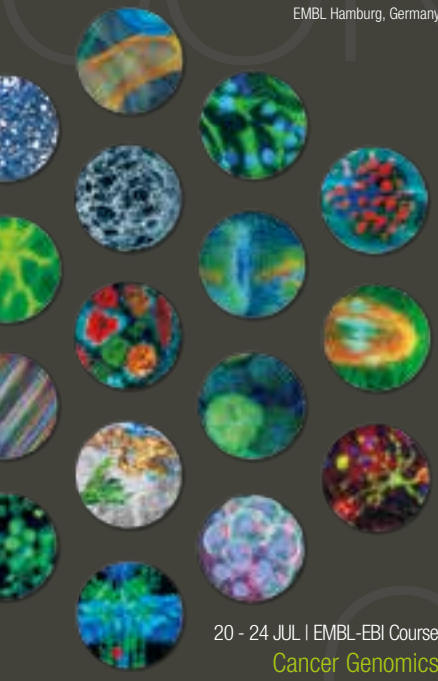
Conferences

10 - 12 JUN | EMBL Conference
Human Microbiome
M. Arumugam, P. Bork, C. Huttenhower | EMBL Heidelberg, Germany

14 - 17 JUN | EMBO | EMBL Symposium
Mechanisms of Neurodegeneration
K. Dumstrei, T. Golde, C. Haas | EMBL Heidelberg, Germany

15 - 17 JUN | EMBL Workshop
BioStruct-X Industrial Workshop
T. Lundqvist, S. Monaco, A. Meents, J. Márquez, E. Pereiro, T. Schneider, E. Shotton, D. Svergun, Weiss
EMBL Hamburg, Germany



20 - 24 JUL | EMBL-EBI Course
Cancer Genomics
G. Rustici | EMBL-EBI Hinxton, UK

20 - 25 JUL | EMBL Advanced Course
Super-resolution Microscopy
M. Lampe, S. Liebe, R. Pepperkok, U. Schwarz | EMBL Heidelberg, Germany

25 - 31 JUL | EMBO Practical Course
Analysis of small non-coding RNAs: per aspera ad astra
V. Benes, A. Enright, D. O'Carroll, M.L Baudet, M. Castoldi, S. Pospisilova | EMBL Heidelberg, Germany

24 - 27 AUG | EMBL Introductory Course
Next Generation Sequencing: Amplicon Based Clinical Resequencing
V. Benes, J. Dreyer-Lamm, B. Farnung | EMBL Heidelberg, Germany

21 - 23 JUN | EMBO | EMBL Symposium
Enabling Technologies for Eukaryotic Synthetic Biology
M. Fussenegger, J. Nielsen, K. Patil, C. Smolke | EMBL Heidelberg, Germany

9 - 13 SEP | EMBO Conference
Protein Synthesis and Translational Control
F. Gebauer, M. Hentze | EMBL Heidelberg, Germany

16 - 19 SEP | EMBO | EMBL Symposium
The Mobile Genome: Genetic and Physiological Impacts of Transposable Elements
O. Barabas, D. O'Carroll, D. Odom, E. Miska, J. Moran | EMBL Heidelberg, Germany

6 - 10 OCT | EMBO | EMBL Symposium
Seeing is Believing - Imaging the Processes of Life
J. Ellenberg, J. Lippincott-Schwartz | EMBL Heidelberg, Germany

11 - 14 OCT | EMBO | EMBL Symposium
New Approaches and Concepts in Microbiology
P. Cossart, K. C. Huang, M. Laub, N. Typas | EMBL Heidelberg, Germany

18 - 21 OCT | EMBO | EMBL Symposium
The Non-Coding Genome
D. Bartel, E. Izaurralde, J. Rinn, J. Vogel | EMBL Heidelberg, Germany

22 - 24 OCT | The 17th EMBL PhD Symposium
Just by Chance?
How Randomness & Variability shape Biology
EMBL PhD Students | EMBL Heidelberg, Germany

1 - 4 NOV | EMBL Conference
Cancer Genomics
A. Biankin, P. Campbell, L. Chin, J. Korbel | EMBL Heidelberg, Germany

5 - 6 NOV | The 16th EMBO | EMBL Science and Society Conference
From Research and Technology to Health and a Sustainable Environment
S. Bendiscioli, M. Garfinkel | EMBL Heidelberg, Germany

12 - 14 NOV | EMBO | EMBL Symposium
Biological Oscillators: Design, Mechanism, Function
A. Aulehla, M. Elowitz, U. Schibler | EMBL Heidelberg, Germany

16 - 19 NOV | EMBL | Stanford Conference
Personalised Health
P. Bork, J. Ellenberg, W. Huber, M. Snyder, L. Steinmetz,

Courses

14 - 18 SEP | EMBL-EBI Course
Metagenomics Bioinformatics
L. Emery | EMBL-EBI Hinxton, UK

14 - 22 SEP | EMBO Practical Course
Current Methods in Cell Biology
J. Schwab, P. Neveu | EMB Heidelberg, Germany

5 - 9 OCT | EMBL-EBI Course
Introduction to Next Generation Sequencing
T. Hancocks | EMBL-EBI Hinxton, UK

12 - 16 OCT | EMBL-EBI Course
Computational Structural Biology - From Data to Structure to Function
T. Hancocks | EMBL-EBI Hinxton, UK

12 - 15 OCT | EMBL Introductory Course
Statistical Bioinformatics using R and Bioconductor
S. Anders, B. Klaus | EMBL Heidelberg, Germany

14 - 15 OCT | EMBL Introductory Course
Microinjection into Adherent Cells
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19 - 24 OCT | EMBL-EBI Course
Analysis of High-Throughput Sequencing Data
G. R ustici | EMBL-EBI Hinxton, UK

22 - 23 OCT | EMBL Advanced Course
Digital PCR
J.Dreyer-Lamm | EMBL Heidelberg, Germany

2 - 6 NOV | Joint EMBL-EBI Wellcome Trust Course
Resources for Computational Drug Discovery
A. Gaulton, T. Hancocks, A. Hersey, J. Overington, G. Papadatos | EMBL-EBI Hinxton,UK

2 - 6 NOV | EMBL-EBI Course
Networks and Pathways
L. Emery, S. Orchard | EMBL-EBI Hinxton, UK

6 - 11 DEC | Joint EMBL-EBI Wellcome Trust Course
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