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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: Salt ponds at San Francisco bay. Red colors are due to halophilic microbes. **(see also article page** 58 ff)
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welcome note

Dear Reader,

Research and innovation are ensuring that Germany and Europe can keep their competitive edge on the global knowledge market. The common European Research Area is a significant key to progress in this context. The European Union is giving an important signal for the internationalization of research and innovation in Europe by introducing its new research framework programme “Horizon 2020” with a funding volume of over 70 billion euros.

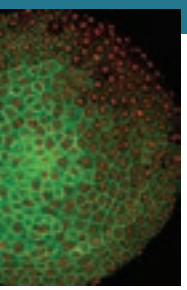
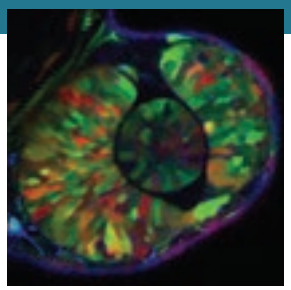
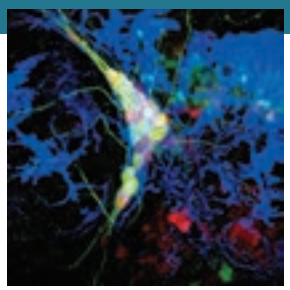
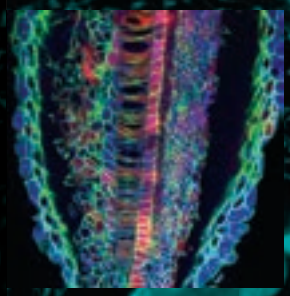
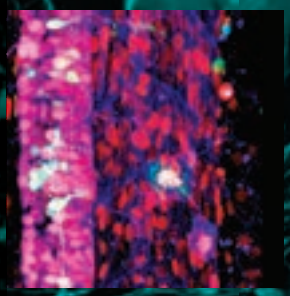
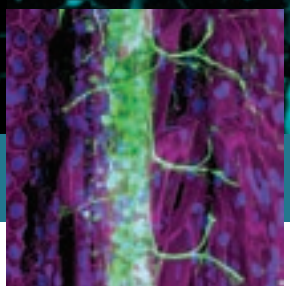
As a relatively young field of research, systems biology adopted the path of European and international cooperation from the very start. Its iterative approach of scientific experimentation and mathematical modelling calls for the involvement of widely varied disciplines and often for cooperation between experts located all over the world. The Federal Ministry of Education and Research (BMBF) recognized the potential and particular needs of systems biology over ten years ago and focused its support on European and international initiatives from an early stage.

The ERASysBio coordination measure, which ran for five years until 2011, and its spin-offs SysMO and SysMO2 made great advances in networking European systems biology research and giving it a common direction. Not only did ERASysBio involve funding measures with a budget of around 70 million euros, it also triggered some of the most important initiatives in international systems biology. These include among others the current ERA-Nets and coordination measures in the fields of applied systems biology (ERASysAPP) and systems medicine (CASyM).

This issue of *systembiologie.de* provides more information on the successful work and future development of the research networks in the systems biology sector.

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

INTERNATIONAL CONFERENCE ON SYSTEMS BIOLOGY OF HUMAN DISEASE

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Leonidas Alexoupoulos – National Technical University of Athens
Grégoire Altan-Bonnet – Memorial Sloan-Kettering Cancer Center
Chris Bakal – The Institute of Cancer Research, London
Bernd Bodenmiller – University of Zurich
Markus Covert – Stanford University
Dirk Drasdo – INRIA, Paris/IZBI, Leipzig
Roland Eils – German Cancer Research Center (DKFZ)
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Doug Lauffenburger – Massachusetts Institute of Technology
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foreword

Only hard work makes a genius

This, or something similar, is the sentiment expressed in the last line of a poem that the writer Theodor Fontane dedicated to his friend Adolf Menzel. The two men were members of a nineteenth-century literary society, the *Tunnel über der Spree* (Tunnel over the River Spree), that was a dominant influence on literary life in up-and-coming Berlin for over 50 years. The name of this exclusive circle, of which Theodor Storm was also a member, was inspired by Isambard Kingdom Brunel's project to build the first tunnel under the River Thames in London. True to the society's motto – "Endless irony and endless melancholy" – its name was an ironic reference to the lamentable fact that its members' city of Berlin could boast no such structure.

Fontane paid several visits to the real tunnel at Rotherhithe in London. Although the world's press had lauded it as a masterpiece of civil engineering, the tunnel in reality was a disappointment to him. Despite the hard work and genius that had gone into building it, Fontane felt like he was in an elongated gateway to a fortress. He was unable to appreciate its genius.

Could the same be true of reports from the fascinating world of systems biology? Dear Reader, you will find testimony to a great deal of hard work in this eighth issue of systembiologie.de. Yet the genius behind it is not always easy to appreciate. Is it due to the sheer complexity of systems biology that this genius lies hidden, often manifesting itself only to a small group of initiated scientists? To many, one report or the other may also seem like an unspectacular fortress gateway. I would like to counter this impression by citing Fontane's comment on his first, rather uninspired visit to the Thames Tunnel: "Only what strikes the senses at the moment makes a great impression, one has to feel the greatness directly. If, however, one is forced to work out this greatness for oneself, one arrives only via detours and with the help of all kinds of imaginations at the realisation: 'Yes, that really is something great (...)'."

This quotation sounds as if Fontane was using the example of the Thames Tunnel to describe the credo of systems biology. An experimental observation may at first sight appear somewhat unexciting: only when it is mapped in a computer model is this superficially unspectacular finding transformed into a small sensation. Read, for example, in this issue how two biophysicists are using high-throughput sequencing in combination with computer simulation to decode access control to the genome. Or how a team of inexperienced systems biology students amazed synthetic biology experts with their seminal work on the use of bacteria to recover gold particles. There is ample scope here for refreshing insights into the sometimes obscure world of systems biology.

So how did the tunnel story end? Thanks to extensive reports on the construction of the Thames Tunnel, the English word "tunnel" became firmly anchored in the German language. The last recorded meeting of the *Tunnel über der Spree* society was held in 1898, a year before Berlin finally acquired its first tunnel beneath the River Spree – a tram tunnel between Stralau and Treptow. It was abandoned following severe damage in World War II, whereas the genius of the Thames Tunnel is still around to be admired.

I hope you enjoy reading the journal!

Yours truly, Roland Eils

Editor in Chief

index

light from the dark matter of the genome

The functional relevance of non-coding RNAs

by Sven Diederichs

One of the early surprises of the Human Genome Project was the fact that only a very small part of the genome information – less than 2 % – is sufficient to produce all proteins of the human organism. People soon started calling the rest "junk DNA", or the dark matter of the genome. The functional significance of these sequences was unknown or even doubted (except for a few regulatory sequences).

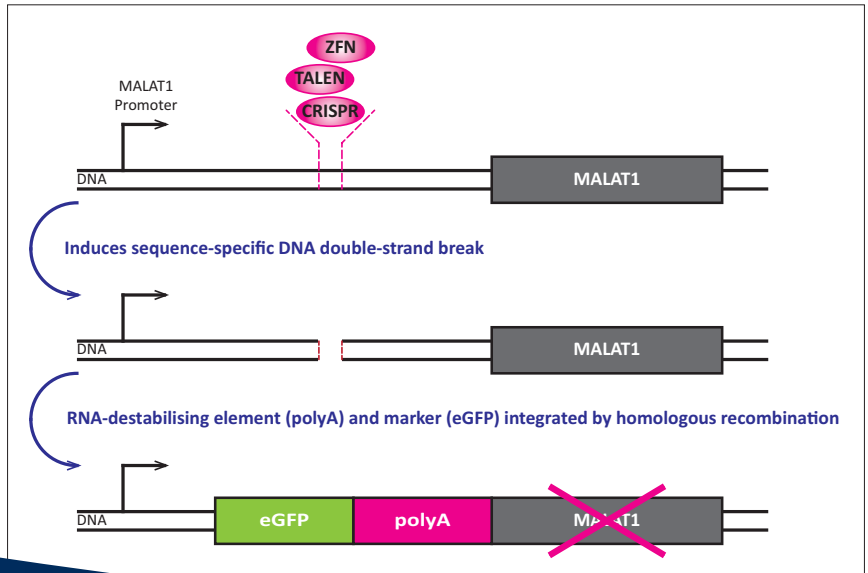
Not until the last decade did it become possible to cast light on this area of the genome by high-throughput methods such as deep sequencing and tiling arrays, which proved that it is not so dark after all. On the contrary, a very large part of the human genome is transcribed into RNA, but not translated into proteins (Djebali *et al.*, 2012). Initial studies showed that at least 70 % of the genome is transcribed into RNA. The latest methods such as capture sequencing even give cause to speculate that, if sequencing extends deep enough and includes all cell types in an organism, transcripts of every nucleotide in the genome may be found.

However, this has only shifted the question about the functional relevance from the genomic level of DNA to the transcriptomic level of RNA. Given the large number of non-protein-coding RNAs (ncRNAs), which now outnumber the protein-coding genes, there is even more reason to ask which actually play a relevant role in the cell. At present, it is not even possible to estimate what proportion of ncRNAs might be of fundamental significance for the cell. For each individual molecule, a detailed molecular biology analysis in a relevant system must prove whether this ncRNA is functionally active, a random product of the transcriptional background, or a by-product of a regulatory process.

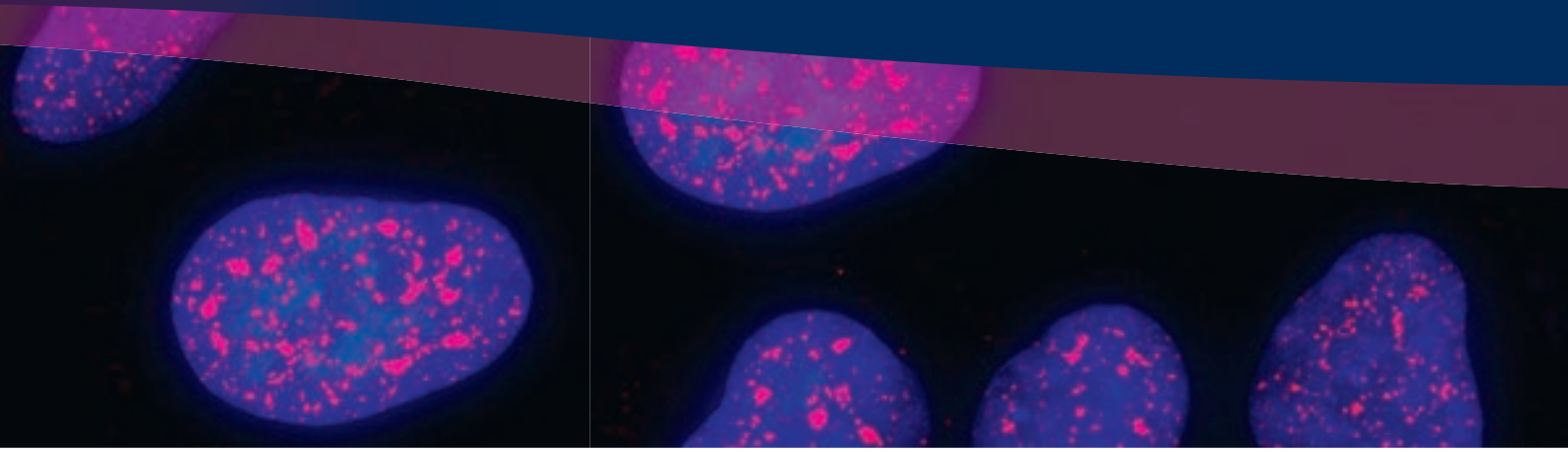
The function of non-coding RNAs: a new level of regulation ... and more?

The first examples of functionally active ncRNAs show without a doubt that they can perform relevant functions in the cell. Small ncRNAs called microRNAs are the best-studied group of ncRNAs and are important regulators of gene expression. However, long ncRNAs (lncRNAs) can also perform significant functions: the lncRNA *XIST* and other neigh-

Figure 1: Strategy for a quantitative *MALAT1* loss-of-function model



Genome editing achieves a quantitative loss of *MALAT1*: sequence-specific nuclease technologies such as ZFN, TALEN or CRISPR cut the human genome immediately after the *MALAT1* promoter. The cellular repair system for this double-strand break is used to integrate an RNA-destabilising element (e.g. polyA) together with a CMV-driven GFP marker gene. This leads to a more than onethousandfold reduction of *MALAT1* in lung cancer cells (Gutschner *et al.*, 2011) (Graphics: Sven Diederichs).



bouring lncRNAs mediate the inactivation of the second X chromosome in female cells for dosage compensation. The lncRNAs *HOTAIR* and *HOTTIP* influence the expression of *HOX* genes, which are important for individual development. The lncRNA *linc*-MD1 controls muscle cell differentiation.

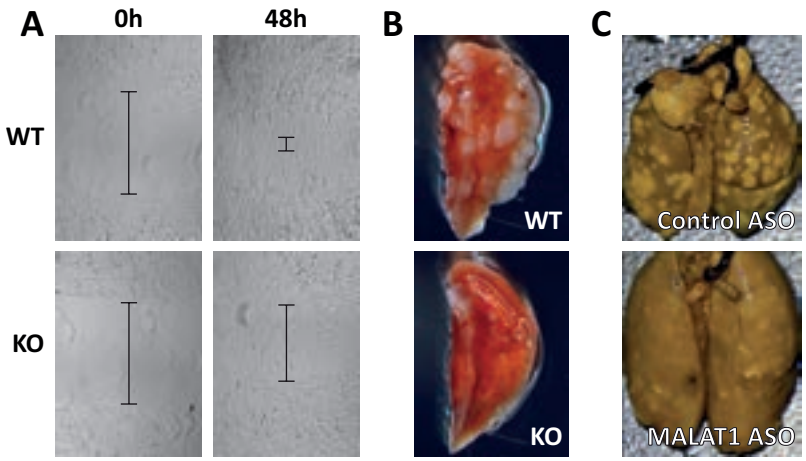
Mechanistically, ncRNAs can bind to DNA, RNA or proteins, so they use a large number of different mechanisms of which probably only a small part has been discovered so far. Known ncRNAs mediate gene regulation at the level of transcription, mRNA processing, mRNA stability or translation, but can also directly influence the activity of proteins or the composition of protein complexes. Hence one thing about ncRNAs, from short to long, is clear beyond a doubt – they form a significant new level of regulation of a wide range of cellular activities and must therefore not be omitted from any systems biological consideration of a physiological or pathological process.

lncRNAs as products of the cancer genome

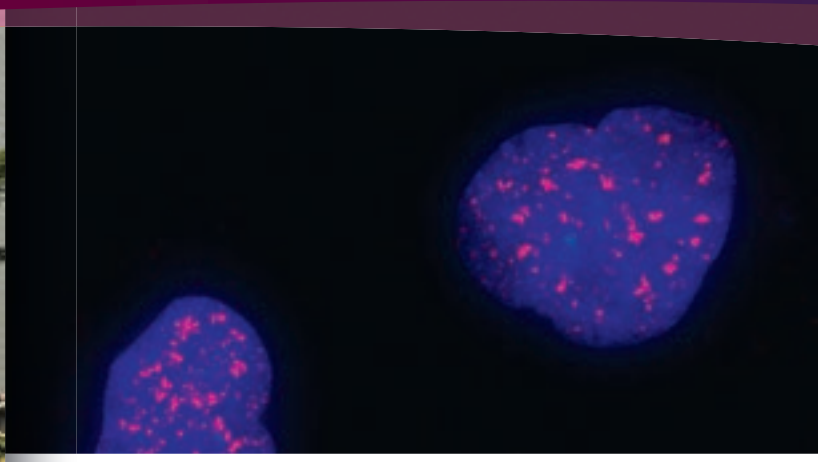
Cancer, at least the tumour diseases that are not triggered by infectious agents, is also known as a "disease of the genome", caused by changes in the genome, the activation of oncogenes and the inhibition of tumour suppressor genes. However, if cancer is a disease of the genome, all products of the cancer genome must be investigated, and the majority of the genome is transcribed into ncRNAs. Our research therefore concentrates on analysing long non-coding RNAs in tumour diseases (Gutschner *et al.*, 2012). Like the entire field of ncRNA biology, our research centres on two fundamental challenges:

First, the identity of most ncRNAs is unknown. Deep sequencing studies have quickly led to the finding that large parts of the genome are transcribed into RNA. However, for the majority of the genome, we still do not know which short RNA pieces are linked as a transcript, which splicing isoforms exist,

Figure 2: *MALAT1* as an essential factor in lung cancer metastasis



The loss of *MALAT1* in human lung cancer cells (KO) leads to epigenetic inhibition of a metastatic gene signature, which results in decreased cell migration (A) and fewer metastases in a xenograft mouse model (B). Inhibition of *MALAT1* by antisense oligonucleotides (ASOs; C) as a therapeutic approach can also block metastasis of subcutaneous xenograft tumours (Figure: adapted from Gutschner *et al.*, 2013).



The Helmholtz-University-Junior Research Group "Molecular RNA Biology and Cancer" (Source: Sven Diederichs).

how primary transcripts are processed, which transcripts are stable in the cell, and which are present in a functionally significant quantity. A further unknown for most of the transcriptome is the coding potential of newly discovered transcripts, that is, whether a new transcript is really non-coding or whether it can be translated (also) into a short peptide.

Second, the function of the vast majority of known transcripts is completely unknown. Fewer than 100 of what are now tens of thousands of lncRNAs have been functionally investigated to date, and we understand the molecular mechanisms of even fewer.

Our research therefore aims to examine the expression and regulation patterns of lncRNAs in lung and liver cancer, to confirm their exact identity and to unravel their function at the cellular and molecular level.

MALAT1: model lncRNA in tumour diseases

MALAT1 is a perfect example for a long non-protein-coding RNA in tumour diseases. We discovered *MALAT1* as a marker that is significantly associated with the metastasis of lung cancer, hence, *MALAT1* stands for *metastasis-associated in lung adenocarcinoma transcript 1*. Early-stage lung tumours that develop metastases despite complete resection of the primary tumour express considerably more *MALAT1* than tumours that do not recur after the surgery (Ji, Diederichs *et al.*, 2003). *MALAT1* was thus one of the first lncRNAs to be linked to cancer. After the initial discovery of *MALAT1*, researchers found a deregulation of *MALAT1* in many other tumour entities. Nonetheless, the function and mechanism of *MALAT1* was for a long time unknown.

With more than 8,000 nucleotides, *MALAT1* is quite a long transcript among the lncRNAs. However, it has only a very short open reading frame of 53 amino acids. Moreover, it has no relevant Kozak sequence and generates no peptide *in vitro*. It is therefore a non-coding RNA. In addition, *MALAT1* is evolutionarily highly conserved, possibly a further indication of its functional relevance. Initial functional studies have linked *MALAT1* to the regulation of alternative splicing or epigenetic gene regulation in the cell cycle.

One special challenge in *MALAT1* research is its extremely high expression in all tissues examined so far and its nuclear localization, both of which hamper the generation of effective loss-of-function models using RNA interference (RNAi). We therefore sought a new way of shutting down lncRNAs quantitatively in human cells rather than just reducing them by means of RNAi. We further developed the technique of genome editing, which permits lasting modifications of the genome, to shut down lncRNAs (Gutschner *et al.*, 2011). Using sequence-specific nucleases (ZFN, TALEN or CRISPR/Cas9), which induce a double-strand break at a specific site in the genome, we integrated RNA-destabilising elements such as polyadenylation signals (polyA) or RNase P cleavage sites into the *MALAT1* locus (fig. 1). While RNAi reduced the extremely abundant *MALAT1* by a factor of five, the new approach achieved a more than onethousandfold inhibition and hence a true loss-of-function model for *MALAT1* (Gutschner *et al.*, 2011).

Lung cancer cells that have quantitatively lost *MALAT1* migrate significantly less *in vitro* and can also form considerably fewer metastases in a xenograft mouse model *in vivo* (fig. 2) (Gutschner *et al.*, 2013). Inhibition of *MALAT1* by antisense oligonucleotides (ASOs) can also interrupt the metastatic cascade in the mouse model, thus making *MALAT1* an interesting therapeutic target for the prevention of lung cancer metastases (Gutschner *et al.*,

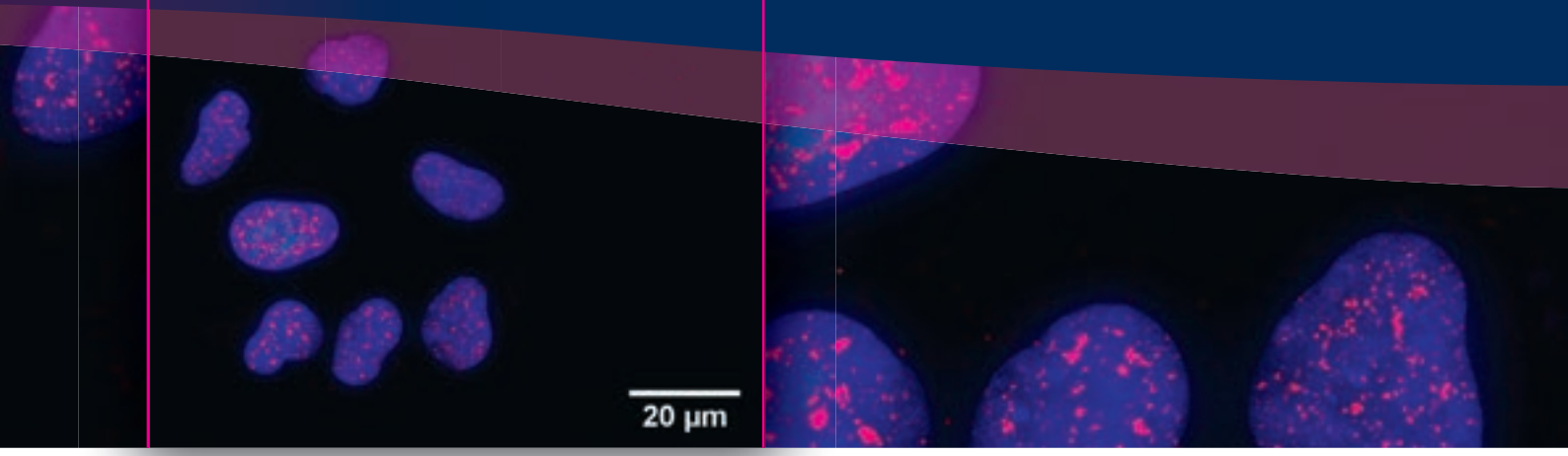


Figure 3: Nuclear localisation of *MALAT-1* in human lung cancer cells. With the help of a fluorescent probe, *MALAT1* (red) can be rendered visible in the cell nucleus (blue) (Source: Anna Roth, Sven Diederichs).

2013). At the molecular level, nuclear *MALAT1* does not influence splicing in lung cancer cells, but rather the transcription of a signature of target genes that play an essential role in migration, invasion and metastasis (Gutschner *et al.*, 2013).

In summary, *MALAT1* is a marker for the development of metastasis in lung cancer, but it is also an active and essential factor in this process as an epigenetic regulator and an attractive target structure for therapeutic intervention. *MALAT1* is thus one of the few lncRNAs whose identity, pattern of expression, cellular function and molecular mechanism have been characterized.

Nonetheless, even this lncRNA still holds some surprises. Despite the impressive phenotypes that the loss of *MALAT1* causes in human cells, its strong expression and high evolutionary conservation, and its link with fundamental processes such as cell migration or cell cycle, the quantitative loss of *MALAT1* in three independent mouse models has so far not revealed physiological or developmental phenotypes. Consequently, the investigation of murine knockout models with regard to tumour-related questions and analysis of *MALAT1* in other human organs, in which this ubiquitously expressed lncRNA is also present, are exciting challenges for the future.

lncRNAs as a new regulatory level in the cellular system

MALAT1 as a model lncRNA illustrates the fundamental functions that this class of molecules can fulfil. Many lncRNAs examined to date play an active role in gene regulation and can thus control a large number of different signalling pathways. As with microRNAs, we can expect that lncRNAs can be found to influence every physiological or pathological process. This new level of regulation between genome and proteome is therefore indispensible to a global understanding of molecular interrelationships in a cell.

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enigmatic RNAs

Circular RNAs (circRNAs) are a widespread class of molecules

by Sebastian Memczak, Ulrike Ziebold and Nikolaus Rajewsky

Circular ribonucleic acids were long thought to be sporadic, exotic types of RNA whose function in higher organisms was unknown so far. However, high-throughput analyses have recently cast light on these circular RNAs (circRNAs). The studies show that circRNAs are a surprisingly large and widespread class of RNA molecules. Their frequency, tissue-specific expression and in particular their extraordinary stability make them attractive for basic and applied medical research. Analysis of the expression and biogenesis of circRNAs also holds the promise of new insights into the general control of splicing and RNA-RNA as well as RNA-protein interactions. The possible molecular and physiological functions of circRNAs in humans are still largely unexplored. In the future, circRNAs could play an important role in diagnosis and research into the cause of diseases. The research team around Nikolaus Rajewsky, is tackling this complex subject at the Berlin Institute for Medical Systems Biology (BIMSB), which is funded by the German Federal Ministry of Education and Research (BMBF).

The RNA universe is far from being fully explored, and similar to the many unknowns in the depths of space, the microcosm of human cells is likely to contain more varieties of RNA with new, unknown forms and functions just waiting to be discovered.

Scientists have recently described a large number of RNA molecules that are distinct from well-known RNAs such as messenger RNAs (mRNA) which are templates for protein production, or tRNAs, which are involved in protein biosynthesis. These distinct regulatory RNAs include short micro-RNAs (miRNAs) that control translation of proteins through partial base complementarity to mRNAs and as another example the so far poorly understood long non-coding RNAs (lncRNAs).

Circular RNAs step into the spotlight

Last year, several laboratories published exciting reports that shed light on yet another class of non-canonical RNAs: the largely unknown circular RNAs. The studies uncovered that circRNAs are indeed a new large class of biomolecules, but also revealed possible functional mechanisms of these molecules. CircRNAs, unlike mRNAs or tRNAs, are not expressed as linear molecules with defined ends, but as circularised molecules, *i.e.* RNA rings (Danan *et al.*, 2012; Hansen *et al.*, 2013; 2011; Memczak *et al.*, 2013; Salzman *et al.*, 2012).

Systematic discovery and description of circRNAs has been made possible by new protocols in bioinformatic analysis of RNA sequencing data. These methods take advantage of the fact that circRNAs contain nucleotide sequences that are not present in other RNAs. RNA strands are synthesized from a DNA template by RNA polymerases. Generally RNA introns are removed from primary transcripts by the activity of a complex splicing machinery and the remaining sequences (exons) are joined together in consecutive order. In the case of circRNAs, specific parts of a RNA are back-spliced to themselves by largely unknown mechanisms. This “head-to-tail” splicing results in a covalently closed circular RNA. The circularised molecule contains a unique nucleotide sequence that is a fusion of an upstream sequence with a sequence that was transcribed from a distant downstream DNA region (see Fig. 1). It is these areas of fused RNA sequences that can be traced in sequencing data and that specifically indicate circRNAs (Danan *et al.*, 2012; Salzman *et al.*, 2012).

Until recently these RNA stretches were simply overlooked in sequence analysis, or neglected because they cannot be directly “mapped” onto the genome. However, the research team around Nikolaus Rajewsky made use of them. Using a bioinformatic protocol on large transcriptome datasets, the fused sequences can be detected and serve as specific indicators for

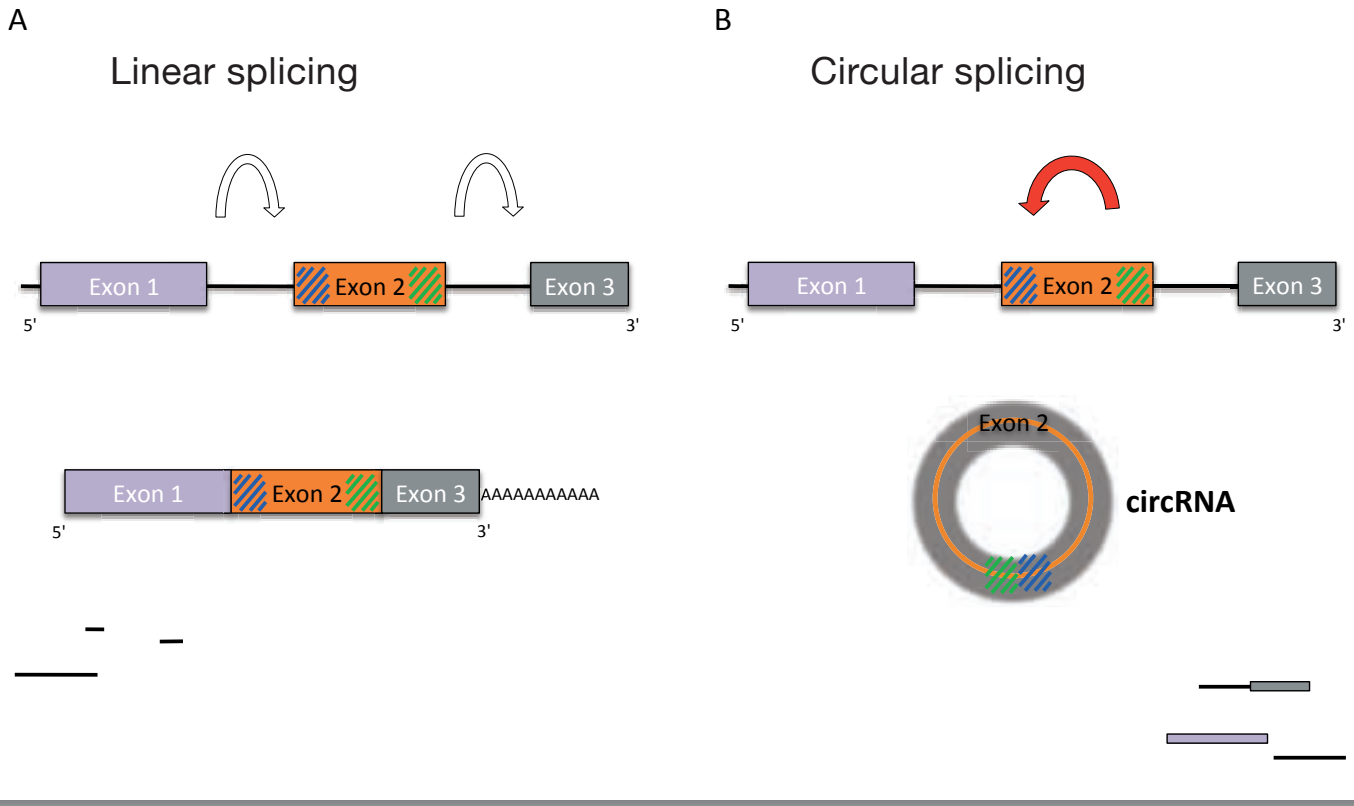

Figure 1:

A) In a complex splicing reaction, the exons of a primary RNA transcript are linked consecutively and the intervening introns are degraded. The resulting product is a linear molecule that serves, for example, as a template for protein synthesis.

B) In some cases, exons are back-spliced: the end of one or more exons (shaded in blue) is linked to the start of an exon (shaded in green). The resulting RNA is circular and has a characteristic sequence (green-blue) that does not occur in linear RNAs and can be detected in sequencing data. The remaining RNA fragments are (most probably) degraded. The function of circular RNAs in humans is still largely unknown. (Source: S. Memczak).

circRNAs. A number of molecular biology methods are then combined and applied to selectively distinguish between circRNA and linear RNA. These methods are very important to characterize circRNAs, since the linear and circular forms of RNA coexist in a cell. Both bioinformatics and biochemical methods are now available for characterising individual circRNAs in more detail and elucidating their function.

Two pioneering studies on the characterization of circular RNAs were published simultaneously in 2013 in Nature: *Hansen et al.* by the laboratory of Jørgen Kjems at Aarhus University in Denmark, and *Memczak et al.* by the research team around Nikolaus Rajewsky of the Berlin Institute for Medical Systems Biology in collaboration with colleagues from the Max-Delbrück-Center for Molecular Medicine in Berlin-Buch (Hansen *et al.*, 2013; Memczak *et al.*, 2013). The interdisciplinary Berlin study was conducted, amongst others, by the wet lab team Francesca Torti and Sebastian Memczak along with bioinformaticians Antigoni Elefsinioti and Marvin Jens. In both articles the researchers characterised individual circRNAs that can function as antagonists to small, regulatory miRNAs. The circular RNAs examined can bind many copies of certain miRNAs, thereby reducing their free concentrations. This novel circRNA function was investigated in a zebrafish model in collaboration with the research team of Ferdinand le Noble at the MDC.

circRNAs are still largely unexplored biomolecules

In addition to analysing individual circular RNAs, the Rajewsky laboratory used computational analyses to describe thousands of these molecules in different organisms: in human and mouse cells and in different development stages of the worm *C. elegans* (*Memczak et al.*, 2013). The researchers found that many circRNAs are differentially expressed in various cell types and several stages of animal development. This specific expression makes circRNAs interesting. In order to better describe and catalogue this new class of RNA molecules, scientists in Rajewsky's laboratory developed a freely accessible database (www.circbase.org, manuscript under revision). The database contains information on circular RNAs themselves as well as computational tools for detecting circRNAs. Many years' experience have shown that such databases are essential for making the variety and depth of genome-wide datasets widely comprehensible, accessible and eventually usable. The "circbase" provides a platform that biomedical research groups can use for disease-relevant questions.

Figure 2: The circRNA team (left to right)
Janna Krüger (postdoc with Ferdinand le Noble), and from the laboratory of Nikolaus Rajewsky: Francesca Torti (PhD student, wet laboratory), Antigoni Elefsinioti (former postdoc, bioinformatics), Sebastian Memczak (PhD student, wet laboratory), Marvin Jens (postdoc, bioinformatics) (Photo: Maimona Id.).

The large number of circRNAs that has been detected until now as well as their biochemical heterogeneity indicate that binding of miRNAs is just one of many possible functions. One prominent characteristic differentiates circRNAs from all other known RNA molecules – their longevity. Since many RNA degradation mechanisms attack the molecule ends, the circular RNAs evade this degradation and are therefore significantly more stable than related molecules. This observation indicates that circRNAs could be used for intracellular transport or serve as a reservoir for RNAs or proteins. These potential functions are part of the ongoing research in the laboratory of Nikolaus Rajewsky.

The Aarhus and Berlin publications triggered numerous international commentaries and review articles (Hentze and Preiss, 2013; Kosik, 2013; Taulli *et al.*, 2013; Toit, 2013; Wilusz and Sharp, 2013).

Recently, other laboratories have also described thousands of circRNAs in various cells and collected data that suggest that the transcription of DNA can be regulated by certain intronic circRNAs (Jeck *et al.*, 2013; Salzman *et al.*, 2013; Zhang *et al.*, 2013).

However, the research of circRNAs is still in its very early stages. As yet, it is unclear whether, or how many, circRNAs have functions on their own. It is also conceivable that the production of "normal" linear molecules is regulated by circRNA biogenesis. The unusual stability of circRNAs also suggests that in the future they could possibly be used as biomarkers in medical diagnostics or even as therapeutic agents (Bak *et al.*, 2013).

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there is no such thing as artificial life

Notes on the ethics of synthetic biology

by Thorsten Moos

In the debate on the ethics of synthetic biology, two strands can be identified. The *fundamental* strand deals with basic questions about the relationship between life and technology, focusing especially on the concept of "artificial life". The other, more *pragmatic* strand concentrates on specific problems relating to research and applied ethics.

The precarious distinction between the natural and the artificial

Synthetic biology – and this probably accounts for much of its allure – plays with the culturally deep-rooted distinction between the natural and the artificial, the living and the technical, the given and the made. The line between the two has never been fixed and has already been moved repeatedly. Ever since human beings first set aside plump grains to sow as seed they have intervened in life as they found it, and modern-day society was not the first to reflect on this. Given life always includes something that is made and in this sense artificial. English landscape architecture, for example, creates especially 'natural' nature as the result of very careful planning. In this sense, synthetic biology is part of a continuum of biotechnologies of domestication, cultivation and genetic modification of life.

So it is nothing new, then? Well, the debate around synthetic biology has given rise to a new awareness of the line between the natural and the artificial. The partly practical, partly programmatic application of engineering methods in the living world is accompanied by a promise to close the gap between the living and the artificial. When top-down approaches will successfully have produced a minimal cell, and bottom-up approaches will have arrived at a protocell, it will – according to the vision of synthetic biology – be possible not only to understand the mechanisms of life, but also to standardise and control them, and thus to design and build living beings for almost any purpose. At that point, the line between the given and the made, the natural and the artificial, will not be moved yet again but will be removed once and for all. That is what is meant by the term *artificial life*, which is at the core of the "hype" (Grunwald, 2013) around synthetic biology. Both the glamour of synthetic biology that generates research funding and the unease about it – an unease articulated in myths such as the golem or Frankenstein, and in religious terms by saying that humans should not play God (Boldt *et al.*, 2012) – have their roots in this guiding vision.

The term "artificial life" is ambivalent. Taken in a broader sense, it can be ethically fertile. In the strict sense, however, I consider it wrong, since it is possible to show that artificial life cannot exist, starting from some quite weak presuppositions. I take "artificial" to mean produced ("hergestellt") as described by Hannah Arendt (Arendt, 2011), that is: modelled and produced for a purpose so that the outcome of the manufacturing process is and remains a product (and can thus be destroyed again without hesitation). What "life" is, I will not try to define. Whatever it is, my only presupposition here is that the access we as human beings have to the living world is distinguished by the fact that we ourselves are life. When we perceive or acknowledge something as living (Rehmann-Sutter, 2013), we recognise in it the essence of life that defines us. So if researchers were one day to succeed in producing self-replicating, metabolising, excitable systems from the *bottom up* in a laboratory (which in principle I see no reason to doubt), and were we actually to recognise them as life, those systems will then cease to be merely products, that is, "artificial" in the above-mentioned sense, precisely because they as life will confront us, who are life. In mythology, the golem and Frankenstein turn against their creators. In science fiction, the female engineer falls in love with the (in a double meaning) *well-built* male robot. For reasons of principle we cannot regard life as merely an artificial product (regardless of how it came into being). In this sense there is no such thing as artificial life.

(Photo: Serg Nvns, Fotolia)

The vision of artificial life and the need for ethical level-headedness

Although there is no artificial life, it may be useful to talk about it as long as we know such talk to be paradoxical. For this distinction between "natural" and "artificial" is about fundamental conditions of scientific and technical action. Everything that is made includes elements that are given. Everything we do is based on preconditions that we did not create ourselves and that are not completely controllable. This insight gives rise to a healthy mistrust of the blueprint metaphor that accompanies synthetic biology, as if an idea alone could seamlessly become reality. This Leonard da Vinci-esque notion of human creativity should prove false when it runs up against stubborn reality, if not before. The practice of synthetic biology has been described as "tinkering" rather than "blueprinting" (Köchy, 2013). (That is also the truth contained in the ambiguous talk about "playing God". It is not as if human beings must not play God, which is theological nonsense, but that they simply *cannot*. The idea of creativity without preconditions or obstacles is naive and it may help to call that to mind occasionally.)

At the interface between science and society we need to adopt an enlightened approach to the great expectations that accompany emerging technologies. Armin Grunwald pointed out that although the production of "technovisions" (Grunwald, 2013) is helpful in generating research funding, it may have negative social consequences if it triggers protest, frustration or simply fatigue. Ethics must not allow this visionary ardour to force it prematurely into a matching moral ardour. We simply know too little about what will really turn out to be ethically problematic. We would be well advised to pay attention, while keeping a level head. One can, however, say something about the directions in which it is worth focusing this attention. That takes us to the pragmatic strand of the debate, concerning which I will mention three points that I believe have received too little attention hitherto.

Who is tinkering?

Major technological breakthroughs are always accompanied by changes in elites. During the Industrial Revolution, the bourgeoisie came along and comprehensively disempowered the aristocracy. Who are the elites in synthetic biology? Something interesting is happening here: the field of players is expanding in two respects. The first relates to academic disciplines. The highly interdisciplinary structure of synthetic biology enables a wealth of disciplines to share in the glamour of life sciences. Even physics, which has suffered something of a decline in relevance since the atomic bomb was finished, can play a role once more. The second expansion is structural. Conventional branches of biotechnology are highly concentrated, with a few major players dominating the pharmaceutical sector or green genetic engineering. In synthetic biology by contrast, it is not only the established large research institutions in universities, research societies, industry and the military that play a role. Even in a garage, almost anything seems possible, as exemplified by the iGEM competition between teams of undergraduates working on new organisms, or even the phenomenon of "biohackers" and DIY biologists. The modular structure of synthetic biology enables large numbers of people to play with its building blocks. I am sceptical about proclaiming a new age of scientific swarm intelligence, like a kind of Wikipedia structure in research. Nonetheless, it is worth keeping a watchful eye on the extent to which the structure of research institutions actually broadens or shifts, because it depends partly on this whether or

not the general public will gain confidence in scientific players and whether the potential to misuse synthetic biology will be curbed.

Who is thinking?

If the tricky aspects of synthetic biology that lie ahead only emerge during the course of time and if science is also restructured, a further question arises. Do the sciences involved in synthetic biology have a functioning early-warning system to alert them to important social questions? Is there an awareness of these questions, are there appropriate places and institutions to broach the issues, and above all is there sufficient confidence in the general public and in politics to make a public debate *worthwhile*? In any case public debate requires not only good institutions to assess the consequences of engineering and examine bioethics, but also a well-developed ethical sensitivity among research scientists themselves.

Who is steering?

Finally, we need to observe and understand the development dynamics of synthetic biology. After all, this has not only to do with scientific genius and successful research, but also with funding opportunities and possibilities for research. Here again there are many players – public, vision-driven funding policy (the EU's in particular), industrial and also military engagement. If synthetic biology holds only a little of the promise that is claimed, it is in the interest of society to understand who is pulling synthetic biology in which direction. I would not go so far as to support naive fantasies that science can simply be steered socially toward desired goals. The mechanisms that steer science are at least as complex as the signalling pathways in a cell. But in both cases we ought to make sure we know where the most important buttons are. As I see it, that should be in the common interest of both science and society.

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in silico hematology

Application of mathematical modeling to predict the outcome of leukemia treatment

by Ingmar Glauche and Ingo Röder

Chronic Myeloid Leukemia (CML) accounts for about 20 % of all leukemias in adults. The malignant cells, which express the BCR-ABL fusion protein, can be targeted efficiently by tyrosine kinase inhibitors (TKI). Due to the specificity of TKI treatment, CML has developed into a show-case example for an efficient, targeted tumor therapy. Applying a single-cell based mathematical model, which describes CML as a clonal competition between normal and leukemic hematopoietic stem cells, we suggest different approaches to further optimize CML therapy. With our model predictions, we particularly address combination therapies and patient-specific treatment cessation protocols.

Chronic Myeloid Leukemia as a model disease

Chronic Myeloid Leukemia (CML) is a fatal disease of the blood forming system accounting for about 20 % of all leukemias in adults. Unlike other leukemia types, the initiating event of CML is known for the majority of patients; about 95 % of them show a characteristic translocation of chromosomes 9 and 22. This translocation results in the formation of the BCR-ABL fusion gene coded on the shortened chromosome 22, which is called "Philadelphia chromosome". Harboring this mutation (which leads to the production of the BCR-ABL protein, a constitutively activated tyrosine kinase) in a hematopoietic stem cell-like cell can lead to an initially slow but sustained expansion of a leukemic cell population. This expansion comes along with a repression of normal hematopoiesis, which is progressively outcompeted, finally leading to the manifestation of a CML (Figure 1A). The primary, mostly symptom-free chronic phase of the disease eventually transforms into an acute blast crisis, in which a majority of undifferentiated peripheral blood cells severely constrain normal blood function and lead to the patient's death in the untreated situation. The abundance of immature "white" blood cells was name-giving for a whole family of blood cancers (greek *leuchaimia*, from *leukós* – white and *haima* – blood).

Since the turn of the last century, treatment and prognosis of CML underwent significant changes, and the disease can now be controlled in many cases. The availability of a specific class of drugs, named tyrosine kinase inhibitors (TKI), allows specific targeting of cells carrying the BCR-ABL oncoprotein. Due to this specificity, TKI treatment – in contrast to classical chemotherapies – widely spares healthy cells (Figure 1B). Already the introduction of the first-generation TKI *Imatinib* significantly improved the treatment prognosis compared to previous therapeutic options, such as the treatment with Hydroxyurea, Interferon-α (IFNα) or bone marrow transplantation. Five-year survival levels increased to values above 90 %. The availability of second-generation (*Dasatinib, Nilotinib*) and third-generation TKIs (*Bosutinib, Ponatinib*) currently further increases therapy effectiveness, especially regarding the successful treatment of a broad spectrum of secondary resistance mutations. Because of the availability and the success of a therapy specifically targeting the tumor cells ("targeted therapy"), CML has developed in a show-case example for the treatment of many tumor entities.

Molecular monitoring of tumor load in peripheral blood, using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), revealed that *Imatinib* monotherapy induces a biphasic decline of BCR-ABL transcript levels in most patients (Figure 2A). It is characterized by an initially steep decline, followed by a second moderate decline. A sensible explanation for the first decline is the rapid depletion of actively cycling BCR-ABL positive, leukemic cells. In contrast, the second decline most likely represents the slow elimination of quiescent residual leukemic stem cells (LSC) owing to their comparatively low turnover.

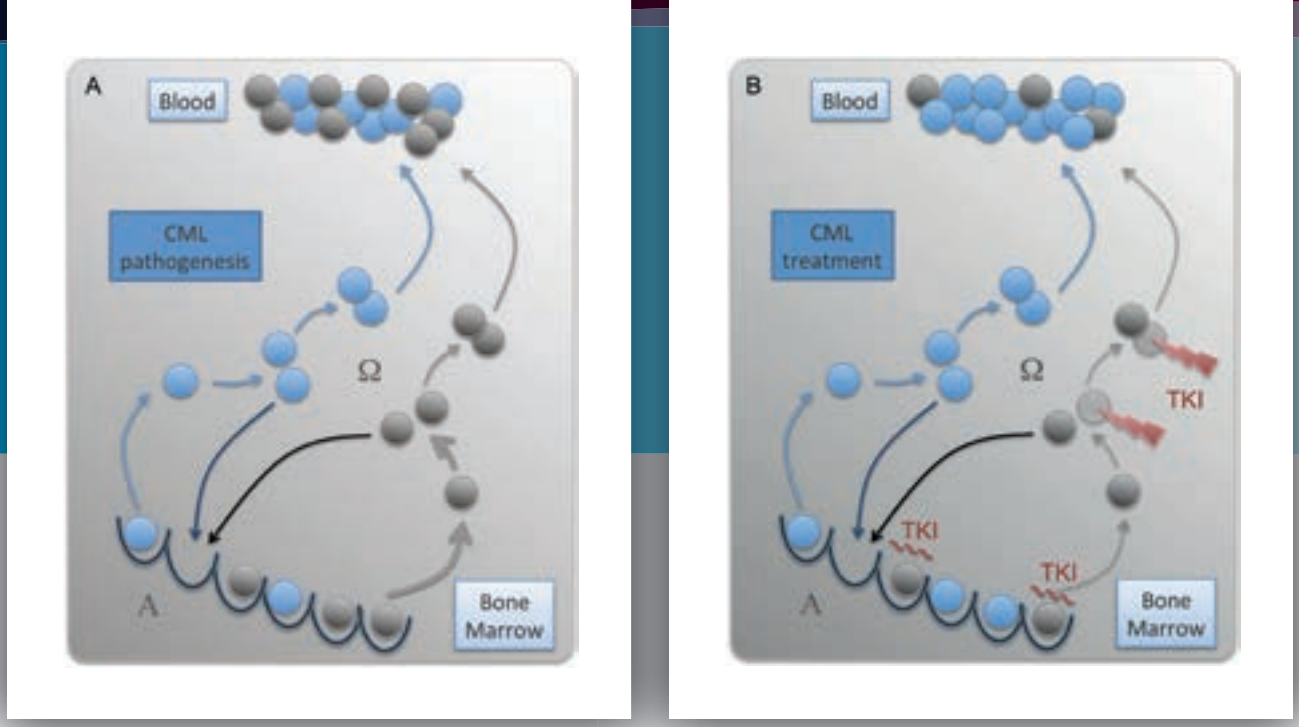

Figure 1: Chronic Myeloid Leukemia

A) Normal (blue) and leukemic (grey) stem cells are regularly activated from their bone marrow niches (bottom, indicated in the model as *signaling context A*) and subsequently divide (indicated in the model as *signaling context Ω*). Some cells return to the niches while others undergo further proliferation and differentiation, and contribute to peripheral blood. Due to an increased activation of the leukemic cells compared to normal cells the leukemic pool slowly outcompetes normal hematopoiesis.

B) TKIs preferentially target activated leukemic cells (indicated by red arrows), thus leading to a significant reduction of tumor load. Furthermore it appears that leukemic stem cells remain largely quiescent under TKI treatment (indicated by the red lines). Therefore, a residual pool of leukemic cells persists over long time scales (Source: adapted from Glauche *et al.*, 2012)

Although most patients respond well to TKI treatment and often reach complete cytogenetic or even complete molecular remission (i.e. hardly any leukemic cell can be detected in the peripheral blood), it appears that even after a massive and persisting reduction of tumor load over many years of treatment a residual disease is retained in the majority of patients. While these patients often relapse after cessation of TKI treatment, a sustained molecular remission after treatment stop has been observed in some patients (e.g. Mahon *et al.*, 2010). Although these cases are not the rule, they suggest that an eradication of the leukemia might in principle be possible and support the view that CML stem cells are not always found in a treatment-protected (potentially quiescent) state, but can in principle be successfully targeted over time.

Although CML has developed into a controllable disease, side effects of TKI therapy persist and especially for younger patients the impact of long-term TKI therapy is not well understood. Furthermore, high costs of TKI treatment exert also economic pressure on the health care system and pose the question, whether drug combinations can further increase treatment efficiency of the disease (with cure as the ultimate goal) or whether discontinuation of TKI therapy at a certain time point is a safe option for CML patients with good treatment response.

Mathematical modeling of CML

Taking a theoretical, systems-biological view, we perceive CML as a clonal competition phenomenon between normal hematopoietic and leukemic stem cells, which can be simulated in the computer using an agent-based modeling approach. This concept and its mathematical representation has originally been developed by Ingo Roeder and Markus Loeffler at the University of Leipzig to describe different phenomena in animal and in vitro models (Roeder and Loeffler, 2002). Later on, this model has successfully been applied to humans, namely to describe CML (Roeder *et al.*, 2006; Glauche *et al.*, 2012; Horn *et al.*, 2013). We could show that small differences in cell-specific parameters of leukemic and normal cells can lead to a slow but sustained outcompetition of normal cells, thus mimicking the clinically observed chronic phase in human CML. Treatment of CML patients with the TKI *Imatinib* is assumed to induce first a cytotoxic effect and second an inhibition of the proliferative activity of leukemic stem cells. Technically, the cytotoxic effect is modeled by a selective kill of a fixed percentage of leukemic cells per time step, while

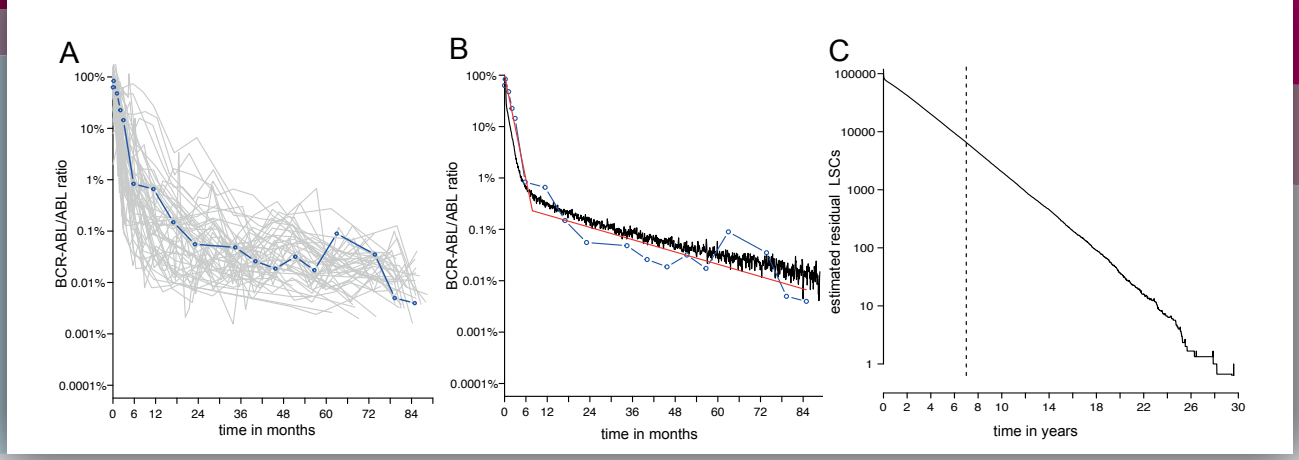

Figure 2: Kinetics of CML under continuous TKI treatment

A) Individual time courses of BCR-ABL transcripts levels for CML patients under *Imatinib* treatment with typical biphasic decline. Time course data for a single patient is highlighted in blue.

B) For the individual patient, a biphasic regression (red lines) provides the necessary input for a simulated predicted time course (black curve).

C) Based on our mathematical modeling approach we provide an estimate of the number of residual leukemic stem cells for this patient, which appears as a major predictor of relapse risk upon treatment cessation. The dashed line corresponds to the time frame of subfigures A and B (Source: adapted from Horn *et al.*, 2013).

the proliferation inhibition is modeled by a reduction of the activation of leukemic cells into cell cycle (compare Figure 1B). We showed that these assumptions are sufficient to reproduce the typical biphasic decline of BCR-ABL transcript levels in TKI-treated CML patients (Figure 2 A,B).

Treatment combinations to increase the long-term success of TKI therapy

Combination of TKI treatment with a cell cycle stimulating drug represents one potential way to increase efficacy of therapy. This idea is based upon the assumption that a cell cycle activation of leukemic stem cells makes them more susceptible to the cytotoxic effect of TKIs, which proved more efficient for cycling compared to quiescent cells, and could, therefore, lead to a faster reduction of the residual clone. Marieke Essers and Andreas Trumpp at the HI-STEM / DKFZ in Heidelberg (Essers et al., 2009) reported about the activating effect of IFNα that directly acts on murine HSCs and induces increased cell cycle activity. Although the findings were obtained in mice, these results again fostered the discussion on enhancing the TKI treatment in CML patients by cell cycle stimulating drugs.

Together with the biological partners in Heidelberg we took up the idea of a combination treatment of TKI and a secondary drug, stimulating the cell cycle activity of hematopoietic stem cells, exemplified for IFNα (Glauche *et al.*, 2012). Specifically, we used our mathematical model to study the potential overall treatment benefit for different activation effects of IFNα on human leukemic stem cells as well as for different treatment schedules. Our model suggests that a successful combination therapy of TKIs with IFNα in CML patients requires the simultaneous application of both drugs in overlapping time intervals. In addition, a less frequent application of IFNα reduces the speed of tumor reduction but might also decrease potential side effects and risks of the combination therapy. We demonstrated that a weekly or biweekly administration of IFNα under optimal conditions still shows a significant advantage compared to Imatinib monotherapy and can reduce the predicted time to tumor eradication considerably. Even in a less favorable situation (i.e. IFNα does not induce activation of leukemic stem cells in humans), a pulsed IFNα therapy under continuous TKI administration is predicted to show no adverse effects compared to standard TKI monotherapy. These findings support recent clinical results that argue in favor of lower doses/longer cycles of IFNα administration in combination therapies to reduce severe side effects while retaining the curative intent.

Estimating residual disease levels and predicting optimal timing for treatment cessation

The question whether TKI therapy can safely be withdrawn after sustained molecular remission, is still controversial. Recent clinical trials report that about 40 % of patients being in complete molecular remission for at least two years, retain their previously achieved molecular responses after Imatinib treatment cessation, while a molecular recurrence of BCR-ABL transcript levels is observed in the remaining 60 %. Relapses can even be observed in patients lacking any measurable BCR-ABL transcripts in peripheral blood. Together with Markus Loeffler and Matthias Horn (Universität Leipzig), we applied our mathematical modeling framework to study whether individual

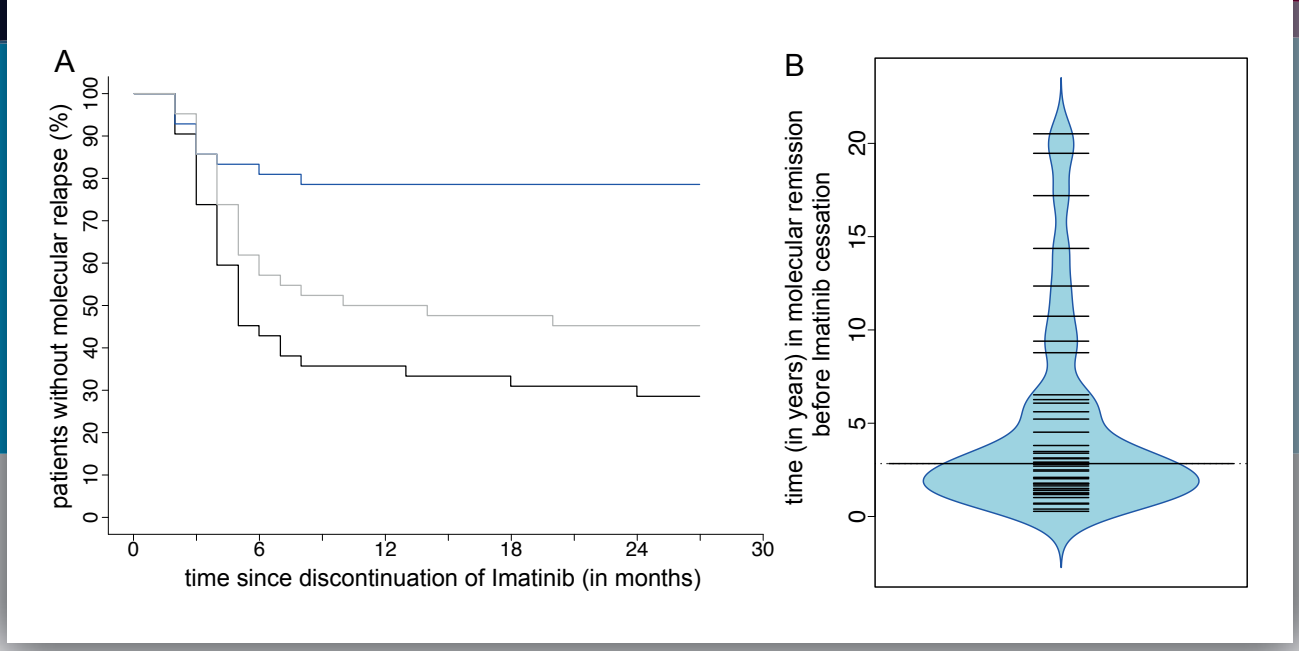

Figure 3:

A) Model predictions for the relapse free survival of *Imatinib* treated CML patients after therapy discontinuation: **black** (standard strategy) – therapy cessation after 2 years in major molecular remission; **grey** – therapy cessation after 2.8 years in major molecular remission (compare median in subfigure 3B); **blue** (individualized strategy) – therapy cessation for sufficiently low LSC numbers based on model predictions.

B) Estimated individual waiting times in major molecular remission until treatment discontinuation is recommended for the individualized, model-based cessation strategy. Median treatment time: 2.8 (range, 0.3-20.5) years (Source: adapted from Horn *et al.*, 2013).

treatment response kinetics are predictive for the relapse risk after treatment cessation (Horn *et al.*, 2013). In particular, we aimed at a sufficiently precise estimate of residual LSC numbers, which are a critical determinant of relapse after therapy discontinuation. Based on seven-year follow-up data of BCR-ABL transcript dynamics from the German cohort of the IRIS trial, provided by Andreas Hochhaus (Universitätsklinikum Jena) and Martin Müller (Medizinische Fakultät Mannheim der Universität Heidelberg), we determined model parameters, which quantitatively characterize the inter-individual heterogeneity of the molecular treatment response. Given a patient's BCR-ABL transcript kinetic, the adapted model generates predictions for patient-specific long-term response to *Imatinib* (Figure 2 B,C) as well as individual times to complete eradication of residual leukemic stem cells. Based thereon, we derived a model-based predictor for the individual risk of molecular relapse upon treatment cessation. A simulation-based comparison of overall relapse-free survival demonstrates that our proposed patient-specific predictor results in a superior clinical decision rule to decide on potential discontinuation of therapy as compared to relying on a fixed (e.g. two years) time in sustained deep molecular remission. Figure 3A indicates that relapse-free survival can possibly be achieved for up to 80 % of patients. Furthermore, our results suggested that there is a high patient heterogeneity with respect to the time in complete molecular remission, which is required to guarantee a sustained remission in case of treatment cessation (Figure 3B). Whereas for some patients a safe treatment stop is predicted to be feasible already after one year in complete molecular remission, for others 10 or even more years appear necessary.

Outlook

Ongoing clinical trials on combination therapies (e.g. CML-V trial) and controlled TKI cessation will yield major insights on a potential operational cure of CML, but will also generate important data to refine the computational disease models. Based on these models, adaptions to individual patient data will allow predicting disease development and thus estimating the risks of clinical interventions. Together with our clinical and experimental collaborators, we plan to develop the current mathematical models to a stage were they can eventually assist clinical decision-making.

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Further information:

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from genotype to phenotype

Metabolomics, a central technology in life sciences

Company profile of Metabolomic Discoveries GmbH

by Nicolas Schauer

Technological developments in recent decades have opened up completely new research opportunities in the life sciences. Research is no longer based solely on examining individual biological building blocks – it also involves a comprehensive analysis of all transcripts, proteins or metabolites. Combined with bioinformatics methods, these technologies permit precise insights into biological systems. Metabolomics is now established as a central component of the life sciences and of research-based industry.

Metabolites are the biochemical end point of genetic information. The metabolome, the sum of all metabolites in a system, forms the basis of communication between biochemical units in cells, tissues and the environment. The development of metabolomic technology more than ten years ago made it possible to analyse and identify hundreds and even thousands of metabolic products in one biological sample. The variety of metabolites and their different physical and chemical properties make metabolomics one of the most complex methods in the life sciences. Metabolites range from highly polar substances such as nucleotides, acids and sugars via sterols to complex lipid compounds. At the same time, concentrations of the substances examined vary, sometimes by several orders of magnitude. This places great demands on the preparation of samples and analytics.

From the analytical side, two technologies dominate the field: nuclear magnetic resonance spectroscopy (NMR) and mass spectometry (MS). Table 1 gives an overview of the advantages and disadvantages of these two often complementary technologies. Due to the large number of chemical compounds and the wealth of information, automatic data analysis is a key prerequisite in these methods. Appropriate software tools and algorithms for extracting metabolite information from raw data have been developed in recent years. In the consequent step chemometric analysis can be used to identify patterns and differences in samples (figure 1).

Table 1: Advantages and disadvantages of mass spectrometry and nuclear magnetic resonance spectroscopy

Technology	Advantages	Disadvantages
NMR	• Metabolite absolutely quantifiable • Non-destructive • Complete structural information	• Low sensitivity • Spectra hard to interpret • High costs

MS	• High sensitivity
	• Very flexible technology
	  • Wide spectrum of instruments
	  • Wide spectrum of metabolites
		• Destructive analysis

Source: Metabolomic Discoveries

Figure 1: Metabolome analysis workflow (Source: Metabolomic Discoveries)

The potential of metabolomics

Metabolomics is now used in all fields of research, with a particular interest being shown in examining biochemical mechanisms and in human diagnostics.

For example, a systems biology approach was successful in reconstructing and modelling human metabolism (Recon 2, Thiele *et al.*, 2013). For this, the human metabolism was first established with the aid of a genome-based network. In combination with information about endogenous metabolites, precise metabolic pathways were determined. Simultaneously, the integration of protein data from cellular units led to further information about biochemical mechanisms at the cell and tissue level. Overall, more than 2,600 metabolites were identified and mapped in more than 7,400 reactions. On this basis, it was possible to predict 77 % of changes to metabolites in 49 metabolic diseases. Hence this approach holds great potential for predicting metabolic changes in diseases and for identifying possible targets for drugs.

With its comprehensive approach, metabolomics enables a better understanding of diseases and action mechanisms of biologically active substances. This opens up new opportunities for investigating diseases and developing new drugs. For example, valid disease models and mode of actions can be developed in cell models and animal experiments during preclinical research. At the same time, metabolomics provides the opportunity to translate results to clinics and thereby develop more specific medical therapy approaches.

That is where Metabolomic Discoveries comes in. Metabolomic Discoveries has established a world-wide unique platform for comprehensive metabolomics and the data interpretation. Metabolite profiling is done on a multi-parallel approach using high-resolution mass spectrometry in combination with liquid chromatography (LC) and gas chromatography (GC). Statistical analysis and visualisation of the data enables a precise insight into metabolic changes (figure 2, Yamada *et al.*, 2011). The integral bioinformatics platform recognises metabolic differences and links them to protein or gene expression data. This makes it possible to model biological systems such as cells and organs, right through to the human system.

Functional testing of cell lines

A better understanding of diseases and the functions of molecular units is the first step towards developing medicines and therapies. Because of this, metabolomics is now an indispensible tool in research. For example, an examination of 60 cancer cell lines showed that each line has a characteristic metabolic profile and that specific metabolites have a functional significance in cancer proliferation (Jain *et al.*, 2012).

Optimising bioprocesses

It is no longer possible to imagine life without the biotechnological production of drugs, vaccines or fine chemicals using microorganisms or cell cultures. Metabolomics can optimise the use of raw materials and process times. For example, identification of nutrient limitations or the excess of metabolites can make it possible to adjust the culture medium optimally to a cell line. In strain development, too, metabolomics helps to identify relevant metabolic pathways and by-products and thus to optimise a strain genetically. Modelling of metabolic flows with the aid of genome-based network maps is highly significant. In this area, we collaborate closely with Insilico Biotechnology AG.

Predicting the development of type 2 diabetes

In epidemiological studies, metabolomics is a very significant tool for investigating the influence of environmental factors and nutrition on quality of life and health. In a long-term study, metabolome analyses using a complex algorithm from the mechanical learning field enabled employees of Metabolomic Discoveries to find a clear metabolic pattern for raised levels in a fasting blood glucose study (Hische *et al.*, 2012). As

a result, it is now possible for the first time to predict, based on the levels of specific metabolites, the development of blood glucose levels towards type 2 diabetes.

Safety and accuracy in developing drugs and early diagnosis of disease

Developing new medicines is a protracted and very expensive process. Often, drug candidates fail to clear the necessary hurdles due to low effectiveness, intolerance or toxicity. Metabolomics can be used in the experimental and preclinical phase to identify possible risks and toxicities better and more accurately than other methods. In clinical studies, the application of metabolomics leads to better understanding of drug mechanisms and metabolism. Thus, metabolic markers can recognise outliers among test subjects, identify toxicity effects, or distinguish between responders and non-responders at an early stage.

Outlook

In the years ahead metabolomics will become increasingly significant, especially in the fields of applied and industrial research. Metabolomics, in combination with other "omics" technologies and high-performance computing, will also be used in personalised and precision medicine. Metabolomic Discoveries will further develop its technology for these purposes and will continue to play an active role.

Profile Metabolomic Discoveries GmbH:

Metabolomic Discoveries GmbH is an internationally leading contract research company in the biotechnology and pharmaceuticals field. The company was established in Potsdam, Germany, in 2009. Its work is based on high-resolution metabolomics technology combined with bioinformatic analysis of large data sets. Metabolomic Discoveries also develops diagnostic markers for diseases and therapeutic effectiveness.

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Figure 2: Visualisation of metabolic changes on the basis of iPath

Source: EMBL, Metabolomic Discoveries

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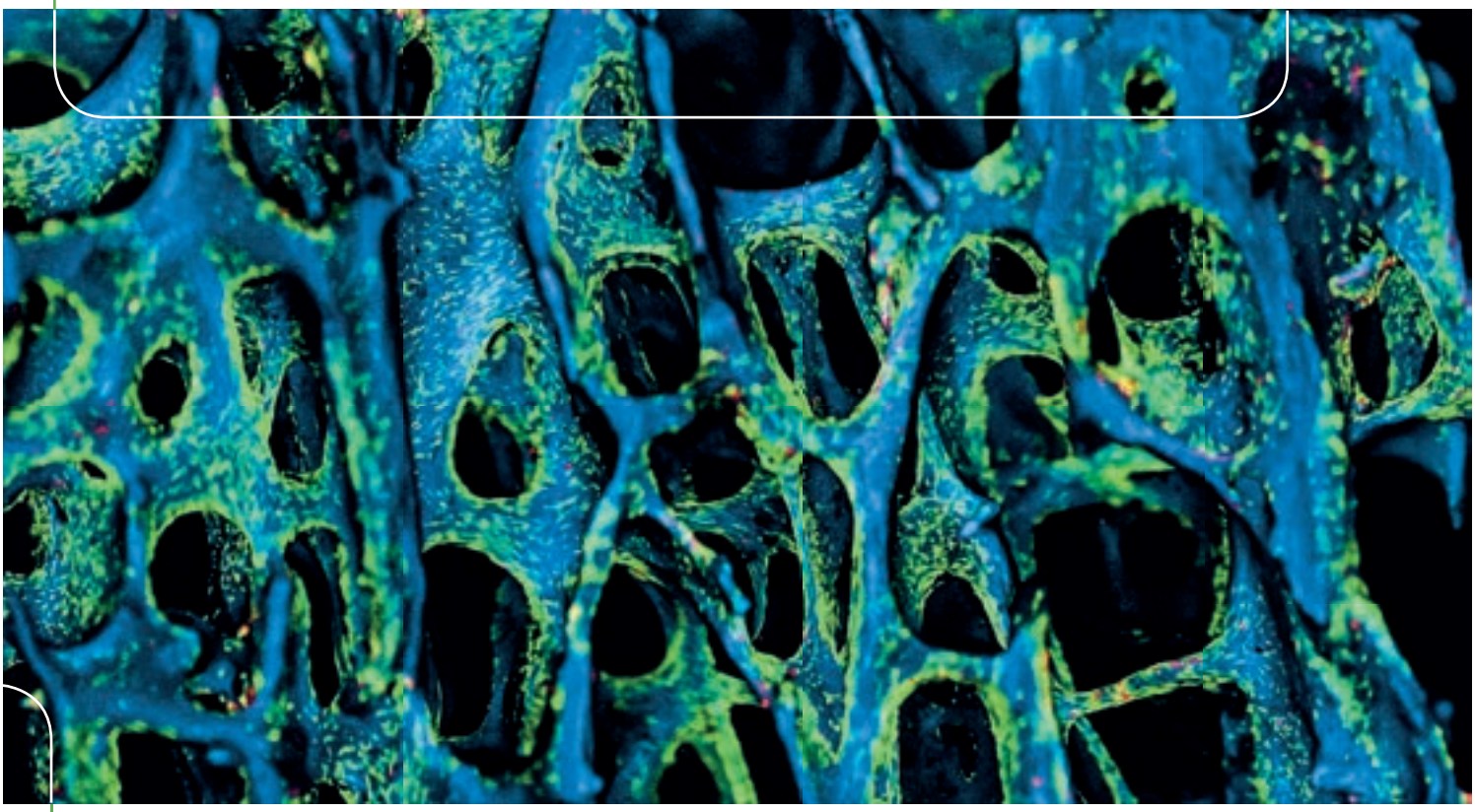

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BRICS, the braunschweig integrated centre of systems biology

Systems biology for infection research and biotechnology

by Dietmar Schomburg, Ida Retter and Dieter Jahn

Systematic health research – with this as their guiding motto, only three years ago the Technische Universität Braunschweig (TU Braunschweig) and the Helmholtz Centre for Infection Research joined forces and established BRICS, the Braunschweig Integrated Centre of Systems Biology. The Braunschweig scientists' shared objective is to apply systems biology methods in order to develop therapies to counter infections and biotechnological production methods for therapeutic agents. Their work draws on an ultra-modern, comprehensive infrastructure in genome, transcriptome, proteome and metabolome analyses, along with bioinformatic tool development and expertise in quantitative modelling of biological systems. A new building for the BRICS research groups is under construction on the TU Braunschweig campus.

Examining bacteria using systems biology methods – the basis of infection research and biotechnology

BRICS is a joint interdisciplinary centre of the Technische Universität Braunschweig (TU Braunschweig) and the Helmholtz Centre for Infection Research (HZI). These two institutions are linked by a shared research interest in combatting infections and developing anti-microbially active substances. In the case of the HZI, its dedication to this as its main focus is evident from its name. A bottom-up strategy process initiated by the TU Braunschweig in 2013 defined "infections and active substances" as one of the university's three strategic research foci. BRICS was established with the aim of making a significant contribution towards this area of research by applying systems biology methods.

Biotechnology also plays an important role in the field of infection and natural product research. It is used to develop and optimise sustainable production methods for active substances, with the main focus on anti-infectives. For instance, defined growth conditions in bioreactors enable reproducible biological data for quantitative metabolism models. In the last 14 years, the special research area "From Gene to Product" (SFB 578) at the TU Braunschweig has merged systems biology with biotechnology, thereby coining the term "systems biotechnology". The SFB has collaborated closely with the HZI, which emerged eight years ago from the Gesellschaft für Biotechnologische Forschung (GBF).

The central biological objects of BRICS research are bacteria (figure 1). Bacteria are relevant both as pathogens and as producers of active substances. Dieter Jahn, Microbiologist, Vice-President for Research at the TU Braunschweig and Spokesman of the BRICS Board of Directors, is an expert in bacteria. Dietmar Schomburg is the Board's representative for bioinformatics and biochemistry, while systems immunologist Michael Meyer-Hermann focuses on the host aspect of infections and uses mathematical models to examine *inter alia* the development of antibodies by the immune system (figure 2). Further BRICS members are research scientists from the TU Braunschweig, the HZI and the Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures).

Collaborative projects at BRICS: Clostridial infections, systems biology of *Roseobacter*

BRICS is involved in numerous collaborative research projects. Two of the larger ones are described here. The CDiff research project is centred around the bacterium *Clostridium difficile*, which is responsible for many infections acquired in hospitals and communities that can have life-threatening consequences (figure 3A). The aim of this project is to understand proliferation pathways and pathogenicity mechanisms in order to identify new points of attack for medicines. The systems biology approach enables holistic

Figure 1: Molecular biology and microbiological work being completed by Dieter Jahn's BRICS study group
A) Cutting strips of agarose gel.
B) Checking a plate with bacterial growth (Photos: Frank Bierstedt).

recording of the biology of the organism, which because of its sensitivity to oxygen is difficult and time-consuming to cultivate. The project falls within the scope of the North German Center for Microbial Genomics. Project spokesman Dieter Jahn and the BRICS office coordinate the 13 participating research teams, from the Universities of Greifswald and Göttingen, Hannover Medical School, the HZI, the Leibniz Institute DSMZ and the TU Braunschweig. The project has been granted € 3.9 million in funding over a three-year period by the Federal State of Lower Saxony (Niedersächsisches Vorab fund).

Another major collaborative project at BRICS, the DFG-funded transregional collaborative research centre SFB TRR-51, is investigating bacteria of the *Roseobacter* group, which are among the most commonly found organisms in the world's oceans (figure 3B). Because they adapt to a wide range of habitats they have an astonishing diversity of metabolic processes. Together with the University of Oldenburg, research scientists from the TU Braunschweig, the HZI and the DSMZ are exploring the evolutionary, physiological and genetic principles that make this group of bacteria so successful. The goal of this SFB is to achieve a systems biology understanding of this globally important group of marine bacteria. The SFB TRR-51 and the associated graduate school are entering the second funding period this year.

Technology and infrastructure for systems biology and bioinformatics

Systems biology depends on close dovetailing of experimental research and model development. BRICS is very broadly established in this area. Genome sequencing, transcriptome, proteome, metabolome and fluxome research are conducted in separate groups with state-of-the-art instrumental equipment. In future four bioinformatics teams will provide the corresponding theoretical basis to this area. Naturally, this infrastructure is put to use for BRICS' own projects, but it also makes BRICS study groups sought-after cooperation partners in national and international networks.

Probably the most-used TU Braunschweig "product", the BRENDA enzyme information system is of international significance for the further development of research in the life

Figure 2: The BRICS Board of Directors in front of an artist's impression of the new building
(left to right) Dietmar Schomburg (bioinformatics and biochemistry), Dieter Jahn (microbiology), Michael Meyer-Hermann (systems immunology) (Photo: TU Braunschweig).

sciences. It has more than 60,000 users per month. BRENDA is now being integrated into Dietmar Schomburg's study group as a BRICS development project and is also an important partner in the emerging Deutsches Netzwerk für Bioinformatik Infrastruktur (German Network for Bioinformatic Infrastructure).

A further infrastructure project of major future significance is currently being created in Jörg Overmann's research group at the Leibniz Institute DSMZ. This is the BacDive database, which provides detailed information about the strains of more than 23,000 microorganisms.

A new research building on the TU Braunschweig campus will enable the close cooperation that is essential for BRICS. Construction work on this 3,500-square-metre building began in November 2013, the symbolic foundation stone-laying ceremony having taken place in August 2013.

TU Braunschweig, HZI and BRICS networks

Beyond the duration of individual funding projects, as part of the TU Braunschweig and the HZI, BRICS is linked into long-term networks with local, national and international cooperation partners. Within the TU Braunschweig, BRICS benefits from collaboration with a further interdisciplinary centre, the Zentrum für Pharmaverfahrenstechnik (PVZ). The main focus of the PVZ, which is likewise newly established, is on developing process principles for the production of drugs for personalised medicine. BRICS has close ties with the Leibniz Institute DSMZ, whose Managing Director Jörg Overmann is a member of BRICS.

BRICS also belongs to TRAIN, the translation alliance for Lower Saxony. This is a biomedical alliance bundling the know-how and infrastructure of university and non-university research in the Braunschweig-Hannover-area in order to develop active substances up to therapeutical applications. Another important alliance is the above-mentioned North German Center for Microbial Genomics, a cooperation network in the field of genomics that supports collaboration between North German research institutes and enables projects such as CDiff.

Studying systems biology at the TU Braunschweig

Systems biology research activities are reflected in a corresponding teaching offering at the TU Braunschweig. Students on biology and biotechnology master's courses are offered various systems biology modules focusing mainly on OMICS technologies and bioinformatics. Closely integrated modelling and experiments efforts are typical of systems biology modules in Braunschweig. A module begins, for example with a week for annotating a bacterial genome and developing the corresponding metabolic network on a computer. In the second week, an analysis of the metabolome of the native organism and mutants is developed in the laboratory. Finally, in the third week the experimental data is integrated into the genome-wide flux balance model of the cell. The module is supplemented by a literature seminar that goes into the

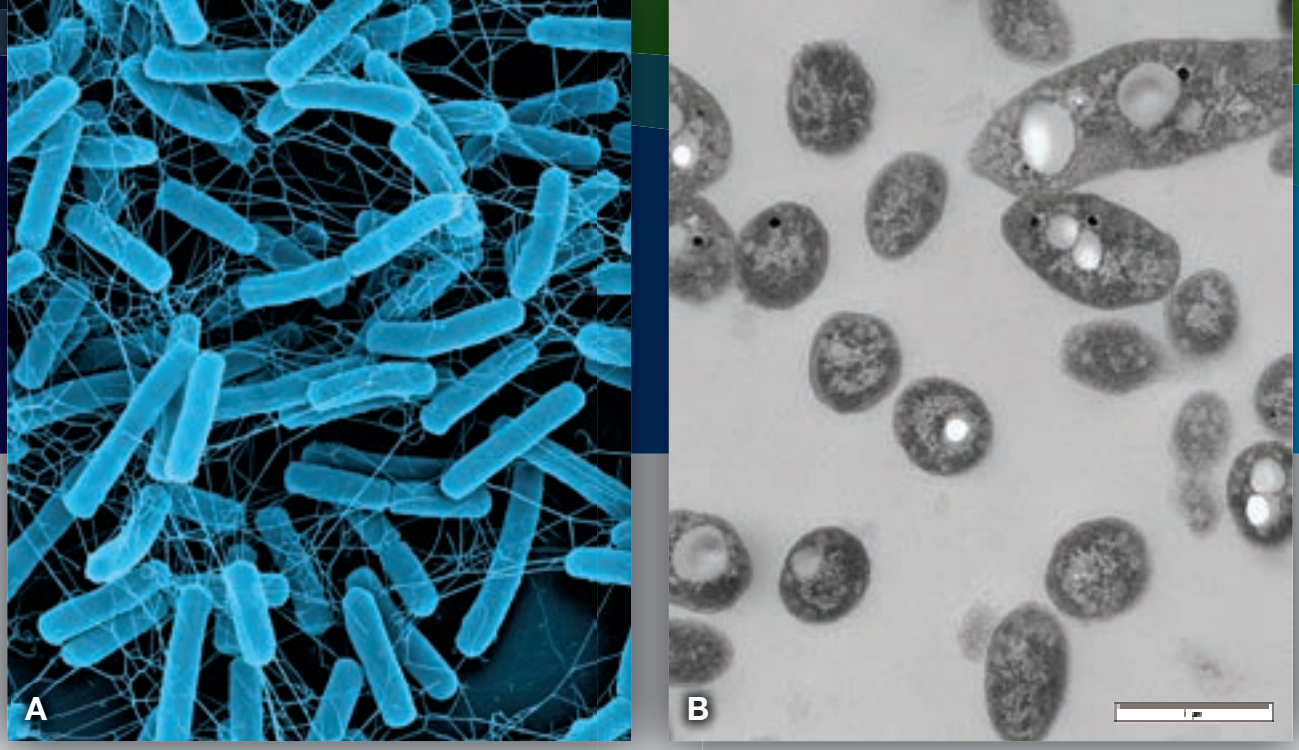

Figure 3: Bacteria undergoing investigation at BRICS
A) *Clostridium difficile*
B) *Dinoroseobacter shibae* (Photos: Manfred Rohde, HZI)

biological and theoretical backgrounds to the practical part. Along with this integrated approach, characteristic features of systems biology teaching at the TU Braunschweig are inclusion of biotechnological downstream production processes under the heading "systems biotechnology".

The bases for this are laid for students during the preceding bachelor programmes, which include compulsory courses in bioinformatics, for example. With its variety of research areas, BRICS offers a wide range of possible subjects to explore in depth during a doctorate, which usually follows the master programme. To summarise: BRICS in Braunschweig is the right place for anyone interested in modern systems biology and anti-infective production.

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hepatomasys

Evaluating cancer's metabolome for diagnosis and therapy

by Stefan Kempa, Thorsten Cramer and Hergo Holzhütter

Cancer is a complex disease resulting from alterations at the genetic, epigenetic and metabolic level. Thus it is evident that it cannot be studied and understood mechanistically on a single layer – making the evaluation of cancer biology a prime example for systems biology. The in depth analyses of cancer *in vitro* led to important discoveries, however, the *in vivo* situation adds multiple more layers of complexity and represents a pivotal reason why current and novel therapies often fail in the clinic. The HepatomaSys consortium has taken on this challenge and tries to connect the present knowledge using state of the art technology, engineering and mathematical modeling in order to decode the metabolic program of hepatocellular carcinoma.

The group of Thorsten Cramer at the Hepatology and Gastroenterology Department of the Charité is focusing on the role of the microenvironment for solid tumor progression. Lack of oxygen (hypoxia) and infiltrating immune cells are of special interest to the research group. Collaborations with various clinicians enables the Cramer group to analyze tumor samples from patients with well-characterized clinical courses, ultimately establishing a translational research approach covering bench-to-beside as well as bedside-to-bench dynamics.

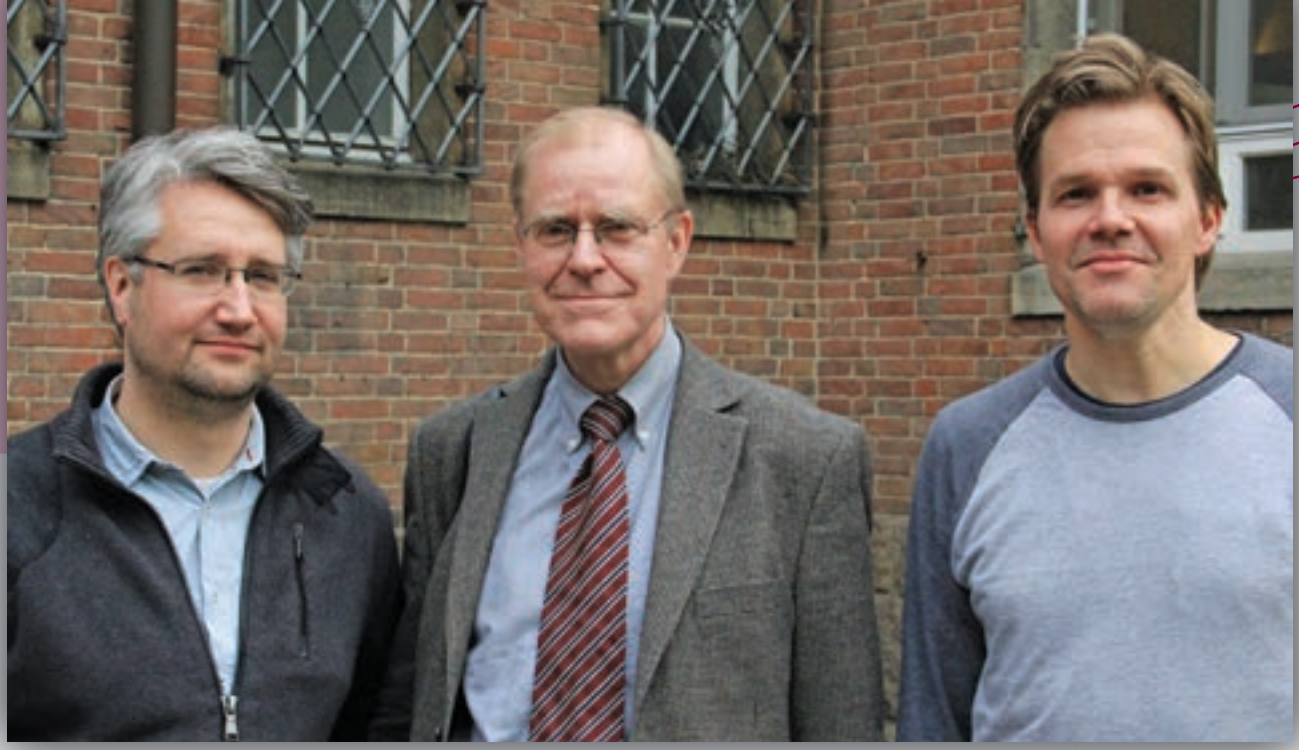
Hergo Holzhütter and his group have a longstanding experience and tradition in mathematical modeling of the human metabolism and have developed a large scale kinetic model of the hepatocellular metabolism in the frame of Hepatosys and virtual liver. The group has started to adapt this model to the specific metabolic signature of HCC by including cancer-specific iso-enzymes. Adjusting the wild-type and cancer-specific variants of the kinetic model to experimentally determined protein profiles, metabolite concentrations and stable isotope-tracer distributions will allow to quantify the fluxes trough central metabolic pathways of the energy-, lipid- and carbohydrate metabolism in both cell types and to reveal how the observed differences are brought about by changes in the activity and regulatory properties of the underlying enzymatic reactions.

The group of Stefan Kempa from the Berlin Institute of Medical Systems Biology (BIMSB, hosted at the MDC in Berlin-Buch) developed metabolomics and proteomics techniques allowing for quantitative and dynamic analyses of metabolism from tissue cultures and biopsies and is therefore filling the gap between clinical research and mathematical modeling.

The HepatomaSys projects aims at comparing *in vitro* and *in vivo* data to understand the impact of the microenvironment on metabolic reprogramming of cancer. The results will be used for mathematical analyses and predictions. Finally, we aim to decipher concepts of therapy resistance and to evaluate tumor metabolism for tumor stratification and as a target for therapy.

Metabolic reprogramming

The metabolic reprogramming is a hallmark of cancer (Hanahan *et al.*, 2011). The first description of tumorspecific metabolic alterations was noted almost a century ago, when Otto Heinrich Warburg made his landmark observation of enhanced aerobic glycolysis in cancer (termed the Warburg



Principal investigators of the HepatomaSys project:
(left to right) Stefan Kempa (BIMSB/MDC, Berlin-Buch), Hergo Holzhütter (Charité and Humboldt University, Berlin) and Thorsten Cramer (Charité, Berlin) (Photo: Fabian Bindel).

effect) (Warburg *et al.*, 1927). It was not until the post-genome era, however, that Warburg's observation received widespread international attention. We know today, that the action of numerous transforming oncogenes and tumor suppressor genes is associated with marked alterations of cellular metabolism, most prominently glycolysis and mitochondrial activity (Levine *et al.*, 2010). On a molecular level, the Warburg effect is largely mediated by upregulation of glycolysis-related enzymes (e.g. hexokinase II (HK II), phosphofructokinase 2 (PFK-2) and pyruvate kinase M2 (PK-M2)) as well as glucose transporters (e.g. glucose transporter 1 (Glut-1)). Functional inactivation of glucose transporters and glycolytic enzymes, respectively, has shown anti-tumor activity in human and murine cancer cell lines from various organs. Enhanced glucose uptake and utilization is such a robust feature of cancer that it has been translated into clinical application in the form of FDG-PET ([18F] fluorodeoxyglucose positron electron tomography) imaging 7. FDG-PET exploits the enhanced glucose uptake of tumor cells, has a > 90 % sensitivity and specificity for the detection of metastases of most epithelial cancers and is now clinically established for tumor detection as well as monitoring responses to treatment (Mankoff *et al.*, 2007). Remarkably, the functional significance of tumor-specific metabolic reprogramming for therapy resistance, one of the main obstacles in clinical oncology, remains largely elusive.

It was shown that inactivation of lactate dehydrogenase A (LDH-A) could enhance the *in vitro* antiproliferative efficacy of taxol in human mammary carcinoma cells and that of novel multikinase inhibitors in hepatocellular carcinoma cells. To the best of our knowledge, these are the only reports pointing towards a functional importance of metabolism for chemoresistance. Furthermore, the precise role of mitochondria for tumor formation remains elusive and under intense discussion until today (Wallace *et al.*, 2012).

Role of the microenvironment

Metabolic reprogramming in tumors can develop due to intrinsic or extrinsic mechanisms or a combination thereof. Activated oncogenes or inactivated tumor suppressor genes are examples for tumor intrinsic means to flip the metabolic switch. Experimental evidence from the past decade shows that oncogenes like *c-myc, akt, k-ras* and *b-raf* as well as various tyrosine kinase receptors (epidermal growth factor receptor, EGFR; insulin-like growth factor 1 receptor, IGF-1R; etc.) very potently enhance the transcription of genes that mediate glycolysis and glutaminolysis4. On the other hand, the tumor microenvironment can serve as an extrinsic and very potent inducer of the metabolic switch. Malignant tumors are characterized by extremely hostile microenvironmental conditions such as hypoxia, nutrient starvation and acidosis. These

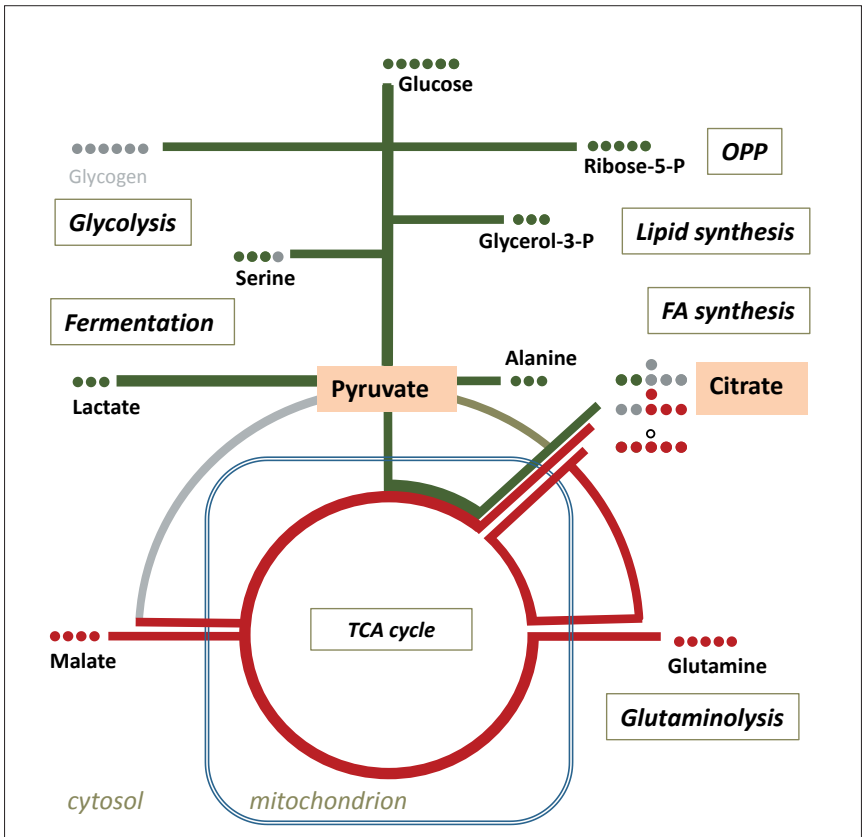
conditions exert an extensive selection pressure on the tumor and, ultimately, only well-adapted neoplastic cells will survive and enable tumor progression. In this scenario, the Warburg effect is believed to confer a substantial growth advantage by reducing oxygen dependency, enabling the neoplastic cells to thrive in hypoxic microenvironments.

Intratumoral hypoxia activates the hypoxia-inducible factor 1 (HIF-1), a transcription factor that mediates cellular adaptation to hypoxia. HIF-1 is considered to be a central pro-tumorigenic factor, because it is expressed by the majority of human cancers and their metastases, activates a transcriptional program closely related to malignant progression and functional inactivation of HIF-1 results in reduced tumor growth and enhanced therapy efficacy in various animal tumor models. HIF-1 stabilization results in elevated glucose transport and enhanced glycolysis, thereby conferring a metabolic growth advantage to tumor cells that closely resembles the Warburg effect. In fact, HIF-1 target genes control glucose transport, glycolysis, mitochondrial activity, intracellular pH regulation and lactate extrusion, making HIF-1 a pivotal molecular mediator of metabolic reprogramming in cancer.

Hepatocellular carcinoma (HCC)

Hepatocellular carcinoma (HCC) represents the fifth most common cancer and the third most common cause of cancer-related deaths in the world. While the majority of cases still affect regions like Africa, South America and Southeast Asia, the incidence of HCC in Europe and the United States is constantly rising, turning HCC into a pivotal threat to general health also in Germany. HCC is characterized by robust therapy resistance and very poor prognosis. Chronic liver diseases such as liver cirrhosis, chronic viral hepatitis and non-alcoholic fatty liver disease (NAFLD) represent important risk factors for HCC development. Early detection of HCC in patients at risk relies primarily on ultrasound and the HCC serum marker α-fetoprotein (AFP), resulting in insufficient surveillance. Ultimately, the majority of HCC cases get diagnosed at advanced stages without the possibility of curative intervention. In summary, the development of innovative and more effective options for surveillance and therapy of affected patients is urgently needed. Detailed molecular characterization of HCC pathogenesis and therapy resistance represents an essential prerequisite to achieve this goal. Interestingly, the precise role and clinical exploitability of metabolic re-

Figure 1: Scheme of the central metabolism of a cancer cell



The Scheme shows the central carbon metabolism and indicated stable isotope incorporation into metabolic intermediates derived from stable isotope resolved metabolomics experiments (SIRM). The data represent a proliferating cancer cell with high activity of the glycolytic and glutaminolyitc pathway. (Graphics: Stefan Kempa)

programming for HCC pathogenesis and therapy resistance is largely elusive. The HepatomaSys project will characterize the functional importance of metabolic alterations for human HCC with an integrated systems biology approach using state-of-the-art metabolomics, proteomics and targeted sequencing methodology.

Medical systems biology

Medical systems biology marks a new field in biology combining systems biology approaches as mathematical modeling with high throughput genome-wide methods as genomics, proteomics and metabolomics; ideally, these approaches are applied on clinical specimens and compared to patient data.

The combination of mathematical modeling and quantitative proteomics will allow the understanding of how the expression of alternative isoforms and splice variants of metabolic enzymes reprogram HCC metabolism *in vitro* and *in vivo.* Although tumor-specific metabolic alterations in liver tumors were first described almost a century ago (Lo *et al.*, 1968), no clinical application based on metabolic reprogramming has been established thus far. One reason for this obvious lack of clinical translation is given by the robust heterogeneity of liver diseases and the enormous complexity of metabolic pathways in hepatocytes. It is therefore reasonable to assume that a comprehensive and "translatable" understanding of the role of metabolic reprogramming for the many aspects of HCC pathobiology can only be achieved by a systems biology approach integrating all levels of molecular biology.

The research project in brief:

The HepatomaSys projects aims at comparing *in vitro* and *in vivo* data to understand the impact of the microenvironment on metabolic reprogramming of cancer. The close interaction of the groups of Thorsten Cramer (Charité), Hergo Holzhütter (Charité, HU-Berlin) und Stefan Kempa (BIMSB/MDC) it is possible to combine clinical expertise, high throughput methods and mathematical modeling to address this complex question using a medical systems biology approach.

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News from the BMBF

Most foreign qualifications are recognized

Since April 2012, the possibilities of gaining recognition of vocational qualifications obtained abroad have improved considerably. For the first time, the Assessment and Recognition of Foreign Professional Qualifications Act, or Recognition Act, establishes a legal claim to a review whether a foreign qualification is equivalent to a German reference occupation.

About 8,000 reviews were conducted in the first nine months and the vast majority (82 per cent) resulted in the full recognition of a vocational qualification obtained abroad. “The large number of full recognitions is a very positive sign,” said Federal Education Minister Johanna Wanka. No more than 6.5 per cent of the cases were rejected in total.

There was a particularly great interest in gaining recognition in the area of regulated professions for which recognition is a prerequisite for exercising the profession. More than 90 per cent of the positive decisions were made on applications submitted by physicians and nurses – professions of which Germany has a considerable shortage. “The figures highlight that the Recognition Act is helping significantly to achieve the goal of securing skilled labour,” Wanka said.

Further information is available at:
www.bmbf.de/en/15644.php

Growing old and staying healthy

Humankind has come much closer to the aim of growing as old as possible while staying healthy. Life expectancy in the OECD member states has risen by 11 years since 1969. However, about 60 per cent of people aged 65 and over suffer from at least three chronic diseases. The BMBF therefore provides targeted support for research for better medical care of the elderly and has been funding six research collaborations on “Health in Old Age” since 2007. Funding volume is about 35 million euros.

Federal Research Minister Johanna Wanka explained: “We all hope to spend our additional years in good health and with a high quality of life. The projects we fund provide important insight for such a development. They facilitate targeted counselling and help to diagnose health risks at an early stage and to contribute to preventing them”.

The Berlin project AMA, for example, has demonstrated that autonomy in old age can even be secured in the case of existing multimorbidity. ESTHER-Net in Heidelberg has studied frailty prevention and care strategies. The Munich-based research collaboration KORA-Age demonstrates that social contacts are a decisive factor for well-being in old age. On the basis of a long-term study, the LUCAS collaboration in Hamburg has developed recommendations for targeted counselling and health promotion. Interactions between different diseases are in the focus of the Hamburg project MultiCare. The so-called “Priscus Study” gives a list of medical drugs which are potentially unsuited for the elderly.

Further information is available at:
www.demografische-chance.de/ and
www.bmbf.de/en/10849.php

Number of BAföG recipients at the highest level in 30 years

The number of BAföG recipients (Federal Training Assistance Act) in 2012 reached the highest level of the past 30 years. At the presentation of the 20th BAföG report, Federal Education Minister Johanna Wanka said: "Federal Government and *Länder* have invested even more in education opportunities and equity in education with BAföG. The reforms of 2008 and 2010 are showing results: They have benefited more students and pupils and have brought about tangible improvements".

630,000 students and pupils received grants on average for the year, which is 7.7 per cent more than in 2010. The number of students receiving grants even rose by 14 per cent in the reporting period to a total of 440,000; the rate of students receiving grants thus reached 28 per cent.

In addition to the greater number of recipients, the average amount of monthly grants also rose. Furthermore, grants for studies abroad and grants for pupils and students from other countries were also increased considerably. Expenditure by the Federal Government and the *Länder* for BAföG has further increased from 2.84 billion euros in 2010 to 3.34 billion euros in 2012.

Reliable training assistance and student support will be guaranteed in future too. "The report shows that we need to develop BAföG further. We will start the necessary talks right away and pursue the aim of presenting an amendment act speedily," Wanka said.

Further information is available at:
www.bmbf.de/ and
www.bmbf.de/press/3568.php

Europe invests strongly in research and innovation

The new EU research framework programme Horizon 2020 was launched with a funding volume of about 77 billion euros. It is the largest coherent research and innovation programme worldwide. From 2014, it pools all research funding programmes at European level and is geared more than previous programmes towards cooperation between science, research and industry.

At the national launch event, Federal Minister of Education and Research Johanna Wanka said: "Horizon 2020 facilitates an additional boost of investment across national borders. This means progress and development for Europe. We now have the opportunity to jointly forge the future of Europe and to considerably improve people's quality of life".

Máire Geoghegan-Quinn, Commissioner for Research, Innovation and Science of the European Commission, stated: "Horizon 2020 will close the present gap between research and innovation. It will make an important contribution to greater competitiveness and therefore more jobs and greater prosperity in Europe and will raise Europe's global attractiveness for science and industry."

The new programme enables funding of research and innovation from basic research to commercial launch. German universities of applied sciences and small and medium-sized enterprises (SMEs) will benefit from better funding opportunities due to a stronger application orientation.

Further information is available at:
www.horizont2020.de and
www.bmbf.de/en/959.php

German-Turkish year of research, education and innovation 2014

"Research transcends borders. More than ever, it is internationally organized and interlinked and thrives on mutual exchanges of information and cross-border networks. Germany and Turkey are linked by a long tradition of fruitful cooperation in research and education – which is a good opportunity for a joint year of science", said Federal Minister of Education and Research Johanna Wanka on the occasion of the festive launch event with her Turkish counterpart Fikri Isik, Minister of Science, Industry and Technology of the Republic of Turkey.

Deutsch-Türkisches Jahr der Forschung, Bildung und Innovation 2014
Türk-Alman Araştırma, Eğitim ve İnovasyon Yılı 2014

The Science Year is taking place under the motto of "Science Bridging Nations". Topics range from application-oriented research in key technologies to challenges of global change to the humanities and social sciences. Particular attention is being paid to cooperation between research and industry, initial and continuing vocational training in industry, science and research and to the interface between science and society.

A large number of conferences and expert meetings are planned during the Science Year. The "Turkey Weeks" at German institutions of higher education and the promotion of new projects with a competition of ideas are among the highlights of these events.

Further information is available at:
www.german-turkish-scienceyear.com and
www.bmbf.de/de/22881.php

Launch of the Science Year in Berlin: Federal Minister Johanna Wanka and her Turkish counterpart Fikri Isik sign an agreement on bilateral cooperation.
Copyright: BMBF, Jessica Wahl / Wahluniversum

Erasmus+ boosts mobility in Europe

More than 4 million people in Europe – in particular pupils and students, apprentices, teachers and young volunteers – will receive scholarships and grants by 2020 that facilitate study periods abroad. The new EU programme Erasmus+, which was launched in January of 2014, has a duration of seven years and a budget of 14.7 billion euros.

"We are providing young people in Europe with new education opportunities and helping to prevent unemployment. More young people will have the opportunity to study abroad or do a part of their training in another European country", said Federal Education Minister Johanna Wanka. More international orientation in vocational and academic education is important to secure skilled labour in Germany. "Companies will benefit from Erasmus+ because it will become easier to find skilled labour from abroad and because they can profit from the international experience of their apprentices", Wanka stressed.

Young people who have spent time abroad develop a new perspective on other cultures and themselves. Europe must come alive for young people if they are to carry on the idea of the European Union.

Further information is available at:
www.bmbf.de/en/23262.php

R&D investment has reached 3 percent of GDP objective

Expenditure in research and development (R&D) in Germany reached a record level of over 79.5 billion euros in 2012. The R&D share of gross domestic product (GDP) has therefore peaked at 2.98 per cent for the first time in Germany, the Donors' Association for German Science said. Germany has thereby visibly strengthened its position as one of the internationally leading sites for innovation, also due to its successful High-Tech Strategy.

"Germany is investing in the future like never before. Together with industry and science we have achieved the 3 percent objective for the first time. We now have to secure this positive development in the long term", said Johanna Wanka, Federal Minister of Education and Research.

Between 2010 and 2013, the Federal Government provided additional funds for education and research to the amount of over 13 billion euros while consolidating its federal budget. The original plan was for 12 billion euros. In a European comparison, Germany is now among the leaders in R&D expenditure. Only the Scandinavian countries Finland, Sweden and Denmark invested more in R&D as a proportion of GDP in 2012.

Further information is available at:
www.bmbf.de/en/6075.php

Alexander von Humboldt's diaries return to Berlin

The Stiftung Preußischer Kulturbesitz (SPK) has acquired the famous "American Travel Diaries" of Alexander von Humboldt. Travel diaries are among the scientifically most revealing documents of the 19th century. The purchase was made possible by a broad consortium of public and private donors including the BMBF. Furthermore, the BMBF is facilitating the analysis of content and material of the diaries. All in all, the BMBF will provide 3 billion euros.

"I am very happy that Humboldt's travel diaries are returning to Berlin. They are a valuable part of our cultural heritage. However, the travel diaries are not only a valuable cultural asset – they are also of greatest interest to science", Federal Education Minister Johanna Wanka commented on the return of the documents.

The aim of the funded project is to analyse the travel diaries in the context of the entire estate, to pursue new approaches in research and place them in the context of culture, politics, society and, above all, research of the 19th century.

Further information is available at:
www.bmbf.de/en/21592.php

Contact

Information on these and other interesting topics on the High-Tech Strategy for Germany is available at **www.hightech-strategie.de**

HELMHOLTZ ASSOCIATION

ANTICIPATING THE FUTURE OF TECHNOLOGY AND EXPLORING OPTIONS TO SHAPE IT

The Institute for Technology Assessment and Systems Analysis (ITAS) at KIT

It is nowadays taken for granted that the impacts of scientific and technological progress must be explored and considered at an early stage: both to enable its opportunities and potential to be realised and to prevent risks and avoid unpleasant surprises. That, very briefly, is the task of technology assessment and thus of the Institute for Technology Assessment and Systems Analysis (ITAS) at the Karlsruhe Institute of Technology (KIT).

The challenges arise in many different areas: Staff at the institute analyse new branches of science and key technologies (such as synthetic biology or service robotics) as well as the development and transformation of modern society's key infrastructures (such as the energy system or the internet) and the handling of technological legacies such as highly radioactive waste. The aim is to look ahead and consider, classify and assess the relevant "technological futures". This is followed by an evaluation, based for example on criteria related to responsibility, political or societal objectives. Finally, the work strives to develop options for constructive action. Technological assessment translates acquired knowledge into advisory expertise. It therefore explores impacts for the purpose of providing scientific advice to policymakers and society.

ITAS was founded in 1995 based on previous institutional activities on systems analysis. Since 2012, the institute is located in a venerable art nouveau building close to Europaplatz in the inner city of Karlsruhe.

Source: © ITAS

Accordingly, the task of ITAS is to generate and combine three different types of knowledge:

SYSTEMS KNOWLEDGE

A sufficient understanding of the systems studied (technological developments and their conditions, possible applications, stakeholder constellations and interests, conditions for success and factors inhibiting innovations, assessments of future markets and acceptance levels, processes at the interface between science, engineering, politics, environment and society, etc.) and their temporal dynamics is a necessary prerequisite for an anticipatory analysis of the impacts of science and technology.

ORIENTATION KNOWLEDGE

The evaluation of scientific and technological developments and their impact is not based on factual knowledge alone, but requires normative premises and relevant arguments, through to ethical considerations. The main focus here is on questions of responsibility (a central concept in the EU's HORIZON2020 research framework programme is "responsible research and innovation") and of sustainable development.

ACTION KNOWLEDGE

To develop options for shaping processes, action knowledge is required: for example knowledge of regulations and research funding, but also about economic incentive mechanisms or social processes such as participation.

Service robotic systems are one of the topics at ITAS. It analyses opportunities, risks and restrictions as well as appropriate funding schemes for such systems to develop recommendations and policy options for their application in society.

Source: © KIT

Prospective knowledge is a notoriously precarious type of knowledge. Its usually fundamental uncertainty means that it is frequently impossible to make forecasts. Different options for the future are considered instead, often in the form of scenarios. At the same time, system boundaries must not be drawn too tightly. A wide range of consequential dimensions, including ethical, cultural, economic, environmental and political aspects, must be considered. In addition, different points of view must be taken into account. Analysis and evaluation must not only consider the decision makers' points of view, but also the views of those affected, and even the needs of future generations. Finally, amid this complexity one must "think through to the end", which means following the life of technological innovations through to the end in order to draw an overall balance.

Naturally, this programme can only be processed in an interdisciplinary fashion, and it often requires trans-disciplinary cooperation with non-scientific stakeholders. For this reason, the ITAS does not fall within the scope of any traditional faculty: humanities, economic and social sciences are just as much at home here as natural and environmental science and engineering.

In this interdisciplinary diversity, we work in four areas of research:

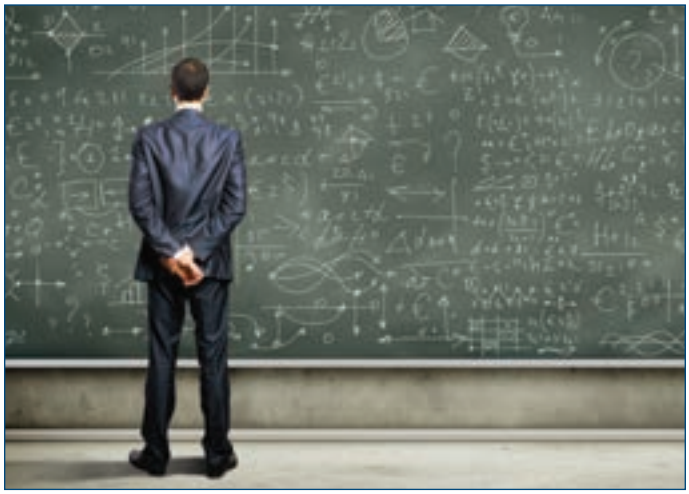
SUSTAINABILITY AND ENVIRONMENT

The main focus in **Sustainability and Environment** is the sustainable development of urban systems, global changes and the sustainable treatment of renewable and non-renewable resources, primarily in relation to land and water.

Source: © vichie81/Fotolia

KNOWLEDGE SOCIETY AND KNOWLEDGE POLICY

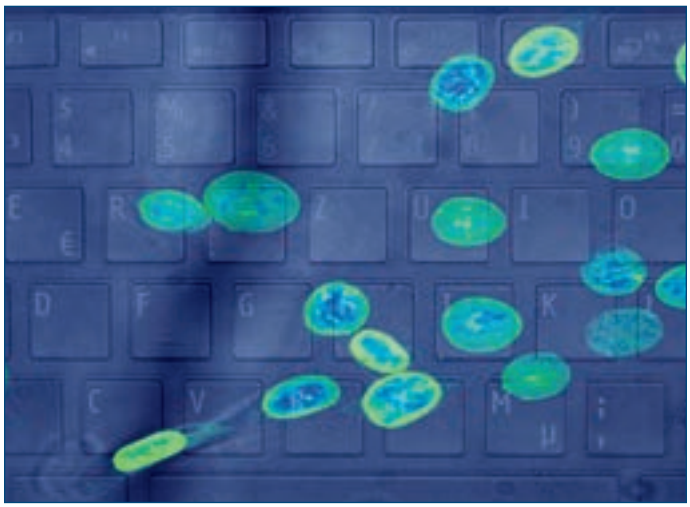
The **Knowledge Society and Knowledge Policy** research area is concerned with knowledge and technology policy, technological change and social dynamics, along with concepts and methods of assessing technological impacts.



Source: © Serg Nvns/Fotolia

INNOVATION PROCESSES AND IMPACTS OF TECHNOLOGY

Innovation Processes and Impacts of Technology concentrates on new biotechnologies such as synthetic biology, information and communication technology, nanotechnology and key technologies for future mobility and transport concepts.



Source: © ITAS

ENERGY – RESOURCES, TECHNOLOGIES, SYSTEMS

In **Energy – Resources, Technologies, Systems** the main emphases are on energy from biomass (including microalgae), high-efficiency fossil power plants, new energy technologies, crosscutting and efficiency technologies, and carbon management strategies.

Source: © mentaldisorder/photocase.de

ITAS provides a variety of advisory services in these areas. It is the leading parliamentary advice institution and works with the German Bundestag (see also on top of page 43) and the European Parliament. A wide range of work for national and local government ministries in Germany, the European Commission and other authorities, along with involvement in a large number of scientific and non-scientific bodies testify to the diversity of its scientific policy advice.

Synthetic biology: an important subject for technology assessment in the life sciences

Within the framework of the tasks outlined above, ITAS has in recent years been involved with synthetic biology in several projects. Major aims of synthetic biology include the production of new kinds of biological systems, functions and organisms that do not occur in nature. Until now, it has not so much been a strictly demarcated field of research but rather an engineering-type approach to changing life forms in a precisely foreseeable way or even designing new ones. As a result, synthetic biology often involves various disciplines such as molecular and systems biology, chemistry, (bio)physics or computer-assisted modelling. While we are seeing a discussion of the great opportunities this field offers for solving key problems in areas such as energy, health and the environment, we are increasingly also experiencing academic and public debates – both in Germany and internationally – about the potential risks. These relate to protection from misuse (biosecurity), possible risks to human health and the environment (biosafety) and the socioeconomic risks of some applications. The possible impacts of synthetic biology on traditional notions of life are another feature of these discussions. Artists, too, are paying increasing attention to the subject. Hopes and concerns converge in activities aimed at enabling responsible research and innovation (RRI, see above).

Synthetic biology, like other new and emerging science and technologies (NEST), places special demands on technology assessment. For example, it is required to provide orientation knowledge at a time when developments are in their infancy, but when there is already far-reaching discourse about impacts, technological visions and political and societal expectations.

Source: © ITAS

ITAS has operated the **Office of Technology Assessment at the German Bundestag** (TAB) for more than 20 years. During that time it has produced more than 150 studies commissioned by a variety of parliamentary committees. Subjects tackled in recent years include gene doping, the impacts of an extended electricity supply blackout, the future of the German automotive industry and synthetic biology.

Source: © KIT

Through its participation in various projects, ITAS began to analyse the opportunities and risks of synthetic biology, including ethical challenges, to examine public discourse in this subject area and to contribute toward highlighting options for action at a relatively early stage. It was involved in "SYNTH-ETHICS" (under the Seventh Framework Programme of the EU, until 2011), the "Engineering Life" project (German Federal Ministry of Education and Research, BMBF, until 2013) and the "Synthetic Biology" project (Office of Technology Assessment at the German Bundestag, ongoing). In the Helmholtz Initiative on Synthetic Biology launched in September 2012, which is coordinated by the German Cancer Research Center (DKFZ) in Heidelberg, and in which five Helmholtz centres, along with the Universities of Freiburg and Heidelberg, are participating, ITAS has assumed responsibility for sociological and humanities research and technology assessment. In this case it is concerned specifically with the ethical and societal aspects of synthetic biology and with research policy and funding strategy questions. ITAS also supports dialogue with the general public. Since September 2013, ITAS and KIT have been coordinating the "SYNENERGENE" project, which is part of the European Commission's "Science and Society" programme. More than 25 institutions and networks from Europe, the US and Canada have joined forces in this project to bring together scientists, companies, political decision makers, civil society organisations, artists and other groups to discuss synthetic biology. The project's objectives are to foster public discourse relating to synthetic biology, for example in order to analyse developments or to identify critical questions. It also aims to develop and begin to implement agendas in the areas of policy, research and public participation. These should influence the way the field develops beyond the end of the project. The overall intention is to help establish the guiding principle of responsible research and development of new technologies in alignment with the goals, needs and expectations of society at the European level and beyond.

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systems biology twinpack

Ursula Klingmüller and Jens Timmer in the spotlight

by Svantje Braun

Ursula Klingmüller and Jens Timmer first met in 1999. At the time Klingmüller, a molecular biologist, was head of a junior research group at the Max Planck Institute for Immunology in Freiburg. Intrigued by the complex processes that control the transduction of signals within a cell, she was looking for someone she could collaborate with to bring "some order" to the molecules. A phone call to Timmer – who had recently qualified as a university lecturer and was working at Freiburg University's Institute of Physics – was the first step in a long journey they have since taken together. Klingmüller and Timmer believe that new knowledge can be acquired by combining biological data with dynamic mathematical modeling. As a result, over the past 15 years they have played a key role in developing data-based dynamic modeling as a systems biology approach.

Timmer became involved in theoretical physics by chance. After graduating from high school he was even considering studying philosophy, medicine, or economics. He opted for physics at the University of Oldenburg because of the laid-back atmosphere in a research group investigating renewable sources of energy. It seemed the best way for him to fulfill his wish to support the anti-nuclear energy movement. However, the courses in Oldenburg soon began to seem too technical, and he discovered his love of theoretical physics. At the University of Freiburg he learned that it is mathematical physics that "...holds the world together at its core," to quote Goethe. He soon made contact with experimental research groups, initially in medicine and subsequently in biology.

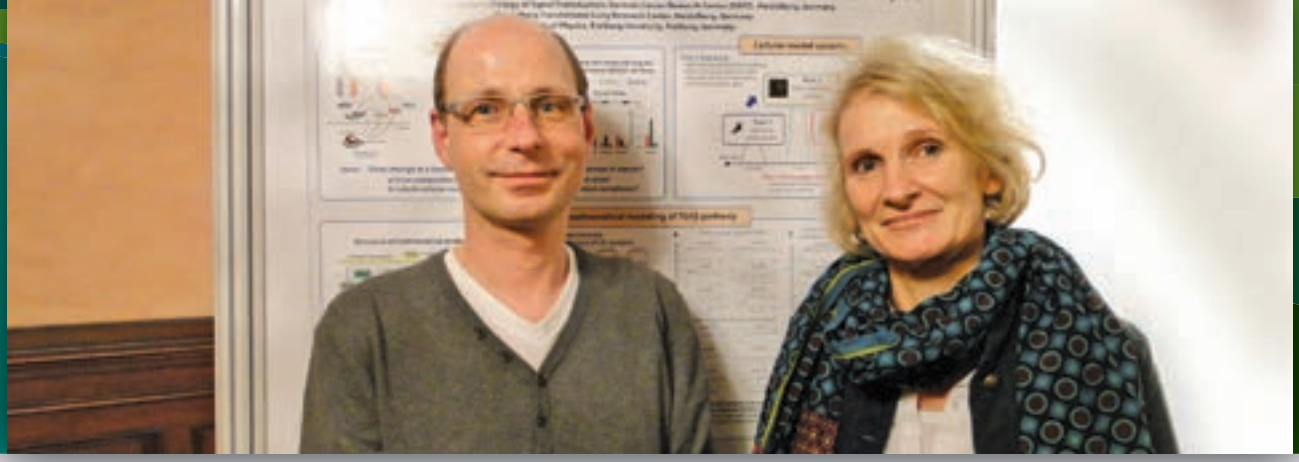
In Klingmüller's case, by contrast, an interest in science ran in the family. Her father was a geneticist and from an early age she wanted to emulate him and study biology. The only likely alternative, graphic art and design, remained a hobby. She deliberately chose to study in Heidelberg, where molecular biology was particularly advanced. While preparing her diploma and doctoral thesis in the laboratory of chemist Professor Dr. Heinz Schaller at Heidelberg University's Center for Molecular Biology (ZMBH) she soon became acquainted with a very accurate style of conducting research. Her area of research in Heidelberg was the interaction of hepatitis viruses with liver cells. Eventually, this work turned full circle and now a large section of the Klingmüller laboratory is engaged in research on liver functions, damage, and infections.

"A sound foundation is everything"

As a young scientist at Harvard Medical School and the Whitehead Institute for Biomedical Research in Boston and Cambridge, Massachusetts, Ursula Klingmüller benefited from her sound basic training in biology in Heidelberg. This training, along with the opportunities she had in the United States, made for a highly successful period as a postgraduate. Jens Timmer, too, emphasizes the importance of a sound foundation. He says he would study pure physics again because the basic knowledge of mathematics and physics acquired by doing so enables one to develop "truly new" methods later on. "Otherwise you are just a user," Timmer says. The two scientists advise students against early specialization. They say you can build on a thorough study of the basics to branch out successfully in other directions at a later stage.

Modeling already starts with data collection

"At first I simply had no feel for the other discipline," Timmer says, recalling the early years of collaboration in Freiburg. He had given up biology in his mid-teens at school. It took several months for the experimental data from Klingmüller's laboratory to match up to his expectations as a modeler. Calibrating a mathematical model requires a large number of data points, i.e., a high temporal resolution with only the smallest possible errors. Klingmüller was the right partner in this regard: the temporal course of activation of JAK2 kinase in the erythropoietin signaling pathway featured in the very first article published during her time in the USA. Klingmüller always endeavored to quantify the activity of signaling proteins in the cell extremely precisely. Together with her colleagues



Ursula Klingmüller and Jens Timmer presenting joint projects in Heidelberg (January 2014) (Photo: Ulrike Conrad).

and in constant communication with Timmer she developed methods for adjusting biological measurements better to what the modelers required. "We had to standardize many things. Standardized cell systems and methods were not commonly established in biological laboratories," Klingmüller explains. "At the beginning that took up a great deal of time." In return, the mathematical tools were adjusted to the biological questions and mathematical equations made their way into Klingmüller's group. "There were times when a differential equation was explained on an airplane napkin," Timmer recalls with a smile. Now, biologists are no longer the sole factor determining overall speed because the computing effort required for the very large mathematical models has become enormous.

Systems biology as a new discipline

Mathematical models have long existed in biology. What is new is their closeness to the actual data. Moreover, when the model is finished, the project is far from being over. The model is nice to have, but it primarily provides a more in-depth understanding of biology and can now be tested thoroughly in further experiments. However, the idea that data-based mathematical modeling of signaling pathways could lead to new insights first had to take root in the minds of their peers. Without any footsteps to follow, Klingmüller and Timmer entered this new territory. It was a move that demanded considerable courage and a willingness to take risks because they were both on fixed-term contracts and their professional futures were at stake. As luck would have it, the German Federal Ministry for Education and Research picked up on the combination of biology and mathematical modeling very early on, in 2004, with its HepatoSys funding program. The expertise of the Klingmüller/Timmer partnership, which had already published the first data-based dynamic model for the JAK-STAT signaling pathway, was now sought after.

The JAK-STAT signaling pathway as a starter model

The JAK-STAT signaling pathway, which is activated by the hormone erythropoietin (Epo) in precursors of red blood cells, was ideal as the first subject for data-based dynamic modeling. Signal transduction from cell surface to cell nucleus is mediated by just a few components – the cell surface receptor EpoR, the kinase JAK2, and the transcription factor STAT5, which activates target genes (figure 1). Building on data from Klingmüller's laboratory, Timmer and his colleagues were able to generate a model for the signaling pathway that gave insights which pure experimental work alone could not have provided. It was shown that, as a fundamental characteristic of the signaling pathway, the continuous transport of STAT5 between cytoplasm and cell nucleus constantly links events on the cell surface with the activation of target genes in the cell nucleus (Swameye *et al.*, 2003).

Model-based investigation of the signaling pathway led to further successes in the years that followed. How precursors of red blood cells exposed to a very wide range of Epo concentrations can nonetheless react appropriately to each concentration was a question that had long puzzled researchers. Klingmüller, Timmer, and their colleagues jointly decoded the interaction of Epo with its receptor EpoR and developed several models, each representing a particular biological hypothesis. Only one of the different models fitted the experimental data, though: It showed that Epo binds to the receptor EpoR and both are subsequently absorbed by the cell. The cell surface is then equipped with new EpoR molecules that come from large EpoR reserves inside the cell (Becker *et al.*, 2010).

Further studies focused on intracellular events. The two researchers were able to establish a quantitative link between the activation of the MAP kinase ERK after the action of Epo on the cells and subsequent cell division (Schilling *et al.*, 2009). They also showed how the two molecules SOCS3 and CIS down-regulate the JAK2-STAT5 signaling pathway at different Epo concentrations, thereby influencing cell survival (Bachmann *et al.*, 2011).

Systems biology not only in blood

Klingmüller's research into erythropoiesis dates back to her time in Boston, and she is still in the vanguard in this field. Building on this, a large section of her group is investigating

the significance of Epo in lung cancer. In addition, HepatoSys and the current Virtual Liver Network funding program brought her back to the liver, an interest from her days as a doctoral candidate. These fields of research, in which she also collaborates with Jens Timmer's group, may appear very disparate, but Klingmüller emphasizes that they are of great mutual benefit. "The basic principle – that is the transduction of a signal from a cell surface receptor to the cell nucleus – is common to all projects. We explore the different strategies by which cells have managed this task in different contexts," she explains.

Interdisciplinary research at a distance

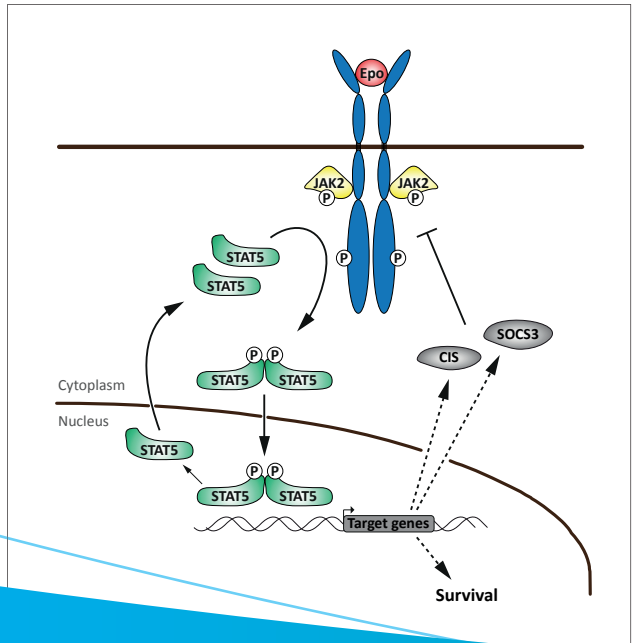
Timmer is now Professor of Theoretical Physics at the University of Freiburg and heads the division "Data Analysis and Modeling of Dynamic Processes in the Life Sciences". In 2003 Klingmüller moved back to Heidelberg, where she is head of the division "Systems Biology of Signal Transduction" at the German Cancer Research Center (DKFZ). As Professor of Systems Biology she also co-coordinates the master's program "Major Systems Biology" jointly with Ursula Kummer. There is lively interaction between the two research groups. Modelers from Timmer's team always have the opportunity to pour gels and wield pipettes during annual student internships in Heidelberg while experimental researchers from the Klingmüller team sit at computers in Freiburg. Nonetheless, distance is the greatest handicap. "If we were in the same city, or even the same building, we could make even greater and faster progress," Klingmüller says. Interdisciplinary institutes are ideal, she adds. However, thanks to their long-standing cooperation, there are no longer any communication problems between the two teams. A "couple" consisting of an experimental researcher and a modeler usually work together on each project. Issues that cannot be resolved via Skype are discussed at regular meetings or on a joint retreat such as the one held last year in the Black Forest.

From basic research to medical application

Recent years have been dominated by basic research and the development of methods. Now, "a window is opening in the direction of medical application," Timmer says. Several projects involve cooperation with clinical partners and the two researchers would still like to develop at least one approach that actually helps a patient in a hospital bed. "The only opportunity for us to really contribute something is to build things up from the bottom, step by step," Klingmüller says. "And that takes time." She adds that dynamic modeling has now reached the stage where it can be linked with other modeling approaches. Describing this new challenge, she says: "We wonder whether there is a limit." That is because the resulting models are very large. In Klingmüller's group a new mass spectrometer is helping researchers to quantify even more components very precisely, thereby giving the modelers what they require. Measuring biological phenomena on different timescales – signal transduction is measurable after a few minutes, cell division only after many hours or even days – is still challenging, however.

The two researchers think that, in the future, systems biology will be more widely applied in basic research, and in clinical practice as well. The perspective of this interdisciplinary approach, which no longer only focuses on one single molecule, will continue to grow because the advantages are obvious. Not

Figure 1: Diagram of the JAK2-STAT5 signaling pathway activated by erythropoietin (Epo) binding



Epo binds to its receptor EpoR (blue) on the cell surface. The receptor-bound kinase JAK2 inside the cell is then activated. It first phosphorylates (P) itself, then the EpoR. This creates binding points for STAT5. After binding to the receptor, STAT5 molecules are likewise phosphorylated and migrate in pairs into the cell nucleus. There they activate target genes that help the cells to survive. Two of these target genes, CIS and SOCS3, can down-regulate the signaling pathway. STAT5 pairs are dephosphorylated in the nucleus and degrade into individual STAT5 molecules. These can be transported back into the cytoplasm where they are once more available for signal transduction (Graphics: Svantje Braun and Lorenz Adlung).

Ursula Klingmüller and Jens Timmer with their teams at a joint retreat in Durbach in the Black Forest (May 2013)
(Photo: Frédérique Kok).

only are systems biology methods being further developed. Indeed, the biologists' specific questions require answers from modeling, and solving these problems leads to progress in the theoretical field. Conversely, mathematical methods leading to quantitative predictions that have enabled important insights in physics could also find promising uses in biology. In any case, this mutual stimulation has taken the two research groups much further than if each had worked on their own behind closed doors.

Science and family

The good organization that close cooperation at a distance between Heidelberg and Freiburg requires is reflected in the family life of the two scientists. Timmer has set aside two evenings a week for his daughter and travels less than he used to. Clearly, their partners need to have a lot of patience, but their professorial work routine permits them to adapt flexibly to spontaneous events involving their children. Science doesn't leave time for much more than weekend activities with the family and an occasional concert. Klingmüller is also involved in a campaign to get more women into executive positions at DKFZ. "There is still a long way to go, but we are connected via the DKFZ Executive Women's Initiative," she says, describing her involvement. More women have been recruited but, like learning to appreciate systems biology, the process requires patience.

Klingmüller's favorite way of relaxing is to walk around her laboratory and discuss new findings directly with her colleagues at the bench. Timmer, too, feels most relaxed when he is able to work in peace and quiet.

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NGFN – national genome research network

12 years of outstanding genome research – for our future health

by Silke Argo

"We had to start by learning another language," is how *in silico* and *in vivo* researchers, in this case computer scientists and biologists, recall the beginnings of the German National Genome Research Network (NGFN). The NGFN was a stroke of genius of forward-looking and sustainable research funding which has had a lasting impact on the research landscape in Germany. Clinicians and basic research scientists now work closely together as a matter of course to clarify disease mechanisms and to incorporate newly acquired knowledge into healthcare provision as quickly as possible. Computer scientists, biologists and medics using first-class, state-of-the-art technologies joined forces within the network to probe highly complex problems of human health.

Networking brings success

The outstanding success of the NGFN network soon became apparent and was evident throughout the funding period. Between 2001 and 2013, more than 5,200 publications in well-known specialist journals – 280 of which were published by the most highly respected journals – shared the results of this top-rate research with the global scientific community. Each research finding is a contribution to our understanding of the complex molecular interplay which keeps us healthy when it runs smoothly. "Many research findings not only led to significant gains in knowledge and a better understanding of disease but also enabled patients to benefit in concrete terms from the medical application of those findings," Professor Dr. Johanna Wanka, Germany's Federal Minister of Education and Research, told the NGFN Project Committee.

With the NGFN, Germany picked up where the German Human Genome Project (DHGP) left off. The genome had been decoded and the next pressing questions were: How are structure and function connected? How many genes are there and which ones play a key role in our health? Which gene variants are critical to the onset of a disease and how can they be used as starting points for therapy? Tenacious pursuit of these questions together with colleagues with complementary interests was the NGFN researchers' recipe for success.

Bioinformatics and systems biology point the way

"We need integrated bioinformatics," said Professor Dr. Annemarie Poustka, founding member of the NGFN, who died in 2008. Thus ten years ago, IT was already an essential component of the "research pipeline" along which molecular disease interrelations were explored. In addition, bioinformatics was of critical importance to the NGFN's success as key platform technology. Burgeoning systems biology modelled both cellular processes at the highly precise sub-molecular level and intercellular interconnections such as molecular signalling pathways. In the NGFN, these two disciplines developed their analytical power as medical researchers and clinicians, biologists, chemists, physicists, engineers and technicians worked together.

Order in the data jungle

The NGFN can therefore count among its successes bioinformatics and statistical methods such as those developed for analysing and annotating data from high-throughput experiments using the sequencing technologies current at the time, that is molecular and tissue microarrays or automatic cellular screening. For this, for example, regulation networks were systematically generated or calculated in reverse by means of modular response analysis. Nested effects models were produced for high-dimensional phenotyping, or support vector machines for predicting classes of protein folding and algorithms for protein interaction were integrated. Various R and bioconductor software packages were developed in NGFN projects, such as for sequence comparison of microarray samples or visualisation of signalling pathways from the KEGG. Comprehensive databases and data integration platforms for experiments in functional genomics, proteomics and systems biology encouraged cooperation in the NGFN. Various methods

Figure 1: High-throughput technology: Application and development are crucial for medical genome research (Photo: NGFN/J. Lampert).

Figure 2: Translating the findings of genome research into medical application gained the focus of interest for NGFN research (Photo: NGFN/University Hospital Heidelberg).

were further developed for use in clinics, for instance for characterisation, prediction and genetic diagnostics in cardiac insufficiency, neuroblastoma or leukaemia. Thus a test developed in the NGFN facilitates accurate risk prediction when neuroblastoma is first discovered, thereby enabling improved decisions on patient therapy. This method is clearly superior to the standard methods previously used. The test records the activity of a selection of genes in the tumour tissue and uses an algorithm to evaluate this information that involves a shrunken centroid-based classifying algorithm developed by NGFN scientists. The test is currently being included in the NB2012 neuroblastoma study.

Biomarkers for individual medicine

NGFN scientists identified and analysed the function of numerous biomarkers that have since been incorporated into the individual treatment of patients. Individually or as a signature they are used in the diagnosis, prediction and treatment of disorders such as obesity, alcoholism, allergies, breast cancer, Crohn's disease, epilepsy, leukaemia, myocardial infarction, neuroblastoma, oligodendroglioma, pancreatic cancer, Parkinson's disease and prostate cancer. For example, a biomarker identified by NGFN researchers is used in companion diagnostics for the management of allergy therapy. In a joint venture with a company, a testing system for medical *in vitro* labora-

Statement international

by Ivo Glynne Gut, Member of the Scientific Advisory Board of NGFN

Key to success: From isolation to consortia

It has indeed been a great honour for me to have been involved in all three cycles of the NGFN programme and seeing the tremendous transition that has taken place on so many levels. The transition from research teams working in isolation to consortia consolidating their efforts to resolve a common goal has been key to success in medical genome research, as the problems are too large in scale for individual researchers to resolve. The NGFN programme has been crucial for this transition in Germany. The evolution of projects from basic research in genomics to applied research and translation, with the development of clinical tests to better diagnose disease and support treatment decisions, to the understanding of disease mechanisms and development of drug candidates in a broad palette of pathologies is highly visible from the output of the NGFN-funded projects. In order to succeed consortia included very diverse expertise from medicine to computation and needed to find a common ground to communicate. It has brought diverse specialists closer. The programme has helped the evolution of a new breed of researcher who is aware of his or her societal responsibility to give value back to the citizen who ultimately provides the funding for the research. The NGFN is a funding programme that is an example that should be followed in other countries. Germany has put itself at the forefront in this field with the help of this programme.

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Figure 3: Deciphering the human genome allowed a better understanding of the function of genes and their role in various diseases (Image: NGFN/J. Lampert).

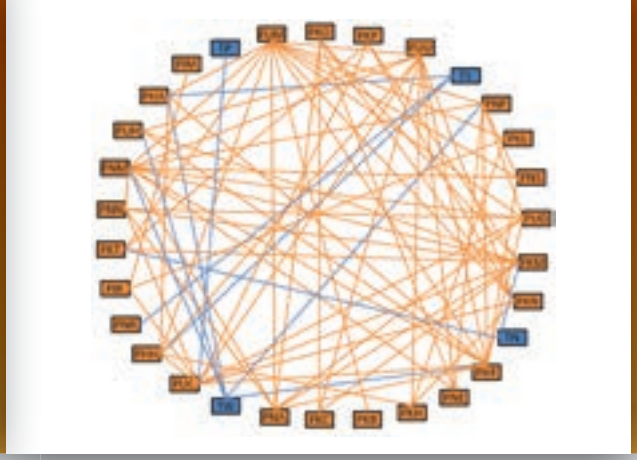

Figure 4: A dense network of scientific cooperation linked the various consortia in the NGFN, documented by joint publications (Chart: NGFN).

tory diagnostics was developed to improve risk assessment for individuals from families with a history of heart attacks. A new, sensitive diagnostic method enables the detection of activated molecules in various types of tumour biopsies. Biomarkers that provide cross-disease information have special potential because the signalling pathways concerned play an important role in several diseases. Thus NGFN researchers have shown that some Crohn's disease genes are also relevant in disorders of other barrier organs such as the skin and lungs. The dynamics of translating NGFN research into clinical practice are also demonstrated by the initiation of various clinical studies, for example into more specific treatment of patients with breast cancer, lung cancer, neuroblastoma or Crohn's disease.

Technologies for research and clinics

The NGFN's success in advancing knowledge was closely interlinked with the issue-driven development of technology. For instance, *esiRNA* technology was developed with the support of the NGFN, and more recently *TALENs* was used to develop disease models in mice. An industrial joint venture developed an innovative method of isolating intact proteins from tissue samples that are fixed with formalin and embedded in paraffin. Since this process is of hospital standard the method is already in use. These are just a few examples. Innovative technology platforms were also developed within the framework of the NGFN. The German Mouse Clinic is of particularly far-reaching significance here as relevant player in the international research area. Extensive projects such as the *popgen* and *KORAgen* population biomaterial banks were – and remain – important resources for medical genome research.

The funding provided to NGFN also made a key contribution to the formation of 13 industrial companies with a total of nearly 100 employees. Ten of these companies are still in operation. More than 130 patents were registered as a result of NGFN funding.

International and collegial

The culture of interdisciplinary cooperation at the highest level has enabled German NGFN scientists to take up key positions in international projects and to establish cooperation arrangements in over 40 countries all over the world. German participation in global projects such as the 1,000 Genome Project or German contributions to the ICGC are also fruits of the NGFN. A unique process of collegial self-management undertaken by the Project Committee as the NGFN's governing body progressively enhanced the network's outstanding scientific standard. In addition, the NGFN's development was critically accompanied by professional advice given by a high-level external advisory board and the Competence Centre Technology Transfer was a key partner in utilisation support. The BMBF funded the National Genome Research Network for over a decade, providing more than €600 million. Without this long-standing financial and professional support, the development of such a successful collaboration between basic research scientists and clinicians would not have been conceivable.

90 groups link 130 locations by means of 1,000 projects

Hundreds of outstanding scientists in laboratories and at computers made these successes possible. During the 12 years of the NGFN, their input linked more than 130 locations all over Germany in a multiple and interdisciplinary way. Universities, clinics, research institutes and companies jointly pushed forward the molecular basic research of a wide range of diseases in a total of over 90 groups and 1,000 sub-projects, covering the areas of neurology, cancer, cardiac/circulatory complaints and infection/inflammation-related illnesses. The successes, visibility and sustainability of this trend-setting programme motivated young scientists abroad to return to Germany and continue their excellent work here. The NGFN community was strengthened by joint workshops and annual conferences with international participation that also gave rise to a high

Figure 5: The NGFN Annual Meetings with up to 700 participants – The central event for the presentation of results, the scientific exchange within the NGFN, the discussion with international experts and the information on new technologies (Photo: NGFN).

level of industrial interest. The NGFN brand soon became internationally known. 34 NGFN members are amongst the 50 top-cited scientists of human genetics in the German-speaking area as found by the publication analysis of the magazine "Laborjournal" for the years 2007-2010 (http://laborjournal.de/rubric/ranking/R13_12/index.lasso). The fruits of NGFN funding can be expected to enrich the community for years to come in the form of outstanding publications.

Knowledge at first hand

The NGFN's achievements were presented to the general public in a number of high-quality information resources. Over 60,000 print products alone were distributed. Use of the NGFN information stand, material and projects for schools and the Genome Research Day held in Berlin in 2011 are further components of intensive public relations work. The NGFN thereby contributed to awakening interest in medical genome research among members of the general public by means of first-hand knowledge and to generating soundly based acceptance.

The road to systems medicine

So what do the protagonists in *in silico* und *in vivo* research say after 12 years of the NGFN? "We learnt how to make our language comprehensible and to understand our partners in

Internal statement

by Stefan Schreiber, long-standing spokesman for the NGFN Project Committee

"What did we gain from 12 years of the NGFN?"
The NGFN: a leading international research network with an interdisciplinary approach

NGFN funding enabled us to establish research into the biology of the human genome and, especially, the genomics of human diseases in Germany in such a way as to gain a leading international position in many areas. This was achieved by means of a funding initiative that is unique in German biosciences both in its sustainability and in its interdisciplinary breadth. A culture of discussion was created that closely connected scientific high-flyers in both basic and clinical research at German universities with non-university basic research institutions. For a while, with intensive pharmaceutical industry participation, a process of self-management was established that enabled both an intensive exchange among specialists and an internal quality control system. The NGFN demonstrates that it is possible to bundle by subject matter the centres of research excellence which are distributed throughout Germany because of its federal system and to merge them into a joint critical mass. As a result, it took us just a few years to make good our backlog in molecular biology and genomics and to start competing successfully with much better-funded programmes such as those in the United States. In addition to its successful scientific research, there is no doubt that the NGFN shaped a new generation of young academics to think in interdisciplinary terms and positioned them to further develop university and non-university research.

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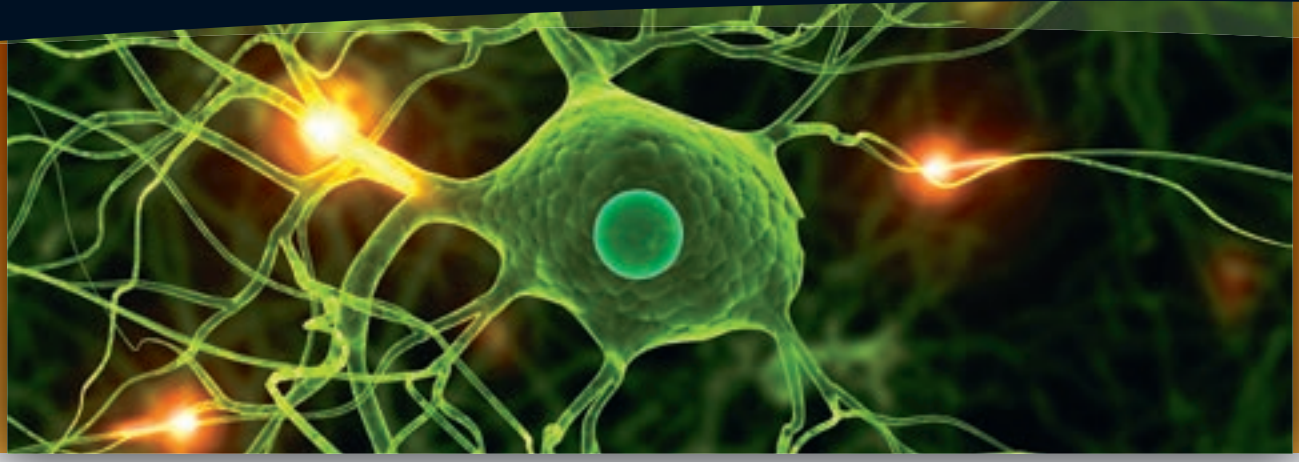

Figure 6: The genetic basis of neuronal function – one out of many questions pursued by NGFN researchers (Image: Fotolia).

other disciplines. It is a great process when this leads to a forward-looking success." Systems biology expertise, large-scale computing power, basic research, technology development and clinical research interlock in funding programmes like the NGFN. One success is that functional analyses of highly complex molecular processes and their modelling have found their way into systems medicine. Researchers are increasingly succeeding in recognising individual characteristics and in stratifying patients into molecular sub-types. The current development of personalised medicine is an opportunity for the individual to come by medication with a more precise effect and above all to stay healthy by means of targeted prevention. Large networks will continue to offer ideal preconditions for this comprehensive and interdisciplinary research.

The research project in brief:

Project name:

National Genome Research Network
(Nationales Genomforschungsnetz/NGFN)

Funding:

The NGFN was funded by the German Federal Ministry of Education and Research (BMBF) in three phases: NGFN-1: 2001–2004, NGFN-2: 2004–2008, NGFN-Plus and NGFN-Transfer 2008–2013.

More information at www.ngfn.de

Partners:

NGFN projects were undertaken throughout Germany at over 60 locations by hundreds of scientists who cannot be named here but whose names can be found on the website, especially in the list of publications. Partners in NGFN cooperation included research departments, large-scale research establishments, clinics, universities and commercial enterprises.

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homeostasis of blood glucose

Computer simulations of central liver functions

by Matthias König and Hermann-Georg Holzhütter

The liver performs a multitude of important physiological functions such as synthesising plasma proteins, producing bile and hormones, detoxifying toxic compounds and precisely regulating the concentration of various substances in the blood (homeostasis). In particular, the liver plays a crucial role in the regulation of blood glucose levels.

Modelling hepatic glucose metabolism helps to understand central liver functions and basic regulation mechanisms and thereby provides insights in the role of the liver in systemic diseases such as diabetes.

Glucose regulation is vital

The concentration of glucose in the blood is maintained within a narrow range between 3 millimolars (mM) after prolonged fasting or extensive muscle activity and 9 mM after food intake. This homeostasis is crucial for the body. Too high concentrations of plasma glucose (hyperglycaemia) for an extended period result in protein modifications due to non-specific binding of sugar molecules to proteins (glycation), ultimately leading to tissue damage, especially in smaller blood vessels. On the other hand, too low concentrations (hypoglycaemia) result in under-supply of tissues and organs, first and foremost to the brain, which derives its energy almost entirely from glucose. Without the contribution of the liver to maintain the glucose levels under hypoglycaemia, we could only survive for a couple of minutes.

The blood glucose level – a dynamic quantity

The concentration of blood glucose is a dynamic quantity which is influenced by nearly all organs (figure 1). Its regulation is a challenge, since both utilisation (e.g. by muscle activity) and intake of glucose via food are subject to major fluctuations in the course of the day.

The hormones insulin and glucagon mainly regulate glucose homeostasis by adjusting both hepatic production and utilization of glucose and utilisation of glucose by muscle and fatty tissue. While insulin lowers the concentration of glucose, glucagon helps to counteract hypoglycaemia. The blood concentrations of insulin and glucagon change as direct response to alterations in glucose as their secretion by the pancreas is adjusted. An increase in blood glucose levels leads to an increase of insulin and a decrease in the glucagon concentration. Both hormones bind to receptors on the target organs, thereby activating signalling pathways that adapt glucose consumers and producers in a concerted manner to one another.

The liver's dual role in systemic glucose metabolism

The liver plays a dual role in glucose homeostasis (figure 2). On one hand, it produces hepatic glucose when the blood glucose level is too low (hypoglycaemia), for instance during extensive physical activity or prolonged fasting. On the other, if the levels are too high after meals (hyperglycaemia), the liver functions as a store and consumer of glucose. The liver can thus counteract systemic changes in blood glucose due to fluctuations in utilisation (sleep versus physical activity) and in glucose intake (fasting versus food uptake).

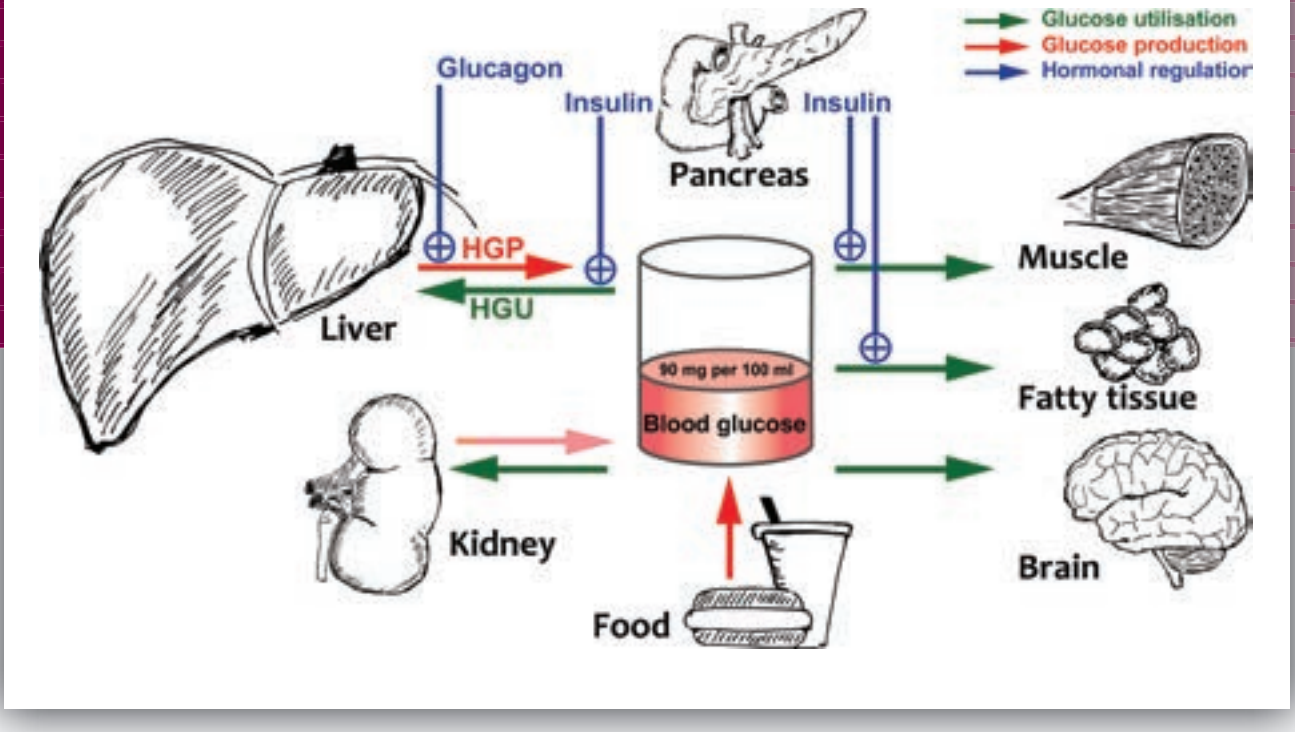

Figure 1: Homeostasis of the blood glucose level
The blood glucose concentration is decreased by glucose utilising organs and tissues (green) or increased by food uptake and glucose production (red). The glucose consumption of the brain is relatively constant in the course of the day, whereas the glucose requirement of muscles is subject to major fluctuations depending on activity. The liver can switch between production (hepatic glucose production, HGP) and utilisation (hepatic glucose utilisation, HGU) under normal physiological conditions. During extended periods of fasting the kidneys too can produce glucose. Insulin and glucagon, hormones secreted by the pancreas, play a central role in blood glucose regulation, firstly because control of utilisation by muscles and fatty tissue is insulin-dependent and secondly because HGU and HGP are adjusted by insulin and glucagon (Graphics: M. König).

Hepatocytes, the main liver cells, account for the bulk of the hepatic metabolic activity. Glucose metabolism in hepatocytes consists of the reactions of glycolysis (glucose degradation and subsequent conversion into fat or energy), gluconeogenesis, formation of glucose from precursors such as lactate, and the ability to store glucose as a polymer carbohydrate (glycogen). Hepatocytes form glycogen when blood glucose concentrations are high (glycogen synthesis) or release glucose from the glycogen store when glucose levels are low (glycogenolysis).

Due to the ability to either newly synthesize glucose or release from buffer stores, the body has implemented a system that can react quickly to fluctuations and counteract them.

The switch between glucose utilisation and production is controlled both by the hormones insulin and glucagon and by glucose itself in its role as a substrate, product, inhibitor and activator of reactions. In the liver insulin increases the activity of glucose-utilising metabolic pathways (HGU, glycolysis and glycogen synthesis) and reduces glucose-producing pathways (HGP, gluconeogenesis and glycogenolysis). Glucagon has the opposite effect. Insulin and glucagon act by changing the activity of key enzymes in glucose metabolism, such as pyruvate kinase or glycogen synthase.

Insulin and glucagon activate signalling cascades which result in changes of the phosphorylation state of key proteins that take effect within seconds or minutes accompanied by changes in activity, thereby permitting fast reaction to alterations in glucose utilisation or food uptake. Furthermore, over longer periods of hours, days or weeks modulation at the gene expression and protein levels take place that enable adaptation to states of fasting or to day and night rhythms.

Mathematic modelling of liver metabolism

Modelling liver metabolism is a central task of the Virtual Liver Network (www.virtual-liver.de), a large-scale national initiative of the German Federal Ministry of Education and Research (BMBF) which is of particular significance for establishing systems biology and systems medicine. The network aims to create a series of dynamic models of the human liver that, while not fully replicate its physiology, morphology and function, will represent key aspects in models (Holzhuetter *et*

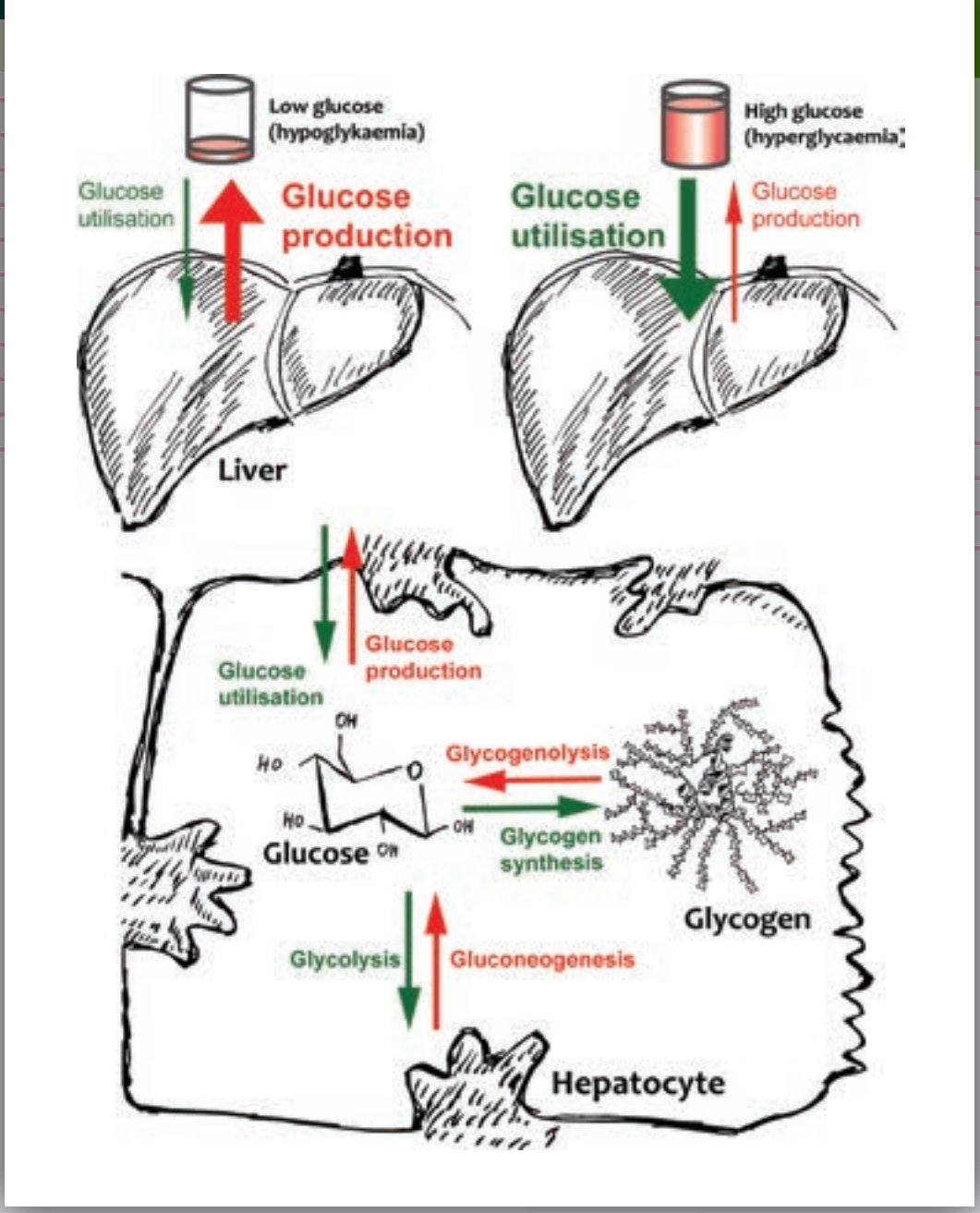

Figure 2: The liver's dual role in systemic glucose metabolism
The liver is able to switch between glucose production when blood glucose is low and glucose consumption when blood glucose is high. In hepatocytes, glucose can be used for energy production by means of glycolysis, or be synthesised *de novo* from precursors (gluconeogenesis). Furthermore, glucose can be stored as glycogen when the blood glucose levels are high (glycogen synthesis) or be released from glycogen when the blood glucose is low (glycogenolysis) (Graphics: M. König).

al., 2012). During this process, quantitative and qualitative data from all organisational levels will be integrated. One milestone in modelling the liver metabolism was the reconstruction of the hepatic reaction network HepatoNet1 (Gille *et al.*, 2010).

With the aim of gaining a better understanding of the central role played by the liver in glucose homeostasis, a detailed kinetic model of glucose metabolism was developed within the Virtual Liver Network (Koenig *et al.*, 2012; figure 3). The model includes hormonal control of glucose metabolism by insulin and glucagon via changes in the phosphorylation state of key enzymes, thereby linking hormonal control with metabolism. The mathematical core of the model is a description of individual processes by means of customary differential equations that make it possible to simulate the behaviour of metabolism over time.

Based on the kinetic properties of individual enzymes, the model describes the dual role of the liver as a producer (HGP) and utilizer (HGU) of glucose, depending on dynamic changes in blood glucose level and the hormones insulin and glucagon. It thus describes the systemic function of the liver in glucose metabolism (figure 3).

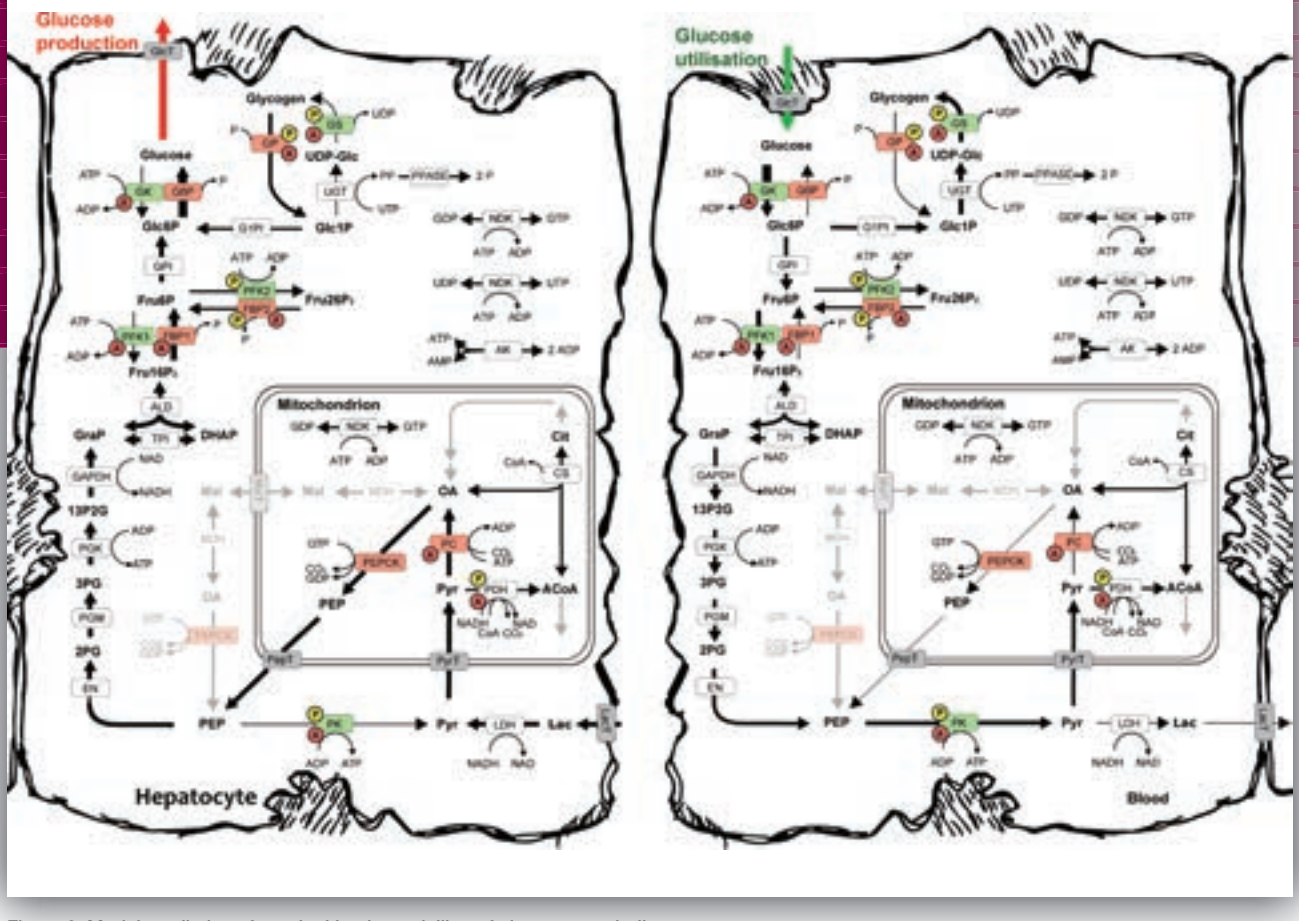

Figure 3: Model predictions from the kinetic modelling of glucose metabolism
Left: the liver works as a glucose producer when glucose concentrations are low. Part of the glucose released into the blood comes from glycogen stores, the remainder from glucose synthesis (gluconeogenesis). Right: the liver functions as a glucose consumer. Part of the utilised glucose is stored in the form of glycogen, the remainder enters glycolysis (Graphics: M. König, from König *et al.*, 2012).

The contributions of the liver are essential to the understanding glucose metabolism in the body. The developed model provides a module that can be used to model whole body glucose metabolism, thereby giving insights in disorders of glucose homeostasis such as diabetes (Ajmera *et al.*, 2013).

Application of the model to type 2 diabetes

Glucose homeostasis is impaired in type 2 diabetes mellitus (T2DM) because the hormonal signals do not match existing glucose concentrations in the blood and consequently lead to significantly increased blood glucose levels. Both the increase in insulin (relative insulin deficiency) and the decrease in glucagon as a consequence of increasing glucose concentration are reduced, resulting in higher glucagon concentrations. As a result of these wrong signals the liver produces too much glucose, which contributes additionally to the increased blood glucose level in diabetes. The administration of insulin to patients with T2DM becomes necessary if alternative therapies such as changes in lifestyle or treatment with the drug metformin fail to normalise blood glucose concentrations. One side effect of insulin therapy is an increased risk of hypoglycaemic episodes that, if untreated, can lead to confusion, loss of consciousness, and in serious cases to cramps, coma or even death.

The detailed kinetic model of glucose metabolism was used to examine the contribution of the liver to hypoglycaemia in T2DM (Koenig *et al.*, 2012). Our model predicts that the hepatic capacity to react to a falling blood glucose levels by increasing glucose production will be impaired because of changed enzymatic activities in gluconeogenesis as a result of incorrect hormonal signals. That could explain the increased number of hypoglycaemic episodes during strict insulin treatment. Furthermore, the model predictions regarding the normalization of the altered hormonal signals showed the great potential for normalising raised glucagon profiles in the treatment of T2DM.

Mathematical modelling over spatial and temporal scales

Mayor challenges in modelling the liver are the bridging of spatial and temporal scales from single hepatocytes to whole-liver in the range from seconds to days, as well as the integration of heterogeneous qualitative and quantitative data from clinical studies to animal experiments, from studies of the entire organism over isolated liver to experiments in cell cultures or on isolated proteins. Our research team is currently developing a multi-scale model of this kind.

The research project in brief:

The Computational Systems Biochemistry research group at the Charité Berlin is involved in developing computer models that simulate and predict complex biological processes. The modelling of glucose metabolism in the liver is a sub-project of the Virtual Liver Network funded by the BMBF, the German Federal Ministry for Education and Research (funding code 0315741).

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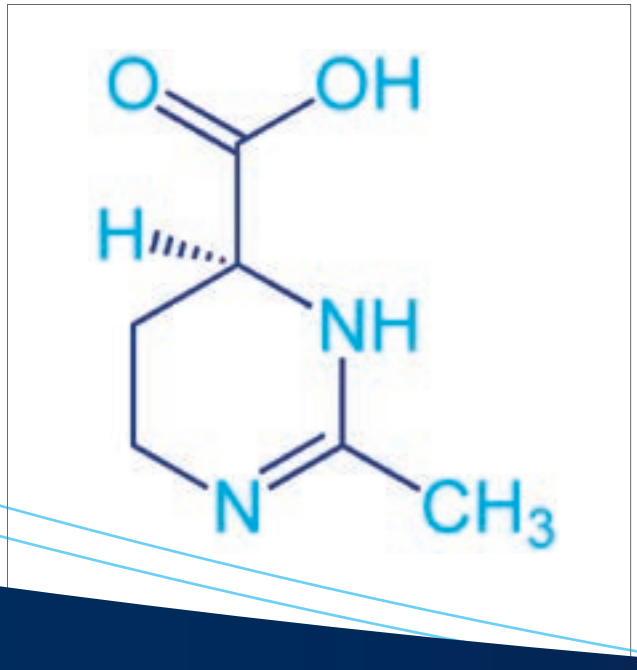
OpHeLIA – optimization of *halomonas elongata* for industrial applications

Towards metabolic engineering of halophiles

by Alberto Marín-Sanguino

Turning water into wine is not a big deal; all that is needed is a grapevine, sunshine, and a few cells of *Saccharomyces cerevisiae*. These kinds of biotransformations are often miraculous yet at the same time unspectacular. Microbes are extremely sophisticated factories that convert the most mundane substrates into all kinds of products. Some of them, like citric acid, have been produced by microbes for half a century and the processes and microbial strains used to obtain them have been highly optimized. This has led to the establishment of a handful of microbes as industrial workhorses. But in order to take the next step towards a real bioeconomy, a greater diversity of industrial microorganisms is needed, and that is not going to be achieved by traditional methods of random mutation and artificial selection.

Figure 1: Chemical structure of ectoine



Source: A. Marín-Sanguino

Systems biology as a key technology to optimize microbial productivity

A rational approach for the optimization of microbial productivity should enable us to enhance existing processes and to re-purpose known microbes. However, such a scenario requires a better understanding of the general principles governing metabolic networks as well as a deeper insight on the differences and similarities between microbial strains. Metabolic networks are typically highly complex and dynamic systems that evolved over millions of years. Single parameter changes will usually result in either no reaction because of redundancies in the network guaranteeing the robustness of the system or in fatal errors that finally may lead to death of the cell. Increasing the productivity without risking the viability of the organism is thus a complex manipulation that requires a deep understanding of the system. The most promising basis upon which such an understanding can be built is Systems Biology. To put it in simple terms, trying to reroute metabolic fluxes is more similar to hacking a computer network than to turning on a tap. Now the question remains open: How do we do it?

Our approach to "hacking a microbe" starts with the composition of the group itself. Projects in systems biology are often the result of cooperation between highly specialised groups, each focused on a given technique (modelling, proteomics, metabolomics, etc.). Instead, we have opted for a small group of scientists with mixed backgrounds working together on a problem oriented basis to ensure a more effective communication and a faster cycle between theory and experiment. A valid analogy for this would be the evolution of software engineering. Traditional approaches based on a long initial phase of heavy planning have been substituted by concepts such as radical programming: pairs of programmers start writing code very early on, maintaining a very fast and flexible

Figure 2: Salt ponds at San Francisco bay. Red colors are due to halophilic microbes.
(Licensed under CC Attribution-NonCommercial-ShareAlike 2.0 Generic license by Kevin Kemmerer (fanlgelman en Flickr))

testing-coding cycle. By bringing this philosophy to Metabolic Engineering, we expect to shift the focus from the techniques to the organism. Our goal is to take a microbe that has not been extensively studied and try to bring it up to speed as an industrial platform.

Halophilic bacteria as salt-tolerant production strains

Halomonas elongata is perfect as a case study. This bacterium grows in seawater, salt lakes and salterns, withstanding salt concentrations more than tenfold that of the sea. To protect itself from osmotic stress, *H. elongata* accumulates high concentrations of ectoine (Figure 1). The ability of this substance to protect membranes and biomolecules leads to applications in medicine and cosmetics. As a high value commodity, ectoine is a worthy goal by itself but the potential uses of *H. elongata* go far beyond, thanks to its ability to operate in sea water and salinized environments. By creating a knowledge base of the metabolic capabilities of *H. elongata* and optimizing the techniques for its genetic manipulation, the first steps will be made, not only to optimize ectoine production, but also to advance towards further industrial and environmental applications.

The starting point for the OpHeLIA project was the sequence of the genome encoded by the collaboration between Max Planck Institute in Martinsried and the Federal Institute for Material Research and Testing (BAM) in Berlin (Schwibbert *et al.*, 2011). Additional high-throughput and expert feedback from these two cooperation partners provides the omics component of the project. The initial steps on manual annotation of the genome provided a valuable opportunity to involve every member of the team to ensure that they all started on common ground before they moved on to separate work packages.

Figure 3: The Systems Biotechnology subdivision at TU München
From left to right: Dagmar Rother, Viktoria Kindzierski, Christiana Sehr, Alexandra Iovkova, Susanne Kuchenbaur, Adrian Rauschenbach, Katharina Pfüger-Grau, Andreas Kremling, Alberto Marín-Sanguino
(Source: TU München)

From gene annotation to dynamic modelling

The initial annotation round led to the reconstruction of the central metabolic pathways and ectoine synthesis, providing a "core model" based on flux balances and thermodynamics. From there on, the model has been growing organically through extensions of the parts that proved to play a relevant role. Literature review, sequence homology and standard biochemical experiments all took turns to validate the predictions of the model and correct its inconsistencies. Once an overall balance of the main biosynthetic pathways had been added, the model could make steady state predictions to be compared with highly reproducible experiments in a bioreactor.

The constraint based models presented so far produced a snapshot of the metabolic fluxes for potential steady-states. Setting this still image into motion entails serious difficulties. Even though modern computers can simulate very complex dynamic models, calculating their parameters remains extremely difficult. A model that is complex enough can explain any kind of data, but there is always the possibility that the errors incurred in estimating parameter x get compensated by introducing errors in parameter y. There are many strategies for coping with this problem but no generally valid solution. Here is where a close cooperation of experimentalists and theoreticians excels with models suggesting the experiments that provide the biggest amount of information.

The structure of the model is also a key in achieving a good description of the system. Using rate laws that are as simple as possible, like those based on certain formalisms such as power-law kinetics, will result in models that have a standard (or canonical) form. The use of mathematical models with a regular structure and well known properties enables theoretical analysis beyond simple simulation, which may provide mathematically sound foundations for bridging the gap between steady state models of fluxes and dynamic models where metabolites are included (Marin-Sanguino *et al.*, 2010). This structural regularity can also be exploited to find optimal manipulation strategies (Pozo *et al.*, 2011). Such strategies should consist of modified media compositions and altered expression levels for a series of enzymes. The tools that enable such manipulations in *H. elongata* are being developed in our lab. This is an important part of the project with one of the options being the creation of a synthetic promoter library, a set of semi-random promoters that can be tested with report-

er genes like GFP in expression libraries. By measuring the fluorescence of the different colonies in the library a correspondence can be established between sequence and intensity of expression, so all the promoters can be ordered accordingly and stored for future use.

There is still a long way to go before a clean bio-industry can fully dispel the Dickensian nightmares left by the industrial revolution. It will be a slow transit made by many small groups of scientists, engineers, mathematicians and a wide cast of microbes like *Halomonas elongata*. It will not be spectacular, but it will be a miracle indeed.

The research project in brief:

The OpHeLIA project is funded by the BMBF (grant number 0316197) as part of the e:Bio initiative and supports the junior research group Metabolic Engineering of Halophiles (M.E.H.). The group is embedded in the department of Systems Biotechnology led by Prof. Andreas Kremling (Technische Universität München). The work of the group is possible thanks to a close collaboration with the emeritus group of Prof. Dieter Oesterhelt at the Max-Planck Institute in Martinsried, Prof. Jörg Kunte's group in the Federal Institute of Materials Research and Testing and BITOP AG in Witten, a pioneering company with respect to the industrial application of extremophiles.

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http://www.biovt.mw.tum.de/fg-systembiotechnologie/forschung/nachwuchsgruppe-ophelia/

german network for bioinformatics infrastructure (de.NBI)

A new initiative of the German Federal Ministry for Education and Research (BMBF)

by Yvonne Pfeiffenschneider

A distinguishing feature of modern biological and medical research is the increasing use of data-intensive technologies such as 'omics' approaches, sequencing and high-throughput imaging techniques. This rapidly advancing development is supported in Germany and elsewhere by targeted funding measures. The high complexity of the datasets generated presents new challenges to bioinformatics in respect of adequate annotating, standardised archiving, systematic analysis and optimal exploitability of data. Furthermore, efficient use of experimental data requires structured concepts to ensure long-term operation and interoperability of data resources.

In response to these challenges, the BMBF published a statement in June 2013 announcing the development of the German Network for Bioinformatics Infrastructure (de.NBI). The goal of this new infrastructure initiative is to expand, improve and ensure the availability both of hardware (computing and storage capacity) and of data resources and bioinformatics tools in the life sciences.

In establishing the German Network for Bioinformatics Infrastructure, the BMBF aims to develop a virtual community comprising a number of service centres that are already well equipped and networked and a superordinate coordination unit. The service centres have specific areas of bioinformatics expertise and resources that they will provide within the framework of this infrastructure initiative. With the support of the coordinating unit, a network will be built that pools the expertise of the individual service centres, thereby delivering a thematically comprehensive and sustainable IT basis for all research within the life sciences.

The network will be developed in two consecutive application and funding phases – a six-month design phase and a five-year establishment phase. Twenty-one individual and collaborative projects responded to the call to submit project outlines for the design phase. In mid-October 2013, nine international experts selected six service centres, four local databases and a data management component (Table 1). Alfred Pühler from Bielefeld University's Center for Biotechnology (CeBiTec) is to head the coordination unit.

An initial meeting between representatives of the BMBF, Project Management Jülich, the selected projects and the coordinator was held in December 2013. At his meeting, an eight-headed team of authors was appointed to work together to draw up a comprehensive concept for a German network during the subsequent six-month design phase.

The committee of experts will evaluate this concept in June 2014. If it is assessed positively and approved, the establishment phase, when work on building the network will commence, is expected to start in November 2014.

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Table 1: Selected service centres, local databases, data management

ACRONYM/SUBJECT	INSTITUTION
SERVICE CENTRES	
HuB – The Heidelberg Center for Human Bioinformatics	German Cancer Research Center (DKFZ), Heidelberg
BiGi – Bioinformatics Resource Center of Microbial Genome Research relevant for Biotechnology and Medicine	Bielefeld University
BioInfra.Prot – Infrastructure Center Bioinformatics for Proteomics	University of Bochum
CiBi – The Center for Integrative Bioinformatics	University of Tübingen
RBC – RNA-Bioinformatic Center	University of Freiburg
GCBN – German Crop BioGreenformatics Network	IPK Gatersleben
LOCAL DATABASES	
PANGAEA	University of Bremen
SILVA	Jacobs University Bremen
BRENDA	Braunschweig University of Technology
DSMZ	Leibniz Institute DSMZ Braunschweig
DATA MANAGEMENT	
NBI-SysBIO data management	Hits GmbH, Heidelberg

ERASynBio

Promotion and responsible development of synthetic biology in European and international networks

by Annette Kremser

„I think the biggest innovations of the twenty-first century will be the intersection of biology and technology. A new era is beginning…"
Steve Jobs (Isaacson, 2011).

Synthetic biology, at the intersection of gene technology and engineering, is a key technology with the potential to fundamentally change approaches, tools and techniques in modern biotechnology. It is based on biotechnology and gene technology methods that have been further developed – above all, on the technical capacity to analyse and synthesise genetic information at increasingly high speed.

Synthetic biology: the development of a scientific discipline

In defining synthetic biology, ERASynBio essentially follows the definition proposed by the European Academies Science Advisory Council (EASAC policy report 13, 2010): "Synthetic biology is the engineering of biology: the deliberate (re)design and construction of novel biological and biologically based parts, devices and systems to perform new functions for useful purposes, that draws on principles elucidated from biology and engineering." Based on this definition, it is evident that synthetic biology is not an entirely new, emerging branch of science, but merely a further development of existing fields such as gene technology, biosensorics and biomimetics, all of which likewise develop technical applications based on biological principles. In the same way as systems biology, synthetic biology is evolving from a combination of different disciplines such as genetics, molecular biology, information technology and engineering. While systems biology delivers the necessary quantitative data and parameters for modelling natural processes, synthetic biology – as a logical further development – goes one step further in using these principles and findings to construct, for example, new and optimised enzyme cascades, genetic circuits or "biological building blocks" such as promoters. At present, the development of new kinds of pharmaceuticals, diagnostic products and biomaterials, environmentally friendly and resource-efficient chemicals, and alternative production methods for bio-based raw and intermediate materials are emerging as potential applications. According to studies by McKinsey & Company and BBC Research, the estimated economic potential is huge.

Objectives of ERASynBio

To utilise the opportunities presented by this key technology and to actively shape its further development, European and US funding providers have joined together in ERASynBio, a research promotion network financed by the European Commission. Germany is represented in this network by the Federal Ministry of Education and Research (BMBF) and Project Management Jülich (PtJ), and is also coordinating the initiative. The network comprises a total of 16 partners from 14 European countries, along with the United States as an observer. One of ERASynBio's principal activities is to plan and implement joint calls for research projects. The 55 applications received in response to the first joint call in 2013, for a funding volume of approximately € 16 million, are currently being assessed, and planning for the second joint call in 2014

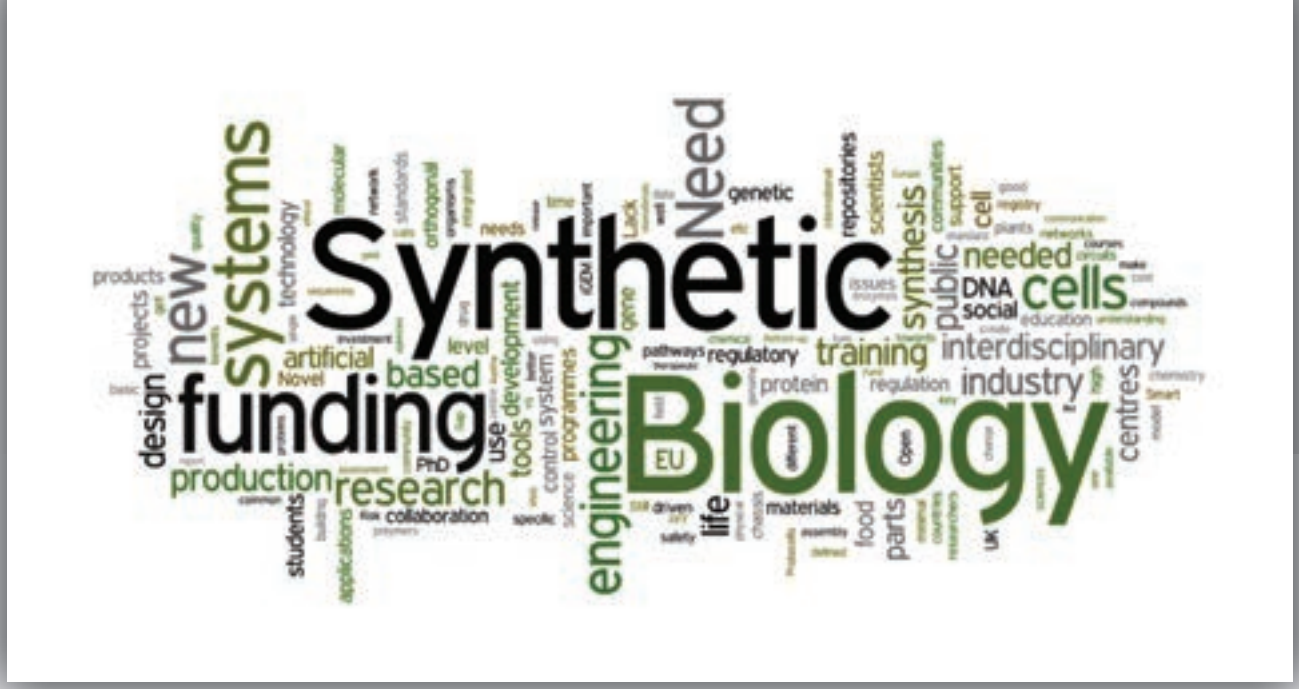

Figure 1: Synthetic biology word cloud (Graphics: ERASynBio).

has begun at the same time. Preparatory meetings for project initiation, summer schools for doctoral candidates and sponsorship for the iGEM student competition help the players to network and provide the necessary interdisciplinary training and education in a field that is still young. iGEM in particular is a good indicator of Europe's strong position in university-level education. At the 2013 World Jamboree in Boston, the Heidelberg (undergraduate) and Paris Bettencourt (graduate) teams won the main prize, the Technical University of Munich (undergraduate) and Bielefeld (graduate) teams came second, and Imperial College London (undergraduate) was third. In addition, 16 European teams won other awards.

Another central objective of ERASynBio is to develop synthetic biology in a coordinated and responsible way. Society and policymakers need to keep abreast of synthetic biology from an early stage in order to appreciate its enormous opportunities and potential in many branches of industry, but also so as to foresee possible risks and confront them. In close consultation with participating researchers and scientists and the wider public, the network partners have therefore devised a joint strategic vision that is intended to serve as the basis for further European research funding in synthetic biology in the years ahead.

Developing a research strategy

This strategic vision will be published on the ERASynBio website in the first quarter of 2014. Next to examining the scientific challenges in this field, it deals in depth with the different subject areas important to the development of synthetic biology, such as its ethical and societal implications, risk analyses, the legal framework, and training and education measures. These subjects have been addressed nationally and internationally in recent years by a wide variety of bodies, publications and events (in Germany, for example: Statement of the German Research Foundation [DFG], acatech und Leopoldina, 2009; Deutscher Ethikrat – Werkstatt Leben Tagungsbericht, 2011; Thesenpapier des DECHEMA Arbeitskreises Systembiologie und Synthetische Biologie, 2011; Supplement of the interdisciplinary research group "Gene Technology Report", 2012). These statements in their entirety, supplemented by the results of two ERASynBio strategy conferences (January 2013 in Basle, October 2013 in Edinburgh), formed the basis for developing the joint strategic vision and set the framework of reference for future joint calls and activities.

The central element of the strategy is forward-looking, transparent research and innovation funding that needs to be supported systematically and embedded in relevant social structures. The success of synthetic biology depends not only on technological innovations by natural scientists and engineers but also on its acceptance by the public and on significant utility of its applications and products. Due to the depth of research intervention on the one hand and the rapid pace of advances in synthetic biology on the other, it is urgently necessary for all stakeholders to keep constant track of ethical and legal aspects. One good example of this is the development of alternative genetic codes and non-canonical amino acids that affect fundamental principles of genetics. The further significance of this as regards possible applications, but also potential risks, is hard to assess at present.

In the individual subject areas listed above, such as potential misuse for military purposes or the ethical evaluation of gene

manipulation of higher organisms, the ERASynBio research strategy mentions various control and monitoring options that should ideally accompany this development process. Only by adopting an integrated approach of this kind will it be possible to exploit the entire innovation potential of synthetic biology in a socially responsible way.

ERASynBio profile:

ERASynBio is an ERA-NET from the Seventh Framework Programme for the development and coordination of synthetic biology in the European Research Area.

- **Duration:** January 2012 to December 2014
- **Consortium:** 16 funding organisations from 14 countries; the US National Science Foundation
- **Coordination:** Project Management Jülich
- **Budget:** € 1,997,022
- **Coordinator:** Annette Kremser (a.kremser@fz-juelich.de)

www.erasynbio.eu

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Figure 2: European ERASynBio partner countries

14 European countries are involved in ERASynBio through national funding organisations. The United States participates in ERASynBio as an observer through the NSF (Graphics: ERASynBio).

nucleosomes – gatekeepers of the genome?

Computer simulations and high throughput sequencing reveal how genome access is controlled

by Karsten Rippe and Gero Wedemann

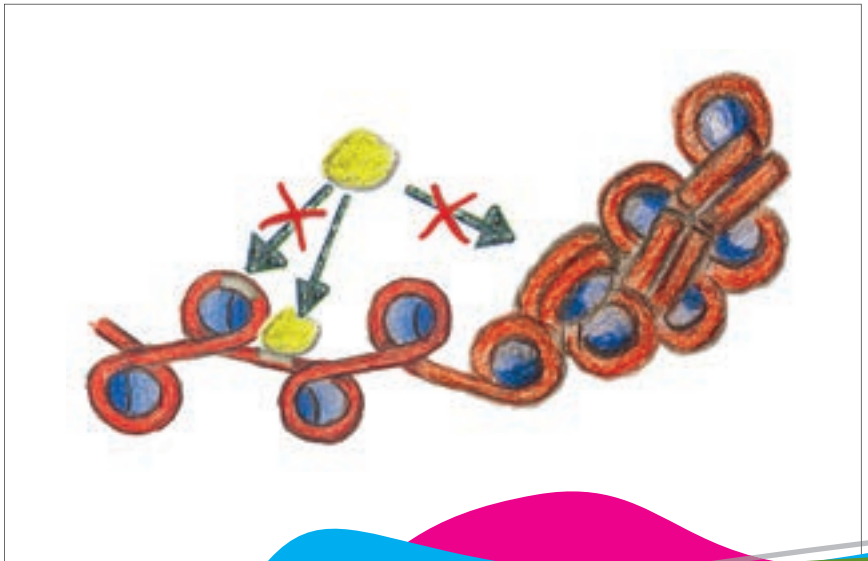
The human genome is packaged into a chain of nucleosomes — complexes of a protein core with DNA wrapped around. Many transcription factors can bind much better to the linker DNA between nucleosomes, and thus their positioning can directly determine access to the genome. Nucleosomes control genome access also indirectly: a regular spacing promotes folding of the chain into higher order fibers in which also the linker DNA can be occluded. Thus, predicting gene expression from the binding of transcription factors becomes dependent not only on DNA sequence and protein concentration but also on the spatial organization of the genome by nucleosomes.

What chapters to read in the book of DNA?

All the cells of the human body contain essentially the same DNA sequence and with it the identical genetic information. The DNA sequence encodes the construction and maintenance plan of the organism. However, each cell 'reads' only selected chapters of the bodies 'DNA book', and uses only some of its plans and recipes to exert specific functions. But how does a undifferentiated stem cell establish the appropriate DNA program during development that makes it for example a muscle cell, a liver cells or a skin cell? One of the sophisticated mechanisms involved in this process is the packaging of the DNA genome in the cell nucleus into a structure called *chromatin* (the term originates from the Greek word *chroma*, i.e. color, because it can be easily stained for imaging by light microscopy): The chromatin in a human cell nucleus contains DNA chains that are almost 2 meters in length when put together. About ¾ of this DNA is part of the ~30 million nucleosomes that consist of DNA wrapped around a histone protein core in almost two turns.

Nucleosomes are connected by segments of protein-free linker DNA between them. As depicted in Fig. 1, DNA within the nucleosome is less accessible for other protein factors than linker DNA. Hence, the positions of the nucleosomes determine whether certain DNA sequences are in the more easily acces-

Figure 1: Nucleosome chain with a protein binding to the linker DNA between two nucleosomes



Nucleosome chain with a transcription factor (in yellow) binding to the linker DNA (red) between the nucleosomes (protein core in blue). The open 'beads-on-a-string' conformation of a nucleosome chain can associate into a chromatin fiber. Thus, both the position of a nucleosome along the DNA, as well as the higher order folding of the nucleosome chain, control access to the DNA (Source: Gernot Längst).

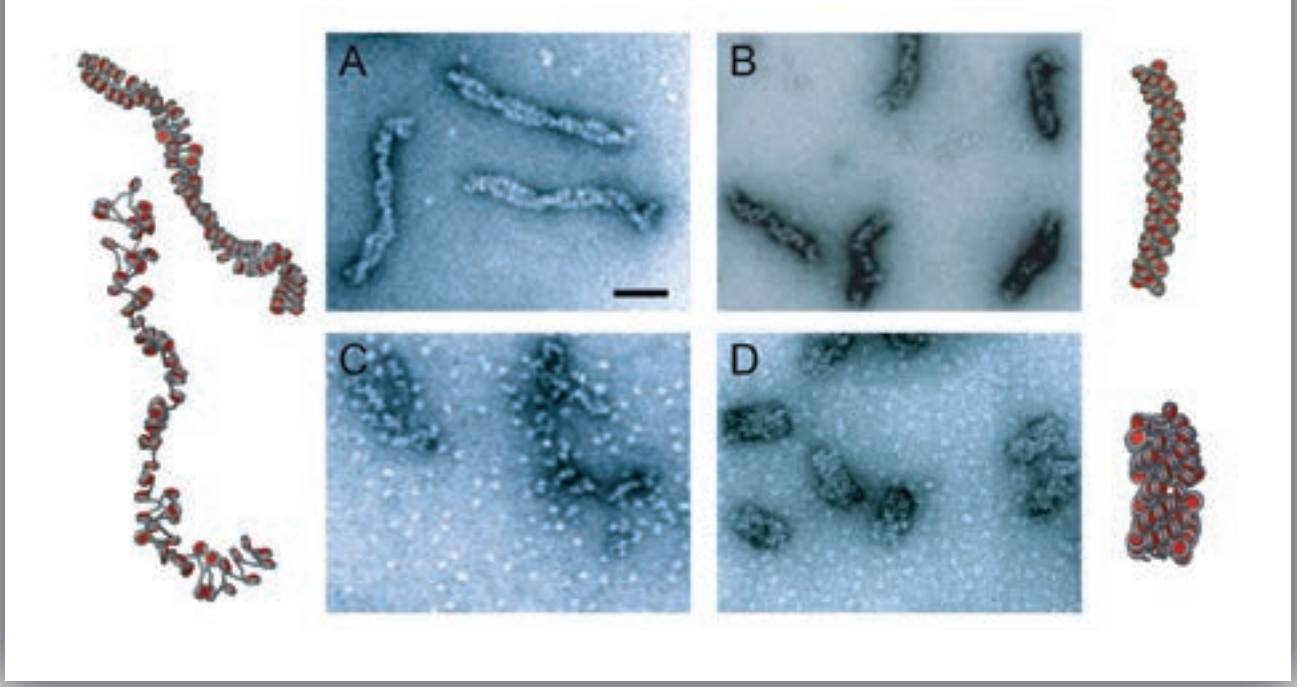


Figure 2: Chromatin fibers observed on electron microscopy images and model structures from Monte Carlo computer simulations
Samples were different with respect to the spacing of nucleosomes, i.e. the nucleosome repeat length (NRL) and the presence or absence of the linker histone H1. Scale bar: 50 nm.
A) Unfolded 167-bp NRL fiber in the absence of linker histone H1.
B) Addition of H1 induces the folding of the 167-bp NRL fiber.
C) Unfolded 197-bp NRL nucleosome chain with more irregular structures in the absence of linker histone.
D) Fully folded 197-bp NRL fibers form in the presence of saturating linker histone concentrations.
[Source: Daniela Rhodes (electron microscopy images), Nick Kepper (chromatin fiber simulation images)].

sible DNA linker region between nucleosomes or are masked by histone-DNA interactions. Thus, differences in nucleosome position, in conjunction with other regulatory mechanisms, decide whether genes are active or inactive and different cellular functions can be selected.

From models to experiments and back

Our theoretical studies at the level of a single nucleosome revealed a complex linkage between the dynamic structure of the nucleosome, DNA interactions of the unstructured tails of histone proteins and the binding of transcription factors (Ettig *et al.*, 2011; Teif *et al.*, 2010). Likewise, we developed and applied coarse-grained models to predict accessibility of the linker DNA as well as partial unwrapping of nucleosomal DNA for larger chains of up to 1000 nucleosomes (Rippe *et al.*, 2012). These models were designed and parameterized with experimental data obtained from the analysis of short nucleosome chains with regular nucleosome spacing studied *in vitro*. As illustrated in Fig. 2, the comparison of fiber structures observed experimentally by electron microscopy for different nucleosome repeat lengths and their predicted conformation from the Monte Carlo computer simulations is very good.

However, until recently it was not possible to investigate the linkage between nucleosome positions and transcription factor binding in a systematic manner for mammalian genomes. Rather, most studies focused on the analysis of simple model organisms like yeast and the fruit fly *Drosophila* or were restricted to studies of nucleosome positions at a few selected genes. With recent advancements in high-throughput DNA sequencing methods it became possible to map all individual nucleosome positions in mammalian genome at single base-pair resolution and to link models and experiments (Fig. 3). Taking advantage of this exciting new possibility the *EpiGenSys* consortium supported by the BMBF set out to map for the first time all nucleosome positions in mouse embryonic stem cells in comparison to their differentiated counterparts (Teif *et al.*, 2012). The results revealed a wealth of features of nucleosome positioning at DNA sites that are important for cell differentiation. The start and the end of active genes displayed nucleosome-depleted regions as compared to silent genes where these sites were covered with nucleosomes.

Interestingly, the nucleosome positioning profiles were found to change according to specific modifications of the histone proteins. These modifications include posttranslational modifications with methyl and acetyl groups that can be attached and removed from the histones and represent so-called 'epigenetic' signals that serve to transmit a certain chromatin state through cell division.

As predicted, many proteins that play a central role for cell development were indeed found to bind to the free linker DNA between nucleosomes in embryonic stem cells, supporting the gatekeeper model for the role of nucleosomes in regulating DNA access. However, some proteins like three of the master regulators of the stem cell state, the proteins Nanog, Oct4 and Sox2 showed a different binding pattern. They were found also at regions with high nucleosome occupancy, suggesting that they act as 'pioneering' factors that can bind to the nucleosomal DNA. Thus, the simplified view that nucleosomes always impede transcription factor binding needs to be revisited.

An additional challenge for modeling work that uses the experimental nucleosome positions as input for the Monte Carlo simulations for 3D genome folding arises from the heterogeneity of the cell populations studied. In many instances the experimentally determined peaks that demarcate nucleosome positions were incompatible with a single nucleosome chain conformation. To obtain specific nucleosome configurations and optimized solutions for the complex positioning patterns from experimental data, we developed a scheme that combines binary-variable analysis and a Monte Carlo approach with a simulated annealing method (Schöpflin *et al.*, 2013). With this framework we are now conducting a new iteration of computer simulations based on the experimental data from the population average for specific genomic loci of up to 1000 nucleosomes to investigate their spatial organization and DNA accessibility (Fig. 3).

Where to go from here?

Nucleosome positioning is a dynamic process that is regulated in the cell by energy consuming molecular machines that are referred to as chromatin remodeling complexes (CRCs). They can move or evict nucleosomes along the DNA chain to switch between 'on' and 'off' states of the DNA. Interestingly, genome-wide sequencing studies, as conducted for example in the International Cancer Genome Consortium (http://dcc.icgc.org), reveal a surprisingly high number of mutations in different CRC types in cancer. Potential 'driver' mutations were identified for members of the CHD and SMARCA type CRC families in breast, ovarian, kidney, lung, colon, uterus and liver cancer. Furthermore, it is known that aberrant epigenetic patterns can misguide CRC activity to establish pathological chromatin states in tumors.

Thus, important new questions emerge from the insights already gained within the *EpiGenSys* project: How is active nucleosome translocation regulated in the cell? Our current model proposes that signals targeting CRCs and modifying their activity are encoded by the nucleosomal DNA sequence and the posttranslational modifications of histones H3 and H4, as well as other chromatin features like DNA cytosine methylation. It appears that a yet unknown 'chromatin remodeling code' exists that targets CRC to genome sites, where they change nucleosome positions. In this manner, CRCs can establish distinct local chromatin structures, rather than solely being unspecific nucleosome-moving entities that make chromatin more 'fluid'. In addition, our unpublished modeling work points to another linkage between nucleosome positions and chromatin structure: Already by changing a single nucleosome position the higher order folding of the nucleosome chain can be dramatically altered. Thus, local nucleosome positions modulate access to the linker DNA via changing the chromatin fiber conformation and, at the same time, can also promote or impede long-range interactions between proteins bound at distant sites.

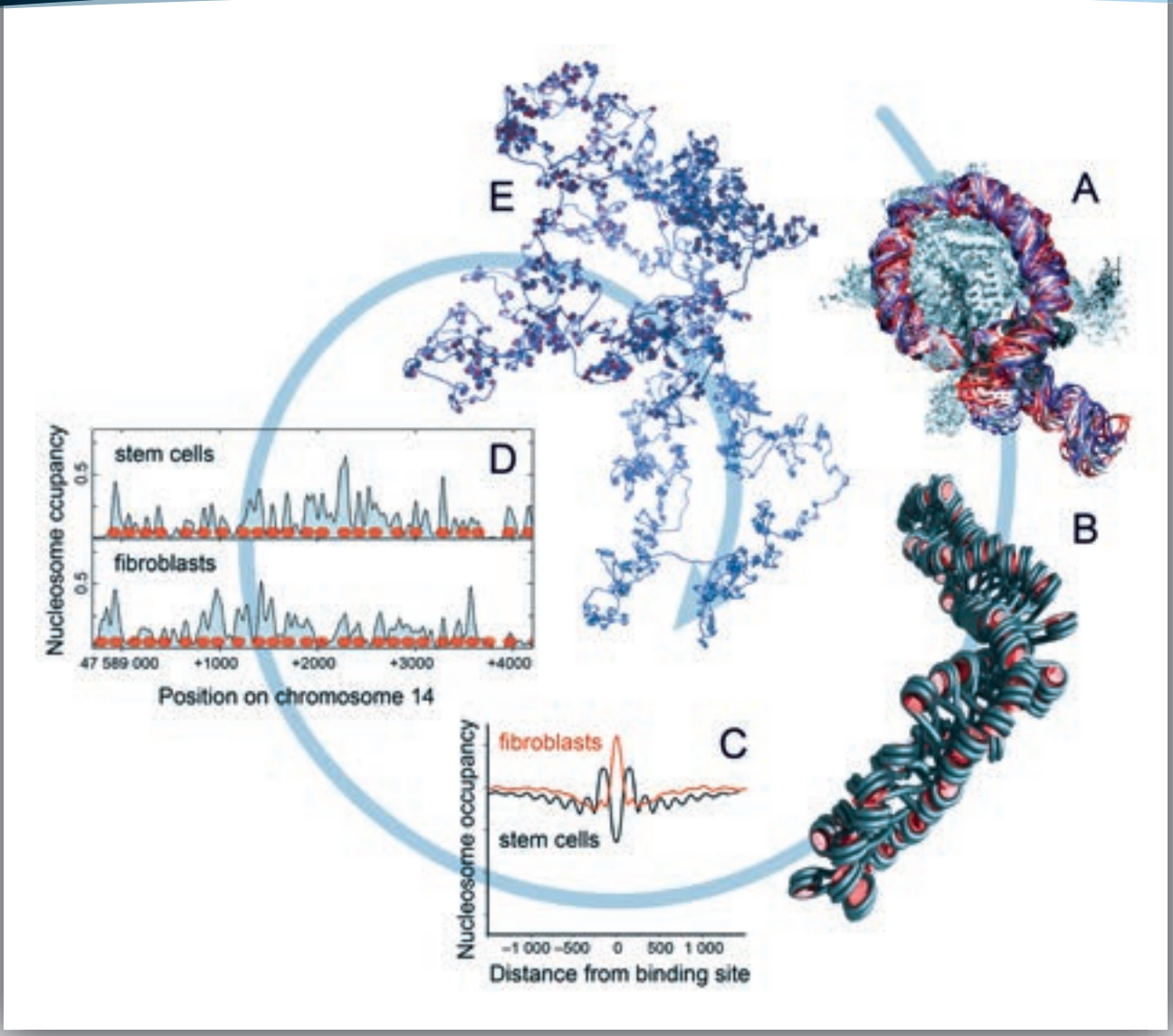


Figure 3: Iterations between modeling and experimental studies dissect the relation between nucleosomes and DNA accessibility for transcription factor binding

A) By molecular dynamics simulation access of nucleosomal DNA was studied with respect to the competitive DNA binding of the histone protein core and transcription factors (Ettig *et al.*, 2011; Teif *et al.*, 2010). In the image nucleosome conformations are overlayed in 0.2 nanosecond time intervals. The DNA is color coded with increasing simulation time from red to white to blue. The core histone proteins are shown in white. Already during the very short simulation time period of 2 nanoseconds, the nucleosome conformation is very dynamic.

B) Monte Carlo simulations of nucleosome chain folding provide information on access to linker DNA in chromatin fibers [reviewed in (Rippe *et al.*, 2012)].

C) Experimentally mapped nucleosome occupancy profiles at binding sites of the CTCF transcription factor (Teif *et al.*, 2012). In embryonic stem cells, bound CTCF introduces a regular spacing of nucleosomes in the region flanking its binding site. In differentiated fibroblast cells, nucleosomes occupy some of the sites that had CTCF bound in stem cells.

D) Unique nucleosome positions were extracted from experimental data sets for subsequent modeling of chromatin at specific genomic loci (Schöpflin *et al.*, 2013).

E) Monte Carlo simulation of chromatin organization at the 200 kb large SAMD4 gene locus based on experimental nucleosome position maps. (Source: Robert Schöpflin)

Given the importance of nucleosome positioning for genome access and readout, another exciting question is about deregulation of nucleosome positioning in cancer cells. This issue is addressed within the work of the consortium *CancerEpiSys* (www.CancerEpiSys.org) in the BMBF CancerSys program. Nucleosome positions and epigenetic modifications are mapped in primary tumor cells from patients with chronic lymphocytic leukemia to identify patterns of deregulated chromatin organization that can be related to the cancer disease state.

Based on the results obtained so far, it is emerging that integrating the various epigenetic signals attached to the genome with changes of DNA access provides valuable information for novel personalized medicine approaches. Thus, including nucleosome positions becomes an important part of quantitative descriptions that predict how cells select their active genetic program and that rationalize how this process is deregulated in cancer cells.

The research project in brief:

The project on nucleosome and chromatin modeling as well as the experimental mapping of nucleosome positions was part of the three-year BMBF funded consortium project *EpiGenSys* – System Biological Determination of the Epigenomic Structure-Function Relation (www.EpiGenSys.org) within the European ERASysBioPlus initiative in the EU FP7 ERA-NET Plus program (grant numbers 0315712A to KR and 0315712C to GW).

In *EpiGenSys* (epi-)genomic structure-function relationships between chromatin and transcription were dissected and modeled with a multi-scale approach. The work integrated the single nucleosome structure, folding of the nucleosome chain into higher order fibers and the larges scale 3D architecture of the genome. Within the consortium, the groups of KR and GW conducted modeling work on the single nucleosome level with respect to accessing nucleosomal DNA (Ettig *et al.*, 2011; Teif *et al.*, 2010), developed coarse grained models for the nucleosome chain by Monte Carlo simulations [reviewed in (Rippe *et al.*, 2012)], mapped nucleosome positions experimentally by deep sequencing (Teif *et al.*, 2012) and developed bioinformatical approaches to link experimental data and modeling (Schöpflin *et al.*, 2013).

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bridging the gap

Virtual Liver Network

Student and Postdoc Retreat

by Saskia Sperber, Lorenza A. D'Alessandro, Jonathan Fuller, Philippe Lucarelli, Madlen Matz-Soja, Christian Priesnitz, Julia Sanwald, Maria Thomas and Sebastian Vlaic

The Virtual Liver Network is a systems biology flagship programme in Germany that involves 69 work groups across the country. The project is an integrated approach to investigate the physiology of the liver with a scale-bridging mathematical model of the liver as its final objective.

The greatest challenge faced by systems biology work in general and by a project of this size in particular is communication. To ensure the greatest possible precision in planning experiments and the usability of data generated, detailed agreements between work groups and, above all, between modellers and experimenters are essential. Young scientists are offered a good opportunity to communicate at the annual Virtual Liver Network Student and Postdoc Retreat. This meeting, organised by PhD students and young postdocs themselves, provides plenty of opportunities for participants to communicate with each other directly.

One of the initial obstacles that must be overcome is that PhD students and postdocs from the different groups generally do not know each other personally. To make introductions easier for everybody, the annual get-together begins with a kind of "scientific speed dating". This enables participants to gain an overview of the expertise that is available in the network, as well as making their first contacts. To intensify this further in the course of social activities, the retreat is intentionally held in a degree of seclusion.

Participants are able to present their work in several series of lectures on specific subject areas. Unlike many other gatherings of this kind, however, it is not solely a matter of presenting scientific findings but above all of outlining problems that have arisen. In this way new solutions can be developed jointly in group discussions. This process is supplemented by poster sessions enabling those who were not selected to give a lecture to engage in debate. New cooperation partners find each other every year in this way, contributing significantly to the success of the network. At the retreat, PhD students and postdocs also gain an opportunity to acquaint themselves with career paths outside the scope of a classic academic career and to get to know and make contact with invited speakers from these areas of activity. Free workshops on subjects such as scientific writing or self-presentation offer an additional opportunity for personal development.

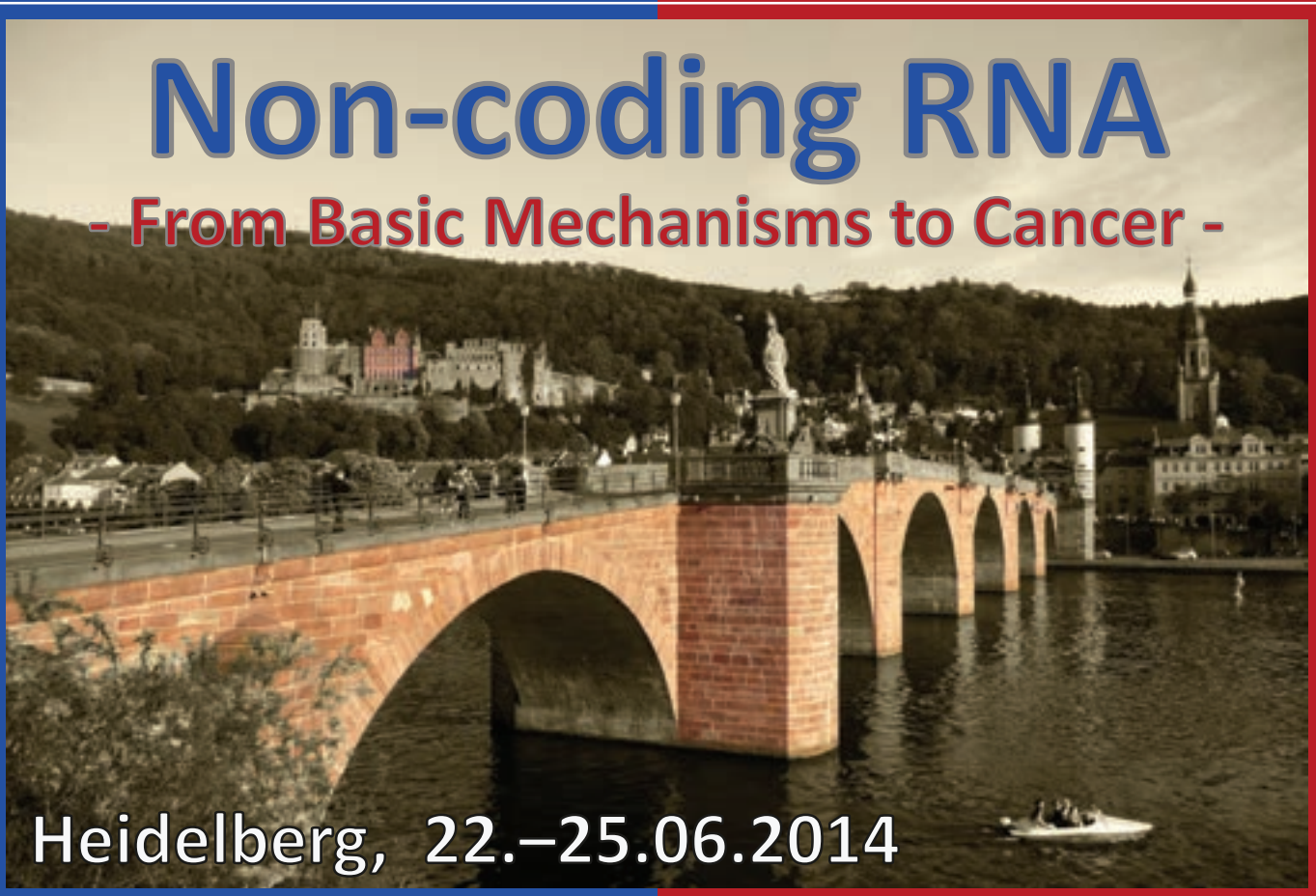
To improve the retreat from year to year and adapt it to young scientists' needs, participants evaluate the gathering. The surveys show that the annual meetings have contributed to a lively exchange of knowledge between work groups and to new approaches to problems that arise. A specific feature is, without a doubt, the informal way in which participants interact with one another, without having to contact the head of the group first. This makes the retreat an extremely effective resource and a mainstay of the Virtual Liver Network.

Participants at the Virtual Liver Network Retreat held in Hünfeld, Germany, in October 2013

Photo: Andreas Hoppe

Non-coding RNA

- From Basic Mechanisms to Cancer -



Heidelberg, 22.–25.06.2014

Speakers:

Keynote Speakers:

Arul Chinnaiyan - Ann Arbor
Jeannie T. Lee - Boston
John Mattick - Sydney
Thomas Tuschl - New York

Organizers:

Sven Diederichs - Heidelberg
Georg Stoecklin - Heidelberg
RNA@DKFZ & CellNetworks EcTop5

Contact: rna-meeting@dkfz.de
Website: www.dkfz.de/rna-meeting

Reuven Agami - Amsterdam
Anja K. Bosserhoff - Regensburg
Irene Bozzoni - Rome
Antonio J. Giraldez - New Haven
Myriam Gorospe - Bethesda
Shiv Grewal - Bethesda
Ingrid Grummt - Heidelberg
René Ketting - Mainz
Leonard Lipovich - Detroit
Anders Lund - Copenhagen
Gunter Meister - Regensburg
Nicholas Proudfoot - Oxford
Nikolaus Rajewsky - Berlin
Michael G. Rosenfeld - San Diego
Alexander Schier - Boston
Gerhard Schratt - Marburg
Frank Slack - New Haven
Francoise Stutz - Geneva
Andrea Ventura - New York
Mihaela Zavolan - Basel

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EXZELLENZCLUSTER
CellNetworks

UNIVERSITÄT HEIDELBERG
ZUKUNFT SEIT 1386

award-winning gold recovery using bacteria

A team of students from Heidelberg that took part in the international iGEM synthetic biology competition in 2013 has developed a method for recycling gold from electronic waste by biomineralisation using a bacterial peptide. To achieve this, the peptide's entire synthesis pathway was inserted into *E. coli* by means of recombinant transfer. The team won the Grand Prize in the iGEM competition for its method, which can compete with the conventional chemical processing of gold.

No other chemical element has held such a fascination for people over the millennia as gold – from the pharaohs of Ancient Egypt to the Incas and Spanish conquistadores to the Gold Rush in California and Australia and to modern-day speculation in gold. In the 1920s the distinguished chemist Fritz Haber spent years searching for ways to isolate gold from seawater, which Germany could have used to pay reparations imposed by the Treaty of Versailles after World War I. Haber, a Nobel laureate who discovered how to synthesise ammonia from atmospheric nitrogen and hydrogen, gave up looking for "this dubious needle in a haystack," as he wrote in his 1926 research report, after finding that the gold content of seawater was about a thousand times lower than initially assumed.

Since then, there has been no shortage of efforts to extract gold from places other than the normal deposits. Gold has traditionally been used as a national reserve by central banks as well as for jewellery and art. Nowadays, there is also an increasing demand in the electronics industry as gold is an essential component of modern computers and mobile phones, for instance. In recent years, the dramatic rise in the price of gold, and the new technical possibilities have further intensified efforts to extract gold from unconventional sources.

World champion in the 2013 iGEM competition

A team of 13 students from the University of Heidelberg and the German Cancer Research Center (DKFZ) has now developed a method for recovering pure gold as part of the prestigious iGEM synthetic biology competition. Gold is extracted from electronic waste using a peptide made by the bacterium *Delftia acidovorans*. Now in its ninth year, the iGEM (international Genetically Engineered Machines competition) held by the Massachusetts Institute of Technology (MIT) in Cambridge, USA, has become an international name. In 2013, 204 teams from all over the world took part, including 11 from Germany.

Of the six teams that qualified for the iGEM finals at MIT in early November 2013, five were from Europe and three from Germany. The Grand Prize was awarded to the team from Heidelberg under the scientific supervision of mathematician and systems biologist Roland Eils and Barbara Di Ventura, head of the synthetic biology research group at the BioQuant systems biology centre in Heidelberg. Along with the team from Freiburg, the Heidelberg students also won an award for the "Best Foundational Advance Project", a special award for projects that have made the greatest advances in the basics of synthetic biology.

The winning iGEM team from Heidelberg (left: Prof. Roland Eils)

Photo: UUniversity of Heidelberg

Contact pins from an old processor that contain gold (Photo: iGEM Heidelberg 2013).

A solution containing gold from the pins of an old processor (Photo: iGEM Heidelberg 2013).

Solid gold particles precipitated by *Delftia acidovorans* in Petri dishes (Photo: iGEM Heidelberg 2013).

The award-winning project

The project that the young scientists from Heidelberg presented at the iGEM competition involves using a substance from the *Delftia acidovorans* bacterium to precipitate and recover elemental gold from a gold solution. Among other places, this bacterium has been found in gold mines. Like many other bacteria that live in extreme environments, it grows relatively slowly. *Delftia* protects itself against the toxic effects of gold chloride ions by producing a peptide consisting of ten amino acids which precipitates gold. This unusual molecule, known as delftibactin, is a non-ribosomal peptide (NRP) and is not synthesised by way of a special messenger RNA on the ribosome but via the polyketide pathway, in a reaction chain of specific enzymes.

A team of US researchers found recently that these synthesising and modifying enzymes in the *Delftia* genome are encoded by a coherent cluster of 21 genes. The iGEM team isolated this large DEL cluster from *Delftia* and inserted it into the easy-to-handle and fast-growing laboratory bacterium *Escherichia coli*. The researchers also inserted two further enzymes from *Bacillus subtilis* into *E. coli*, one to improve activation of the polyketide synthase and one to produce the substrate required for reaction (methylmalonyl-CoA), which *E. coli* cannot metabolise naturally.

The students from Heidelberg were able to show that their recombinant *E. coli* strain produces the NRP delftibactin in sufficient quantities to make industrial application seem possible. To improve the visibility and purification of NRPs, the students developed a dye marker built into the peptides. They have filed a patent for this innovative labelling method, which is based on the NRP indigoidine. Using the recombinant delftibactin, the team succeeded in recovering a good yield of the precious metal from solutions with gold concentrations as low as those extracted from electronic waste.

The philosopher's stone

So has the team from the University of Heidelberg and the German Cancer Research Center (DKFZ) found the philosopher's stone, the substance sought by alchemists for centuries in their quest to transform base metals into gold? Whatever the answer, the jury at the 2013 iGEM World Championship Jamboree were won over not just by the team's elegant synthetic biology approach and the quality of the project in terms of execution and presentation, but also by the students' feasibility study showing the potential of delftibactin for industrial recycling of gold.

Millions of tonnes of electronic waste containing many tonnes of gold and other rare metals are generated each year. According to a study carried out by the Technical University of Berlin, in 2007 more than two tonnes of gold worth $92 million were discarded in Germany alone. The most common chemical method currently used for extracting gold from electronic waste is based on electrolysis. This does not involve the use of the highly toxic cyanide solution that is still frequently used in gold mines, but electrolysis consumes a lot of energy and is inefficient. It is estimated that no more than 10 to 15 per cent of the metal can be recovered in this way.

Estimates suggest that biomineralisation of gold using delftibactin, as described here, costs around €180 per mole, so it is already able to compete with conventional gold processing. Moreover, it causes significantly less environmental pollution, said Roland Eils, coach of the iGEM team from Heidelberg, who is also head of the department of Theoretical Bioinformatics at the DKFZ, Professor of Bioinformatics and Functional Genomics, and managing director of the BioQuant Center at the University of Heidelberg. He has also previously supervised other teams of students who won iGEM awards. However, the internationally acclaimed gold recycling project surpassed all previous achievements. Maybe it is the needle Fritz Haber looked for in vain in the haystack.

First published at www.bio-pro.de (EJ - 25.11.2013)

Further Information about iGEM: www.igem.org

communication is about delivering your message

Conference Report from the Comm4Biotech 2013

by Fanny Georgi

Getting to the bottom of current issues and solving pressing problems is not only motivation for most scientists, but at the same time it is also the justification for research in general. The prospect to improve or even save lives justifies public funding. Moreover, the success of the suggested solutions depends substantially on the society's approval. Last but not least, the general public has a final say with respect to the legal environment in which research takes place. It is obvious that scientific players should not withdraw into their ivory tower and instead engage society in their work in a clear and easily understandable manner, so that the general public can appreciate scientific research and its importance.

Preparing tomorrow's scientists for this challenge is the aim of Comm4Biotech (comm4biotech.eu). The conference was held on November 29th and 30th 2013 for the second time, organized by the German Biotechnologische Studenteninitiative e.V. (btS) and the French Nouvelle Génération des Biotechnologistes (NGB). Both student initiatives have set their sight on facilitating the exchange between students, academia, industry, and the public.

COMM4BIOTECH 2 international congress 29th-30th November 2013
communicating life science & technology

After Comm4Biotech's debut in 2011 in Strasbourg, more than 200 scientists gathered at the German Cancer Research Center (DKFZ) in Heidelberg. Participating in the Comm4Biotech was free of charge thanks to the support of sponsors and the generous funding by the Franco-German University.

Referees from Germany, France and the USA pointed out how scientists can engage politicians and the public in discussions on their research and how complex issues could be elaborated comprehensively. Sebastian Olényi, PhD student in science communication at Den Haag, explained how the

Source: btS e.V.

More than 200 future scientists debated on approaches to discuss their research with the public in a clear and comprehensive manner at the Comm4Biotech (Source: btS e.V.).

usage of genetically modified organisms (GMOs) in green biotechnology encounters vigorous public resistance.

Furthermore, he discussed both reasons and communication measures to counteract this development. Prof. Dr. Marcel Kuntz from the University of Grenoble went even further and stated that the discourse of GMOs was a 'political war' rather than a 'scientific debate', considering that the driving motor for all players were mostly money and votes.

This year's conference focused on synthetic biology. As an emerging field of research it offers the unique opportunity to study a young science's self-defining process. During interactive workshops, Dr. Harald König from the Institute for Technology Assessment and Systems Analysis (ITAS) at the Karlsruhe Institute for Technology (KIT), Prof. Dr. Bernadette Bensaude Vincent from the University of Paris and 2013's iGEM (international genetically engineered machines competition) winners from Heidelberg discussed experiences and the latest data on synthetic biology's public perception with the participants.

All referees encouraged the listeners to make substantial efforts and to leave their laboratory comfort zone. It is essential that researchers engage the public in addition to their colleagues with regards to their own research. If tomorrow's scientists become aware of this challenge during the Comm4Biotech and approach it with open mindedness, raises hope for a new generation of communicative researchers.

Further Information:

www.comm4biotech.eu
www.btS-eV.de
www.biotechnologistes.fr
www.dfh-ufa.org

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f.georgi@stud.uni-heidelberg.de

events

International Congress on Stem Cells and Tissue Formation

July 8 – 11, 2014, Dresden

For the 5th time, stem cell researchers from all over the world gather in Dresden for the International Congress on Stem Cells and Tissue Formation. What separates this conference from others is the strong focus on developmental biology, an interdisciplinary perspective and the translational ambitions. A reasonable number of participants allows effective direct interaction.

International Congress on Stem Cells and Tissue Formation

As in previous years, the congress combines symposia on basic aspects of stem cell biology with more specialized topics such as hematopoetic stem cells, neural stem cells and diabetes.

A large number of excellent speakers encourages the participating scientists to involve in interdisciplinary discussions beyond their own area of research and networking. Ph.D. students and postdocs are explicitly invited to present their work on posters or in short presentations.

For additional information see:
www.stemcellcongress-dresden.org

ICSB 2014 – 15th International Conference on Systems Biology

September 14 – 18, 2014, Melbourne Convention and Exhibition Centre, Melbourne, Australia

The International Conference on Systems Biology (ICSB) is the highly anticipated main event for the global systems biology community. The 15th ICSB continues the annual series of conferences launched by the International Society of Systems Biology (ISSB) in Tokyo in 2000, by Hiroaki Kitano.

ICSB attracts top systems biologists from all over the world to an environment that encourages integration of biology, computer science, engineering and chemistry, and that spans leading areas of biomedical research. This year, the conference is taking place at the Melbourne Convention and Exhibition Centre and brings together interdisciplinary researchers in order to advance biomedical research, health care, and drug development.

Registration is now open, visit www.icsb14.com for more information on the internationally renowned speakers, the dynamic and advanced program, and the amazing city of Melbourne, Australia.

For more information see poster on page 88.

Contact:
info@icsb14.com

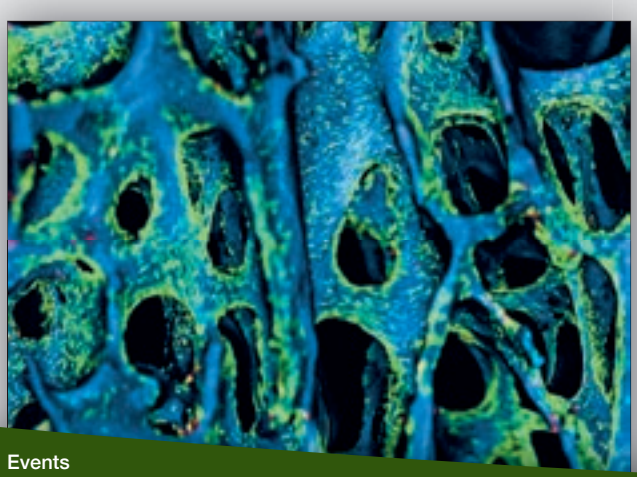

Photo: Falk Milan

Biology feat. Engineering

17th – 28th November 2014
International Autumn School on Synthetic Biology

www.synmikro.com/biofeateng2014

Joint Autumn School Marburg – Heidelberg – Jülich – Karlsruhe

Workshops on experimental and theoretical approaches
Guest lectures and literature seminars

Registration starts in June, 2014

Local Coordinators: Anke Becker, SYNMIKRO Marburg
Roland Eils, DKFZ and Heidelberg University
Harald König, KIT-ITAS Karlsruhe
Wolfgang Wiechert, Forschungszentrum Jülich

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SPONSORED BY THE Federal Ministry of Education and Research

German Conference on Bioinformatics (GCB)

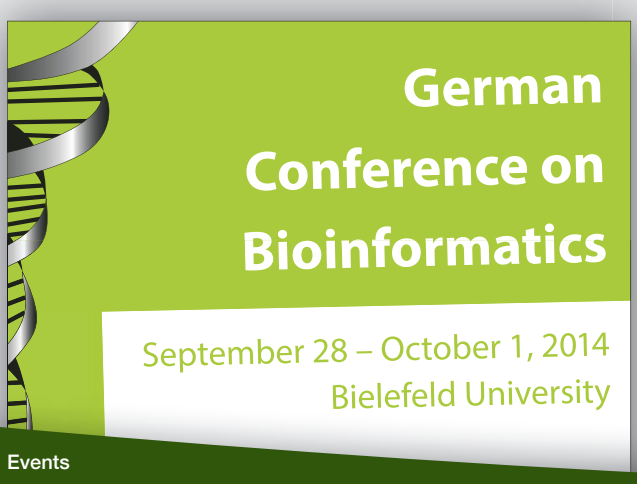
September 28 – Oktober 1, 2014, Bielefeld, Germany

GCB is an annual international conference, which covers all aspects of bioinformatics: genomics, metagenomics and polyomics, as well as visualization and processing of medical data, regulatory networks and metabolic pathways, structural modeling, personalized and translational medicine, management and interpretation of big data and bioinformatics in the cloud. The event is organized by the bioinformatics research groups of Bielefeld University together with the Society for Chemical Engineering and Biotechnology (DECHEMA), the special interest group BIOINF of the German Informatis Society (GI) and the Society for Biochemistry and Molecular Biology (GBM). The event takes place at Bielefeld University and is accompanied by satellite workshops on September 28.

Scientists from all over the world are not only invited to participate, but also to contribute by submitting recent advances in bioinformatics. After a review procedure, selected topics will be presented by the authors during the conference and published in a conference book of *GI Lecture Notes Informatics*. Moreover, highlight papers, poster abstracts and suggestions for satellite workshops can be submitted.

Renowned speakers, as Theodore Alexandrov (San Diego), Rolf Apweiler (EMBL Hinxton), Ellen Baake (Bielefeld), Cenk Sahinalp (Vancouver), Peter Stadler (Leipzig) and Tanja Woyke (Walnut Creek, CA) will contribute to the interesting program.

More informationen and registration on:
www.gcb2014.de
Contact:
contact@gcb2014.de



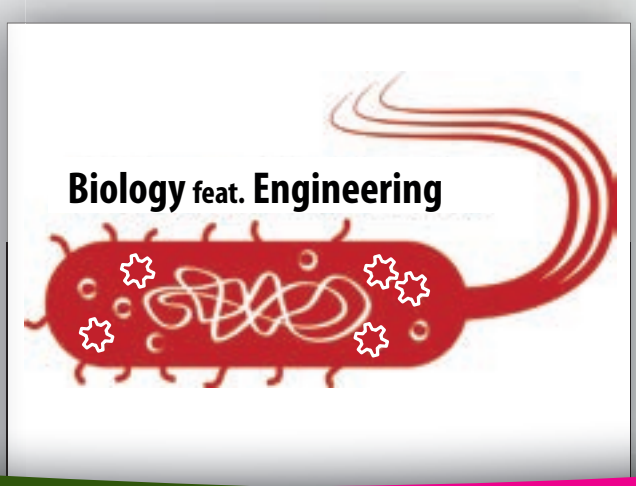
Biology feat. Engineering: International Autumn School on Synthetic Biology

November 17 – 28, 2014, Marburg/Heidelberg/ Jülich, Germany

Synthetic Biology exploits the concepts of engineering to investigate the functional principles of cells. Thereby, this young scientific area makes for ground-breaking insights in fundamental research and also has an enormous potential for applications in biotechnology and medicine.

"Biology feat. Engineering", the International Autumn School on Synthetic Biology of the LOEWE Center for Synthetic Microbiology (SYNMIKRO) and the Helmholtz Initiative on Synthetic Biology, will take place from November 17 to 28, 2014. Participants can choose optional workshops on experimental and theoretical approaches relevant to synthetic biology, e.g., laboratory automation, microfluidics, super resolution live cell imaging, structural biology, design of viral vectors for mammalian systems, or mathematical modeling. These workshops will take place in Marburg, Heidelberg and Jülich during the first week of the autumn school. In the second week, attendees and internationally renowned scientists will meet in Marburg to discuss both the latest developments in synthetic biology and the participant's own projects.

Registration opens in June, 2014.
Detailed information on program and registration is posted at:
www.synmikro.com/biofeateng2014



Conference Report

International Symposium on Synthetic Biology – from understanding to application

December 9 – 11, 2013, DKFZ Heidelberg, Germany

Synthetic biology is an emerging research field – also in Germany! This was the convincing message for more than 230 participants of the international symposium "Synthetic Biology – from understanding to application". The three-day high-ranking scientific program took place at DKFZ in Heidelberg.

Leading organizer of this symposium was the Helmholtz Initiative on Synthetic Biology coordinated by Roland Eils, which also celebrated its first anniversary with this event. The initiative of the Helmholtz Association results in the establishment of a sustainable network in synthetic biology in Germany, proven by the participation of numerous scientific partners: the LOEWE Centre for Synthetic Microbiology (SYNMIKRO Marburg), BIOSS Centre for Biological Signalling Studies (Freiburg), the network biotechnology of DECHEMA (Frankfurt), Heidelberg-Karlsruhe Research Partnership (HEiKA) and the Interreg-network on synthetic biology in the upper rhine area (Strasbourg) contributed to the high-ranking scientific program. 15 international leading scientists, e.g. John Glass (J. Craig Venter Institute, USA), Eckard Wimmer (Stony Brook University, USA) and Luis Serrano (Centre for Genomic Regulation, Barcelona, Spain) made their way to winterly Heidelberg, as well as leading German scientists in synthetic biology. In addition, many young scientists presented their research in posters and short presentations during the conference. An additional highlight were the presentations of four successful European iGEM Teams (amongst them the winning team from Heidelberg). The relaxed atmosphere supported the scientific exchange during the scientific and social program.

Apart from the scientific program, all participants as well as the interested public were invited to a public evening (organized by KIT-ITAS) to discuss ethical and societal challenges of synthetic biology with a mixed panel of politicians (Wolf-Michael Catenhusen, German Ethics Council), scientists (Petra Schwille, Martinsried), ethicists (Thorsten Moos, Heidelberg) and artists (Ursula Damm, Weimar). The lively discussion in the BioQuant Centre was followed by a live performance and the vernissage of the BioArts exhibition "not invented by nature".

Impressions from the international symposium on "Synthetic Biology – from understanding to application" at DKFZ Heidelberg (Photo: T. Schwerdt).

Conference Report

If it says *C. elegans* on the cover, it is actually about systems biology!

by Baris Tursun

European *C. elegans* Conference at the Max Delbrück Center for Molecular Medicine in Berlin-Buch on May 15-17, 2014

It must have taken a considerable amount of intuition, when Sydney Brenner chose the worm *Caenorhabditis elegans* (short: *C. elegans*) as a model organism for his systematic studies on developmental and neurobiology. Since then, *C. elegans* provides continuous successes as an efficient model system for relevant research topics, such as programmed cell death, neurodegenerative diseases and ageing, in classical as well as modern approaches in medical systems biology. *C. elegans* was predestined to a strong role in systems biology research: for once, it was the first metazoan organism with a fully sequenced genome. Even before this achievement, it was possible to examine the worm genome with classical genetic methods and thus, to functionally characterize regulatory elements and genetic networks. As a consequence, the *C. elegans* researchers Sydney Brenner, John Sulston und H. Robert Horvitz could describe complex genetic regulatory mechanisms during the development of organs and tissues and during processes in programmed cell death already during the seventies and eighties of the last century – an achievement, that earned them the 2002 nobel prize in physiology or medicine [1]. More nobel prizes were awarded to *C. elegans* researchers for the identification of RNA interference (RNAi) (2006, Andrew Fire & Craig Mello [2]) and the first application of the *green fluorescent protein* (GFP) (2008, Martin Chalfie [3]). The addition of the modern "omics" technologies (genomics, proteomics, etc.) as well as the recently discovered CRISPR-Cas9 system for genome editing accelerate insights into molecular networks and their physiological phenotypes. Since a while, it seemed as if mainly Northern American researchers appreciated the value of *C. elegans*. This, however, has changed since a while: A strong community of more than 200 research groups in Europe is nowadays using *C. elegans* as their main model organism.

The *C. elegans* meeting was organized at the MDC by researchers from the Berlin Institute for Medical Systems Biology (BIMSB) and gathered the majority of the *C. elegans* community in Europe. Renowned guests and keynote speakers presented different scientific focuses in current *C. elegans* research. Keynote speaker **Julie Ahringer** from the *Wellcome Trust, Gurdon Institute – University of Cambridge* (UK) was the first to construct a genome-wide RNAi library and carry out the first systematic knock-down of all worm genes. Her research group applied this library systematically to examine gene regulation during development and regulation of chromatin structure. She combines different high-throughput RNAi strategies and uses genetic, biochemical and bioinformatics analyses to understand transcriptional and post-transcriptional gene regulation on the basis of chromatin structure. Furthermore,

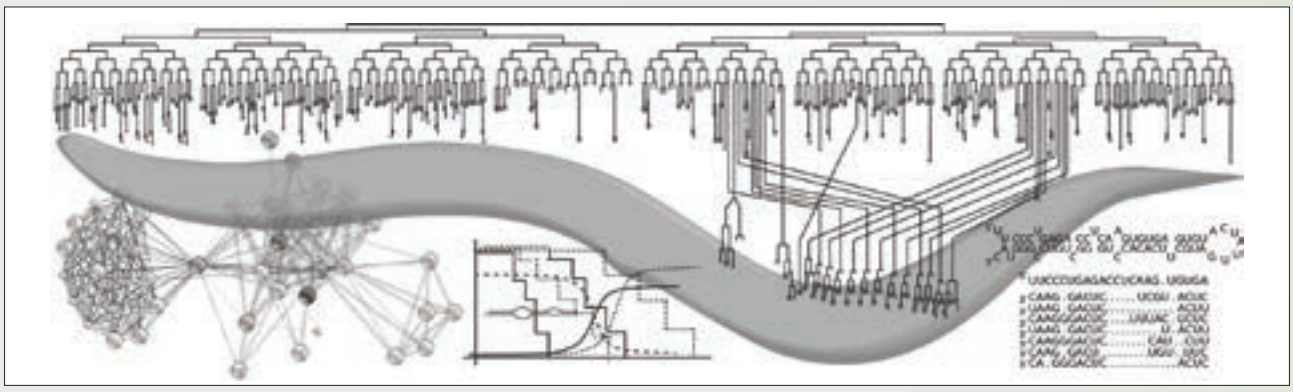


Figure 1: Scheme of *C. elegans* as an efficient model organism in systems biology research. The illustration also contains the sequence of the first ever described microRNA, which was identified in systematic genetic screens in *C. elegans* [4]. (Source: Baris Tursun)

imprint

More than 300 *C. elegans* researchers joined the first European 'worm-meeting' (Photo: Jutta Steinkötter, MDC Berlin).

Adam Antebi, director at the Max-Planck-Institute for the Biology of Ageing in Cologne and coordinator of *Sybacol – Systems Biology of Ageing Cologne* aims to understand the molecular mechanisms of signal transduction and protein quality control and their role in ageing. He reported about a novel finding that implicates a systemically acting worm miRNA in regulating ageing of different tissues and which is conserved in mammals.

Another highlight was keynote speaker **Barbara Conradt** from the *Ludwig-Maximilians-University München* (GER) who worked in the nineties with H. Robert Horvitz on programmed cell death (apoptosis) regulation and continued until now to further contribute important insight into regulation of apoptosis.

The *C. elegans* conference from May 15-17, 2014 provided an interactive forum for European and international *C. elegans* researchers and thus stimulated the formation of new networks and cooperation. By enabling scientific exchange and discussion between researchers from different disciplines and nations, it prompted the development of new technologies, for example at the workshop on genome editing using the CRISPR-Cas9 and transposon systems. Furthermore, it was a seeding point for regular European *C. elegans* meetings.

More information:
www.wormmeeting-berlin.de

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[3] Zamore PD. RNA Interference: Big Applause for Silencing in Stockholm, vol. 127. 2006.
[4] Lee RC, Feinbaum RL, Ambros V. Cell 1993;75:843.

systembiologie.de – International Edition

The magazine for Systems Biology Research in Germany – International Edition Issue 08, June 2014

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news

Who's in your Network?

You like networks? You want to scrutinize nodes and edges, understand relationships. Are you already part of a network as well? If so, there is no excuse: Find out who's who in systems biology (even if you do not consider yourself a systems biologist), get connected to the network, meet your neighbours, discover who shares your interests and expertise, unravel information coded in the network, and build and shape the systems biology community.

The systems biology community website promotes the development of a scientific network: http://community.isbe.eu
Graphics: CRG, Barcelona, Spain

Have a look at the European Systems Biology Community website (community.isbe.eu), a community building tool, designed to help connecting the multidisciplinary and disperse population of systems biology scientists, and to define the European landscape of systems biology. This community site has been fostered by the EC-funded ISBE project (Infrastructure for Systems Biology – Europe, www.isbe.eu) and needs your input!

Help us to build the community network by editing your own information online: http://community.isbe.eu

Just start with searching for your name!

Contact:
Joaquim Calbó, Centre for Genomic Regulation (CRG Barcelona) and ISBE team

Announcement:

Coming soon:
systembiologie.de School Edition

Spreading results of systems biology research to a wider public is the mission of the magazine systembiologie.de. However, as a relatively young scientific discipline, systems biology has only barely arrived in the curricula of schools. In order to raise interest also in younger generations, a special school edition tailor-made for the final year of the high school curriculum will be released at the beginning of the upcoming school year 2014/2015.

To assist teachers in conveying systems biology knowledge, the school edition of systembiologie.de will be based on current topics from previous editions of the magazine, e.g. stem cell and cancer research or epigenetic mechanisms giving students insights into the interdisciplinary approaches of modern systems biology research. Each article will come with scientific questions to test comprehension of the topic (as well as a separate brochure with answers for teachers). The publication aims to provide scientific knowledge, promote scientific discussion among students and increase awareness of scientific and medical problems. The project is supported by the German Federal Ministry of Education and Research (BMBF).

Expected release date: September 2014,
check www.systembiologie.de for updates

Photo: contrastwerkstatt / Fotolia

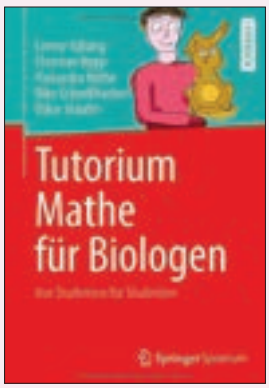
Book Reports

"Tutorial – mathematics for biologists"

From students for students

This German book addresses beginning students in molecular biology and aims at guiding them safely and without awe for this discipline through exam preparations. For this purpose, the young authors adapt their explanations in a colloquial style to the needs of students without sacrificing technical correctness. The material contains, next to the basics of analysis and linear algebra, exactly those methods that are most frequently used in molecular biological practice. With regard to recent technological developments, the book also covers the increasingly important simulation of biological systems. In contrast to other teaching books, the mathematical topics are continuously explained in the context of current research projects from molecular systems biology so that the student automatically dives into science while reading this book. In their descriptions – e. g. in the section for statistical methods – the authors furthermore explain specific functions in common software packages such as R or Excel. With this book, the students would in principle already be able to evaluate a data set from the laboratory.

Conclusion: This book fills a long standing gap on the market for mathematical teaching books, while preparing students equally for exam and laboratory practice.



L. Adlung, C. Hopp, A. Köthe, N. Schnellbächer, O. Staufer: Tutorium – Mathe für Biologen, 2014, Springer Spektrum

Source: systembiologie.de editorial team

Compendium "Computational Systems Biology"

From molecular mechanisms to disease

In the latest edition of this book, the two experienced authors Roland Eils and Andres Kriete use their extensive scientific network for covering the complete methodical as well as scientific scope of computational systems biology by a thorough choice of topics. Since the 19 chapters are exclusively written by leading scientists of this rapidly developing research area, the rich content creates a view into the future in comparison to the first edition eight years ago. Apart from methods on dynamic modeling and network analysis, the book also accounts for technological developments concerning databases and modeling platforms. Irrelevant of the degree of specialization, the reader quickly discovers the fascination for a whole range of highly relevant research topics – such as disease – relevant signal transduction pathways, circadian oscillations, ageing, metabolic networks and many more. The very comprehensive and thorough perspective helps to quickly understand a matter, which is otherwise only difficult to access.

Conclusion: To every interested scientist and student, this compendium offers not only a solid overview with possibilities to deepen insights via abundant literature references, but also a possibility to interconnect between the individual topics of computational systems biology.

R. Eils, A. Kriete: Computational Systems Biology, 2nd edition, 2014, Elsevier

Source: systembiologie.de editorial team

about us

Presenting the systembiologie.de editorial team

systembiologie.de would like to make the success of German systems biology accessible to a wider public in an illustrative way. The magazine, which is published twice per year in German and once in English, is produced jointly by the Helmholtz Association, Cross Program Topic Systems Biology and Helmholtz Initiative on 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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