



Systems Biology

Results, Progress and Innovations from BMBF Funding



Imprint

Published by

Forschungszentrum Jülich GmbH
Project Management Jülich (Ptj)
52425 Jülich

Orders

In writing to the publisher
Project Management Jülich (Ptj)
Außenstelle Berlin
PO Box 610247
10923 Berlin

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Translation

Language Services, Central Library, Forschungszentrum Jülich GmbH

Layout

FOCON GmbH, Aachen

Print

Bonifatius GmbH, Druck Buch Verlag, Paderborn

As of Jülich, Berlin 2008

Photo credits

Derichs Kommunikation GmbH, Jülich: Cover picture
Further photos and figures are provided from the respective authors (q.v.)

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Federal Ministry
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Systems Biology

Results, Progress and Innovations from BMBF Funding

Preface

Systems biology is working



Systems biology began well before the turn of the century in the USA and in Japan. Its empirical molecular genetics/genomics roots were more American, its physical chemistry/mathematical biology roots, including non-equilibrium thermodynamics and metabolic control analysis, more European. Hybridization arrays, QPCR, 2-D electrophoresis, chromatography plus 2-D mass spectrometry, and quantitative microscopy enable the quantification of changes in concentrations of molecules and thus represent an additional basis for systems biology. Even if Europe has a historical lead in some of these, the major initiatives in systems biology started in the USA and Japan. There the time was ripe for systems biology whilst Europe was more sceptical with respect to new research and development.

This barrier was overcome by the first major coherent systems biology research programme in Europe, which started in 2004. The programme was 'HepatoSys', funded by the Federal Ministry of Education and Research (BMBF). In the following year, the UK BBSRC and EPSRC funded research centres and doctoral training centres for systems biology. The BMBF then funded four such research centres (FORSYS) in 2007. Setting up further funding priorities (QuantPro, FORSYS Partner), BMBF continued to propel German systems biology forward, also by supporting the training of young scientists. At the same time BMBF announced a transnational research programme on microbial systems biology (SysMO) with the Netherlands, the UK, Austria, Norway and Spain. In 2008 many additional systems biology research programmes are now running in Europe, including Germany, where the new funding priority MedSys will fund applied systems biology in medical research.

The functioning of living organisms does not only depend on their individual components but also on the interactions between these components, i.e. on dynamic networking. For good reasons, molecular biology focuses on individual macromolecules. One paradox is therefore that systems biology needs to interface actively with molecular biology, which itself shies away from studying interactions and networking. Systems biology also needs to interface strongly with physiology, which itself frowns upon an analytical approach based on individual components. Tuned to the simplest possible systems and linear approximations thereof, mathematics and physics consider biology a mere set of special cases, too complex to resolve. Systems biology needs to integrate and add to these three paradoxical approaches.

Quite a number of research programmes throughout the world call themselves systems biology, but do not integrate these three approaches. Some merely calculate theoretical behaviour that may not actually function. Others collect data without interpreting how functions arise from interactions. The BMBF research programmes, and certainly HepatoSys, integrate the three approaches, and with appreciable success. The preparatory committees and the international steering committees worked hard to bring about this integration. The committees had to reject excellent research that lacked integration perspectives, and they insisted on the integration of distinct proposals. Both types of action are unusual in evaluating and advising on scientific research.

The paradigm shift effected by systems biology implies that the success of systems biology programmes should be judged by more stringent criteria than the success of traditional research

programmes. Of course, the research should be excellent, as judged from the discoveries and applications. In addition, the programmes should be distinguished from the traditional research programmes in molecular biology, mathematics and physiology. Research that is excellent in terms of molecular biology but not in terms of mathematics may not be regarded as excellent systems biology. On the other hand, the highest excellence in systems biology may conflict with the paradoxical standards of the two neighbouring disciplines. For the steering committee, this makes life difficult, as the quality of research proposals/reports cannot be assessed from the number of publications in journals with high impact in molecular biology, or in conference proceedings in engineering.

Another paradox relates to the involvement of industry in systems biology programmes. The pharmaceutical industry understands why one should look at disease and drug safety from the perspective of networks. However, since systems biology deals with entire networks, the best expertise needs to be engaged; involving too many research groups for the intellectual property to remain exclusive. The pharmaceutical industry will only become involved when research starts to become applicable. Then they plan defined bilateral projects with academic research groups. These kinds of projects are expected for the funding priority MedSys, and may now also become possible for Hepatosys. Systems biology is a matter for large-scale public funding in order to support new application-relevant research programmes in their early phases.

BMBF is to be complimented on the important role it has played in the emergence of systems biology. Japan and the USA may have been first to engage in systems biology, but BMBF has now put Europe into a leading position with the first and by far the largest, truly integrated systems biology programmes. The integration of the various disciplines dealing with various aspects of the human cell is of tremendous importance for health, disease and drug effectiveness. BMBF has also promoted the standardisation that is absolutely essential for the life sciences and for the 'silicon human' of the future. In addition, it has enabled scientists to make scientific discoveries that could not otherwise have been made.

I invite you to study this brochure, and to assess and enjoy the progress made by systems biology in Germany. Systems biology is working, also in Europe.



Hans V. Westerhoff

Hans V. Westerhoff is AstraZeneca Professor of Systems Biology and Director of the Doctoral Training Centre for Integrative Systems Biology from Molecules to Life (ISBML), Manchester, UK. Furthermore, he is Professor of Molecular Cell Physiology at the Free University Amsterdam and is Chairman of the "HepatoSys" Steering Committee.

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Progress and Innovation through Systems Biology

In all societies, innovations form the basis for progress and development. Innovations ensure continuous growth, prosperity and international competitiveness. The Federal Government's High-Tech Strategy for Germany is therefore specifically promoting research fields with a high innovation potential. This also includes the relatively young discipline of systems biology.

After the widespread introduction of the methods of molecular biology in medicine and biology, systems biology is regarded as the second key technology for achieving progress in the life sciences. At the same time, it forms the basis for exploiting new innovation potential in the knowledge-based bioindustry.

What is systems biology?

In the past, the individual research disciplines in the life sciences primarily focused on investigating process flows down to the molecular details. In a descriptive approach directed at achieving high quality and molecular details, a wealth of data was generated concerning single cell components or functions. However, the interaction of these molecular structures is highly dynamic and is controlled by cross-linkages with all cellular hierarchies. In order to understand such a biological system as a whole, it is necessary to have a quantitative understanding of the processes taking place in it. This is the starting point for systems biology. The aim of the systems biology research approach is to understand the behaviour, the dynamics of a biological system, for example a metabolic pathway, a cell organelle or - in the distant future - a whole cell or organism in its entirety. This requires the linkage of all molecular biology data from the level of the genome, through the transcriptome and the proteome, up to and including the metabolome, the analysis of interaction patterns and also data integration with the aid of mathematical methods. A basic prerequisite for systems biology approaches is therefore the interdisciplinary collaboration of researchers from the fields of biology, chemistry, medicine, computer science, mathematics, systems science and also engineering. The heart of the systems biology research approach

is an iterative process between laboratory experiments and mathematical modelling. The result of this process is an optimised mathematical model describing the behaviour of a given biological system in a defined environment. This thus facilitates predictions about the behaviour of the system under the influence of internal and external factors.

What is systems biology?

Systems biology is characterised as the quantitative analysis of dynamic interactions between the components of a biological system with the aim of understanding the behaviour of the system as a whole and enabling predictions of its behaviour to be made. To this end, mathematical concepts are applied to biological systems so that an iterative process takes place between laboratory experiments and computer modelling.

Benefits of systems biology

With its new concept, systems biology has the potential to radically change the life sciences and to provide completely new findings for biomedical research and for biotechnology in industry and agriculture. Working with models and computer simulations offers the opportunity of proceeding in a targeted manner. Instead of looking for the proverbial needle in a haystack, the most probable processes can be calculated and experiments tailored accordingly. Systems biology thus offers the opportunity of raising knowledge of dynamics and the interaction of vital functions to a completely new plane and of exploiting new potential for innovation in medicine, the pharmaceutical industry, the chemical industry and the biotechnology industry. The application of computer models may in future, for example, serve to find new targets for treating diseases or forecasting possible side effects of new active substances. Drug development will thus become more effective and safer and, moreover, permit animal experiments to be restricted to a minimum. In the same way, biological applications can be specifically optimised, for example increas-

ing the productivity of cell systems for certain systems and also the development of novel synthesis techniques. First applications are already beginning to emerge in the ongoing research projects.

Support measures implemented by the Federal Ministry of Education and Research

Systems biology requires altered research structures in science and industry, new cooperation models and a new quality of interdisciplinary and interindustrial collaboration in a national and international framework. The Federal Ministry of Education and Research (BMBF) recognised this at an early point and is reacting to these constraints. As part of the Federal Government's High-Tech Strategy, it is undertaking a selective expansion of systems biology support measures and the establishment of relevant research and funding structures on a national and international level. These measures are being taken within the context of lines of action planned or implemented by the federal states, the Helmholtz Association, the Max Planck Society and other research and funding organisations in this field.

Back in 2001, BMBF initialised funding of this innovative research field in Germany with its call for proposals for "Living Systems – Systems Biology". The resulting pilot project "Systems Biology of the Liver Cell - HepatoSys" has now developed into a nationwide network of expertise which also enjoys international recognition.

The support programme "Research Units of Systems Biology- FORSYS" created the decisive basis for systems biology in Germany. The establishment of the four FORSYS centres in Freiburg, Heidelberg, Magdeburg and Potsdam in 2007 improved the situation for systems biology, with respect to both structure and content. The FORSYS centres ensure that the interdisciplinary collaboration essential for systems biology is available "under the same roof", and that there is a local concentration of research expertise that also provides training opportunities for young scientists.

The support measure announced in 2007 "Partners for Research Units of Systems Biology – FORSYS Partners" further strengthened systems

biology in Germany. This support measure consists of two components. The "FORSYS Cooperations" provide support for a transfer of know-how between the existing FORSYS centres and partners from academia and industry and lay the foundation for the establishment of further competence nodes for systems biology in Germany. The "FORSYS Young Investigators Groups" give young scientists the opportunity to conduct independent research and thus to exploit their creative potential.

Peter Gruss, President of the Max Planck Society

"The systems biology research approach in the life sciences will have a decisive influence on progress in biology and medicine."

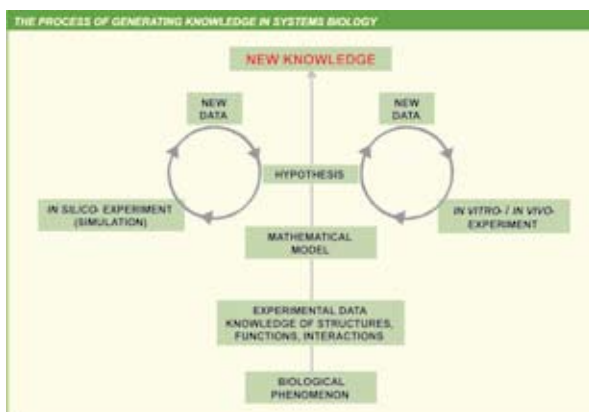
The support measure "Medical Systems Biology – MedSys" announced early in 2008 focuses on the application potential of systems biology for medicine and drug development. Apart from academic research groups, it therefore primarily targets corporate research departments in the pharmaceutical and biotechnology industries, which are concerned, among other things, with the development of patient-related tools for diagnosis and treatment or the application of systems biology approaches for increasing the efficiency of clinical trials.

Systems biology also plays a not inconsiderable part in the support measure on the topic of "BioEnergy 2021 – Research on Utilising Biomass" announced at the beginning of 2008. The "Systems Biology for Bioenergy" module will fund research projects that contribute towards optimising crops as energy plants.

The discussion is currently focusing on further research measures including the expansion of the methodological and technological basis for systems biology, for which the foundation was laid by the research priority "Quantitative Analysis to Describe the Dynamic Processes in Living Systems – Quant-Pro" that started in 2004. In addition, attention is also being paid to exploiting the potential of systems biology research for other fields of application (e.g. for health in ageing).

Apart from national commitments, the Federal Research Ministry is also involved in the development of European support measures for systems biology. For example, in 2006 as part of the ERA-Net ERASYSBIO, the transnational support measure SysMO (Systems Biology of Microorganisms) was agreed jointly with six European partners. Funding of the transnational collaborative projects began in 2007 and due to the success of these projects will be continued beyond 2010.

Schematic representation of the iterative cycle of experiment and modelling in systems biology.



Bilateral support measures for Medical Systems Biology were started with Slovenia in 2007. Other cooperative projects are planned with Austria from spring 2008.

Furthermore, another European support measure for systems biology is under preparation as part of ERASYSBIO, which is receiving major support from Germany and the UK. With these support measures, BMBF is pursuing the goal of specifically strengthening national research and funding priorities in the field of systems biology by international networking and of promoting cooperation between systems biology centres, which are at the moment mainly located in the Netherlands, the UK and Germany.

The Federal Research Ministry is also involved in planning a large-scale European project on the “Systems Biology of the Metabolic Syndrome” that is scheduled to be launched in 2009.

The strategy paper “Systems Biology in the European Research Area” was published in April 2008 by ERA-Net ERASYSBIO as a basis for the further harmonisation of funding policy for systems biology in Europe.

Karl-Heinz Maurer, Henkel KGaA Düsseldorf

“Systems biology is becoming increasingly important in Henkel research in terms of the technological application and control of microorganisms, and in the long-term, in order to establish in vitro test systems on the basis of skin cells. We use systems biology approaches to optimise the efficiency and quality of microbial fabrication processes for technical enzymes. We look at microorganisms that colonise our bodies and use methods from systems biology to work on the influences that the growth and formation of relevant metabolites have as a result of specific principles. In the long-term, we want to use the basic principles of the systems biology of skin cells in tissue models and cell cultures to develop alternative methods to replace animal experiments.”

Summary

With its early and comprehensive support for systems biology, BMBF has helped Germany to establish a leading position in this important research field that has great future potential. The total BMBF funding of systems biology is € 37 million per year for national and international support measures.

The aim of the BMBF's coordinated research and funding measures is to support the establishment of the research infrastructure required for systems biology together with major actors in Germany and Europe. It is taking up central research fields and topics which are of major significance for progress in the life sciences and for exploiting new innovation potential and contributes significantly to the training and encouragement of young scientists.

In this way, the competitiveness of Germany will be sustained in the field of the life sciences and cooperation between academic and industrial research specifically promoted.

Practical applications of the systems biology research approach are already foreseeable in the fields of biotechnology and medicine. Since 2004, the first companies – mainly small and medium-sized enterprises (SMEs) – have already become involved in this research as pioneers in systems biology. The increasing maturity of systems biology is manifested in the growing number of research partners from the pharmaceutical industry, the biotech industry and other sectors who recognise opportunities for the medium- and long-term development of new areas of business.

Gisela Miczka, Projektträger Jülich (PtJ)

On the Internet: www.fz-juelich.de/ptj/systembiologie

Fascination Systems Biology

Dr. Vytaute Starkuviene-Erle has been head of the Young Investigators Group "Screening of Cellular Networks" since autumn 2007, which is part of the VIROQUANT centre in Heidelberg. She spoke about her enthusiasm for systems biology and the excellent opportunity to work in such a research centre.

When did you become interested in systems biology?

It was during my time as a postdoc at EMBL in Heidelberg. I had established high-throughput assays for studies on trafficking mechanisms in mammalian cells. In doing so, I began to understand the significance of being able to analyse many components at the one time instead of concentrating on single proteins, which is what I was familiar with from my earlier work. So that's why I decided to orient myself in the direction of systems biology.

What is so fascinating about systems biology?

Systems biology throws light on a specific issue from all sides – both from the level of the gene and the protein right up to the effects in the system and vice versa. It accounts for a wide variety of factors in this process, and can be expanded to areas where classical biology was not admitted in the past, such as biophysics, bioorganic

chemistry or even quantum physics. This gives us an overall picture of what's going on – it doesn't just show us how a single protein behaves, but rather how the whole system or at least a part of it behaves. That's absolutely fascinating. Systems biology has the potential to help us understand every system, every organism in its entirety and complexity at some point in time. At the moment, we've only just begun, but the process of getting to this stage alone is very captivating.

What does it mean for you to head a Young Investigators Group in the VIROQUANT centre?

As soon as they have penetrated the host cell, viruses become involved in a number of cellular processes and therefore influence the entire system of the cell. VIROQUANT investigates these processes and this information is what attracts me. In the BIOQUANT building in Heidelberg, I work side-by-side with virologists and cell biologists, as well as with mathematicians, bioinformaticians, and modellers. We have lively discussions and our cooperations are extremely fruitful. This is the only way that systems biology can really function well. I believe that my research benefits from this to a large degree and I hope that I too can contribute a great deal.

HepatoSys

Systems biology studies of liver cells

With its call for proposals for “Living Systems – Systems Biology” in 2001, the Federal Ministry of Education and Research (BMBF) gave the go-ahead for the funding of systems biology in Germany. HepatoSys – a national network of expertise for research into liver cells – was initiated in 2004. Today, HepatoSys is the largest systems biology consortium working on interdisciplinary principles anywhere in the world.

The liver is the central metabolic organ in vertebrates and is in many respects a very special organ. Each day, it synthesises, converts or degrades more than 10,000 substances and thus contributes to the utilisation of food and purifying the body of metabolic products, drugs, alcohol and other harmful substances. As the largest gland in the body, in 24 hours it produces almost a litre of bile and thus assists the body’s digestive system. The organ serves to store glucose and vitamins and also produces vital proteins such as the blood clotting factors. The liver is furthermore characterised by its unique ability to regenerate itself almost completely after damage by injury or toxic agents. Hepatocytes express more genes than most other types of tissue in the mammalian organism. They therefore have a very wide range of enzymes and metabolic networks.



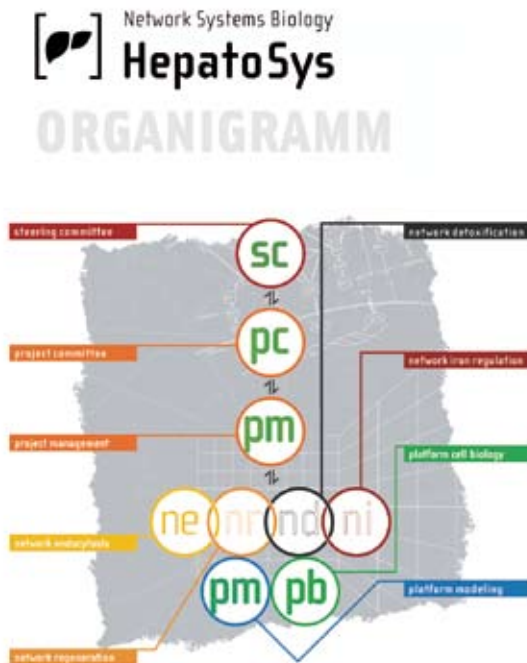
Othmar Pfannes, Genedata AG Basel

“In the decades to come we will see many innovations that are based on systems biology research. These novel products and processes will have a global impact on our way of life. Information and communication technology in particular will have to solve enormous challenges, but also benefit from very promising business opportunities. As a growing company with global focus, Genedata concentrates on establishing a strong position in this emerging systems biology based market. The Hepatosys project is a key element in this strategy.”

The HepatoSys network of expertise investigates regeneration, differentiation, endocytosis, detoxification and the iron metabolism in liver cells. To this end, experimental research teams from biology, chemistry, pharmacology and medicine work hand in hand with representatives of theoretical physics, mathematics and with computer scientists and engineers. The long-term objective is to create a model for predicting vital processes in the liver, which would represent an enormous increase in knowledge for medicine and pharmacology. Drugs can be developed more efficiently and economically with the aid of such tools. In silico models open up new possibilities of individualising treatments and significantly reducing the number of animal experiments in drug development.

No content without structure – the development of HepatoSys

During the first funding phase of the HepatoSys consortium from 2004 to 2006, attention was initially focused on creating a functioning infrastructure. Today, more than 40 teams work in HepatoSys in the four regional networks of detoxification, iron metabolism, endocytosis and regeneration in addition to the two platforms of cell biology and modelling using comparable cells according to jointly agreed lab protocols.



The important results, laboratory regulations and background information are made accessible to all members of the network by means of central data and knowledge management. The second funding period started at the beginning of 2007. Until 2009, all activities are focused on result-oriented research.

The project is monitored by an international panel of high-calibre experts who provide valuable stimulus for further developments. HepatoSys is steered by a project committee consisting of the coordinators and representatives of the networks and platforms. The project committee takes up the recommendations of the steering committee and is responsible for implementing the milestone planning. The project committee coordinates the interdisciplinary collaboration, organises the dates and keeps itself informed of the scientific progress made in the consortium. In order to handle the wide range of organisational tasks, the project committee has a central project management unit. In addition, each network and platform has its own local project management.

The other side of the fence

There is a very high level of knowledge transfer in the HepatoSys consortium due to the interdisciplinary collaboration and the numerous external cooperation. A steadily increasing number of companies are joining the association. This development is encouraged by the fact that the regional networks are located at important biotechnology sites throughout Germany. For example, the regeneration network has its main sites in Freiburg and Heidelberg in the immediate vicinity of the world-famous hospitals in Heidelberg and Freiburg and also close to BioValley e.V., an association bringing together industrial companies and research institutions in Germany, France and Switzerland. The detoxification network maintains close contacts with the process engineering industry in the Stuttgart region. The iron regulation network collaborates closely with the Charité university hospital in Berlin and the Heidelberg university clinic.

The network of expertise is also very well known outside Germany. In 2006, HepatoSys attracted international attention as the organiser of the first “Systems Biology of Mammalian Cells” (SBMC) conference. At the beginning of 2008, the Health Programme of the European Union granted funding for a HepatoSys project devoted to studying cancer of the liver. This project, which will be starting in October 2008, means that the HepatoSys network of expertise is extending its activities on the European level.

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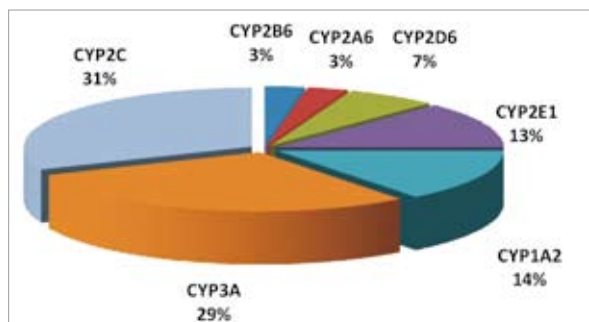
Robustness of the Drug Detoxification Metabolism in Liver Cells

Compensation for genetic polymorphisms and environmental influences in degrading xenobiotics in the liver

Humans and animals are subjected to permanent exposure to different kinds of xenobiotic substances, including plant toxins, medicinal drugs, and environmental poisons. In vertebrates, the liver has the task of making these substances water-soluble and thus preparing them for excretion. In hepatocytes, this process consists of a complex sequence of reaction steps (biotransformations) that are catalysed by an extensive enzyme system, in particular by the cytochrome P450 monooxygenases (CYP450).

Caused by a number of variations in genetic make-up, so-called genetic polymorphisms, and as a result of illness or environmental factors such as the intake of food and drugs, these enzymes exhibit a pronounced interindividual variability in their expression and functionality, in other words in how much of the effective enzyme is available in the cell.

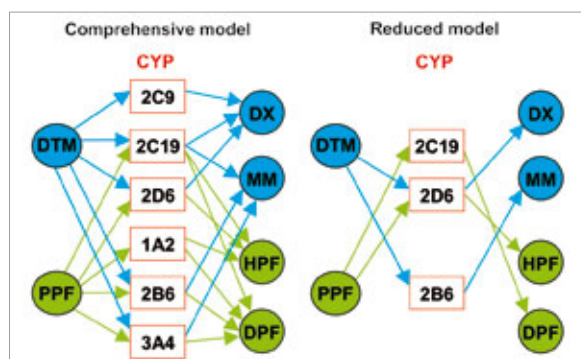
So that the metabolism of xenobiotic substances progresses smoothly, it must be “robust” compared to the individual factors of influence. Robustness is a central concept in the systems-theory analysis of networks. It describes how well a system can compensate for disturbances that have either been caused internally, for example as a result of mutations, or externally, for example through environmental influences. Experimental and theoretical groups within the HepatoSys Competence Network are working on our project, which aims to mathematically model and simulate the detoxification metabolism of drug substances. As part of this project, we are studying how the large variability of the CYP450 system is compensated.



In the liver, cytochrome P450 monooxygenases are responsible for the degradation of xenobiotic substances. Here, the percentage distribution of different CYP450 agents is shown.

The metabolic network structure

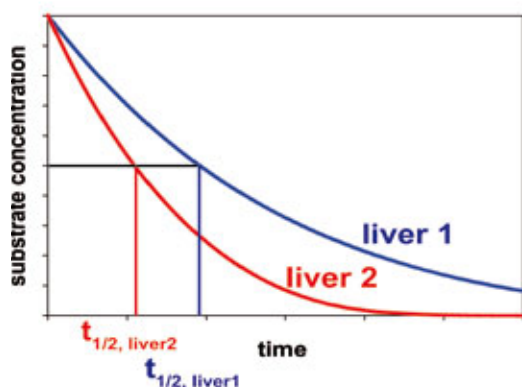
For the characterisation of the catalytic properties of the CYP450 system, we chose dextromethorphan, a cough suppressant, and propafenone, an anti-arrhythmic agent as model substances. In order to identify the CYP450 variants involved in converting these substances, we first conducted activity measurements of recombinant enzymes. On this basis, we established the topological structure of the metabolic system: it is a multi-reactive system in which individual isoenzymes have an overlapping substrate specificity.



Model structure of the drug detoxification metabolism of the substrates dextromethorphan (DTM) and propafenone (PPF) for the phase I metabolites and CYP450 enzymes involved in the complete isoenzyme model (left) and in the reduced model (right).

Both of the model substrates were degraded by a number of enzyme variants. This redundancy is extremely important for the robustness of the system. It increases the number of possible degradation pathways and reduces the risk that the loss of an individual CYP could endanger the functional performance of the degradation. This becomes clear through a comparison with a reduced model in which each reaction step is only realised by the most active “master” isoenzyme. In this case, the loss of an individual enzyme would have a more drastic effect on the degradation of xenobiotic substances.

In order to identify the parameters of the mathematical model, we conducted experiments on microsomal fractions, membrane-limited vesicles from human liver tissue, in which CYP450 agents were anchored. We determined the formation rates of the degradation products for different starting



The inter-individual differences in the CYP isoenzyme concentration is reflected in the half-life for substrate degradation. The example shows the simulation profiles for two different livers.

concentrations of the model substrates - individually or in combination. On the basis of these data, model parameters such as the maximum enzymatic degradation rate were estimated with the aid of what is known as an evolutionary algorithm.

The results show that the parameters - comparable with the interindividual differences in CYP concentrations and activities - may vary strongly in the isoenzyme model. Despite high variable parameters, a constant good adaptation was achieved, which can be interpreted as the maintenance of the functionality of the drug detoxification metabolism.

Robustness with respect to inter-individual variability

Using model simulations of substrate degradation, we investigated how well protected the CYP450 system is against inter-individual variability. In order to do so, we selected the data of 150 individual isoenzyme concentrations in the liver cell from a comprehensive liver bank and integrated them into the reaction kinetics in the model simulations.

The following is true of the robustness of the metabolism of drug substances: the smaller the deviations of the half-lives of the substrate degradation, the more robust the system. It was shown that the complete isoenzyme model exhibited a lower variance of half-lives than the reduced model, which lacked isoenzymes that appeared to be less important but were critical for redundancy. The redundancy of the isoenzymes therefore represents a decisive factor for the compensation of individual differences.

A variety of possible applications

That the CYP450 system represents an extremely diverse and robust chemical defence system is not

new. The advantage of systems biology analyses lies in the fact that many different conditions can be simulated with the models created. In the future, this could help us to gain a better understanding of the circumstances under which the robustness of the system is no longer sufficient to perform an adequate detoxification, or to simulate the degradation of new substances under different conditions in early phases of drug development.

Furthermore, we want to link other systems biology model assemblies, which contain the gene regulation of the CYP450 enzymes, or relevant aspects of the central carbon metabolism of the cell, and thus achieve a comprehensive modelling and simulation of the degradation processes of drug substances in the liver cell.

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Prof. Ulrich Zanger is head of the research field of Molecular and Cell Biology at the Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology in Stuttgart.

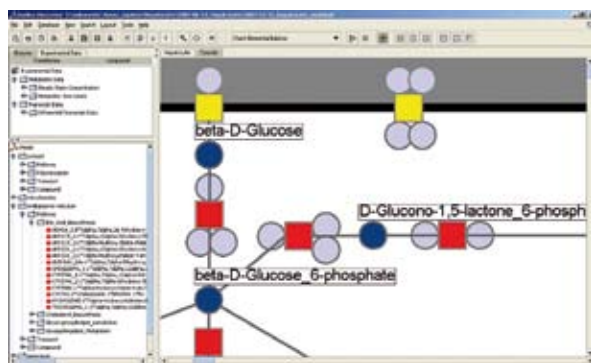
For the last 20 years, he has been working on drug metabolism and genetic polymorphisms.

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A Circuit Diagram for Biotransformation

Dynamic flux analysis of the central metabolism in human hepatocytes

The enzyme system of the central metabolism plays an important role in the functioning of an organism. It supplies energy equivalents that make it possible for vital processes to occur and it also allows the biotransformation of endogenous and foreign substances so that these can be eliminated from the body. The mathematical description of this enzyme system represents a decisive basic principle for predicting the dosage of medications. The objective of our research project is therefore to create a type of circuit diagram that quantitatively records the transformation of the components involved in the central metabolism. Coordinated by Insilico Biotechnology AG, Stuttgart, a team of chemists, biologists, engineers, and computer scientists analyse the activities of the central metabolism and integrate the data into a computer model before they simulate the behaviour of the metabolism with the aid of supercomputers.



Metabolic network of human hepatocytes (circles symbolise metabolites, enzyme-catalysed reactions are represented by rectangles). From the modelling and simulation environment, precise mathematical equations for the components are formulated, evaluated and compared with experimental data.

Following metabolic pathways

We have already succeeded in integrating a few hundred enzyme-catalysed reactions and more than 400 metabolites, in other words the degradation products and intermediate degradation products of biochemical metabolic processes, into the model system. In quantifying intracellular metabolite concentrations, it is essential that the metabolism of cells be stopped immediately after sampling. This is the only way of ensuring that our

results reflect the actual situation at a selected point in time. For this purpose, researchers at the Institute of Biochemical Engineering in the University of Stuttgart apply an ingenious procedure which involves briefly treating hepatocytes that have been quickly separated from the medium with water at a temperature of over 90 degrees Celsius. This inactivates enzymes ensuring that the metabolites are not degraded further. With the heat treatment, we simultaneously achieve a cell disruption that releases intracellular metabolites.

Following this, colleagues in the Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, determine the amount of metabolites in the samples. In order to do so, they combine gas chromatography and high performance liquid chromatography with mass spectrometry. The highly sensitive measuring procedure does not just guarantee a precise determination of the metabolites; it also allows the detection of isotopes, which are labelled compounds that only differ from unlabelled compounds by their mass numbers. In this manner, it is also possible to investigate conversions in parallel metabolic pathways.



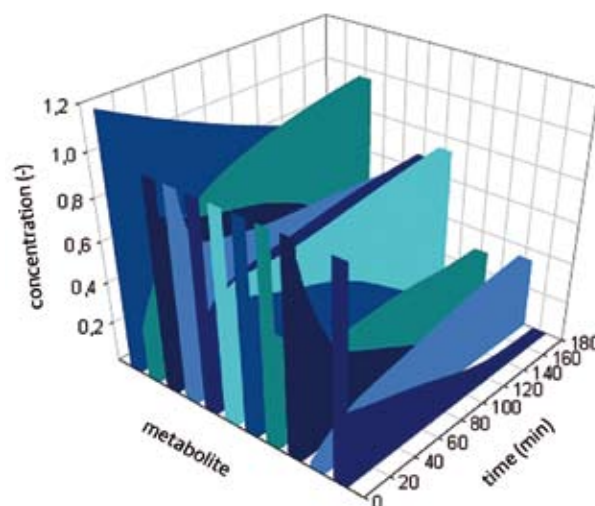
The determination of intracellular metabolite concentrations requires precise analysis. A combination of high-pressure liquid chromatography and mass spectrometry is, for instance, used for this purpose.

For example, we use glucose labelled with ^{13}C carbon atoms and trace the distribution dynamics of the heavy isotope in the glucose degradation products. Computer simulations on the basis of these studies then allow us to calculate metabolic fluxes in parallel pathways and reaction cycles. Using this method, we succeeded for the first time in determining the production rates of NADPH - an important cosubstrate for medication degradation in the liver - in hepatocytes using the pentose phosphate pathway. We now know that NADPH is not a limiting factor for the application and disposal of therapeutic agents.

Metabolic dynamics after the administration of medication

During the first project phase in the period 2004 to 2006, the focus was on the analysis of stationary metabolic fluxes in the central metabolism of hepatocytes. On this basis, we will direct our attention in the second phase from 2007 to 2009 to the metabolic dynamics after the administration of cholesterol-lowering drugs. The aim is to describe the effect of these therapeutic agents mechanistically on a metabolic level and to predict the influence of the medication dosage. We want to know how the enzymes involved control the production of cholesterol and how side effects can be avoided. Furthermore, we are interested in the effect the substances have in people with different genetic backgrounds so that we will be able to take steps towards creating individualised treatments. In this way, we are making an economically relevant contribution to reducing the amount of time and the high costs involved in studies that aim to determine the appropriate medication dosage.

In cooperation with other partners in the Network Detoxification from the Stuttgart group involved in HepatoSys, we are working on a model system which can be used to predict the optimum dosage of medication using a computer. The first step requires experiments for recording the dynamics of the central metabolism after the administration of the active ingredient and then imaging them using the computer. Since the kinetic parameters for the majority of the enzyme reactions involved are unknown, we use sophisticated estimation



Computer simulation of the time course of selected metabolites after excitation of the system by a sudden reduction in the external nutrient concentration.

procedures known as evolution strategies in order to determine the unknown parameters. The results previously obtained with this procedure are encouraging. With one of the first dynamic models, we thus succeeded in achieving good agreement between the simulated and measured metabolite concentrations. This model is available to HepatoSys project partners so that further models can be incorporated.

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High Tech for Liver Cells

Automated image analysis of endocytosis

As part of the HepatoSys initiative, the Endocytosis Network (EndoSys) focuses upon analysis of endocytosis and its influence on signal transduction using systems biology. The members of the consortium, for instance, investigate the formation of vesicles (cell compartments enclosed by the cytoplasmic membrane with which membrane proteins or nutrients are ingested into the cell), and how the vesicles are transported within the cell. These investigations produce large quantities of very heterogeneous image data, such as two- or three-dimensional microscopic images of hepatocytes and their components, as well as simulated image data of different biological processes. Relevant parameters for investigation are simultaneously determined using both experiments and simulations.

As the technology partner of the HepatoSys network, Definiens AG Munich has the task of generating automated image analysis so as: to enable these heterogeneous datasets to be combined; to generate parameters from the experimental image files; and, conversely, to produce simulated images from the experimental measurements.

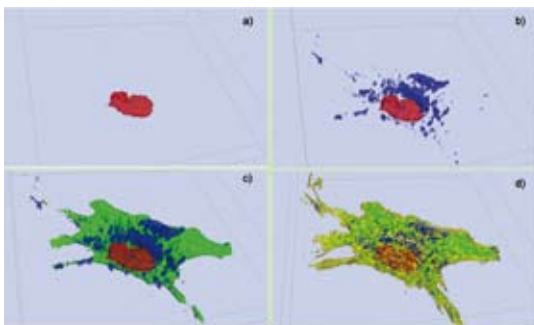


Image analysis of three-dimensional (confocal) pictures of a hepatocyte based upon Definiens Network Technology (red: cell nucleus; blue: marker; green: cytoplasm; yellow: cell boundary for the membrane representation; image data: Steven Dooley).

Recognition technology

Originating from the idea of mapping elementary mechanisms of human perception simply and naturally into an image analysis process, Gerd Binnig and his team developed Definiens Cognition Network Technology. It is based upon the concept of information processing through cognition networks. This image analysis process involves gen-

erating semantic networks that describe objects. In simple terms, a cell, for example, is represented together with its properties such as size or shape, and these attributes are linked hierarchically (“the cell is large, elongated and granulated”). These hierarchies are then in turn linked and they collectively form a network - the cognition network.

Considering the example of a liver cell, imaged with the aid of a confocal microscope, the network has the following structure: the lowest level contains pixels as objects, which are combined on the next higher hierarchical level to form larger units such as cell nuclei, endosomes and cytoplasm; these are further combined into objects representing individual hepatocytes, which are finally combined into groups of liver cells. This procedure can be further continued, for example, by combining clusters of cells into organs and organs into organisms. If the necessary data is not present in a single image, it is possible to relate the contents of several images. Metadata such as measurement information can also be utilised.

From the image to the parameter and simulation – and back again

Cognition Network Technology can be used to analyse both image data (e.g. three-dimensional images of hepatocytes) and results from modelling and simulations (e.g. the modelling of endocytotic processes).

In the case of microscopic images, information is recorded such as the following:

- **the segmentation and classification of individual hepatocytes, cell nuclei, vesicles, endosomes and cytoplasm;**
- **the detailed description of hepatocytes and their contents;**
- **the quantitative description of the mutual relations between the objects involved, such as the distance of endosomes from the cell nuclei.**

In simulations like those involving vesicles during endocytosis, point coordinates are available which describe the image e.g. its extent and orientation in space. Similarly, data from

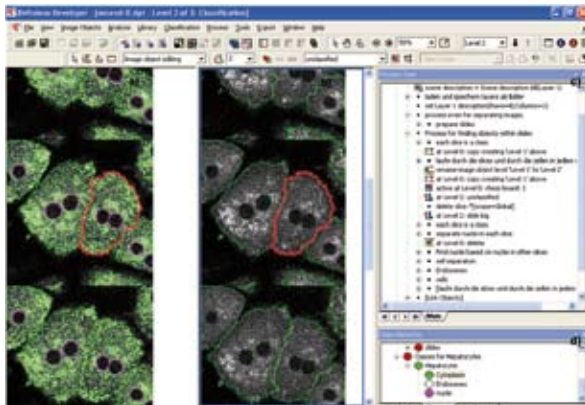


Image analysis of three-dimensional (confocal) pictures of hepatocytes (white: individual endosomes; magenta: cell nuclei; green: cytoplasm; image data: Marino Zerial).

experiments and statistical analyses can be used to compile simulated images.

Since information from “real” and simulated images and also experimentally-obtained data can be processed at one common level, these different types of information can be interconverted e.g. information from a confocal image can be converted into experimental data in order to produce a simulation. Experiment, modeling and simulation can be linked in this way and the parameters resulting from the analysis can be used to optimise experiments and models.



Image analysis of three-dimensional simulated image data. The figure shows a time sequence from the development of vesicles during a simulation. Each individual vesicle is represented by a different colour. In the Definiens image analysis platform, images are automatically generated from the point coordinates of the simulated vesicles and then analysed via Cognition Network Language (CNL) rules (Simulation: J. S. McCaskill).

Large volumes of data and various types of cells

In order to investigate the endocytotic processes in liver cells, Endocytosis project partners have developed assays that have to be performed in extensive screening programmes, thereby generating large quantities of data sets. The algorithms

developed by Definiens are used to analyse these data sets, to record them quantitatively and to extract information from them. The algorithms, which were first developed for small volumes of data, can then be adapted to the requirements of large volumes of data. Benefits of this approach using Cognition Network Technology include: good transferability and high precision in applying the image analysis approach to a large number of image files; full-automation; user-friendly software that is easy to operate; and an implementation that is flexible and adapts easily to changing usage patterns. The algorithms that Cognition Network Technology employ for image and table analysis will in future also be used to investigate other cell types.

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The Endocytosis Transport System

Molecular switches control material transport inside the cell

Endocytosis is a central cellular process in which membrane components and dissolved substances are taken up by the cell surface. In this process, the cell membrane folds around the object thus forming vesicles which transfer their cargo to a set of intracellular membrane compartments that constitute the endosomal transport system. Depending on the purpose, the endocytosed cargo, it is either recycled or degraded within the cell. Endocytosis controls processes such as nutrient uptake, protein transport within the cell and the signalling response to growth factors and hormones. Diseases such as Alzheimer's, asthma or viral and bacterial infections have been associated with defects in this transport system, which makes endocytosis important and interesting from a biomedical perspective.

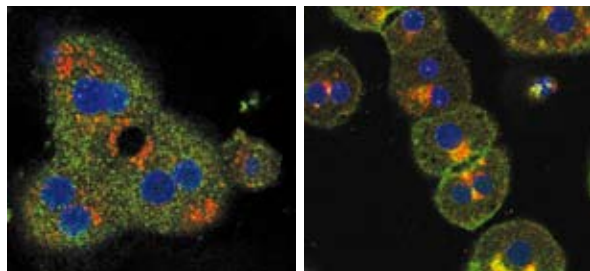
To date, the mechanisms underlying endocytosis remain largely unexplored. At present, there is no possibility of predicting the course of endocytosis under different physiological and pathological conditions. Within the general frame of HepatoSys, the aim of the EndoSys network is to analyse endocytosis and its influence on cellular signalling networks by a systems biology approach, focusing on liver cells. The ultimate goal is to develop both specific mathematical models and a general simulation platform. This will serve for a quantitative prediction of endocytotic processes and signal transduction in hepatocytes under predefined physiological or pathological conditions.

Simulation platforms for endocytosis

In systems biology, the analysis of endocytosis in liver cells presents us with entirely new challenges. Current studies demonstrate that molecular reactions as well as changes in transport and shape in cellular compartments such as endosomes are closely coupled. The necessary integration includes chemical activities on several spatial levels - starting with individual molecules, via supramolecular processes, up to the dynamics of compartments, such as protein sorting by vesicle budding and finally the entire cell.

In order to improve our understanding of these processes, within the EndoSys network at the Ruhr University in Bochum we are developing a novel hierarchical simulation platform. Complex objects –

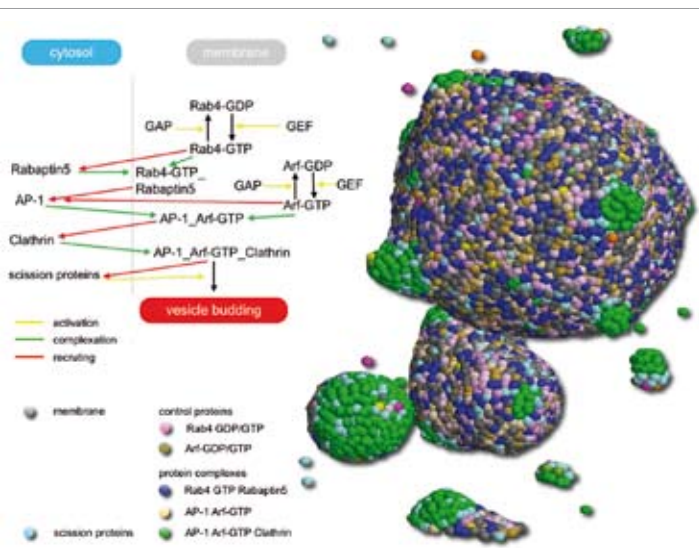
for example, collections of proteins at the molecular level and surface elements of vesicle membranes at the next higher level – are regarded as a hierarchy of container systems. The individual containers are loaded with molecular structures of the next lower level. The simulation therefore bridges the gap between molecular processes such as the interaction of proteins on the vesicle membrane and the dynamic processes on the level of complete endosomes, for instance for the processes of membrane deformation, membrane fusion and protein exchange. For the first time, a systematic and molecular-based simulation platform has been established combining the chemical kinetics and physical self-organisation of structures for a spatially and temporally resolved investigation of cellular processes. This method will be of major importance for future computer-based studies of endocytosis in liver cells in systems biology but the simulations can also be systematically adapted to many different problems.



Primary mouse hepatocytes with endosomes under the microscope. Early endosomes are stained green, late endosomes red and the cell nuclei glow blue.

New organisation principle

To gain a full understanding over and above this, we must also unravel interested in the precise molecular mechanisms underlying endocytotic material transport. To this end, at Dresden University of Technology we have translated the molecular switches that regulate the transport between early and late endosomes into a system of partial differential equations. These can be used to represent, for example, the concentration of typical key regulatory proteins – Rab5 for early endosomes, Rab7 for late endosomes – as a function of time and position on the vesicle membrane.



Simulation (mprDPD from BioMIP) of budding vesicles in liver cells. The model shows the self-organisation of different proteins and membrane lipids which induce cascades of protein recruitment processes. The coat protein complexes (green) show the formation of distinct domains and the budding of new vesicles.

Rab5 and Rab7 are molecular switches that can each recruit a specific ensemble of partner effector proteins which undertake different tasks in the sorting, recycling and degradation of transported material up to and including vesicle movement and deformation. Fluorescence microscopy investigations at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden have shown that absorbed material of endocytosed cargo destined for degradation is first concentrated in Rab5 endosomes and then collected and transferred to a Rab7 endosome. In this process, Rab5 appears to play two conflicting roles. On the one hand, the protein controls the accumulation of cargo by fusing several Rab5 vesicles. This requires a sufficiently high Rab5 density on the vesicle membrane which is regulated via a positive feedback mechanism.

With the aid of simulations, we sought an answer to the question of which organisation principle enables Rab5 to best fulfil its task in accumulating material before the protein is displaced from the surface of the vesicles. The model analysis supplied an astonishing answer. Rab5 does not “defend” itself against its supposed opponent Rab7 but rather actually activates the Rab7 protein. As a consequence of vesicle fusion, the density of Rab5 increases with time, which initially promotes the accumulation. However, at the same time more Rab7 is also recruited on the membrane – until it displaces its predecessor Rab5 through a negative feed-back mechanism.

By a combination of modelling, model analysis, simulation, living cell microscopy and observation of individual endosomes in image sequences we succeeded in unravelling the organisation principle that enables the directed and effective transport of material via the endocytotic pathway. We term this principle the “cut-out switch”, and it may also play a part in other biological contexts.

Our simulations and the detailed investigation of the mechanisms and organisation principles of endocytosis will make a contribution towards a better understanding of this phenomenon. On this basis, it is possible to identify new targets for treating such diseases as Alzheimer’s, asthma, bacterial or virus infections such as tuberculosis, HIV and flu and even cancer, in which endocytosis plays a decisive part.

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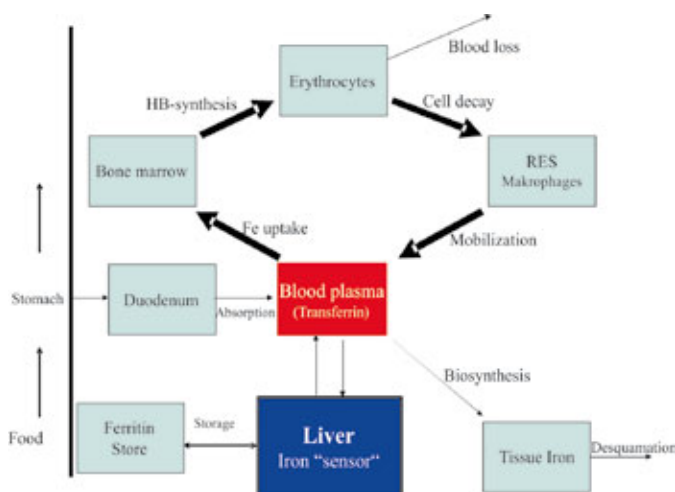
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Iron Network

Systems analyses of iron metabolism in the liver

Ionic iron is an essential trace element but also a dangerous poison. Ionic iron mediates the electron transfer during cellular respiration, as required for energy supply to the body. Moreover, it is indispensable for detoxification of foreign substances by the liver. Above all, however, iron is an important component of hemoglobin, the red blood pigment, without which oxygen could not be supplied to the body's organs.

Iron depletion - as a result of illness, of unbalanced diet, or during growth phases and pregnancy, as well as after repeated blood donation - is therefore a serious health problem which afflicts about 500 million people world-wide. However, excess of iron is also problematic, for example in patients who due to certain other diseases depend on regular blood transfusion therapy, or even in the case of a certain genetic disease, an iron overload disorder, which causes excessive accumulation of iron in the liver, and can lead to liver cirrhosis, liver cancer, and ultimately even to death. Nowadays, iron can be flushed out by application of certain drugs but then again an iron deficiency must be avoided.



The iron storage metabolism is controlled by a complex system. The figure shows the flowchart of body iron. The thickness of the arrow symbolises the conversion rate (small arrow: 1–2 mg iron per day; thick arrow: 20–30 mg iron per day; Hb: haemoglobin, red blood pigment; transferrin: iron-transport protein; ferritin: iron storage protein)

Leroy Hood, President of the Institute of Systems Biology in Seattle

“The new era of predictive, preventive and personalised medicine made possible by systems biology represents a radical change in medicine and will have an impact on many aspects of our lives.”

The liver as control centre of iron metabolism

Iron metabolism is therefore of central significance. It is controlled by a complex regulatory system that steers absorption, distribution and excretion of the trace element. The intestinal tract, the liver, the spleen, several kinds of macrophages and also the muscular system play a key role in this system. A special coordination task is performed by the small intestine as the organ that absorbs iron, and the liver as the control centre. The liver has sensors for the iron requirement of the entire organism and sends an appropriate dose of the peptide hormone hepcidin as a signal to the small intestine and macrophages, which fine-tunes absorption and distribution of iron in accordance with the overall requirements of the organism.

The “IronLiver” collaborative project combines a theoretical working group (Max Delbrück Centre, Berlin) with two experimental working groups (EMBL and University Clinic Heidelberg). Their objective is to study the regulatory processes of the iron metabolism in more detail. We are developing a computer model of iron regulation which reflects the interactions of the liver with other organs of the body in the form of a dynamic network integrating absorption, transport, inter-conversion and excretion of iron-related proteins.

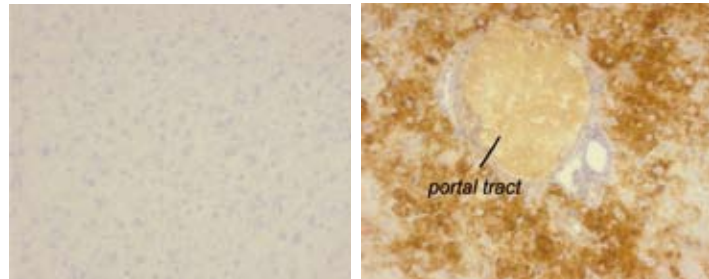
The mouse as model animal

We use mice for the physiological studies - the basis for developing the model. Using genetic modifications, we selectively inhibit or switch off certain components in the iron metabolic system. We then analyse the iron content in the various organs involved in the network in these animals, for example in the liver and the intestines, as well as in the blood. In this way, we elucidate the regulatory roles of the components of the iron system. This will allow us to modify, for example, the ferroportin gene, which codes for the iron transport protein at the entry port from the intestine into the blood, such that it can no longer react to the hepcidin signals from the liver. This will result, of course, in iron excess of the body. It is of great interest to discover how the iron metabolism system and the complete organism of the mouse react to this drastic perturbation of the system.

The physiological data acquired from the experiments with genetically modified animals are incorporated into a flux model. Basis of comparison is a model of iron content and distribution rate of the healthy mouse which we have already developed.

In parallel to our work on genetically engineered animals, we also prepare an analogous model for humans. The basis here is the flux model of the mouse into which we feed literature data on human iron metabolism. The aim is to qualitatively and quantitatively simulate the physiological human iron turnover, as well as its pathological deviations - on the basis of interactions between the levels of cellular and organismic system hierarchy.

This type of overall model serves as basis for a detailed study of iron-related human diseases. It is also hoped that it can be used for computer-controlled therapy planning in conditions of either iron deficiency or iron overload. With the aid of computer simulations, it could become possible to wash out or replete iron as required – thus avoiding excessive as well as insufficient dosage of iron.



Liver tissue of a healthy mouse (wild type, left) and of a knock-out mouse (right), where the *Hfe*-gene that is involved in the production of the iron sensor Hepcidin is switched off. If the gene is absent or damaged, an iron storage disease appears. The iron uptake of the duodenum is out of control and a deposit is build in the liver (brown colour).

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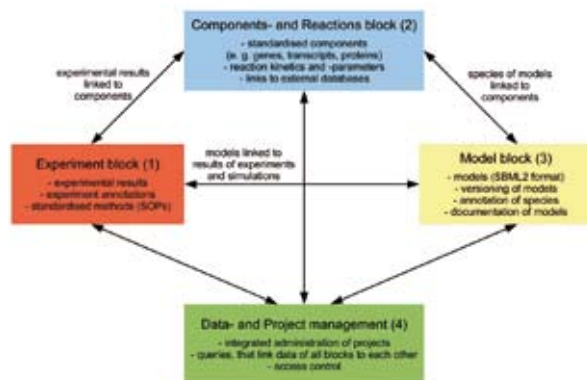
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Central Data Management

The scientific communications platform of the HepatoSys consortium

The main aim of the HepatoSys network is to use the methods of systems biology to achieve as comprehensive an understanding as possible of the cellular processes in hepatocytes. This requires close interdisciplinary collaborations between scientists from widely differing disciplines. More than forty groups from universities, clinics and other research institutions and industry throughout Germany conduct research within the framework of a large alliance. In order to allow the various teams to collaborate efficiently, a central infrastructure is required to collect essential research data and allow it to be exchanged between the groups. This function is fulfilled by the central data management system.



Design of the central data management system: the data within (1) the experiment block, (2) the component and reaction block, and (3) the model block are linked to each other and centrally managed and retrieved.

In the planning phase of HepatoSys, it had already become clear that the volumes of data generated in the research network are considerable, particularly as a result of the application of high-throughput processes. The central data management system therefore had to be designed in such a way that it could scale with large volumes of data.

Furthermore, the central storage of the data in a relational database was essential, as was the systematic structuring and integration of different types of data on the level of the gene, RNA and protein, and also tools in order to biologically interpret the various types of data in the context of the liver cell. The concept of the central data management system was developed by the members of the HepatoSys consortium and then put

into practice by Genedata, a company providing computational systems for life sciences research. The system is administered by the coordinators for central data management within the HepatoSys consortium at the Max Planck Institute for Dynamics of Complex Technical Systems in Magdeburg.

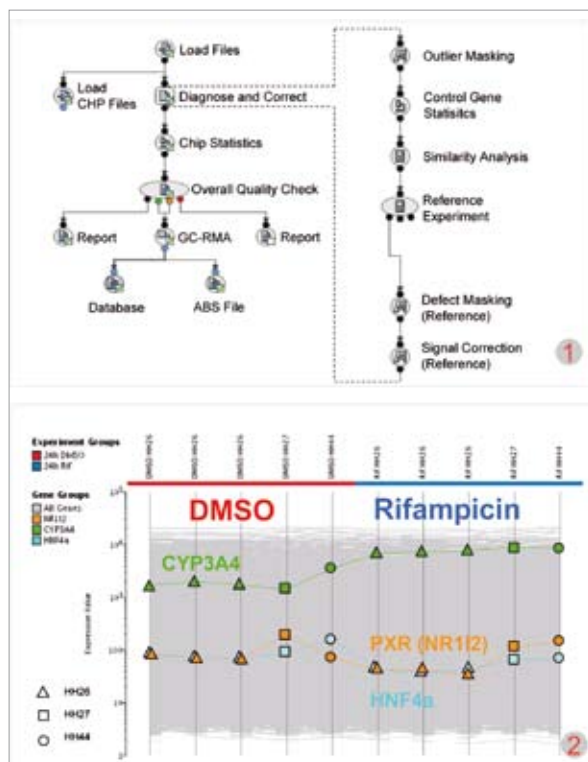
Three components for data management

A significant function of the central data management system is to create a joint communications platform for the partners in the research network through which they can exchange data, findings and information on models. To this end, the application was installed on a central server at the Max Planck Institute in Magdeburg. All HepatoSys groups have password-protected access via the Internet. The system is composed of three modules:

- **The experiment block allows experimental findings obtained within the consortium to be stored centrally.**
- **The component block is used to store information on the genes, mRNAs, proteins and signalling pathways under investigation.**
- **In the model block, in silico models of the simulation of the dynamics of the metabolic and signalling pathways in the liver cells are stored, linked with the individual components and exchanged between the HepatoSys partners.**
- **While the experiment and component blocks are based on software already available from Genedata, the model block had to be developed from scratch.**

Data management for the systems biology research process

Systems biology research requires the definition and application of new standards, such as standard operating procedures (SOPs) for *in vivo* experiments, the unified processing and normalisation of data, and the introduction of joint data formats within the research consortium. The central data management system is therefore



Database in use: a screenshot of the user interface shows the gene expression of hepatocytes stimulated with rifampicin from three patients (HH26, HH27, HH44). (1) The raw data were loaded, processed and the quality of the data was evaluated. (2) The data were then analysed further with the functions of the data management system. The analyses show that the expression of the gene *CYP3A4* is much higher after treatment with rifampicin, while the other genes, for example from *PXR(NR1I2)* and from *HNF4a*, remain largely unaltered. (The diagrams were provided courtesy of Thomas Reichart, ITB, University of Stuttgart).

based on established IT Community Standards, such as Systems Biology Markup Language (SBML) for the exchange of mathematical models that represent molecular biology cellular processes.

The central data management system also has analysis software for processing systems biology data and for interpreting data. Of critical importance here is the automated, computer-assisted data quality and consistency control. Only after quality control and the subsequent steps for normalising and standardising the data, can the different types of biological data be compared to each other. For instance, this allows an informative comparison of the expression of the gene that codes for a particular enzyme with the intrinsic enzyme activity.

The systems biology investigation into liver cells also requires tools for the analysis and interpretation of data within a biological context. This demands a technology-independent data analysis, which is also performed by the central data management system. Specialised “cross-omics” analyses were developed for this purpose, which help us to analyse and gain a better understanding of signal transduction pathways and the underlying regulation processes in hepatocytes.

Data management in the future

The HepatoSys consortium has taken on a pioneering role in developing and establishing data management software for systems biology research processes in cooperation with Genedata. The central data management system currently supports systems biology investigations into liver cells. The functionalities of the system, however, will also be used to address other challenges in systems biology in the future.

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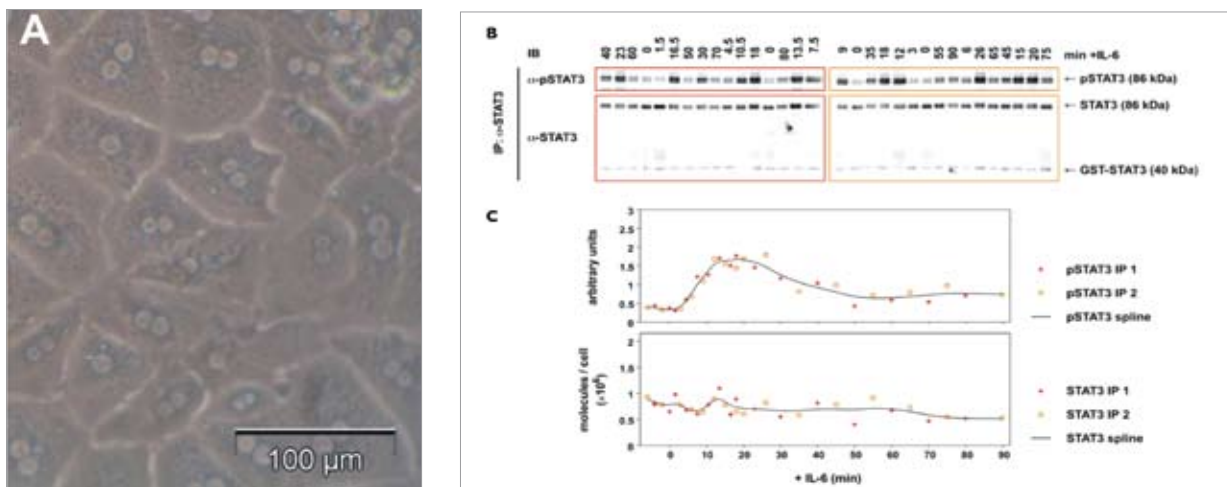
Liver Cells in Culture

Standardised techniques for isolating and cultivating primary hepatocytes

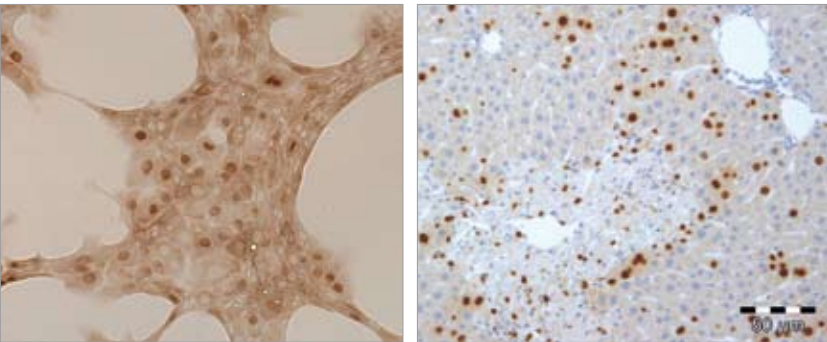
Hepatocytes isolated from liver tissue represent an *in vitro* system that is widely used in the pharmaceutical industry. For example, they are used to investigate the metabolism of a new substance, or to be more specific, how it is converted in the liver in order to be excreted in bile or urine. There is very good agreement between the findings from cell culture experiments and the situation in the human organism here. This was the decisive factor in focusing attention on primary hepatocytes in the systems biology research network HepatoSys. A wide range of prerequisites must first be fulfilled before cells can be used for systems biology investigations. For example, the mathematical modelling of signal transduction networks requires a quantitative and highly reproducible analysis of the signal transducing components and also of their phosphorylation. Against this background, the partners of the cell biology platform and the HepatoSys consortium have developed an *in vitro* system with mouse hepatocytes allowing aspects of the proliferation of liver cells to be investigated - and in such a way that the findings can be applied to the situation in living animals.

Standard requirements for the induction of signal transduction pathways

In order to work in a quantitative and reproducible manner, it is essential that the liver cells first be put into a ground state after they have been extracted and cultured. In this ground state, the signal transduction factors exhibit little basal activity but we should still be able to stimulate them intensively and reproducibly. The cultures are deprived of growth factors for this purpose. This is referred to as cell “starvation”. On this basis, we can use specific simulations to investigate signalling pathways that are relevant for the liver. An example of this process is the phosphorylation of a protein known as ERK1/2 induced by the hepatocyte growth factor (HGF), which can cause the liver cells to divide or increase their motion. A second example is the so-called JAK-STAT signalling pathway, which plays a role in the differentiation and proliferation of cells. Other interesting protein molecules, whose phosphorylation state and activity are important for proliferation and are therefore the focus of our interest, are SMAD, P13 kinase, MAP kinase, NFκB and Wnt/β-catenin.



(A) Primary mouse hepatocytes in a confluent state. (B) If these cells are stimulated with IL6 for example, the phosphorylation of the signal transducing protein STAT3 temporarily increases. (C) This increase can be quantified with the aid of a suitable standard.



Proliferation of hepatocytes *in vivo* and *in vitro*. Cells with brown nuclei (BrdU insertion) have gone through the S phase of the cell cycle and are therefore about to divide. Left: Cultivated hepatocytes 48 h after cytokine stimulation. Right: The culture cells exhibit a proliferation that is comparable with the situation in a mouse liver 48 hours after treatment with the liver toxin CCl_4 .

The use of appropriate standards such as recombinant proteins allows us to calibrate our investigations. This does not just make it possible to determine the proportion of phosphorylated factors but also the number of molecules.

Knowledge and control of interfering parameters, which include cell density for example, are also important for quantitative and reproducible work with this type of system. We discovered that confluent, i.e. densely growing, hepatocytes react in a different manner to stimulation with cytokines than less densely growing subconfluent cells. For example, the phosphorylation of the proteins ERK1 and ERK2 is weaker and briefer in confluent cells after the HGF growth factor has been added. This effect can be attributed to the stronger expression of phosphates in the confluent cultures. In order to be able to generate reproducible results, it is therefore essential that we establish an exactly defined standard protocol for experiments with our cell culture system, which would then hold for all of the working groups involved in the network.

Good agreement with the *in vivo* situation

With the aid of suitable cytokines, such as HGF or the epidermal growth factor EGF, proliferation can be induced in the cell cultures. The percentage of hepatocytes that proliferate in the culture is

comparable with the amount of proliferating cells in a mouse liver regenerating after damage by carbon tetrachloride (CCl_4). The processes of signal transduction also progress in a similar manner in both culture and living animals. We confirmed this using experiments, for example, those with isolated perfused livers - in other words whole organs that were kept in culture. If such an organ is stimulated with the HGF or EGF growth factors, the induced phosphorylation kinetics of factors such as ERK1/2 or the Akt kinase, another important nodal point in the system, are similar to those in cultivated hepatocytes. This confirms that research with primary mouse hepatocytes leads to findings that reflect the situation in living organisms well. The system is therefore suitable for use as an experimental basis for the modelling of signal transduction processes in hepatocyte proliferation. It is applied by the scientists involved in the HepatoSys consortium in accordance with mutually agreed standardised working conditions and culture protocols.

We expect that these studies will lead to a basic understanding of the regulation of signal transduction networks as well as an understanding of the complex and dynamic processes that lead to the decision on whether hepatocytes proliferate or remain dormant. This knowledge provides the foundation for therapies that will improve liver regeneration in the future.

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Feedback for Liver Regeneration

Modelling the control mechanisms for hepatocyte proliferation

The liver boasts the astonishing property of being able to regenerate itself after injury and damages caused by toxic agents so that it is once more in full working order. This process is coordinated by the complex interaction between different growth factors. For the regeneration, differentiated but dormant hepatocytes gain the ability to multiply. Once the liver mass has been restored, the proliferation is disabled in a controlled manner.

A master regulator of this process is the neurotransmitter TGF β . It is responsible for stopping the proliferation of the liver cells and thus preventing excessive growth. An excess of TGF β can lead to inflammatory processes and scarring in the liver right up to fibrosis, a pathological proliferation of the connective tissue, and can thus encourage the formation of hepatocellular tumours.



The generation of quantitative, time-resolved data with the LumilImager. The chemiluminescence detected with the aid of the LumilImager's CCD camera is linear over a large area and therefore particularly accurate.

Interestingly, TGF β is already secreted in an early phase of liver regeneration. This sounds paradoxical and gives rise to the question of what mechanisms are responsible for the fact that hepatocytes can multiply despite TGF β when this neurotransmitter later ensures that the proliferation ends - without the danger of a fibrotic response. We investigate this phenomenon on the basis of data generated experimentally and with the aid of a mathematical modelling of the TGF β signalling cascade.

The best prerequisites for modelling

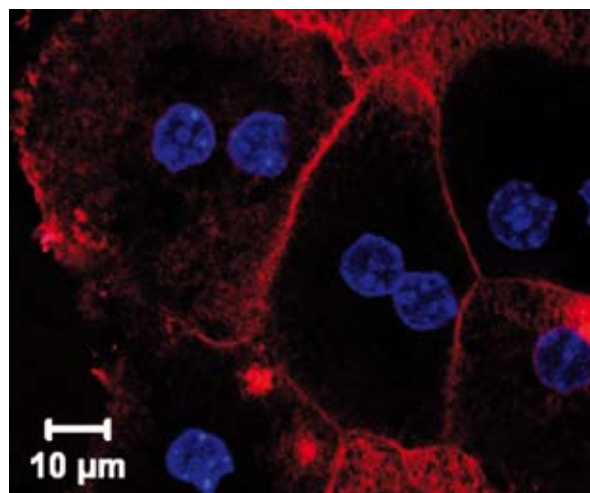
For systems biology investigations, we require quantitative time-resolved data of the highest quality. An important prerequisite for this is working with a standard cell system. In cooperation with other HepatoSys groups, we have developed standard operating procedures (SOPs) with which we can obtain primary hepatocytes from male BL6 mice aged between 6 and 12 weeks as a matter of routine in a two-stage collagenase perfusion. They

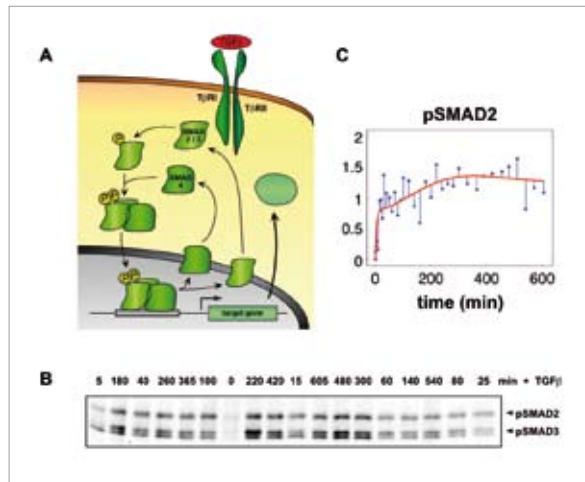
are subsequently cultivated on collagen-coated plates and "starved", in other words, we remove growth factors so that all of the signalling pathways can adopt a ground state. On the first or second day after preparation, the hepatocytes are stimulated with TGF β . They are subsequently lysed at different points in time in order to obtain protein extracts or RNA. We use this standardised sample material to investigate the control mechanisms of the TGF β signalling pathway.

In order to generate appropriate data for mathematical modelling and to quantitatively investigate the TGF β -mediated signal transduction in hepatocytes, we also developed strategies to reduce the errors associated with identifying proteins. One example is that we study the specimens of a time sequence experiment in random order. This prevents deviations, which can occur simply as a result of direct proximity in immunoblotting.

With the aid of GellInspector computer software, the data obtained can be automatically processed and combined. By using the software, we are in a position to produce very extensive data sets for the first time with a high temporal resolution, which represents significant progress for dynamic modelling.

Primary hepatocytes that were cultivated after isolation for 16 hours in collagen-coated cell culture dishes. In order to make the cell nuclei and plasma membrane visible, they were coloured with Hoechst 33342 (blue) and Dil (red) and studied with the aid of confocal microscopy.





Dynamic modelling of the TGFβ signal transduction. (A) Schematic of the TGFβ transduction. (B) Time-resolved analysis of the Smad2 phosphorylation in primary hepatocytes using immunoblotting. (C) Mathematical modelling of experimental data.

The search for feedback mechanisms

The proteins Smad2 and Smad3 are important information carriers in the TGFβ signalling cascade. If TGFβ binds to its receptor on the surface of the cell, these proteins are phosphorylated and they undergo trimerisation with another protein called Smad4, and then migrate to the cell nucleus. A multitude of target genes are activated there, which in turn influence the dynamic behaviour of the signalling cascade. A number of feedback mechanisms for controlling this type of information transfer can be found in the literature. It can therefore be assumed that this type of mechanism also exists for fine-tuning the TGFβ cascade. This is the reason why we stimulate hepatocytes with TGFβ and use microarray analyses to investigate the transcription of the target genes. Genes from potential control proteins, which are more strongly expressed after the TGFβ stimulation, are potential candidates for the fine-tuning of the signalling cascade. The first results suggest that a protein takes over control here, which is something that was not apparent in earlier investigations using the traditional methods of molecular biology.

Quantitative predictions on intervention options

Our data-based mathematical model allows us to investigate processes of the TGFβ signal transduction in silico and to plan promising experiments.

In this way, we have an opportunity of clarifying for the first time the effect that reducing or increasing individual components has on the dynamic behaviour of the entire system. In the long-term, the model will be used to identify approaches that can be used to selectively control the termination of hepatocyte regeneration and to prevent damage.

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Liver Regeneration – A Unique Phenomenon

The regeneration of complex organ architecture in a spatio-temporal model

The liver is a very special organ. It is capable of almost complete regeneration after damage even if more than 50 percent of its total mass is affected. This is of central significance since the liver is a very important metabolic organ – amongst other functions, for the detoxification of the blood. It has a complex anatomy. The lobes of the liver are composed of a large number of lobules with a maximum size of one to two millimetres, which mainly consist of liver cells known as hepatocytes. The special structure of the lobule ensures that during its passage through the liver the blood comes into optimum contact with the liver cells. The portal vein collects the blood and transport it to the liver lobules where it flows through small vessels called sinusoids along one to two cells thick layers of hepatocytes to the central vein.

In liver cirrhosis, caused by drugs, alcohol or virus infections, the complex architecture of the liver lobules is destroyed and the liver can no longer function properly. This also affects the regenerative capacity of this organ. In the case of advanced cirrhosis the only treatment is liver transplantation. Since the transplantation of an organ can involve a wide range of complications there is great interest in developing alternative, drug-based treatments.

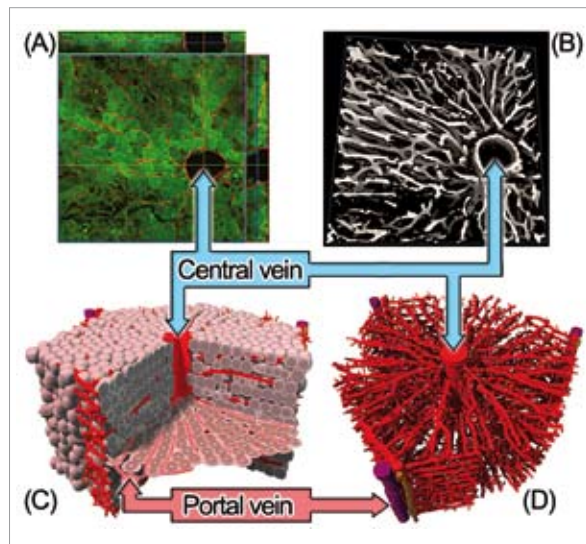
For this reason, it is important to analyse more precisely the processes that take place during the regeneration of the liver on the level of the individual cells. The aim of our project is to reconstruct these processes on the basis of animal experiments and then mathematically model them in order to better understand the underlying mechanisms.

From tissue section to regeneration model

In our detailed investigation of the regeneration processes, we inject mice with the hepatotoxin carbon tetrachloride (CCl_4) by which necrosis of the hepatocytes in the vicinity of the central vein is induced. We then reconstruct the regeneration process from tissue sections, which we evaluate statistically in several image processing steps, and use the data to model the process.

For image processing, we first perform fluorescence staining of the tissue samples according to characteristic cell properties in order to distinguish between hepatocytes and the endothelial cells

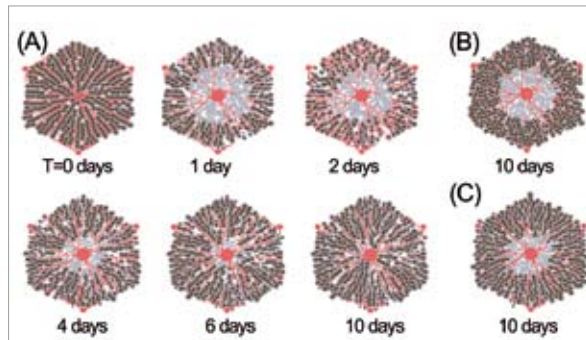
which line the sinusoids. Also those cells which are proliferating can be detected with the aid of suitable staining techniques. In several image processing steps, we then determine the position and size of the cells and blood vessels. We measure parameters such as the radius and density of the sinusoids, the size and direction of the hepatocytes, and also the distance of the hepatocytes from each other and from the central vein.



From tissue section to computer simulation (A) Liver cells around a central vein under a confocal microscope. (B) Visualisation of the sinusoids. (C) Section of a liver lobule in the computer simulation with hepatocytes (pink) and sinusoidal structures (red). (D) Sinusoidal network.

From this information, we calculate a “statistical liver lobule” for the computer simulation. In this simulation, each hepatocyte is represented as an isotropic, homogeneous, elastic object, which interacts with adjacent hepatocytes or sinusoids. This interaction is modelled by forces composed of the forces arising during the deformation, compression and adhesion of the cells. Mathematical equations describe the movement of each cell. We apply the same concept to the modelling of the sinusoid deformations and displacements.

Cell division is simulated in two phases within the model. The diameter of the cell first increases until its volume is doubled. At a constant volume, the cell is then deformed until two separate cell bodies are formed from one cell.



Various models of liver regeneration. Experimental results confirm that complete regeneration requires a reorientation of the daughter cells towards the sinusoids. (A) Complete regeneration scenario after damage with CCl_4 (day 0). If no reorientation takes place (B) or if cell migration is too slow (C), the simulated regeneration is incomplete after 10 days.

In the model, we vary different additional parameters in order to investigate their possible influence on the regeneration process. These parameters include the spatio-temporal cell division pattern, the strength of the cell-cell and cell-sinusoid adhesion, the rigidity of the sinusoids, as well as mechanisms that control the orientation of the daughter cells after cell division, for example, along the sinusoid structures, towards the destroyed area of the liver lobule, towards the central vein, or into a random direction. All the parameters are quantitatively estimated, either directly from the experiments or from the published literature.

Verifying the simulations

The calculated models of liver regeneration are subsequently experimentally validated so that the computer model can be improved in the next step. In this way, we have already been able to rule out an orientation of cell division towards the central vein.

On the other hand, it was possible to experimentally observe another mechanism predicted by the simulations. It is based on the assumption that the sinusoidal cells secrete cytokines, i.e. molecules, which can influence the orientation of the liver cells. In the simulation, the daughter cells re-arrange after cell division in such a way that their connection line orients parallel to the adjacent sinusoid. In this orientation, they

migrate towards the central vein until the destroyed region is completely regenerated.

We are currently working on integrating molecular models of the metabolism into each model cell. It thus becomes possible to directly simulate the influence of regeneration on the liver metabolism. In the next steps, we intend to investigate the role of growth factors in regeneration and also to search for ways of improving the regenerative capacity of the liver by means of molecular agents. It is furthermore planned to extend the simulation model to include the regeneration of the whole liver after surgical removal of a part of it. In future, it will also be possible to use the model to investigate the development of liver tumours from the degenerated cell up to the macroscopic tumour.

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HepatoNet - Modelling the Liver Metabolism

Reconstruction and analysis of the metabolism of liver cells under different conditions

The liver is the central metabolic organ of the human organism and plays a decisive part in the detoxification of ingested foreign substances such as alcohol, nicotine and medicinal drugs. The various tasks are distributed spatially. Depending on their localisation along the bloodstream, the liver cells have different enzymatic properties and metabolic functions. This is known as metabolic zoning. Periportal hepatocytes are those that are located behind the porta hepatis through which the blood enters and they undertake different tasks than the perivenous hepatocytes that are grouped in front of the hepatic vein through which the blood leaves the organ. In this way, the extremely toxic ammonia (NH_3), which arises from the degradation of amino acids, is converted periportally to urea and thus detoxified. Perivenous cells, in contrast, incorporate NH_3 into the amino acid glutamine.

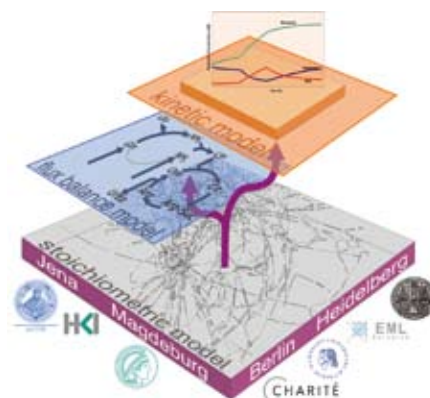
The modelling platform in the HepatoSys consortium, which includes research groups in Berlin, Heidelberg, Magdeburg and Jena, therefore has the objective of mapping all the reactions of the liver metabolism as a comprehensive network in a stoichiometric model. To this end, we are analysing both the structural network properties and static flow distributions and also the dynamic behaviour as a function of different external factors such as diet, pathologies or pharmacotherapy.

Reactions in the network

The metabolic network (HepatoNet) that we are reconstructing in our project currently contains 1139 different substances, which are degraded, converted or synthesised in a total of 1414 reactions. Another 458 reactions describe intracellular transport processes between the different cell compartments and also the exchange of substances with the bloodstream and thus integrate the liver cells into their natural environment. In reconstructing the metabolism, we make use of software (MetAnnoGen) specially developed in the Department of Mathematical Systems Biochemistry at the Institute of Biochemistry of the Charité (University Medicine Berlin), which permits the integration of various sources of information such as biological databases and original publications. In this way – on the basis of genomic sequences, expression profiles and

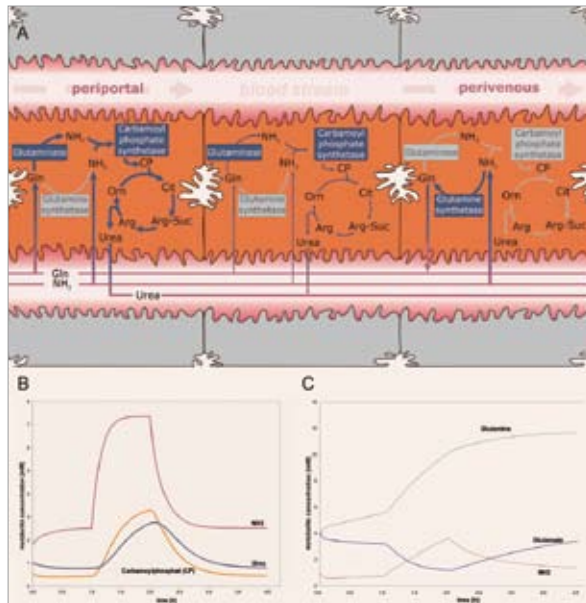
biochemical enzyme assays – we search for evidence of the presence of all known liver cell enzymes.

Starting from the reaction network, we calculate the material flow for each reaction, that is to say the mass transfer per unit time. This results in the flow distribution for the entire network. The network structure itself initially permits a large number of possible flow distributions under physiological conditions. In order to select an optimum flow distribution from the evolutionary perspective, we use an optimisation calculation method, known as a flow-balance analysis (FBA). A suitable criterion is, for example, the economical fulfilment of all liver-specific activities with a parsimonious application of enzymes and nutrients.



Levels of metabolic modelling for hepatocytes. Level 1: Stoichiometric model with comprehensive reaction network of the liver metabolism. Level 2: Calculation of optimum flow distributions in the reaction network. Level 3: Analysis of dynamic behaviour by means of kinetic modelling.

The flow distribution calculated on the basis of this assumption is dependent on the localisation of the hepatocytes relative to the bloodstream and the nutrient availability in the blood, which may vary considerably as a consequence of poor nutrition, hunger or alcohol abuse. Added to this are factors such as the ingestion of medicinal drugs, genetically related enzyme defects or acute liver damage. Using our model calculations we intend to make a quantitative estimate of the impact of such individual influences on liver performance.



Metabolic zonation with the example of ammonia detoxification. Whereas ammonia is converted into urea in periportal hepatocytes, perivenous cells incorporate ammonia into glutamine (Gln). (A) flow distribution; (B) and (C) kinetic simulation of periportal and perivenous hepatocytes.

Simulating the dynamics of reaction networks

In contrast to static flow distribution, changes of the metabolite concentrations and fluxes over time reflect the dynamic behaviour of the liver metabolism. In this way, concentrations of metabolites change after the addition of ammonia – depending on the localisation of the respective liver cells. Whereas the periportal hepatocytes produce increased amounts of urea, in the perivenous region the glutamine concentration rises due to the incorporation of ammonia into the glutamate. In order to simulate such changes over time, kinetic models are indispensable since they permit the mechanism of enzymatic catalysis, which has been elucidated in experiments, to be described as precisely as possible by appropriate formulae. However, at the moment there is a lack of detailed information on liver-specific reaction kinetics for many of the enzymes and transport processes involved. In a novel approach, we therefore intend to identify the reactions which influ-

ence the overall behaviour of the metabolism most strongly and to only set up detailed mechanistic enzyme kinetics for these reactions. The remaining reactions will be described by simplified kinetics.

The aim of this ambitious project is to predict the optimum nutrient supply to the liver even under critical ambient conditions in which all the vital functions of the human organism must be maintained. In future, we hope to be able to develop a computer program that will help doctors to quantitatively assess the severity of a disturbance of the liver metabolism and to select the optimum treatment plan.

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FORSYS

Research Units on Systems Biology

With “FORSYS – Research Units of Systems Biology”, the Federal Ministry of Education and Research (BMBF) aims to expand and strengthen topic-based systems biology research in Germany. Its main objective is to improve the existing infrastructure and thus create the ideal basis for the further development of this pioneering branch of research. Four centres located in Freiburg, Heidelberg, Magdeburg and Potsdam will receive funding for the period 2007 – 2011 amounting to a total of € 45 million. They are heavily integrated into the infrastructure already in place in the local universities and research institutions. An important element in the FORSYS centres is support for young scientists. In addition to establishing Young Investigators Groups, attention will also be devoted to educating and training students and Ph.D. candidates (see table).

FRISYS – Freiburg Initiative for Systems Biology

In their project “Signalling in Growth and Differentiation”, the Freiburg Initiative for Systems Biology (FRISYS) investigates specific aspects of cellular signal transduction in relation to the growth and differentiation of cells. Thirteen working groups are investigating the mechanisms of signal transduction in different prokaryotic and eukaryotic organisms from the animal and plant kingdoms, such as the thale cress *Arabidopsis thaliana*, the moss *Physcomitrella patens*, the cyanobacterium *Synechocystis*, the threadworm *Caenorhabditis elegans*, and the zebra fish *Danio rerio*. They are also using different cell and organ cultures. The variety of the model organisms offers a unique opportunity to investigate the signal-processing systems and their development over the course of evolution.

The FRISYS centre works closely with the Freiburg Centre for Biosystems Analysis (ZBSA). Four other centres are linked to ZBSA, and with their expertise, they contribute to the development of the field locally: the Freiburg Centre for Data Analysis and Modelling, the Bernstein Centre for Computational Neuroscience Freiburg, the Centre for Applied Biosciences, and the Centre for Bioinformatics.

MaCS – Magdeburg Centre for Systems Biology

The Magdeburg Centre for Systems Biology (MaCS) is an initiative of the University of Magdeburg in cooperation with the Max Planck Institute for Dynamics of Complex Technical Systems. MaCS is embedded in the Research Centre for Dynamic Systems in Biomedicine and Process Engineering, which is supported by the federal state of Saxony-Anhalt.

Thirteen working groups, including five Young Investigators Groups, are engaged in MaCS projects. The intensive cooperation between the theoretical and experimental working groups means that molecular cell research is combined with the methods of systems theory and discrete mathematics. The focus of the activities lies on the development of new systems biology approaches and their application for the analysis and reconstruction of molecular networks of signal transduction and regulation on a cellular level. The aim is to establish new, widely applicable concepts for modelling and systems analysis, which work reliably at different levels of molecular and functional complexity.

GoFORSYS: Photosynthesis and Growth – A Systems Biology Approach

On the basis of a cooperation between the University of Potsdam, the Max Planck Institutes (MPIs) in Potsdam – MPI of Molecular Plant Physiology and MPI of Colloids and Interfaces – and Metanomics GmbH as an industrial partner, GoFORSYS unites a unique team of experts in the field of the systems biology of plants in a total of 18 working groups and two Young Investigators Groups. The objective of the initiative is to achieve a better understanding of photosynthesis, its regulation in response to selected environmental factors and the resulting influences on growth. The single-cell alga *Chlamydomonas reinhardtii* has been chosen as a model organism. The evolutionary highly conserved processes of photosynthesis allow the knowledge gained to be transferred to higher plants. In this way, GoFORSYS creates new possibilities for optimising crops.

Centre	Programmes
FRISYS/University of Freiburg	<ul style="list-style-type: none"> • Masters course in bioinformatics and systems biology (from winter semester 2008/2009) • PhD programme with a variety of theoretical and practical courses
MaCS/University of Magdeburg	<ul style="list-style-type: none"> • Diploma course in biosystems engineering (since 2004) • Bachelor course (since winter semester 2007/2008) • Masters course (from 2011) • PhD programme with colloquiums by visiting scientists, workshops and a summer school • Cooperation with the “International Max Planck Research School for Analysis, Design and Optimization”
GoFORSYS/University of Potsdam	<ul style="list-style-type: none"> • Masters programme in bioinformatics with a focus on systems biology (from winter semester 2008/2009) • PhD programme in cooperation with the “International Max-Planck Research School for Primary Metabolism and Plant Growth” • Training for PhD students supported through the Potsdam Graduate School and the Potsdam Welcome Centre
VIROQUANT/University of Heidelberg	<ul style="list-style-type: none"> • Masters course in “Molecular Biosciences” with a major in systems biology (since 2007) • PhD training (in cooperation with graduate programmes offered by the excellence cluster) • Graduate Academy (since 2005)

The training of young scientists is a key component of the FORSYS programme.

All four universities are establishing courses and special PhD programmes.

VIROQUANT: Systems Biology of Virus-Cell Interactions

The Heidelberg research initiative VIROQUANT brings together 31 working groups from the University of Heidelberg and the university clinic, the German Cancer Research Center (DKFZ) and the European Molecular Biology Laboratories (EMBL). VIROQUANT is an integral part of BIOQUANT, the Centre for the Quantitative Analysis of Molecular and Cellular Biosystems at the University of Heidelberg. Furthermore, two industrial partners are involved in the network: the Nikon Imaging Center and the SUN Center of Excellence.

The aim of scientific research in VIROQUANT is the comprehensive characterisation of the cellular networks that are largely responsible for virus replication and propagation in infected host cells. The focus here lies on the human immunodeficiency virus (HIV) and the hepatitis C virus. An improved understanding of the infection cycles of these viruses will benefit the development of new treatment approaches.

FORSYS Partners

With the call for proposals for FORSYS Partners, BMBF added another initiative to the research priority. It exploits the expertise of the research units set up and simultaneously expands this know-how

universities and non-university research institutions as well as those from industry. The aim behind integrating industrial partners is to create clear practical applications for the research at an early stage. Young Investigators Groups support the centres, for example, by developing and contributing outstanding know-how that has been inadequately represented at the various research locations so far.

All FORSYS Partners have the opportunity to fall back on the know-how of the FORSYS centres, to create research networks, and simultaneously to put their own research ideas into practice. They can thus prepare themselves for involvement in future research and funding priorities on systems biology and successfully position themselves on the national and international stage. Ten collaborative projects and thirteen Young Investigators Groups will begin work under the scope of FORSYS Partners in 2008.

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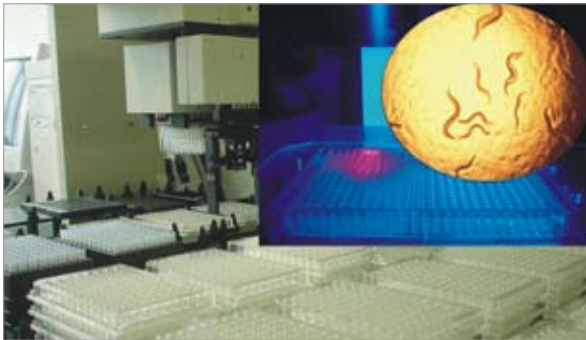
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Targeting Parkinson's Disease

Systems biology of ageing and age-related diseases with the example of Parkinson's disease

Life expectancy is continuously increasing in our society – this is one of the most astounding human phenomena and is undoubtedly one of the great successes of modern medicine. At the same time, the increasing life span can be regarded as the greatest risk factor for a number of diseases such as stroke, coronary heart diseases, various types of cancer and neurodegenerative processes.



C. elegans is ideally suited for systems biology approaches. The 1 mm long nematode can be genetically manipulated in a very easy way and was 1998 the first multicellular organism whose entire genome was sequenced.

As has been convincingly demonstrated by research in the past 15 years, ageing processes and the associated diseases are the result of multifactorial interaction between environmental influences and genetic mechanisms. Thus, for example, more than 100 genes are known that influence age-related changes in the body. Many of these genes were discovered in studies with the nematode *Caenorhabditis elegans*.

The multitude of factors involved represents a problem for an understanding of ageing – especially since in the classical experimental approach scientists so far have typically been restricted to the investigation of individual components, substeps and submechanisms. In order to grasp and correctly assess the involvement of natural ageing processes in the origin and course of age-related illnesses it is necessary, however, to gain an overall picture of events. This could be possible with systems biology since this approach aims to investigate the relations between the manifold mechanisms and functions in an organism – taking into consideration the dynamics of the complex interaction between gene and protein functions.

Parkinson's disease as stress in the brain

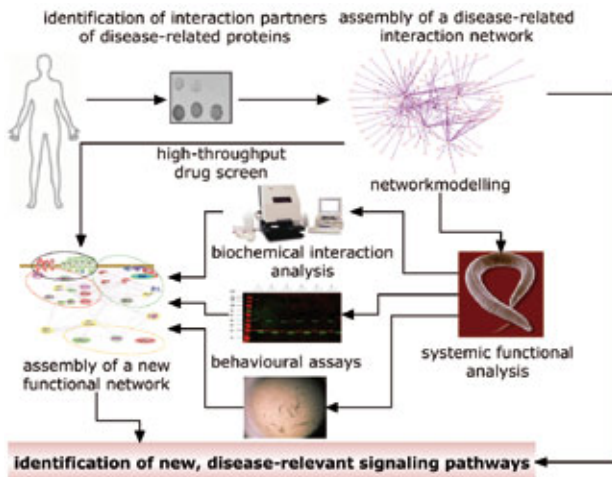
After Alzheimer's disease, Parkinson's disease is the second most common neurodegenerative illness. About four million people are affected worldwide – 200,000 of them in Germany. The progressive loss of dopaminergic neurons in the patient's mid-brain leads to the typical symptoms such as slowing of physical movements, tremor of the hands, mask-like facial expression and stooped, shuffling gait.

In the past ten years, scientists have described mutations in eight genes that lead to genetic forms of Parkinson's disease. Some of the mutated proteins trigger cellular stress or change the reactions of the neurons to stress. They may therefore be involved in the loss of the neurons. In order to investigate the underlying mechanisms more closely, it is necessary to study the precise function of the mutated proteins and their many and varied interaction partners and the dynamic interaction of the various components in the enzyme cascades and signal paths of the organism.

Seeking interaction partners in parkinson's disease

In order to better understand the protein and functional networks and the influence of natural ageing processes on this interaction and thus on the origin of the disease, our working group has designed a technology platform with a systems biology approach in collaboration with the core competence laboratory for proteomics, genomics and imaging at the Centre for Biosystems Analysis (ZBSA) in Freiburg. In this work we are initially concentrating on characterizing the relation between the well-known factors of Parkinson's disease.

The starting point for our project was the protein products of the five most important human Parkinson's genes. In cooperation with GPC Biotech AG, Martinsried, we established a split ubiquitin system in our laboratory in order to make a high-throughput analysis of the interactions of these proteins with the human proteome. We verified the interactions found by appropriate *in vitro* experiments and also in cell cultures. In parallel, we performed all the experiments with the corresponding factors of the model organism



Flow chart for the analysis of signalling pathways involving ageing and disease genes. A reiterative process couples experimental data and modelling processes for the understanding of functional relationships between disease-related genes and their encoded proteins.

C. elegans. In this way, we identified more than one hundred proteins whose interaction is evolutionarily conserved and which thus represent promising candidates for further studies.

With the aid of text and pattern recognition software, we analysed the collected results in a computer-aided network. The aim was to integrate data that had already been published and thus to create a complex interaction network with the aid of which *in silico* predictions could be made for further interconnections. The next step was to experimentally confirm the interactions predicted in the simulation, for example with the aid of suitable high-throughput processes with the model organism *C. elegans*, in the course of which the consequences of a disturbance, for example a mutated gene, or the reinforcing or attenuating influences of other components, were determined quantitatively throughout the genome in order to thus understand the interaction in the network.

One surprising result of the systems biology analysis was that the Parkinson's factors investigated play a joint part in controlling the intercellular dynamics of neurons and thus regulate, amongst other aspects, cytolysis or synapse formation. We expect that a more detailed investigation of the dynamic interaction between the proteins involved will provide us with a better understanding of the pathological process and thus perhaps a new starting point for treatment.

Universally applicable method for network analysis

The resulting network contains factors that play a part in the natural ageing process of the body and it can be expanded without difficulty to include other components. In this way, we intend to extend our understanding of the influence of ageing on age-dependent diseases. Furthermore, on the basis of the predictions of our modelling and with the aid of microtitre-based high-throughput pharmaceutical screening in *C. elegans*, it is possible to search for substances which may help to relieve or prevent the observed neural deficiencies. Finally, our approach can also be modified for the investigation of other biological problems and diseases.

To this end, methods are currently being developed to accelerate the interaction analysis and protein libraries are being generated which also permit investigations of protein networks in other model systems.

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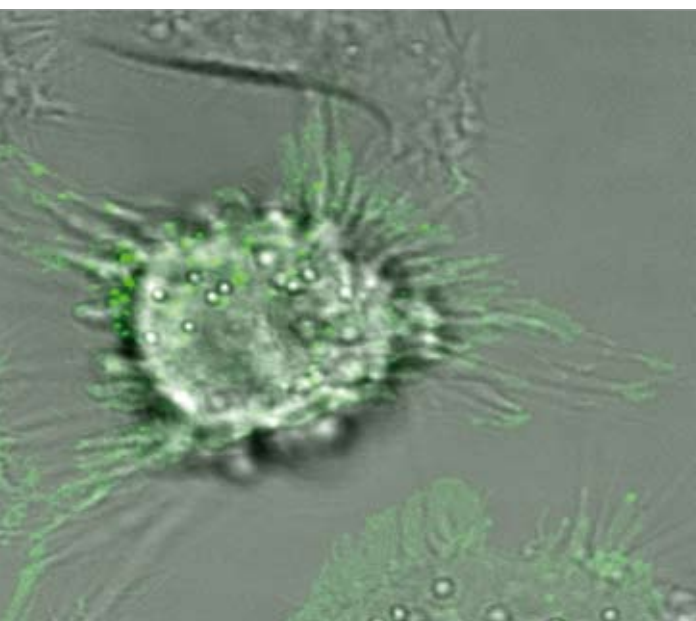
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The Cell's Suicide Programme

Using mathematical models to gain a better understanding of programmed cell death and its contribution to life

A molecular suicide programme, apoptosis, allows cells to die and yet at the same time is a process essential to life. It is of elementary significance, for example, for the development of organisms, for the rebuilding of tissues, and for the correct functioning of the immune system. Scientists assume that all cells are in principle equipped for apoptosis. The process is strictly regulated and follows a predictable pattern, which is why we also speak of programmed cell death. In the embryonic development of the thread-worm *Caenorhabditis elegans*, for example, exactly 131 cells always die in a controlled manner.

During the development of the human foetus, apoptosis also plays a role, and in the adult body, many millions of cells perish in this way every second and are replaced by new cells. Apoptosis makes it possible for superfluous, old, or damaged cells to be removed in a controlled manner. If programmed cell death does not function correctly, it can cause serious diseases. Insufficient apoptosis can lead to cancer or autoimmune diseases, while excessive apoptosis plays a role in acquired immune deficiency syndrome (AIDS) and other infectious diseases, as well as in heart attacks and strokes.



A cell dying as "programmed" under the microscope.

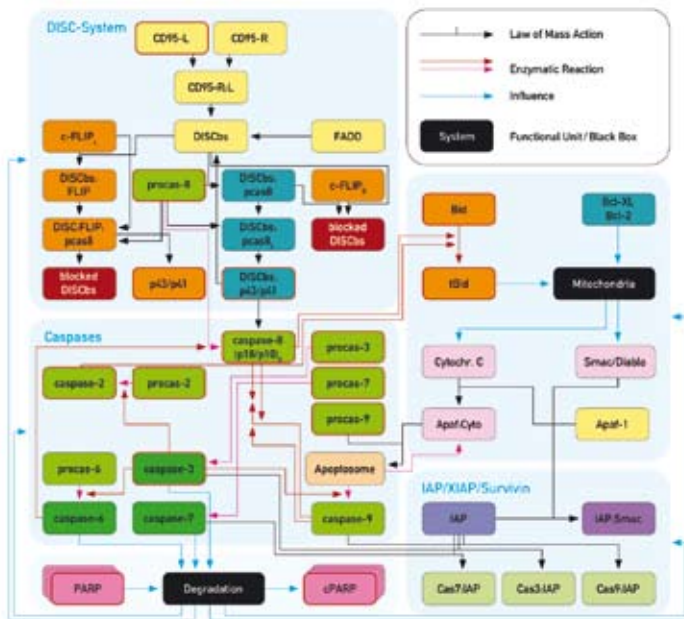
Suicide in a model

The phenomenon of programmed cell death is the object of very intensive research. Despite this, we still do not fully understand it. For example, we still have to answer the question of how apoptosis is controlled. The triggers include ultraviolet rays and X-rays, chemotherapeutic agents, pathogens, the inhibition of growth factors, and the activation of what are known as death receptors on the cell surface. Interestingly, many of these stimuli set apoptosis in motion at a certain intensity. However, at a high intensity, they lead to necrosis - an uncontrolled, pathological form of cell death.

Inside the cell, two different signalling pathways are known to result in programmed cell death: the "extrinsic signalling pathway" begins with death receptors on the cell membrane, while the "intrinsic signalling pathway" leads through the mitochondria, the cell's power plants. In both signalling pathways, certain enzymes known as caspases act as the executive molecules. The apoptosis triggered by the death receptors is one of the signalling pathways in the cell that is best understood. However, a systematic understanding of the complex process that is regulated by many factors acting simultaneously has yet to be achieved.

Mathematics would be useful

Currently, there is no experimental approach that allows the short- and long-term changes of all molecules involved in apoptosis to be observed. A mathematical model of apoptosis summarising the current heterogeneous state of knowledge would be extremely useful here. Computer simulations help to identify critical signalling molecules and to predict an apoptotic active chain - for example, as the reaction of the cell to chemotherapeutic agents. A mathematical model would also be hugely beneficial for designing new experiments in order to investigate apoptosis.



A simplified but mathematically complex model of receptor-induced programmed cell death (graphics by LANGE + PFLANZ Werbeagentur GmbH, Speyer).

Expanding the network

In the future, we want to expand the model of apoptosis currently being developed. In doing so, we will also take into account the interplay with other signal transduction networks and include processes that affect the cellular metabolism, such as those processes involved in the response of the cell to a stress factor. A priority will continue to be understanding the signal transduction pathways of apoptosis in the spatial relation of a single cell. The basis for this will be provided by the considerable expertise available within BIOQUANT in the field of high-resolution microscopy of the smallest structures of the living cell and of quantitative image analysis. In this way, it will be possible to understand the individual responses of cells to apoptotic stimulation and to predict these in the model. In combination with biochemical experiments, mathematical modelling is thus considerably expanding our still currently limited understanding of apoptosis.

Members of the BIOQUANT network have developed an approach for modelling signal transduction networks. This approach made it possible to present a comprehensive model of apoptosis for the first time. It describes the interaction of 41 molecules that interact with each other in 32 reactions. One of the advantages of this model is that despite the large number of almost 60 unknown parameters in the model, it was still possible to achieve precise agreement with the molecule concentrations measured experimentally.

A central prediction was the threshold value behaviour during apoptosis. According to this, a critical concentration threshold of the ligands that trigger programmed cell death must be exceeded for the cell to die. Below this threshold, the cell is in a position to escape programmed cell death. This prediction has been defined and supported by experiments. Furthermore, we identified the key molecule c-FLIP, which plays a decisive role in the regulation of this threshold mechanism.

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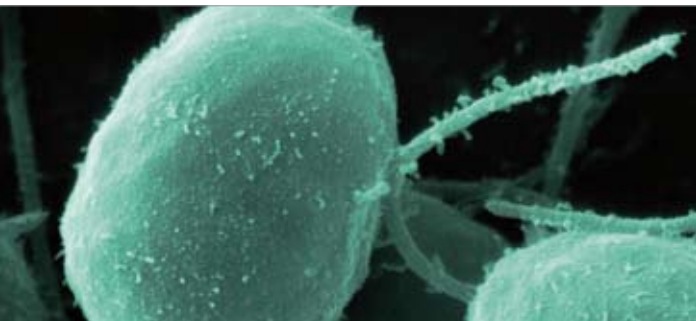
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On the Track of Molecular Synergisms

Metabolomics and data integration for systems biology analysis

The term “metabolome” was coined in analogy to “genome” and “proteome” and relates to the metabolism. The metabolome contains all the characteristic metabolic activities of a cell or tissue and can be described on the basis of conversion rates, enzyme activities and intermediates – the set of metabolites.

In order to study a cell, tissue or an organism in its entirety it is not sufficient to merely consider its metabolome alone. Only the interactions and synergisms between the genome, transcriptome – the set of all gene products - the proteome and finally the metabolome will furnish the key to a functioning living system. We have therefore designed a systems biology approach, in which we analyse and characterise the metabolome, the proteome and the transcriptome of plants in order to integrate the information into one overall model.



The single-cell green alga *Chlamydomonas reinhardtii* is a model organism frequently used in basic plant research.

This approach does not only promise to provide an improved picture of the metabolic processes and the interaction between the proteome, transcriptome and metabolome in different plants. In this way, we also hope to understand the function of genes with hitherto unknown gene products and to characterise substances contained in plants which are of interest for food production and medicine. Finally, computer models for making predictions of the metabolic network and their linkage to experimental data via the interaction of proteins and metabolic processes may contribute to studying fundamental mechanisms of metabolism and resistance – for example biotic and abiotic stresses, certain pests and diseases – and thus help to increase crop yields.

Three platforms: metabolomics, proteomics and data integration

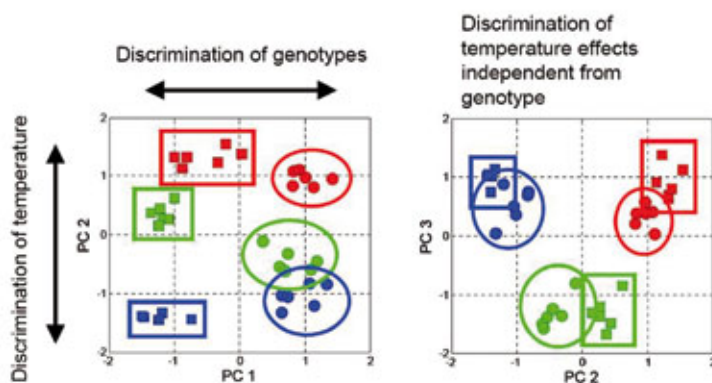
In recent years, we have set up a metabolomics platform based on gas chromatography and liquid chromatography-mass spectrometry, which can be used to measure more than 1000 compounds per sample simultaneously. As a model organism we are first using thale cress (*Arabidopsis thaliana*), in which the metabolome is determined under various stress situations and environmental conditions. Furthermore, we are studying the metabolic products of genetically different plants.

These samples are then evaluated by means of univariate and multivariate statistics and classified on the basis of correspondences in the data. This procedure then also takes into consideration the degree of interlinkage/covariance of all the variables in the metabolism. On the basis of this evaluation, the data can be biologically interpreted. For example, genotypes can be classified and at the same time their sensitivity to temperature stress determined. In parallel to this, we simulate the interactions of the studied metabolites in computer-generated networks, resulting in new findings on metabolic pathways.

On a second level, we consider the plant’s proteome. In order to achieve a high sample throughput, we apply so-called shotgun proteomics. To this end, the total set of all the proteins in the sample are enzymatically cleaved into small pieces, analysed by mass spectrometric methods and identified with the aid of genomic databases.

In the same way as *C. reinhardtii*, thale cress (*Arabidopsis thaliana*) has also been fully sequenced and can therefore be used as a model organism for higher plants, especially crops.





- PGM 4°C
- PGM 20°C
- PGM 32°C
- Col-0 4°C
- Col-0 20°C
- Col-0 32°C

After the integration of metabolome and proteome data, multivariate statistics were prepared for sample pattern recognition. Two different genotypes – the wild type (■) and a starch-deficient mutant (●) – were kept at different temperatures for three days. In the plots, the samples can be classified into distinct groups. On the basis of this visualisation most important metabolite/protein correlations can be determined. (for details Weckwerth, 2008, *Physiologia Plantarum*).

This technology makes it possible for the first time to accurately combine metabolomics and proteomics data since both methods work with a comparable sample throughput. We additionally add results from transcriptome analysis so that we obtain an overall picture of the metabolic processes. In order to avoid technical inaccuracies in integrating these data we have developed a process in which we obtain metabolites, proteins and transcripts from a sample sequentially. These are identified and quantified with the aid of mass spectrometric or other methods and converted into multivariate data matrices which can be analysed as described above.

The result then serves for recognition of the sample pattern. The data enable various genotypes to be distinguished, which, for example, react differently to temperature stress. Pattern recognition identifies certain groups of metabolites, proteins and transcripts, which are responsible

for the respective reaction. They are candidate biomarkers which can be used to determine the reaction to temperature stress or - depending on the properties investigated - other characteristics of the plant such as growth and development.

From technology to increased yields

Our methods for genome-wide metabolite and protein analysis are being applied in a research project on the systems biology of plants at Golm, a suburb of Potsdam (GoFORSYS; www.goforsys.de). With the example of *Chlamydomonas reinhardtii* (model organism for single-cell plants) and *Arabidopsis thaliana* (model organism for multicellular plants), we are investigating the basic mechanisms of the relation between photosynthesis and growth. The aim is to use the findings and the approach for increasing crop quality and yield. The platform we established for data integration is currently also being used for the identification of suitable biomarkers for potato breeding.

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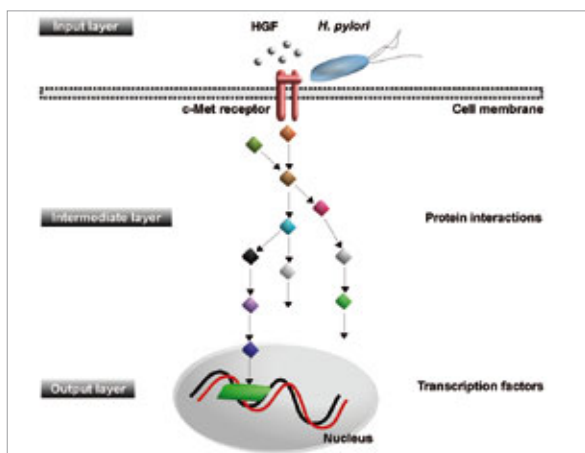
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Microbes and Men – A Complicated Coexistence

Researching the impact of human pathogenic microorganisms on humans using systems biology

When humans become infected with a pathogen, their defence mechanisms kick into action in an effort to fight the invader. These invaders in turn have a number of strategies that they use to avoid or suppress the immune response. For example, microorganisms use their host's proteins to manipulate signalling pathways in order to multiply more efficiently. Our knowledge of these particular interactions between human organisms and pathogens is steadily increasing and we are accumulating very complex information. An enormous amount of data exists on virulence factors which regulate a whole range of host proteins.

In a new systems biology approach, we reproduce the interaction between human and microbe in qualitative models. The knowledge gained contributes to a better understanding of complex pathophysiological relationships. Furthermore, computer-simulated interaction mechanisms help us to find new targets for treatments and to develop new active ingredients for fighting infectious diseases.



The hepatocyte growth factor HGF and the human pathogenic *Helicobacter pylori* activate the membrane receptor c-Met ("input layer") on different ways. The proteins of the "intermediate layer" transfer the incoming signals to the transcriptional factors ("output layer") in a signal cascade.

Humans and Helicobacter – a complex network

Approximately 50% of the world's population are infected with *Helicobacter pylori*. The human pathogen colonises the lining of the stomach and is a significant risk factor for stomach ulcers and tumours. One of its most important virulence factors is the protein molecule CagA. A high cancer risk is associated with the bacterial strains that have this protein. CagA is injected into the host cell and attaches itself to the c-Met receptor inside the cell. The c-Met protein plays a role in controlling the morphogenesis and proliferation of cells and is normally regulated by the hepatocyte growth factor (HGF). In the case of a *H. pylori* infection, the virulence protein modulates the c-Met activity and influences its function. The ERK kinase is modulated in this manner, which leads to a changed morphology and an increased migration of cells.

Within the framework of our network analysis, we reproduced all protein interactions regulated by c-Met after they have been activated by HGF or *H. pylori*. The description of all interactions in the network model is based on Boolean algebra and is realised with the aid of the logical operators AND (two proteins interact with each other), OR (a number of possibilities, for example, the interaction of one protein with different partners), and NOT (no interaction). The studies were conducted in cooperation with the working group headed by Prof. Ernst D. Gilles, Max Planck Institute for Dynamics of Complex Technical Systems in Magdeburg.

The result of this analysis is a model consisting of 54 proteins and molecules, known as nodes in a network, which are linked to each other via a total of 62 different interactions. Here, the elements of the input layer, in other words HGF and *H. pylori*, which initiate the signal transduction, are differentiated from those of the intermediate layer, namely molecules such as the Ca^{2+} ion that are involved in the transmission of the command. A third level is represented by the elements in the output layer. This layer contains the final receptors of the signal such as the transcription factors NF- κ B and c-Jun.



Helicobacter pylori is an important risk-factor for stomach ulcers and tumours. Approximately 50% of the world's population are infected with this human pathogen.

The mathematic modelling of the interaction between host and microorganism on a microbiological level allows complex cellular networks to be visualised. The strengths of this approach lie in what are known as *in silico* analyses, which make it possible to predict different interactions. With the aid of CellNetAnalyzer software, individual network components, for example, can be selectively switched off (knock-out) or activated (knock-in). This allows us to model the possible consequences associated with therapeutic measures. Using a computer, we can make a statement on how a potential active ingredient encroaches upon the process as a whole and what side effects can be expected. The model therefore aids the speedy development of new and effective treatment strategies - an aspect that is particularly important against the background of an increasing resistance to antibiotics.

Our working group succeeded in using this approach to identify the phospholipase Cy1 as a possible target for the treatment of stomach ulcers and tumours caused by infections. This enzyme is responsible for activating ERK - at least in the case of infection with *Helicobacter* - and therefore plays a role in the increased migration of epithelial cells. However, if the c-Met receptor is

stimulated by HGF, PLCy1 then plays a subordinate role in these processes. The simulations showed that PLCy1 plays a key role in *H. pylori* infections and that the inhibition of this enzyme causes decreased cell migration. These *in silico* predictions were confirmed by means of experiment through the pharmacological inhibition of PLCy1.

On the way to the next level

Future activities will focus on expanding our model. The objective is to obtain detailed information on the dynamics of the transmission and processing of signals in the interaction between host and pathogen. An important role is played by the post-translational modification of proteins, which considerably expands the repertoire of different types of protein in the cell. This approach is particularly attractive from a therapeutic point of view because it could lead to new options for targeted medicinal interventions with fewer side effects.

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QuantPro

Quantitative analysis for the description of dynamic processes in living systems

Understanding the processes of life is the challenge that faces the life sciences. In order to rise to this challenge, it is extremely important that we go one step beyond the hitherto more static and systematic analysis of cellular components and ascend to the next highest level: the quantitative analysis of the dynamic interaction of cellular processes. If this is to be achieved, existing technologies must be optimised and new bioinformatics tools must be developed. Against this background, the German Federal Ministry for Education and Research (BMBF) launched the “QuantPro” funding priority in August 2005.

Etienne Sokal, Université de Louvain, Brussels

“Systems biology is one of the most active fields of research in Germany. Since it is not the static genome but the dynamic proteome that regulates the enormous changes occurring in an organism between conception and death, systems biology is the successor to molecular biology. In the long run, integral mathematical models describing functions of the hepatocyte and embedding it in the biological context of the liver will help to understand and successfully treat liver disease.”

QuantPro supports projects that are dedicated to the quantitative analysis of the dynamics of cellular processes. Funded research projects are based on findings from genome, proteome and metabolome research, as well as bioinformatics. A central aim is to build a bridge between the “omics” technologies and systems biology. In the long term, QuantPro projects contribute to combating diseases in humans, animals and plants in a more targeted manner, breeding higher-yielding crops, and developing more effective and more environmentally friendly biotechnological methods and products, and thus replacing chemical industrial processes. In the field of environmental protection, the aim is to develop strategies for accurately targeted bacteriological environmental remediation.

At the same time, the cooperation between industry and science is to be expanded within the framework of the QuantPro funding measure in

order to improve technology transfer and thus the economic performance and competitiveness of Germany. For this reason, close cooperation with partners from industry is supported.

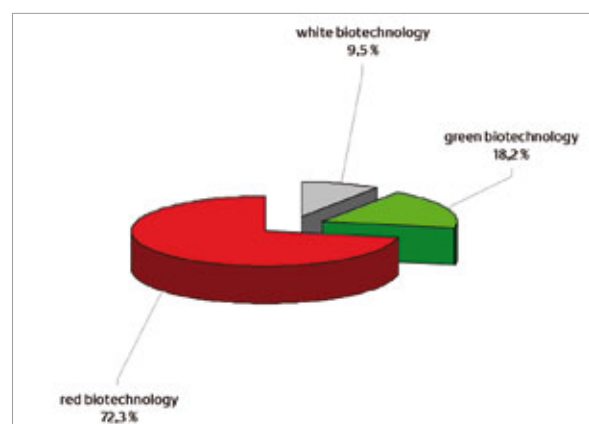
Fourteen interdisciplinary collaborative projects

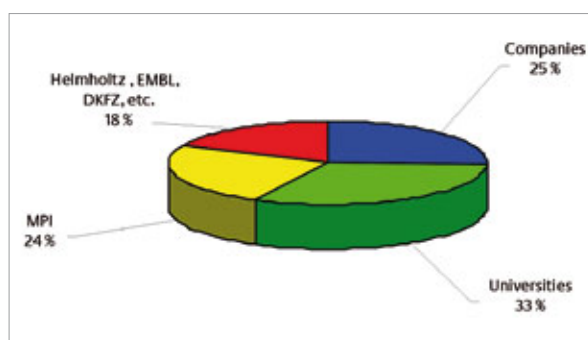
Scientific work on the QuantPro projects began in the middle of 2006/beginning of 2007. An independent panel of experts recommended that fourteen interdisciplinary collaborative projects, consisting of 48 individual projects to be conducted by 63 working groups, be funded initially for a period of three years. BMBF has provided € 26 million for this purpose. An additional € 5.7 million comes from the fourteen industrial companies involved - three of which are large corporations and eleven are classed as small and medium enterprises. Their main focus is on the development of technologies and the development of pharmaceuticals. Furthermore, a plant breeder and a starch-producing company from the food industry are also participating.

Red, green and white biotechnology

In their announcement, BMBF named medicine, agriculture, biotechnology, and environmental protection as possible fields of application, and in doing so left the choice of models for the study open. The projects funded therefore focus on very different areas.

Percentage distribution of funding in the different application areas





Percentage part of the research organisations and companies funded within QuantPro.

MPI: Max Planck institute

DKFZ: German Cancer Research Centre Heidelberg

EMBL: European Molecular Biology Laboratory Heidelberg

Two collaborations concentrate on white biotechnology - industrial production making use of enzymes, cells and microorganisms. One of these projects focuses on the dynamics of membrane proteins in the production strain *Corynebacterium glutamicum* in order to optimise this strain for the production of amino acids. The second research project in this area concentrates on the fabrication of complex oligosaccharides in *Escherichia coli* as the basis for the development of drugs and vaccines.

The three collaborative projects in the field of green biotechnology are mainly oriented towards increasing crop yields and research on herbicides. Using completely new methodological approaches, two of the research projects aim to understand and optimise the breeding characteristics of potato tubers and barley. A third project focuses on the identification of new targets for potential pesticides.

With a total of nine collaborative projects, red or medically oriented biotechnology accounts for the largest part of the QuantPro research priority. The scientists involved pursue a variety of new methodological approaches in order to describe and analyse dynamic cellular processes quantitatively. For example, membrane protein networks, transport proteins, specific cell surface molecules and inhibitors are the centre of intensive investigations. One of the aims is to identify new and better biomarkers for diagnostics, as well as specific surface molecules

as a basis for the development of immunotherapies for cancer. Another project is dedicated to gaining a better understanding of the transport system in the liver, which in turn should help to minimise the risks and side effects associated with drugs. Adult stem cells are also the focus of investigations with the objective of better exploiting their potential for medicine. Another research project concentrates on using 3D-based laser microdissection to significantly improve the preparation of biological samples.

Taking advantage of synergies

Once a year, the coordinators of the fourteen collaborative projects meet in order to present the current results in a small group. Despite the heterogeneity of the investigations or perhaps directly because of this, synergies were identified within the framework of these meetings and new cooperations between individual working groups were created. Already, the first results reveal that the new technologies developed here help make access to new data on dynamic processes a reality. Thanks to new theoretical approaches and mathematical models, biological systems can be better described and understood. In this way, the funding measure makes a significant contribution to issues that arise in systems biology.

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Biomarkers for Potato Breeding

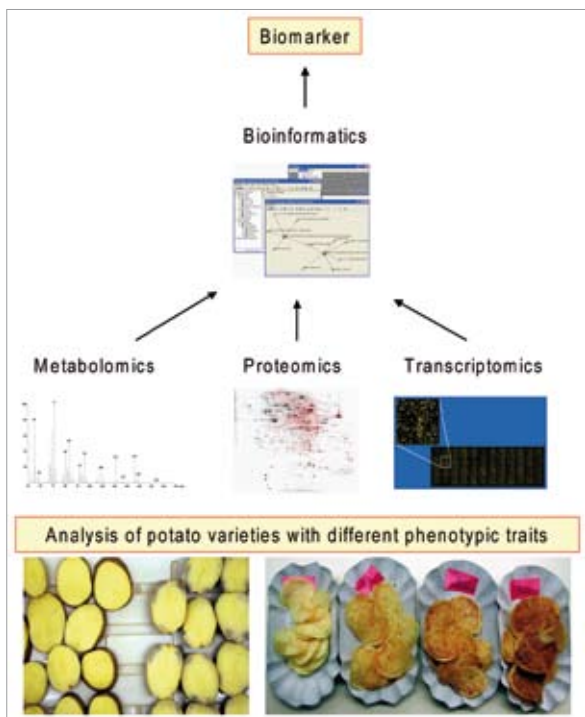
Systems biology approaches for a more targeted breeding process

The potato is one of the most important crops throughout the world. It is extremely important for the economy, since it is used not just as a foodstuff but also as a renewable raw material for industry. It is used to produce starch and high-quality proteins with enzymatic and probiotic properties, as well as fibre that is added to foodstuffs to provide roughage. In order to effectively use the potato, it is important that the varieties planted are not just high-yielding, but that they also produce a high-quality crop that is, for example, resistant to black spot bruise, discolorations that can occur during harvesting, storing, and processing. Other important characteristics are the amount and composition of the substances contained in the potato, such as the protein content, and a resistance to starch conversion to reducing sugars which changes the frying characteristics of the tuber.

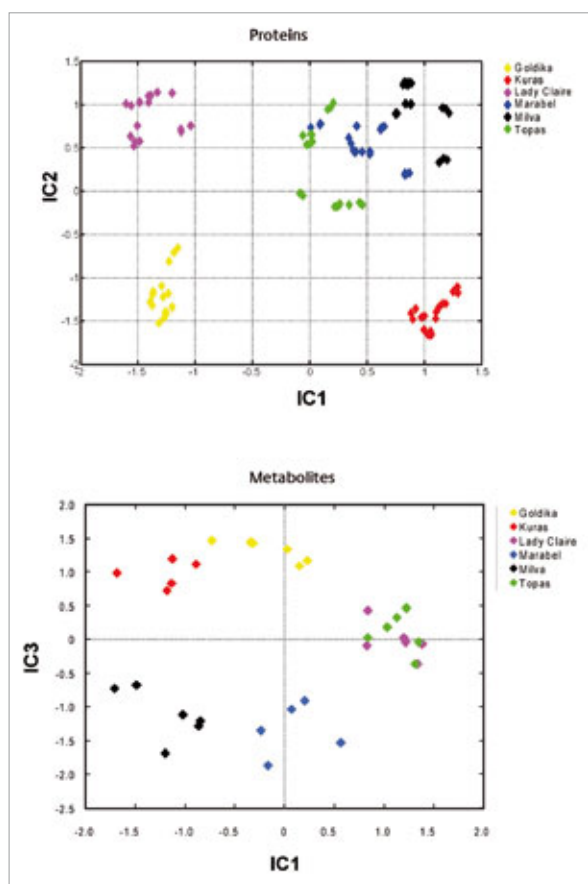
The aim of our project is to identify biomarkers for such characteristics in order to allow a more targeted and effective breeding of potato varieties with the desired characteristics. For this purpose, we have joined forces with breeders (Böhm-Nordkartoffel Agrarproduktion OHG), representatives from consultancy and industry (Lower Saxony Chamber of Agriculture, Proteome Factory AG, Emsland-Stärke GmbH) and academic partners (University of Potsdam, Max Planck Institute for Molecular Plant Physiology, Golm, Institute of Vegetable and Ornamental Crops, Grossbeeren) to form a multidisciplinary alliance. With the aid of a systems biology approach, we investigate metabolic processes in potato varieties which differ in important target features. The basis is provided by proteome, metabolome and transcriptome analyses. The aim is to use bioinformatic methods to identify characteristic proteins, metabolites and transcripts which are associated with a characteristic that is interesting for breeding.

Metabolomic signature for black-spot resistance

In a field study, we cultivated 20 selected potato varieties with different relevant target features in two geographically different locations in Germany (Böhlendorf, Mecklenburg-Western Pomerania, and Ebsdorf, Lower Saxony). With the aid of various “-omics” methods, which are capable of describing as many characteristics of the metabolism as possible in parallel in one measurement, we studied the proteome and the metabolome of the tuber. The analysis of the protein profiles was performed on the basis of a newly developed method known as shotgun proteomics. All of the proteins in a sample are enzymatically digested in this process and analysed using mass spectrometry coupled with liquid chromatography. The analysis of the metabolite profile was conducted after the extraction of a combined soluble fraction of primary and secondary metabolites using mass spectrometry coupled with gas chromatography.



Using systems biology approaches, we identify transcripts, proteins and metabolites in potatoes that are associated with a characteristic that is interesting for breeding, such as black-spot resistance or improved frying characteristics.



In selected potato varieties showing characteristic differences in important target features, we analysed protein and metabolite profiles. A statistical method makes the different variety groupings visible (independent component [IC] analysis). In this way, we can identify candidates for biomarkers in order to distinguish these variety characteristics important for breeding.

In order to be able to describe the large set of experimental data in a condensed form, we studied the metabolome and proteome data separately using what is known as independent component analysis (ICA). ICA is a statistical method that simplifies and reduces the size of large sets of data. The components are based on linear combinations of weighting factors for the proteins and metabolites measured. They can be used to depict the variety characteristics in a two-dimensional coordinate system. This analysis shows that the different variety classes clearly differ from each

other in this reduced coordinate system. In this way, we succeeded in identifying the first proteins and metabolites as potential candidates for biomarkers. Certain metabolite patterns, for example, point towards the characteristic of “black-spot resistance”. It is important that the patterns were identical for both of the locations investigated, so that they are therefore independent of geographical circumstances and growing conditions.

Quick tests for breeders

In order to ensure that the biomarkers that have been identified and are considered ready for practical application will actually make potato breeding more effective, quick and simple test procedures must be developed. ELISA- and PCR-based systems are an option, for example, if they are optimised as an assay kit or a dip stick. These tests allow young plants to be examined and the presence of certain characteristics determined, which in turn will allow breeders to choose clones that have the desired properties at a very early stage. The otherwise long selection process involved in the breeding of crops is significantly reduced in this manner. Furthermore, this type of test system makes better quality assessment of crops a reality for farms, and as a result ensures a more targeted marketing.

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PD Dr. Peter Geigenberger is coordinator of the collaborative project. He is working at the Institute of Vegetable- and Ornamental Crops, Großbeeren / Erfurt e.V. (IGZ). Systems biological analyses are used to investigate the storage metabolism and to improve crops.

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Transport Systems in the Liver

QuantLiver – investigating and modelling transport mechanisms for the excretion of drugs

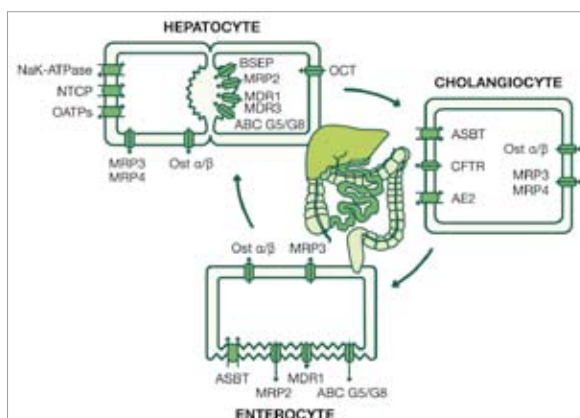
Active transport, above all in the organ systems of liver and intestines – represents a serious problem for drug development. The transport-related excretion of drugs leads to a drop in the level of active substance in the organism. The pharmacokinetic properties of a drug are therefore decisive for its efficacy. Pharmacokinetics describes the concentration profile of active substances and their degradation products in body fluids and tissues. Only a restricted range of high-throughput methods are currently available for investigating the transport proteins involved, which also limits the molecular understanding of the associated processes. The aim of QuantLiver is therefore to develop a method for the quantitative analysis of the transport mechanisms as well as their representation using a modeling software. It is hoped that predictions on pharmacokinetics can be improved thus also assisting the selection of promising candidate active substances in preclinical research.



SURFER Workstation 500.

A further objective is to provide a building block for so-called personalized medicine. Efficacy and tolerability of drugs are different for every individual. This is the result, in particular, of individual genetic variations, so-called polymorphisms, which are frequently restricted to a single base substitution and are therefore termed single nucleotide polymorphisms (SNPs). The aim is to quantitatively describe interindividual differences on the basis of variations in the transporter

genes of the hepatobiliary system, that is to say the liver, gall bladder and bile ducts, on a molecular and functional level. The findings can then be analyzed further using physiologically based pharmacokinetic (PBPK) modelling. This systems biology approach therefore helps to achieve a better understanding of the transport phenomena of drugs in the liver and enables quantitative predictions to be made on the efficacy of medicinal drugs.



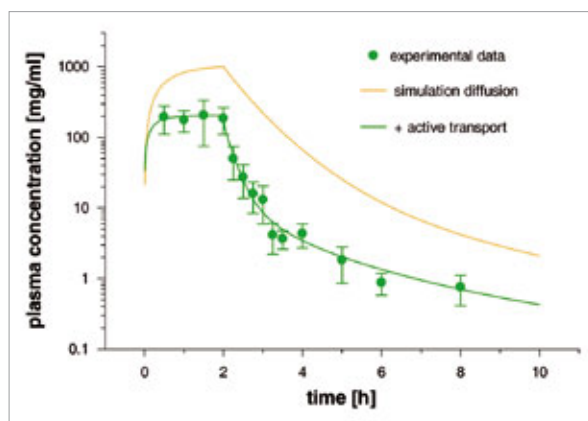
Enterohepatic circulation with the transport systems of hepatocytes, cholangiocytes (bile duct epithelial cells) and enterocytes (epithelial cells of the small intestine).

Tracking down variations

Literature data served as the basis for incorporating the effects of active transport into the pharmacokinetic models that have been generated by Bayer Technology Services with their PBPK software *PK-Sim*[®]. In this way, it has already been successful to create a detailed simulation of adsorption, distribution, metabolism, and excretion of drugs in the human body. Thus, in a next step it is possible to qualitatively predict the effects of polymorphisms on the basis of these validated models.

In the search for variations in transporter genes, we investigated DNA material from 24 individuals of European ancestry (*popgen Project*) and also 12 individuals each of Chinese and African ancestry (*International HapMap Project*). The analysis of the sodium-dependent bile acid transporters, NTCP and ASBT, led to the identification of, respectively, four and two SNPs, which also change the amino acid

sequence of the transport proteins. In the case of OATP2 (organic anion transporting polypeptide 2), there were nine such SNPs and eight for the bile salt export pump, BSEP.



Modeling the plasma concentration of an active substance was optimized by including an active transport process. The time curve simulated with *PK-Sim*[®] assuming a passive distribution of the active substance diverges significantly from the data obtained experimentally. The incorporation of active transport processes into the model leads to good agreement.

In order to effectively analyze any functional effects of these genetic variations, we are currently developing functional high-throughput assays on the basis of the SURFE²R (SURFace Electrogenic Event Reader) sensor technology. This technology enables electrically active membrane proteins to be studied without use of fluorescent dyes or radioactive labelling. In this method, the transport proteins to be investigated are applied to a special sensor surface via vesicles or membrane fragments. A solid-supported hybrid membrane is used consisting of a synthetic lipid layer on a thiolated gold layer. The activation of the transport proteins by charged substrate molecules results in electric signals, so that the activity of the proteins can be detected by electrical measurements. The aim is to use this technology to analyse the effects of the SNPs on transport and to integrate the findings of these functional studies into the models. In a further step, it is planned to investigate pharmaceuticals which have been identified as entering into interactions with the transport systems of the liver and adversely affecting drug efficacy.

Testing active substances and understanding mechanisms

The expertise with respect to genetic, molecular and functional analyses that is represented in this project leads to a unique combination of methods bringing about a significant improvement in the preclinical search for active substances with regard to their pharmacokinetic properties. In this way, it is possible to select suitable candidate active substances already when screening substance libraries. Furthermore, together with targeted mutagenesis the screening technology offers the possibility to characterize transport proteins biochemically and biophysically in order to thus obtain a detailed understanding of their molecular function. In this way, the integrative approach of experiments together with modeling makes a contribution towards understanding the mechanisms by means of which the mutations in the transporter genes lead to diseases of the hepatobiliary system. The PBPK models additionally support personalized medicine by an improved efficacy of drug therapies with reduced side effects.

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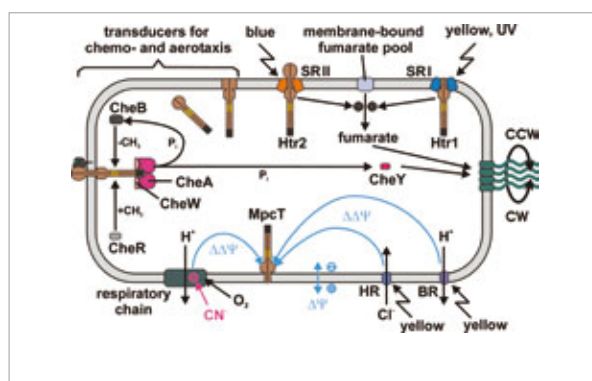
The Light Processing Network

Quantitative proteome analysis of the signal reception and transduction on membrane protein complexes

Membrane proteins are the target structures for more than 70% of all drugs in use worldwide. Investigating these proteins and the dynamics of their signalling pathways is therefore extremely important for R&D in the pharmaceutical industry. The dynamics in signalling pathways are primarily achieved through temporary alterations in protein networks. An important role is played here by protein interactions, changes in post-translational modifications of proteins and alterations in their localisation. These processes are integrated in highly adapted regulatory networks.

Using the example of the signal processing of light – in human sight, as well as in phototaxis, which is the light-oriented movement of archaeobacteria – we investigate membrane receptors and the downstream protein networks. Bacterial and mammalian rhodopsin were the first structurally resolved G-protein coupled receptors and can be considered as an archetype GPCRs. These proteins form the largest family of cell surface receptors and are found in all eukaryotes. They currently account for around 50% of all target structures for drugs. Using proteomic methods we first identified the molecular composition of the protein complexes and network architecture of rhodopsin, define critical post-translational modifications and further on record kinetic data. On the basis of quantitative data thereof, *in silico* models are then generated. They describe network composition (as a scale free network) as well as functional principles (logical network; Petri network) downstream of rhodopsin. A long term goal is to predict network properties and reactions due to intrinsic disturbances caused by mutations or extrinsic disturbances due to environmental influences.

Halobacteriaceae absorb through specific receptors and different environmental signals and induce a signalling cascade whose biochemical reactions control the switching of the direction of rotation of the flagellar motor.



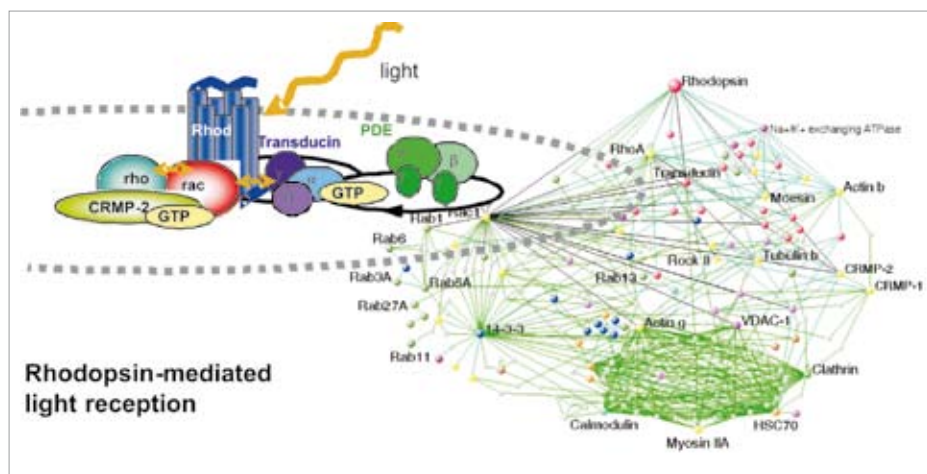
Reactions to light

Halobacteriaceae are a group of archaeobacteria that are capable of employing a single visual pigment, retinene, to utilize light for the production of energy using photosynthesis, as well as for the perception of light and phototaxis. In this process, the two light-driven ion pumps, bacteriorhodopsin and halorhodopsin, are used for photosynthesis, while phototaxis is based on the activity of two photoreceptors, namely sensory rhodopsin I and II. Both of these initiate a type of colour vision – similar to sight in higher animals. Orange represents a positive stimulus and initiates an active movement of the cell towards the light source, while UV and blue light are negative stimuli that induce a reaction away from the light. Both of the sensory rhodopsins are involved in this process: SRI is a receptor for orange and UV light and SRII is a blue-light receptor. The activation state of all receptors is communicated to the downstream protein network by the proteins CheA and CheY. A signalling cascade – mediated by phosphorylations in the network – transmits the light information to the flagellar motor that controls the activity of the flagellum, which is the organ that propels the archaeobacterium. This allows the cell to swim in regions with good illumination for maximum energy generation on the one hand, and to avoid areas with dangerous UV light or suboptimal light conditions on the other hand.

With the aid of quantitative proteomics, the project first focuses on the analysis of regulative protein modifications of the signalling network, for example the attachment of the phosphate or methyl groups. The quantitative measurement of these modifications by mass spectrometry form the basis for modelling the regulative dynamics of the signalling network, which is used to perceive surroundings, consider risks, and control movement.

The principle of sight

Perceiving surroundings is the basic function of the process of vision in higher organisms. In the human eye, specially differentiated photoreceptors make it possible to recognise structures and objects either under dim light conditions (nightsight) or in colour (daysight) with high sensitivity.



Light reaction of photoreceptors in the retina. Rhodopsin absorbs the incoming light and activates a signalling cascade that binds with the G protein transducin and ultimately leads to a change in the membrane potential and the processing of the light signal in the brain.

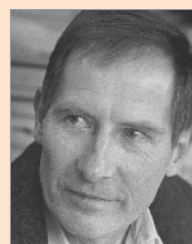
Despite the fact that the signal transduction of light (visual cascade) is already quite well understood, we still lack information on the diverse signalling routes as well as divergent biological outputs resulting thereof apart from the visual cascade.

Considering rhodopsin as a typical GPCR, the in-depth study of its signalling is of principal interest. In addition, rhodopsin as well as other genes expressed in photoreceptors is struck by mutations that lead to hereditary forms of blindness. There is therefore a huge medical interest in understanding signal transduction and disturbances that accompany the mutations.

By combining biochemical separation, affinity based isolation and mass spectrometry we were able to identify the core proteome around rhodopsin comprising 72 different proteins. In collaboration with Gianni Cesareni's group in Rome, this primary dataset was filtered against the human interactome deposited in the MINT database. The resulting network was then merged with own experimental interaction data to further qualify a virtual interaction network containing 150 nodes and 395 edges. In collaboration with Ernst Dieter Gilles and Holger Conzelmann, MPI for Dynamics of Complex Technical Systems, Magdeburg, the focus is now on describing the dynamics involved in the regulation of protein interaction and protein activity starting from the reception of light, wired through this network. A model of discrete signal transduction patterns is to be created on the basis of quantitative proteome data, which are acquired in the QuantPro research priority. Research here concentrates predominantly on the light-dependent regulation of light adaptation as well as photoreceptor structure and polarity mediated by two small enzymes

known as GTPases, rac and rho. In cooperation with industrial partners Becton Dickinson, Toplab and Boehringer Ingelheim, quantitative analytical methods and technology are further developed to meet the specific requirements for model building. A long-term objective is gain of knowledge that supports rational therapy and drug development for degenerative diseases of the eye that could not be adequately treated in the past, for example retinal detachment or age-related macular degeneration.

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SysMO

Systems Biology of Microorganisms

Microorganisms are important in medicine - as pathogens but also as natural inhabitants of skin as intestinal flora. At the same time, they are enormously relevant in economic terms for biotechnological production processes and they play a role in environmental protection, for example, in the degradation of harmful substances. Therefore, microorganisms are the subject of intensive research. In comparison to higher organisms, they are ideal research objects for systems biology. As single-cell organisms, they are organised in a less complicated manner and can therefore be understood more easily than a complete system.

A systems biology understanding of microorganisms opens new opportunities for applications in biotechnology, medicine, and ecology. Production processes can be optimised, mechanisms of pathogenesis and targets for new treatments can be predicted, and microorganisms can be used in a more specific and effective manner to reduce environmental damage.

Expanding systems biology research in Germany and Europe

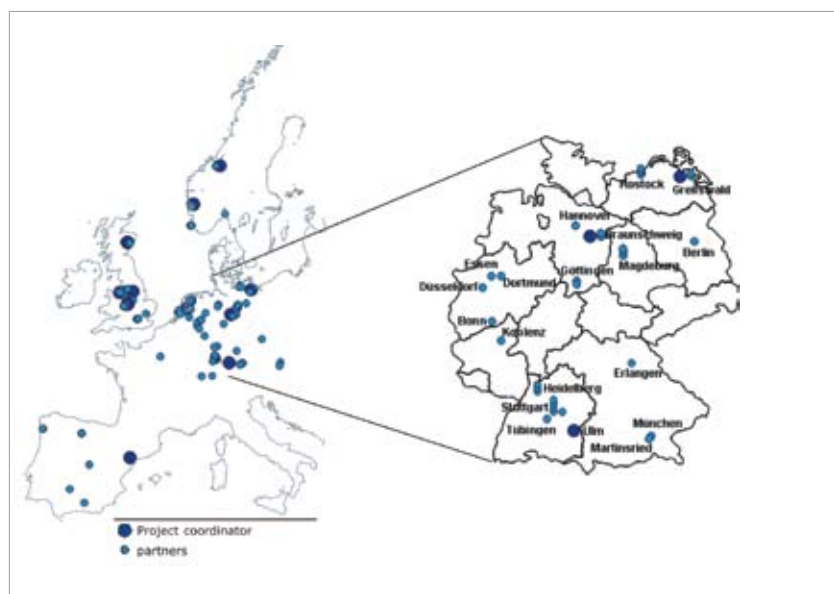
Against this background, the BMBF initialised the funding initiative "Systems Biology of Microorganisms (SysMO)" in 2005. SysMO is a European initiative supported by funding organisations in the United Kingdom, the Netherlands, Norway, Austria, Spain, and Germany. SysMO focuses microorganisms that are highly relevant for biotechnology, health, nutrition, and environmental protection. As a joint activity with six partner countries from the European Research Area on Systems Biology (ERASysBio, see page 58), SysMO pools European research capacities and know-how in this field in order to strengthen Europe's competitiveness in the long-term and to set future trends. Over € 28 million is available within the first funding phase.

In the field of systems biology, SysMO is currently one of the largest and most ambitious funding initiatives in Europe. A specific feature is its transnational structure - not only in terms of scientific cooperations, but in particular at the level of funding and organisation. Together with the partner organisations, the German Federal Ministry of Education and Research (BMBF) granted funding in March 2007 to

eleven interdisciplinary and multinational consortia within the framework of SysMO. These projects unite European scientists from the fields of biology, computer science, physics, chemistry, mathematics, engineering, and medicine in a total of 85 different working groups. Moreover, teams of researchers from France, Switzerland, and the Czech Republic are also involved in SysMO consortia as external partners.

The transnational structure is the key note behind the SysMO initiative: at least three working groups from different partner countries are represented in every collaborative project in accordance with the funding guidelines. German partners from a total of 32 different working groups are represented in each of the eleven SysMO collaborative projects. They will receive funding from BMBF totalling € 11.5 million over a period of three years.

Three of the collaborative projects are being coordinated by scientists of the Federal Republic of Germany. Furthermore, the central coordination office - the SysMO Office - is located at Project Management Jülich in Germany and is funded by BMBF.



Complex networks and central structures

Understanding complex networks in microorganisms is the grand challenge that scientists in the SysMO consortia have set themselves and hope to solve with the aid of systems biology. Besides species often used in biotechnology, namely *E. coli*, *Bacillus subtilis*, and the baker's yeast, *Saccharomyces cerevisiae*, also solvent-producing bacteria (*Clostridium acetobutylicum*), lactic acid bacteria (*Lactococcus lactis*), intestinal bacteria (*Enterococcus faecalis*), and human pathogens (*Pseudomonas*, *Streptococcus pyogenes*) are subject of the systems biology investigations. The long-term goal of the SysMO projects is not only to describe the network characteristics, but also to understand their dynamics in detail, and to use computer models to predict the behaviour of individual microorganisms. This provides the basis for the identification of new starting points for biotechnological production and for the improvement of existing processes. This will in turn lead to a more effective use of microorganisms in the bioproduction of biofuels and antibiotics as well as in the bioremediation of soils and in the improvement of strategies for the treatment of pathogenic species. Whether the systems biology know-how can be used and interpreted across the borders of organisms and strains is also the topic of the SysMO investigations.

To support the transnational work of the SysMO consortia and to make it more efficiently, structures are being established that go beyond the scientific work itself. These structures aim at the direct and continuous exchange of the scientific data and models and the joint usage beyond the borders of projects and nations. The projects and the progress of each participating group are to be made transparent for the entire SysMO consortium so that all partners can profit directly from the results and the know-how acquired. For this reason, the scientists involved in SysMO are jointly developing a central data management, which will be implemented in 2008.

The SysMO consortia:

COSMIC: Systems Biology of *Clostridium acetobutylicum* – a possible answer to dwindling crude oil reserves (coordination: Prof. Peter Duerre, University of Ulm, Germany)

PSysMO: Systems analysis of process-induced stresses: towards a quantum increase in performance of the cell factory *Pseudomonas putida* (coordination: Professor Vitor Martins de Santos, Helmholtz-Centre for Infection Research Brunswick, Germany)

MOSES: MicroOrganism Systems Biology: Energy and *Saccharomyces cerevisiae* (coordination: Professor Hans Westerhoff, University of Manchester, UK)

SUMO: Systems Understanding of Microbial Oxygen Responses (coordination: Professor Robert K. Poole, University of Sheffield, UK)

SysMO-LAB: Comparative Systems Biology – Lactic Acid Bacteria (coordination: Professor Jeroen Hugenholtz, University of Amsterdam, The Netherlands)

BaCell-SysMO: The transition from growing to non-growing *Bacillus subtilis* cells (coordination: Professor Michael Hecker, University of Greifswald, Germany)

Systems biology of a genetically engineered *Pseudomonas fluorescens* with inducible exopolysaccharide production (coordination: Professor Svein Valla, Norwegian University of Science and Technology, Norway)

TRANSLUCENT: Gene interaction networks and models of cation homeostasis in *Saccharomyces cerevisiae* (coordination: Professor Joaquín Ariño, University of Barcelona, Spain)

KOSMOBAC: Ion and solute homeostasis in enteric bacteria (coordination: Professor Ian Booth, University of Edinburgh, UK)

Global metabolic switching in *Streptomyces coelicolor* (coordination: Professor Elizabeth Wellington, University of Warwick, UK)

Silicon cell model for the central carbon metabolism of the archaeon *Sulfolobus solfataricus* under temperature variation (coordination: Professor Christa Schleper, University of Bergen, Norway)

Please see www.sysmo.net for more detailed information about the projects.

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Lactic Acid Bacteria in Comparison

Facilitating model development through comparative systems biology approaches

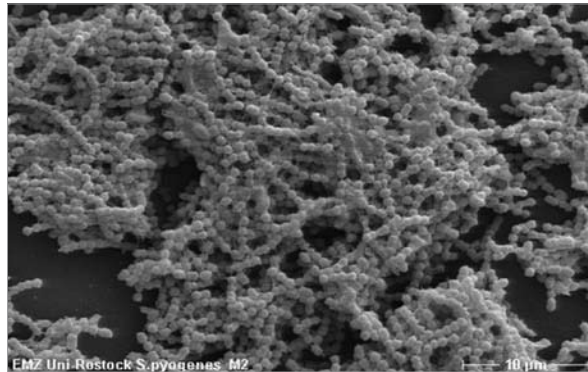
An advantage of systems biology approaches is the integration of both experimental and computational methods. The modelling of biological systems is of particularly great interest. This method makes it possible to simulate relationships and interactions and to predict certain responses in the network, and thus to specifically plan experimental procedures. The development of such a model is very time-consuming. However, the time involved could be reduced if we could fall back on existing models when creating a new one. Therefore, the main goal of the SYSMO-LAB project is to develop methods for easier model development based on a comparative approach.

Lactic acid bacteria as models

We chose lactic acid bacteria for the case study. The different species of this family are very similar, but they are also specialised in many different environmental situations. Moreover, they are of great importance for humans both in a biotechnological and medical sense.

Our approach takes three different homofermentative organisms into account, which exclusively produce lactic acid:

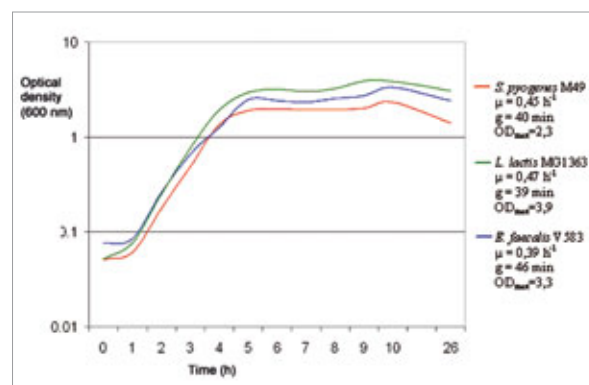
- **Lactococcus lactis, a biotechnologically relevant species, which plays a role in the production of milk products such as yoghurt and kefir;**
- **Enterococcus faecalis, which is biotechnologically important (some strains are used as so-called probiotics), and which is an important part of the human intestinal flora, but can also cause severe infections in people with immunodeficiency;**
- **Streptococcus pyogenes, a lactic acid bacteria that is exclusively pathogenic for humans and causes scarlet fever and other severe infectious diseases.**

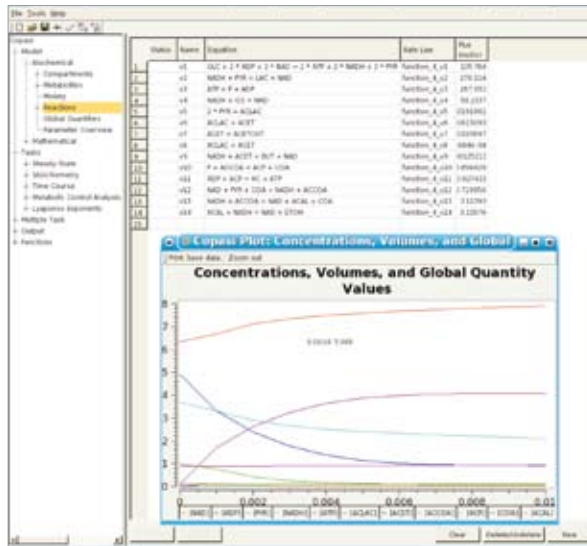


Scanning electron microscope picture of *Streptococcus pyogenes* from a 72-hour old culture.

By developing a basic model for investigating related microorganisms, we aim to explore the differences in the central sugar metabolism between these bacteria and how this metabolism is differentially regulated. Finally, we will also deal with the question of how the findings obtained can be used in biotechnology or for the development of novel therapy strategies against species of human pathogens.

Growth process of *E. faecalis*, *L. lactis* and *S. pyogenes* in aerobic, static cultures in CDM-LAB (chemically defined medium for lactic acid bacteria) at 37 °C. Here, the growth rates (μ) and generation times (g) refer to the exponential growth phase, OD_{max} indicates the maximum optical density of the cultures.





Copasi user interface with a simulation of a pyruvate metabolism model of *Lactococcus lactis*, which was developed in Wageningen (Hoefnagel et al., 2002).

Based on experiments conducted by different partners in the consortium, we will create computer models of the central metabolism of the different lactic acid bacteria for systems biology investigations. Based on fermentation experiments, we will investigate the central carbon metabolism of the three species. Here, we will consider regulatory processes at the genomic, transcription, translation, enzyme and metabolomic levels. They will be quantitatively recorded and integrated into the models in iterative processes. Conversely, we will use experiments to examine predictions that we made on the basis of our computer simulations. This will enable us to refine our models.

Furthermore, we aim to develop algorithms which can be implemented in future for further model development processes. These will then be integrated into existing software solutions, which are used in systems biology. An example of this is COPASI (www.copasi.org), a program that permits the modelling, simulation and analysis of biochemical networks.

Creating a shared foundation

To integrate experimental data from the different consortium members into joint models, comparability of the working conditions and the resulting findings is of major importance. This is achieved by developing *standard operating procedures* (SOP). For this purpose, bacteria from all groups involved will be kept in a similar manner in

collections of strains, and will be grown and treated using the same methods. Experiments will be conducted with standard devices as far as possible.

As an important part of the standardisation process, we developed a joint growth medium for all three bacteria species. This is a chemically defined minimal medium (CDM), which was rebuilt based on protocols from the different laboratories. The establishment of this medium, with which all three investigated species can be cultivated with comparable growth rates, is one of the first important findings gained through cooperation within the SYSMO-LAB project and it forms the basis for further experimental procedures.

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PD Dr. Bernd Kreikemeyer is head of the Molecular Pathogenicity Research working group at the Institute of Medical Microbiology, Virology and Hygiene at University Hospital Rostock. His research activities include the development of systems biology approaches to gain a better understanding of the pathogenicity of *Streptococcus pyogenes*.

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Project partners involved: Prof. Jeroen Hugenholtz (coordinator) and Dr. Joost Teixeira de Mattos (BioCentrum Amsterdam, Netherlands), Prof. Willem de Vos and Dr. Bas Teusink (University of Wageningen, Netherlands), Dr. Isabel Rojas and Dr. Rebecca Wade (EML Research gGmbH, Heidelberg), PD Dr. Bernd Kreikemeyer (University of Rostock), Prof. Hans Westerhoff (University of Manchester, UK), Prof. Ingolf Nes (University of Ås, Norway) and Prof. Ursula Kummer (University of Heidelberg).

Stress in Bacteria

Systems biology analysis of the stress-induced cellular processes of *Pseudomonas putida*

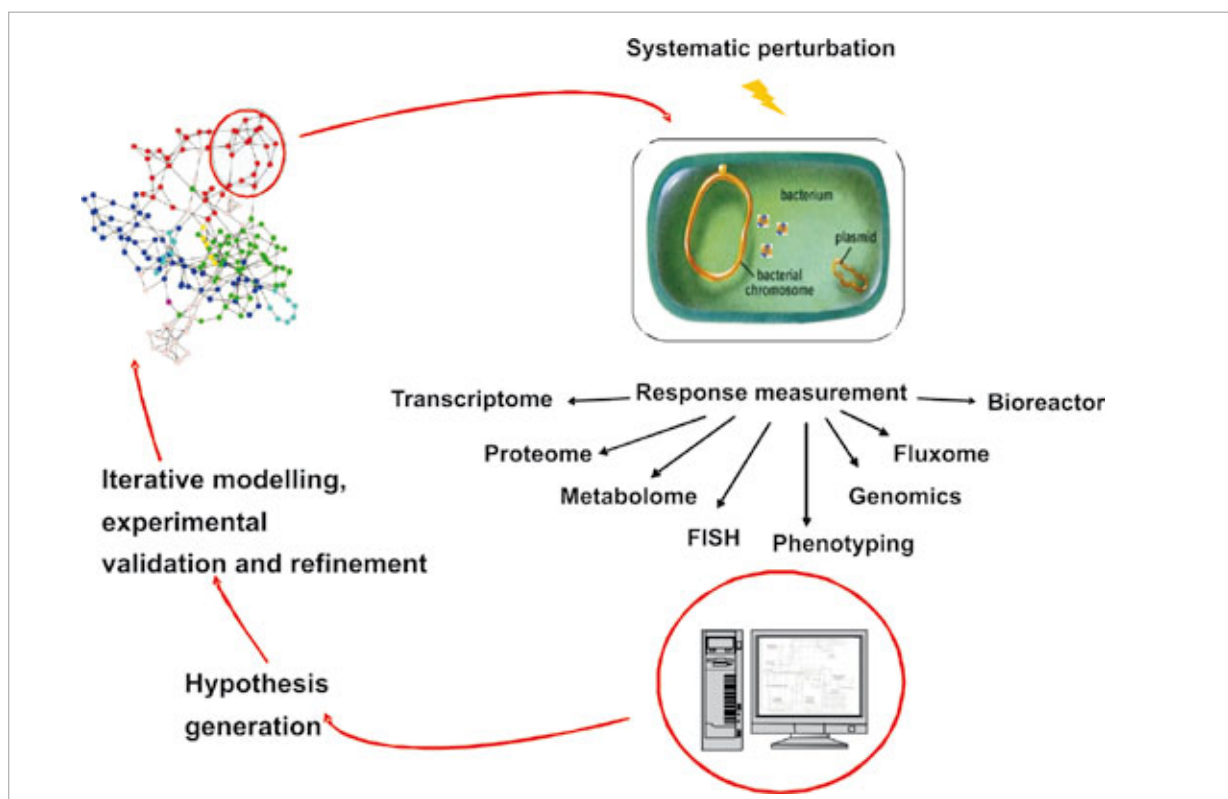
Bacteria react to stress such as extreme temperatures, altered pH, toxins in their environment, the accumulation of metabolic products inside the cell or a lack of nutrients. An understanding of the way in which microorganisms adapt to variable environmental conditions is essential for the development and improvement of biotechnological processes or to find new points of attack against pathogens. The aim of the PSYSMO project is therefore the development of a systems biology strategy for analysing the stress reaction of *Pseudomonas putida*, (strain KT2440). This Gram-negative soil bacterium plays an important part in a whole number of biotechnological processes such as the production of fine chemicals, enzymes and biopolymers, and also for pesticides and the biological degradation of environmental toxicants.

Schematic representation of the systems biology strategy underlying the PSYSMO project. Modelling and experimentation are intertwined at every stage of the iterative process.

PSYSMO is an international and interdisciplinary project – the consortium consists of 17 scientific partners from leading institutions in Germany, Spain and the United Kingdom – which combines functional genomics, bioinformatics and the establishment of mathematical models in order to develop a systems understanding of this key microorganism. Interest is focused on stress factors which occur in a number of biotechnological processes. The aim is to decisively improve the performance of the bacterium.

Analysing stress reactions as a network

Biotechnological applications all represent unnatural and sometimes critical stress situations for the cell and may result in a significant reduction in performance. *P. putida* is unusually resistant to physico-chemical conditions in industrial processes such as toxic solvents, hydrophobic substances, low pH, low or high temperatures or chaotropic compounds such as urea.



Alfred Oberholz, Evonik Degussa GmbH Halle

“For Evonik, systems biology is already a research approach that is indispensable for maintaining our innovative strength in the production of amino acids and other fine chemicals and its significance will continue to increase.”

The PSYSMO project envisages the following steps in order to develop a systems biology understanding of the metabolic and regulatory parameters which control cellular reactions to stress factors:

- **the identification and quantitative analysis of critical structural and regulatory components;**
- **the development of blueprints and new design concepts for the essential and biotechnologically interesting properties of the bacterium under different conditions;**
- **the use of prediction models to selectively improve the model bioprocesses.**

To this end, “classical bottom-up” approaches based on genetics, microbiology and biochemistry will be combined with novel “reverse engineering” methods, in which we analyse the interaction of the regulatory and metabolic networks starting from the bacterium and its reactions to cellular stress – both experimentally and also theoretically. This is done on the basis of an integrated analysis of global cellular reactions by high-throughput methods, transcriptomics, proteomics, metabolomics, interatomics and fluxomics.

Using models for improved yields and novel processes

Genome-wide models of the metabolic network of *P. putida* represent a means of simulating its metabolism under various conditions and also of describing in detail specific sequences that are of interest for biotechnological production. The systems biology analysis of the bacterium provides new insights into its cell functions.

The long-term goal of the project is to link systems biology to synthetic biology. The results of the *in silico* simulations serve to predict the reactions to process-relevant internal stress such as the hyperproduction of metabolites, enzymes and polymers, and also external disturbances. The findings can also be used for *in vivo* reprogramming, that is to say for the design-based genetic modification of the bacteria and their industrially relevant metabolic components. This will lead to improved yields and enables new biotechnological applications to be implemented.

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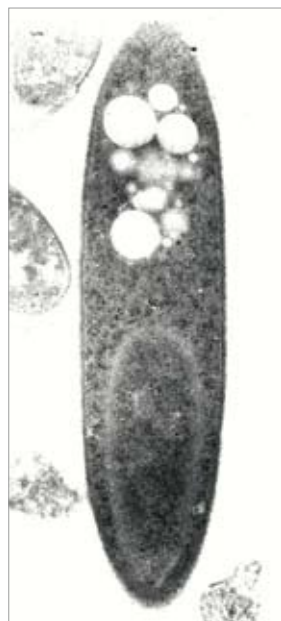
Clostridium acetobutylicum – a Response to Dwindling Crude Oil Reserves

Systems biology approaches for improved biotechnological processes

Clostridium acetobutylicum is an anaerobic bacterium that grows exclusively in the absence of oxygen. It has a fermentative metabolism by which it converts sugar (glucose) into acetic and butyric acid. However, the accumulation of excreted acids in the environment represents a problem for the bacterium and inhibits its bolic activities. In order to avoid this situation it begins to take up the acids again and converts them into acetone and butanol – and in this way it can ensure that it survives for several more reproductive cycles. However, this metabolic pathway ultimately represents a dead end for *C. acetobutylicum* since the butanol attacks the cell membrane.

The bacterium is of great interest for industry due to its ability to produce acetone and butanol. These two solvents are applied in the chemical industry. Butanol is, moreover, also of interest as an alternative and renewable biofuel. It can be mixed with petrol in any concentration without impairing engine operation. Butanol is less corrosive than ethanol, which is already being used for this purpose; it is easier to handle and also has a better mileage/consumption ratio.

Until the middle of the last century, acetone and butanol were produced on a large scale by biotechnological methods. In fact, this is the most important and largest-scale industrial fermentation process that has been applied anywhere in the world to date. After about 1950, crude oil was considered to be a much more economical starting material, which led to biotechnological production being abandoned.



Electron microscopy (thin section) of a *C. acetobutylicum* cell. Clearly visible are the developing endospore (oval structure at one cell pole), the formation of which is induced at the same time as solvent production, and several spheres of the storage compound granulose (bright spheres at the other cell pole), a polysaccharide, whose degradation serves as an energy source for sporulation.

The dramatic rise in oil prices and dwindling crude oil reserves means that the fermentative production of acetone and butanol with the aid of *C. acetobutylicum* is now attracting more interest again. New methodological developments in molecular biology – the so-called “omics” technologies and also systems biology approaches – can be exploited for the selective and effective construction of tailor-made production strains. This thus makes it possible to obtain acetone and butanol as bulk materials for chemical synthesis more economically and, moreover, in a CO₂-neutral manner.

Shift mechanism unknown

The aim of our project is to model and understand on a molecular and cellular level the mechanisms that lead to the switching from acidogenesis to solventogenesis in the metabolism of *C. acetobutylicum*. The individual processes in the cell – i.e. the metabolic pathways from the sugar substrate to the final products of acetic and butyric acid or butanol and acetone – are already well known. On this basis, we create computer models which can be used under certain conditions to make predictions of the metabolic processes. In doing so, we also consider the influence of other cells in the environment, i.e. the communication between bacteria (*quorum sensing*), the glycosylation of proteins in the cell, substrates of different oxidation stages and external stress factors such as heat or the product butanol.

Transcription analyses with DNA microarrays serve as the starting point for the studies. In addition to the other methods of functional genomics, such as proteomics or metabolomics, they are particularly well-suited for identifying the relevant components of a system and also to simultaneously perform the required measurements of as many single components as possible. Work with DNA microarrays makes it possible to quantitatively record the transcription of all the genes of a genome. With the aid of the techniques of proteomics and metabolomics, we also determine which transcripts are actually converted into functional proteins and how active they are.

In order to first investigate the two extreme points in the metabolism, we cultivate *C. acetobutylicum* under defined conditions in a continuous

culture in which the final products are continuously withdrawn. In this way we achieve, on the one hand, sustained acidogenesis and, on the other hand, permanent production of solvents. After identifying the genes, which are expressed differently in the two extreme states, i.e. which belong either to the acid or to the solvent metabolism, the next step is to selectively identify the shift mechanism. To this end, we alter the pH of the cultures, both in distinct steps and also dynamically, take samples and perform transcription analyses. In this way, it is possible to monitor the expression of the genes involved in the switch between the two metabolic pathways.

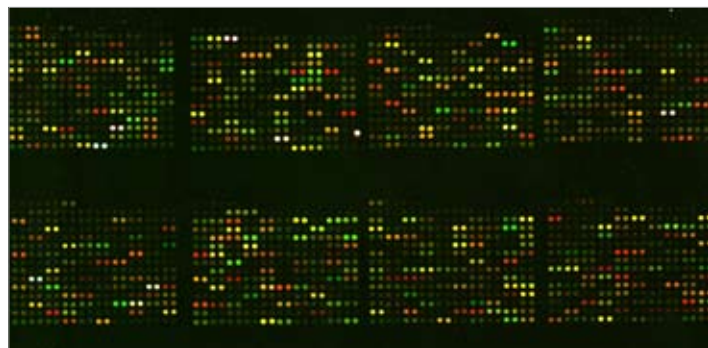
In analogy to this approach, we plan to investigate other factors in addition to the pH, for example the influence of glycerol as a substrate, which is more reduced than glucose, and also different phosphate concentrations.

Increasing the butanol production

Apart from analysing the genes and gene products directly involved in solventogenesis, we are also interested in those genes and gene products that increase the bacterium's tolerance to butanol. This is the property that is of particularly great interest for a high production yield on an industrial scale. To this end, we use transcriptome or proteome analyses to identify those genes and proteins in *C. acetobutylicum* which are of special significance for elevated butanol concentrations. These genes and proteins could possibly have a key function in this organism's tolerance of high butanol concentrations.

From the experimentally determined data we create a quantitative model containing the information we have collected on the transcriptome, proteome and metabolome, and in part also on enzyme activities under different conditions and metabolic situations. We supplement our findings with data already published in the literature.

With the aid of the model, we investigate the shift mechanism in the changeover from acid to solvent production. In this way we hope to identify the central sensors and switches between the two alternative metabolic pathways. Furthermore, a suitable model enables predictions to be made concerning production under altered conditions and thus



Part of a DNA microarray of *C. acetobutylicum*, comparing gene expression during acidogenesis and solventogenesis. Each gene of the organism is represented on this DNA microarray by two adjacent spots. Shades of red mean that the respective gene shows higher expression during solventogenesis, while shades of green indicate higher expression during acidogenesis. Yellow spots indicate genes with unaltered expression under both sets of growth conditions, and blanks result from genes which are not expressed under either of these conditions.

increases the flexibility for commercial applications, in which, for example, alternative substrates are used or the final products are permanently withdrawn during the fermentation process. These findings will contribute to improving the biotechnological production of acetone and butanol by creating ideal culture conditions and possibly also by manipulating the relevant key proteins in the cell.

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ERASysBio

13 countries coordinate their funding activities

Above and beyond the national funding strategy of the Federal Research Ministry (BMBF) in the research field of systems biology, BMBF has also launched two funding initiatives on a European level, namely ERASysBio and SysMO, and has undertaken to coordinate both. In this way, Germany is making a decisive contribution to the further development of systems biology in Europe, as well as to the coordination of the research and funding activities of European funding organisations.



The Strategy Paper of the ERASysBio initiative describes their mission, which is to carry out fundamental and strategic collaboration in the funding of systems approaches to biological research.

ERASysBio is a transnational initiative of funding organisations committed to supporting the establishment the “European Research Area” (ERA) in systems biology.

ERASysBio brings together 16 ministries and funding agencies from 13 countries - EU member states and associated countries - to coordinate their national funding programmes in the field of systems biology. The partners agreed on a common European research agenda, drafted through and in dialogue with the scientific community. The resulting strategy paper “Systems Biology in the European Research Area” represents the views of the scientific community and funding organisations on present and future directions of systems biology in Europe. It was first published in November 2007. The revised and final version can be downloaded from the ERASysBio website www.erasysbio.net since April 2008.

The objective of the ERASysBio partners is to strengthen the ERA in the field of systems biology by

Partner	Funding Management Organisation
Belgium	National Fund for Scientific Research (FNRS)
Germany	Federal Ministry of Education and Research (BMBF) Project Management Jülich (PtJ)
Finland	Academy of Finland (AKA)
France	French National Centre for Scientific Research (CNRS) Agence Nationale de la Recherche (ANR)
United Kingdom	Biotechnology and Biological Sciences Research Council (BBSRC)
Israel	Israeli Science Foundation (ISF)
Netherlands	Netherlands Organisation for Scientific Research (NWO) Netherlands Council for Health and Development (ZonMw)
Norway	The Research Council of Norway (RCN)
Austria	Federal Ministry of Science and Research (BWF)
Slovenia	Ministry of Higher Education, Science and Technology (MHEST)
Spain	Ministry of Education and Science (MEC)
Trento, Italy	Autonomous Province of Trento (PAT), Department of University and Scientific Research
Russia	Russian Foundation for Basic Research (RFBR)
Associated Partners	Funding Management Organisation
Luxembourg	National Research Fund of Luxembourg (FNR)
Switzerland	Swiss National Science Foundation (SNF)

stimulating and facilitating programme coordination and joint activities. The first transnational call for proposals in this framework, in which six partner countries are participating, is SysMO “Systems Biology of Microorganisms”.

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Data and Facts on Funding for Systems Biology in Germany

The funding of research in systems biology in Germany by the Federal Ministry of Education and Research (BMBF) began in 2004 with the funding priority “Systems Biology of Liver Cells - HepatoSys”. A total of 21 research projects - in three collaborative projects and two platforms - were supported in the first phase of funding from 2004 to 2006 with a total of € 14 million. The working groups involved came mainly from university and non-university research institutions as well as from three small and medium enterprises (SMEs).

In the years that followed, the funding of research activities in the field of systems biology was successively expanded. In addition to the approval of a second funding phase for HepatoSys (2007 - 2009), further programmes were launched for systems biology research. Table 1 provides an overview of the current funding of the different BMBF activities on systems biology as well as the breakdown of funds according to funding measures.

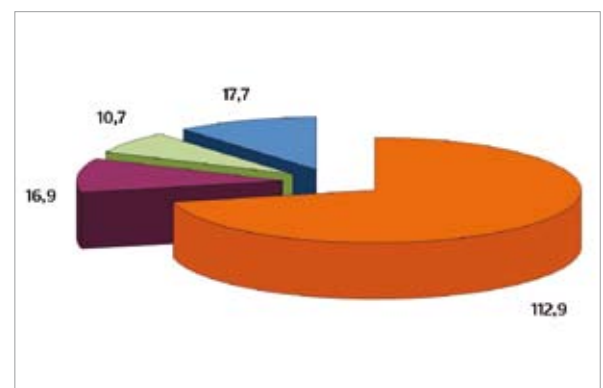
The majority of research activities are organised in collaborative projects which are each composed of a number of individual projects. The only exception is the research priority “FORSYS Partner”. Here, in addition to the collaborative projects, thirteen Young Investigators Groups are supported as individual projects.

The major proportion of the funding - € 112.9 million out of a total sum of € 158 million - is invested in university research. This high proportion can be traced to the establishment of four research centres at the universities within the framework of the FORSYS funding measure. The remaining funds are distributed as follows: € 16.9 million for Max Planck institutes, € 17.7 million for other facilities such as Helmholtz institutes, Blue-List research facilities, Fraunhofer institutes and foundations, and € 10.7 for companies. While BMBF offers projects at university and non-university facilities full financing (100 %), industry must cover an average of 50 % of the total costs from their own funds. A total of 29 companies are supported within the framework of the funding measures for systems biology.

The financial participation of industry varies according to the individual funding measures. Overall, however, particularly for HepatoSys, interest in participating in research projects appears to be increasing on the part of industry. The financial participation of industry in the total funding sum rose from 3.5% in the first phase of funding for HepatoSys to 7.9% in phase two.

Erich Stüttgen, Project Management Jülich (PtJ)

Distribution of grants between different institutions (in million euro)



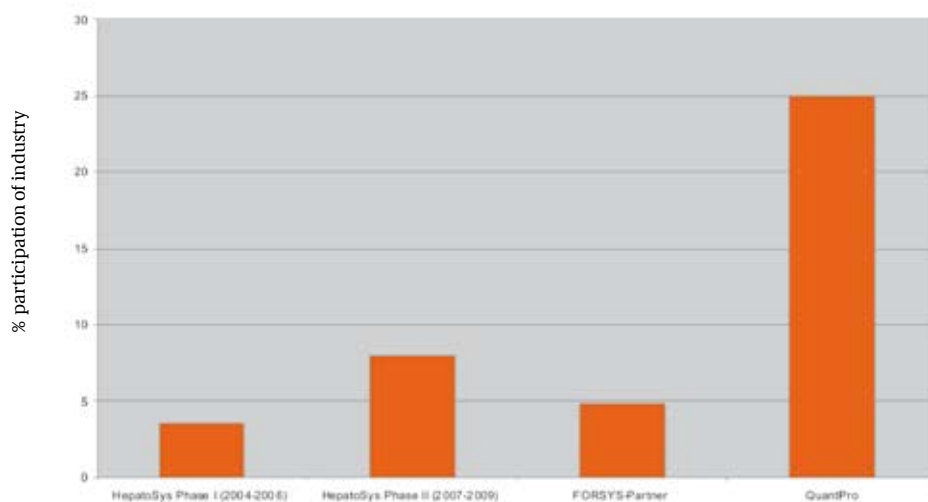
- Universities
- Max Planck Institutes
- Companies
- Other institutions (Helmholtz Centres, Blue List research institutions, foundations, Fraunhofer Institutes)

Table 1: Survey of BMBF funding activities in systems biology

BMBF-funding priority	start	duration (years)	grants (Mio. €)	number of cooperation projects funded
HepatoSys	2004	3+3	36	4 + 2
FORSYS	2006	3+2	51	4
QuantPro	2006	3	27	14
SysMO	2007	3+2	11	11
FORSYS-Partner	2008	3	32	10 cooperation projects 13 young investigator groups

Table 2: Number of projects in systems biology

	HepatoSys	FORSYS	QuantPro	SysMO	FORSYS-Partner
cooperation projects	6	4	14	11	10
subprojects	32	0	48	34	52

Participation of industry in the different funding priorities

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