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SYSBIOMED

Systems Biology for Medical Applications

SSA

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WORKSHOP REPORTS

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1st SysBioMed Workshop on Systems Biology for Medical Applications

26th February – 2nd March 2007
Puerto de la Cruz, Tenerife (Canary Islands)

Participants

Scientist	*Research topic*
Markus Rehm, Royal College of Surgeons, Ireland	*Single cell imaging; apoptotic signalling*
Diego di Bernado, Telethon Institute of Genetics and Medicine, Naples, Italy	*Modelling gene regulatory networks*
Jacky Snoep, University of Amsterdam, Netherlands	*Modelling Enzyme Kinetics*
Jesper Tegner, University of Linkoeping, Sweden	*Reverse engineering of gene regulatory networks*
Jochen Prehn, Royal College of Surgeons, Ireland	*Single cell analysis of caspase activation during apoptosis*
Jörg Stelling, ETH, Zurich, Switzerland	*Robustness and cellular design principles*
Julio Banga, IIM-CSIC, Vigo, Spain	*Parameter estimation and optimal experimental design*
Marta Cascante, University of Barcelona, Spain	*Modelling fragility of robust tumor, metabolism*
Michael White, University of Liverpool, UK	*Real time single cell Imaging; NFk-B pathway*
Nestor Torres, University of La Laguna, Tenerife, Spain	*Power-law modelling in biomedicine*
Nicolas Le Novere, EMBL-EBI, Cambridge, UK	*Modelling neuronal signalling*
Nils Blüthgen, University of Manchester, UK	*Modelling MAPK cascades*
Olaf Wolkenhauer, University of Rostock, Germany	*Statistical vs. Mathematical Models*
Pierre de Meyts, NovoNordisk, Denmark	*Insulin signalling*
Robert Jaster, University of Rostock, Germany	*Signal Transduction in Pancreatic Fibrosis*
Thomas Höfer, Humboldt University, Berlin, Germany	*Molecular immunology and mathematical models*
Rosita Cottone, Federal Ministry of Education and Research, Berlin, Germany	Guest
Maike Heidelberger, Project Management Agency Jülich, Germany	Guest
Veronika Simons, Project Management Agency Jülich, Germany	Guest
Astrid Lunkes, European Science Foundation, Strasbourg, France	Partner
Karsten Schürrle, Society for Chemical Engineering and Biotechnology (DECHEMA e.V.), Frankfurt, Germany	Co-ordinator

Findings

The first preparatory workshop served to gather a group of young scientists from the SB and medical research fields, including clinicians, who discussed the future options of SB approaches in medical research.

There obviously is a need for employing SB in current biomedical research: For decades of efforts in elucidating the mechanisms behind severe diseases the traditional reductionist approach has delivered a huge amount of sound information on the molecular pathologies, the cells, and the signalling and metabolic pathways involved.

However, the more information has been obtained the more the unexpectedly high complexity of many disease conditions has become apparent. The most prominent example is diabetes of both types. Despite more than 50 years of intense research in the field there is still no 'whole picture' of the interplay of the many underlying mechanisms causing the symptoms. For instance, one still does not understand the role of the cell types involved in the disease: ß-cells, adipocytes, muscle cells, not to mention the cells of the immune systems or the role of the brain regulating appetite. Genetic research on 'risk genes' often showed that completely unexpected genes turn out to be relevant. All the knowledge accumulated so far resembles a huge puzzle of partial information on pathways from physiological, genetic, molecular and clinical data. For these reasons the European market leader in diabetes care, NovoNordisk of Denmark, has already embarked on SB projects at its Hagedorn Research Institute. Bayer AG of Leverkusen, Germany, and GlaxoWelcome, Stevenage UK, are also reported having launched SB initiatives in drug discovery.

The participants of the workshop agreed that assembling the puzzle of the pieces of information available from all relevant sources and levels will be key to gain deeper insights into the complex pathologies of major diseases. SB is expected to allow for the definition of mechanisms and the evolution of realistic models of disease conditions. In the discussion the participants looked at different major diseases whith regard to the potential of SB approaches to increase knowledge: tumor diseases, immunological disorders, neurological disorders, infectious diseases, diabetes and ageing (considered a disease).

The outcome of the thorough discussions is best reflected in the proposals for the next round of focused workshops which can be understood as a list of priority projects promising successful applications of SB:

Diabetes: great industrial interest; huge problem that requires a new (systems) approach;

Basal Ganglia Disorders: covers a range of relevant pathologies, including Parkinson's disease, Huntington's chorea, schizophrenia, attention-deficit hyperactivity disorder, Tourette syndrome, drug addiction.

Regulation of Inflammatory Gene Expression: Inflammation is associated with problems that relate to various diseases; allows the study of environmental effects (e.g. epigenetics of T- & B-cells)

Apoptosis & Cell Cycle: chrono-therapy: i.a. very important for understanding and optimising chemotherapies

From Networks to Cell Fate - Colon cancer: a problem for which existing knowledge, data and experimental systems provide an ideal basis to explore systems biology in a translational effort.

Single-Cell Technologies: indispensable tool for generation of high value data on cellular physiology, includes phospho-proteomics; from images to models.

Model-Integration: Looking at the larger picture, we require methodologies to identify (to define the boundaries of) subsystems (modules), explore these in experiments and then integrate models thereof. The techniques should shape the design of experiments. The programme is clearly different but complementary to data integration efforts in bioinformatics, addressing models across (spatial and temporal) scales as well as functional levels. Going from genes to disease pathologies.

The experts agreed on these priorities - i.e. well defined questions that can be investigated within a few years - based on a catalogue of essentials that ideally should be granted to the largest possible extent:

- Established animal models and knock-ins available
- In vitro and ex-vivo models; in vitro measurements
- In vivo imaging
- Single cell experiments feasible
- Data at different levels available
- Possibility to identify subsystems
- Knowledge on environmental aspects in regulation of gene expression (epigenetic vs. genetic factors)
- Quantitative spatio-temporal data (signalling) accessible
- Genes and dynamics should be known
- Ability to cross or integrate levels (model integration)
- Availability of bioinformatics approaches (data integration)
- Opportunities to combine basic research with clinical research
- Need to improve pathological understanding
- Outcome should be therapeutic agents (for which a choice should be available) and understanding why and how they work, options for individualised treatment
- Interest of industry to invest (more)

The organisers plan to invite more clinical researchers from the fields named above to the next round of *exploratory* workshops. The scientists advocating research in the distinct disease areas will care for attracting the experts needed. In addition the SYSBIOMED partners will benefit from the ESF's large network of medical researchers in order to win "the right people" for the task. The second workshop series is tentatively scheduled for the month of July at either Brussels or Frankfurt.

Input from Winter School

The 1st COSBICS Winter School on Systems Biology for Medical Applications (February 27th- March 2nd, 2007) was combined with the SYSBIOMED-Workshop. Some of the scientific lectures confirmed the assumption that SB could deliver valuable insights into medically relevant phenomena. For instance, Katja Rateitschak and Olaf Wolkenhauer from Rostock were able to demonstrate that the modeling of a signalling pathway involved in apoptosis could help to sort out alternative hypotheses based on genetic control and protein-protein interactions. The refined model they presented was well in line with experimental data. Jörg Stelling's group from Zurich is exploring the principles of 'cellular robustness'. He applied the concepts of control theory to the modeling of the the circadian clock. The group arrived at a model that explained the relevance of certain physiological threshold values for the oscillating behaviour – a knowledge that could possibly serve to optimise chemotherapies. Michael White gave an impressive demonstration of the potential of 'real-time single cell imaging' as a source of otherwise inaccessible high value data. His group is able to track individual proteins involved in the NFk-B signalling pathway. Thomas Höfer, Humboldt University Berlin, shed light on the interplay of the cells of the immune system. Focusing at the complex genetic and external factors of the differentiation of naive T cells he presented an silico model which allowed for the study certain combinations. Robert Jaster, Rostock University, Germany, is studying the mechanisms of the signalling pathways responsible for the progression of pancreatic tumors. His talk showed that modelling the Ras-Raf kinase signalling and the (JAK)-signal transducer and activator of transcription (STAT)- pathway could serve to understand the highly prolific growth of the cancer cells and the role of cytokines involved.

Networking with other SB initiatives

The participation of representatives of national and transnational SB initiatives is a key objective of the SSA ensuring that information on ongoing and planned projects and initiatives is widely disseminated and exchanged in the community. The combination of the first SYSBIOMED workshop with the first COSBICS Winter School was especially helpful. Moreover, Maike Heidelberger and Veronika Simons, both of the Project Management Agency Jülich, Germany, and in charge of co-ordinating/managing HepatoSys (a German initiative in SB research on hepatocytes), FORSYS (German Research Centres for Systems Biology) and SysMO (Systems Biology of Microorganisms) were present and informed the audience on these efforts. Astrid Lunkes, European Science Foundation (ESF), Strasbourg, is a SYSBIOMED partner. She presented the ESF networking platforms *Frontiers in Functional Genomics* and *Functional Dynamics in Complex Chemical and Biological Systems.* ESF's forward look *Towards a European Strategy for Synthetic Biology* is currently being drafted and is expected to deliver input for SB research projects. A very interesting new instrument is ESF's *EuroBioFund* programme which was set up in 2006. It seeks to identify future grand challenges in the life sciences which require a coordinated European approach. Identification of these topics is based on ideas put forward by the scientific community. Financing comes from the EC, industry, charities and other public and private funding organisations across Europe. *EuroNanoPar* (European Nanomaterial Proactive Assessment of Risk) is the first example of such a project.

SysBioMed Workshop on Systems Biology for Basal Ganglia Disorders (BGD)

2-3 July 2007, Frankfurt am Main, Germany

Participants

1. Bhalla, Upinder, NCBS, India	bhalla@ncbs.res.in
2. Bjaalie, Jan G., Karolinska Institutet, Sweden	jan.bjaalie@incf.se
3. Bruhn, Thomas, ESF, France	tbruhn@esf.org
4. Gallinat, Jürgen ,Charité Berlin, Germany	juergen.gallinat@charite.de
6. Girault, Jean-Antoine, INSERM, France	jean-antoine.girault@fer-a-moulin.inserm.fr
7. Gurney, Kevin, University of Sheffield, UK	k.gurney@sheffield.ac.uk
8. Le Novère, Nicolas, EMBL-EBI, UK	lenov@ebi.ac.uk
9. Prehn, Jochen, RCSI, Ireland	JPrehn@rcsi.ie
10. Schiffmann, Serge, University of Brussels, Belgium	sschiffm@ulb.ac.be
11. Schürrle, Karsten, DECHEMA, Germany	schürrle@dechema.de
12. Smit, Guus, Vrije Universiteit, The Nederlands	guus.smit@falw.vu.nl

Presentations

First Nicolas LeNovère introduced to the functions and dysfinctions of basal ganglia and the biochemistry and pathology of basal ganglia disorders (BGD). These disorders share the same anatomical substrate, namely a brain structure composed principally of the mesencephalic dopaminergic nuclei, the striatum, and the pre-frontal cortex.
The most important BGDs fall into this category:

- Parkinson's disease (PD)
- Huntington's chorea
- Hemibalism
- Schizophrenia
- Drug addiction
- Depression
- Obsessive-Compulsive Disorder
- Tourette syndrome
- Attention-Deficit/Hyperactivity Disorder (ADHD)

Although both their pathogenesis and their symptoms are very different, the technological bottlenecks faced when developing a systems biology approach are similar.
Concludingly, he briefly looked at the diverse approaches in modelling: electrical modelling (neural-network, cable approximation), chemical kinetics (deterministic, stochastic ...), reaction-diffusion (PDE, finite elements ...), single particle dynamics and others.

Then the participants presented their research focus in concise lectures of 20 minutes each.

Jochen Prehn is developing biological models of neurodegenerative disorders. He presented examples of *in vitro* and *in vivo* models suited to study the degeneration of dopaminergic neurons of the *substantia nigra* which is relevant to PD. *In vitro* models (Primary dopaminergic neurons from mice or rats, cultured as dissociated cells or in organotypic cultures; PC12 cells, neuroblastoma cells or other transformed cell lines; neural stem cells, embryonic stem cells) and single cell imaging deliver good data for systems analyses: quantitative, with good to excellent temporal and spatial resolution. Moreover, significant events can be averaged out in population studies. His *in vivo* studies employ MPP+ / MPTP lesions as well as 6-OH-Dopamin lesions in rodents and other species; alpha-synuclein transgenic animals, and Omi/HtrA2 knockout mice.

Serge Schiffmann presented the current state of the development of *in vivo* models of some neuronal disorders. The utility of any animal model critically depends on the experimental level of analysis, with a multilevel approach more likely to identify novel features. Generating bona fide mouse models for schizophrenia and most human psychiatric disorders is complicated. In comparison to neurodegenerative diseases like Alzheimer's disease or amyotrophic lateral sclerosis, (+PD, HD) where there is a well defined neuropathology, neuropathological markers are not readily apparent in most psychiatric disorders.
Schiffmann presented animal models of drug addiction, KO & transgenic mice with altered responses to psycho-stimulants. Sensitization is achieved by drug self-administration and intracranial self-stimulation. Conditioned place preference (CPP) is regarded as "readout". He also introduced knockouts of mouse orthologues of schizophrenia susceptibility gene candidates which serve as rodent models of schizophrenia. If such models are suited to elucidate the etiology remains to be investigated. Knockout mice with candidate genes affected also serve as animal models of depression. They show physiological changes and patterns of transmitter concentrations and regional brain activity resembling those associated with the disorder in humans.

Guus Smit presented "Novel avenues in high-throughput proteomics and functional analysis of genes". His group performs HT proteomics experiments to study the dynamics of the proteins of tthe synaptic membrane. The synaptic membrane fraction proteome – the proteins mainly synthesized at the synapse from a local pool of mRNA - consists of roughly 900 proteins. Smit investigates drug addiction and the effects of calcium/calmodulin-dependent protein kinase II (CaMKII) at the proteome level. CaMKII is a molecular switch involved in the storage of long-term neural changes. The activity of the CaMKII holoenzyme (the complete enzyme consisting of both regulatory and catalytic subunits) is controlled by its autophosphorylation state. His group is also working on the identification of genes associated with neuronal regeneration by analysis of the effects of transcription factor mutants. As automation is indispensable in standardization and validation of high throughput phenotyping (HTP) Guus Smits laboratory developed Phenotyper, an automated system for the analysis of cage behaviour suited to detect the aberrant behavior of mouse mutants.

Nicolas Le Novère presented the modelling of neuronal function and molecular and sub-cellular levels. After listing the different processes to model to understand basal ganglia disorder: Electrical properties, chemical interactions (metabolic networks, signalling pathways, neurotransmitter receptor function, gene transcription; protein translation), molecule movements, ionic diffusion, protein transport, compartment remodelling, he presented some of these using examples from his group. The model of chemical interactions was illustrated through the interaction of dopamine and glutamate interactios. Several papers were recently published on the topic, showing that this kind o approach is gaining momentum. The problem of small numbers, combinatorial explosion and spatial localisation were tackled using agent-based modelling, through the modelling of calcium/calmodulin multistate behaviour and the diffusion of receptors in the membrane. Many approaches have been developed that allow us to address every questions asked above, and the bottleneck remains the availability of quantitative data of high quality.

Upinder Bhalla discussed the modelling of whole neurons. Neurons are among the best modeled entities in biology. There are five major aspects to consider:
- Passive electrical conduction, - Synaptic input, - Active properties: voltage-gated channels, - Calcium influx, - Intracellular signaling. Different approaches have been made of which passive compartment models that 'chop' the neuron into little cylinders are well suited to study electrical properties (cable equation). They deliver real-time detailed models (100 compartments, lots of channels, Ca) and 10,000 compartment models with ~100,000 ion channel instances. Electrical models also provide input to chemical models where diffusion is represented as flux of molecules between compartments. Further refinement comes from mass-action kinetics (reaction-diffusion model). There are still a lot of technical problems to solve, i.a. the mismatch of time-scales: electrical changes occur within < msec while chemical steps are in the 1 sec range which would require variable timesteps in modelling. However, one of Bhalla's models was able to demonstrate the neuronal signal transduction as the spreading of an activity pattern along the chain of compartments.

Kevin Gurney, one of the pioneers of the modelling of basal ganglia, presented a 'computational hypothesis': The basal ganglia play a critical role in solving the problem of action selection in animals. Mechanistically, the basal ganglia is a central 'switch' that grants access to motor resources from action requests with high urgency or salience where 'actions' can include overt motoric behaviour and 'thoughts' and 'cognitions'
The selection hypothesis is a unifying one: other functions are not excluded, rather they are reinterpreted in terms of selection. In other words: Disorders of the basal ganglia are disorders of action selection. In this model, the input-output characteristics are those required to select, and switch between actions, the dopaminergic modulation being consistent with PD and some other pathologies. Gurney also presented a robot model of a rat that picks up food and hides in a corner to eat. The control architecture of the robot decomposes into 'behavioural units' (wall-seek, corner-seek, can-pick-up), rather than functional units (perception, planning, motor control).

Jürgen Gallinat is investigating the role of dopamin and glutamate in the patho-biology of schizophrenia. He employs nuclear magnetic resonance imaging for the detection of glutamate in the brain and finding of correlations of glutamate concentrations and neuropsychological test performance. One found that the cerebral glutamate concentration in schizophrenia is reduced in the anterior cingulate cortex while increased in the hippocampus. Combining these results with the vivo activity of the ventral striatum ('reward system', associated with euphoric feelings) showed that reward-related activation of the v. striatum is reduced in unmedicated schizophrenic patients, also reduced in patients with typical vs. atypical Antipsychotics, and increased in alcoholics in response to alcohol cues.

Jens Pahnke reported on Parkinson syndromes, histological and clinical subtypes, and new approches in the pathogenesis research of Alzheimer's disease (AD) and PD. One of the most promising therapeutic approaches to PD is deep brain stimulation with micro-electrodes which stimulate the *nucleus subthalamicus* region. It can be considered a systems approach as it affects a key regulatory functional unit of the brain. Pahnke explained that the pathogenesis of AD is likely due to inhibition of protein transport by ABS transporters eventually resulting in aggregation of amyloid proteins and impaired regeneration of brain cells. There is a wide field for the application of bioinformatics methods to investigate the clearance mechanisms at the blood-brain barrier which separates brain cells from blood vessels, especially the kinetics of clearance vs. protein aggregation are subject to modelling.

Jan G Bjaale represented the International Neuroinformatics Coordination Facility (INCF), an OECD initiative. It aims to coordinate and foster international activities in neuroinformatics, to contribute to development and maintenance of database and computational infrastructure and to support mechanisms for neuroscience applications. Main goal is the advancement of neuroinformatics - an interdisciplinary research area, combining research in neuroscience and informatics (including computation) to develop and apply advanced tools and approaches needed for understanding the brain. INCF seeks to enable access to all freely accessible data and analysis resources for human brain research to the international research community. The organisation also develops mechanisms for the seamless flow of information and knowledge between academia, private enterprises and the publication industry. INCF is represented by national 'nodes' which receive funding from the member states. The workshop participants appreciated Jan Bjaale offering the support of INCF to possible projects in SB applied to research in brain disorders.

Discussion and Results

The purpose of the meeting was to evaluate the feasibility of a systems biology (SB) approach to BGDs by exploring the possibilities of the current technology (functional genomics, imaging, in vitro and in vivo physiology etc.), the availability of existing animal models, the access to patients, and the existing modelling efforts.

Based on this evaluation, the participants discussed and eventually agreed on a ranking of disorders suitable for a systems biology approach:

Disease	Significance	Data situation	Modelling
#1 **Drug addiction**	multiple kinds of addiction with common core mechanism; high social impact; targeted treatment required, some clinical ties	good, animal models, large functional genomics projects	common core with neuro adaptation, few quant. models available
#2 **Parkinson's disease**	1% of 60+ and 5% of 80+ affected locomot vs. psychosis, potential clinical ties	strong, good model systems available, many levels accessible, large proteomics networks	ongoing at many levels: metabolic models, dopamin pathway, neurons, MSN models
#3 **Schizophrenia**	Very high, possible links to smoking/alcohol addiction, good access to patients	comparable to drug addiction, few and poor model systems	

However, it was generally agreed that the understanding of schizophrenia is still embryonic, and a systematic and systemic approach was maybe premature. Therefore, the panel suggests that effort should be focussed on **Parkinson's Disease** and **Drug Addiction**

Moreover, the participants discussed in detail the situation of experimental data of all levels with regard to availability, quality and value to SB. The results are concisely summarised in the following table:

Type	Specifications	Quality of curr. data	Remarks
	Molecular		
Gene expression	- which - time - location - data source	B C B B	
Protein	- which - time - location - data source - interaction - dynamics of i. - modification - structure/function	A-C A-C B-C B-C B B-C B A-C	large-scale data should be made available for 3 neuronal cells: dopaminergic (DA) neurons, striatal medium spiny neurons (MSN) D1 and D2
Lipids		C	
Metabolome		C	

Functional	- kinetics - interactions - diffusion constants - cell physiology	C B-C C C	functional data are largely missing
	Cell		
Morphology		A-B	
Electrophysiol.	- passive (Rm, Cm, Vm, Ra) - channel (kinetics, distribution) - transmitter - firing patterns - plasticity - shape (changes)	A-B B-C A A A-C B-C	
	Circuitry		
Connectivity	- statistics - location - type (chem/gap)	B-C B-C B-C	systematic micro-scopy of neuron morphology and brain structures available for recon-struction
Activity	- unit - field - EEG - fMRI (patients)	B B A	
Structure	- MRI - spectroscopy	A A	
	Behaviour		
Disease models		?	
Patients		?	

Considering what is necessary, what is available, where standardisation is mature, and where experimenter's expertise is still the main factor, the panel concluded that a call for funding Systems Biology approaches to basal ganglia should also fund quantitative data gathering along two different lines:

- Large-scale data gathering, centralised in a few experimental facilities should seek to gather information about the protein content of three major cells: mesencephalic dopamine neuron, striato-nigral ("D1") medium-spiny neuron and striato-palidal ("D2") medium-spiny neuron. These large-scale effort should determine at least: the proteome, the interactome, the concentration of the proteins and their subcellular locations. Standardisation and public databases are ready.

- Kinetics information gathering should be funding using classical distributed funding. This information concerns chemical kinetics constants, diffusion constants, electrophysiological parameters of subcellular compartments, dynamics of neurotransmitter release.

The requirements of modelling with regard to computing aspects (standardisation, access, etc.) and options for collaboration shall be discussed separately at the upcoming workshop "Dynamic Principles of Cellular Function" on October 24th, 2007 in Frankfurt/M, Germany.

SYSBIOMED Workshop on Systems Biology and Inflammatory Diseases (IDs)

September 7th, 2007, Frankfurt am Main, Germany

Participants

Ashton-Rickardt	Philip	Imperial College London, UK
Cottone	Rosita	Fed. Ministry of Education and Research, Berlin/Bonn, Germany
Gstaiger	Mathias	Institute of Molecular Systems Biology, ETH Zurich, Switzerland
Höfer	Thomas	German Cancer Research Center (DKFZ) Systems Biology, Modeling of Biological Systems ,Heidelberg, Germany
Klingmüller	Ursula	German Cancer Research Center (DKFZ) Systems Biology of Signal Transduction, Heidelberg, Germany
Löhning	Max	Med. Clinics for Rheumatology and Clinical Immunology, Charité – University Medicine Berlin and Dt. Rheuma-Forschungszentrum (DRFZ), Berlin, Germany
Bruhn	Thomas	European Medical Research Councils (EMRC) European Science Foundation (ESF), Strasbourg, France
Rand	David	Warwick Systems Biology & Mathematics Institute Warwick Systems Biology, Coventry House University of Warwick, England, UK
Schürrle	Karsten	DECHEMA e.V. (coordinator)
Serfling	Edgar	Institute of Pathology Würzburg University, Germany
Swat	Maciej	Biosystems Informatics Institute Newcastle upon Tyne, England, UK
White	Mike	School of Biological Sciences University of Liverpool, England, UK
Wolkenhauer	Olaf	University of Rostock Systems Biology and Bioinformatics Group, Rostock, Germany

Discussion

The half-day workshop consisted of a thorough discussion of the options systems biology (SB) approaches could provide to research on inflammatory processes. The participants believe that the timing is quite right for starting SB projects in medical research since technology, tools and components required for quantitative measurements in biological systems are currently emerging. Depending on the specific question, appropriate methods should allow for reasonable conclusions. In order to elucidate central mechanisms of the immune system across all levels one has to identify key players responsible for IDs, relying on data from *in vivo* models (knockout mice) as well as dish cultures.

Primary immune cells are considered "good models" due to the high standard of available cell culture technologies and the access to *in vivo* models, i.e. KO mice.
The following disease-relevant functions of certain cell types were discussed with respect to the amenability for SB approaches:

- T-cell activation
- T-cell survival/ renewal
- T-cell memory
- macrophage activation
- neutrophil functions

Given the high technological standards of current cell biotechnology T-cells appear as the preferred target system while neutrophils which are involved in host defence were regarded as less suitable for SB projects due to technical reasons, i.a. the limited range of functions and the comparably difficult cultivation *in vitro*.

The participants agreed on research priorities ('big questions') in the ID arena which would benefit most from SB:

1 T cell control/ activation
Elucidate the *decision process* governing cell death and growth, i.a. cell-cycle dependent mechanisms, project would be linking different communication processes involved (external signals, CC). Dynamic gene expression data and proteome information could be provided at high resolution. The factors governing the activation are still not completely identified.

2 Chronic inflammation
apparently is the underlying cause of severe disorders, including the runaway proliferation of mammalian cells. Roughly 15% of human deaths from cancer are associated with chronic viral or bacterial infections. Various disease-related models are accessible, especially when looking at T cell renewal. The long-term regulation of T cells is not well understood: what makes them replicate fast, then remain almost silent? What is the role of the cells of the micro-environment?

3 Key players: COX1/COX2 dependent messengers
Shedding light into the mechanisms triggered by non-steroidal messenger molecules is highly desirable with regard to recent failures in the drug market (Vioxx). SB could help to find very general principles underlying many phenomena, e.g. angiogenesis. Novel insights and sound knowledge could help to alleviate FDA-inspired tight regulations of COX inhibitors.

4 Key players: NF-κB
Signalling pathways via nuclear factor-κB (NF-κB) are central to many cellular processes with pathologic relevance. Since the signalling netwoks vary between different cell types SB research would deliver insights gained from comparing primary and other cells.

5 Immunosupression
What are the mechanisms behind the turning-off of the immune systems in certain, often chronic disease conditions? How do cancer cells induce apoptosis? Which cells are affected? Modelling approaches applied to established model systems are expected to provide deeper insights.

6 Gene transcription
There are still no good models explaining the rates of gene transcription (exeption: the *lac*-operon modeled on the basis of the known repressor proteins). Elucidating the role of key genes and the hirachy of genetic organisation is possible by combining advanced research on gene activities and modelling approaches. Decision processes in the development of T helper cells (T_H1 vs. T_H2) is an example from the ID area that could be investigated.

7 Interaction of antigen presenting cells (APCs) and effector cells
Current efforts to model this important processes are static, only a few players are known.

8 Interaction of ceratinocytes and fibroblasts

This systems constitutes a good model, data are accessible.

9 Proliferation vs. cell death

The dynamics of immune cell populations should be well amenable to modelling approaches since clean data are available at different levels. Questions to be addressed: why does proliferation stop? What is the effect of 'noise', i.e. the robustness of the systems' response to stochastic changes?

Challenges and Obstacles

Data:

Data from assays measuring cytokine profiles are needed, the respective technologies are being currently developed. Quantitative dynamic expression data are hard to obtain, respective technologies (e.g. advanced multiphoton microscopy) are under development. Single cell experiments are considered a valuable source of data. Careful pre-processing of data is considered key to the efficient collaboration of laboratories. Such work requiring sufficient technical staff is usually underestimated in proposals.

Models:

On the theoretical side proper approximation algorithms are indispensable as are tools that allow the interpretation of dynamic imaging data carrying information on gene activities. There is a need for modelling as the current knowledge is low: although 50% of the data of signalling pathways via IL-2 is available, the situation concerning other pathways is far worse (20%). There is good dynamic data on NF-κB pathways. Modelling is expected to help identify the missing components and factors. Modelling is expected to shed light into the secrets of the immune system as listed above.

On research programmes

The *raison d'être* of SB essentially is the added value to science from the productive, complementing interaction of molecular biologists and modellers. On attacking 'Big Qustions' (e.g. How are T cells turned off?) a set of relevant sub-areas has to be defined and teams with complementing expertise must join their forces.

Generally, the duration of SB projects is considered too short. Longer projects with more flexibility would be recommended. People with sound experience are in short supply, SB research projects shall serve to provide training opportunities to young talented scientists. Medical researchers are interested in participating in SB projects, clinicians, however, still stay distant.

Scale issues arising from the type of a research programme needed to answer a question, e.g. the large numbers of people and money systematic knock-in studies would require, were briefly addressed. Future programmes should increase budgets for video-conferences and go with less bureaucracy. On the European level SYBILLA, systems biology of T-cell activation in health and disease, started in April 2008. It is a European Union-funded large integrated project in framework program 7 (FP7). The goal of SYBILLA is to understand the intracellular signalling network of T cells that determines T cell fate.

SYSBIOMED Workshop on
Dynamic Principles of Cellular Function

October, 24th and 25th 2007 Frankfurt am Main, Germany

Participants

Blüthgen, Nils	The University of Manchester	UK	nils.bluthgen@manchester.ac.uk
Bruggemann, Frank	Vrije Universiteit	NL	frank.bruggeman@falw.vu.nl
Fell, David	Oxford Brookes University	UK	dfell@brookes.ac.uk
Girolami, Mark	University of Glasgow	UK	girolami@dcs.gla.ac.uk
Lunkes, Astrid	ESF	France	alunkes@esf.org
Nimmesgern, Elmar	BMBF	Germany	Elmar.Nimmesgern@bmbf.bund.de
Schürrle, Karsten	DECHEMA	Germany	schürrle@dechema.de
Snoep, Jacky	University of Stellenbosch	South Africa	jls@sun.ac.za
Thieffry, Denis	INSERM	France	thieffry@tagc.univ-mrs.fr
van Leeuwen, Ingeborg	University of Nottingham	UK	pmzivl@maths.nottingham.ac.uk
Wilkinson, Darren	Newcastle University	UK	d.j.wilkinson@ncl.ac.uk
Wolkenhauer, Olaf	University of Rostock	Germany	ow@informatik.uni-rostock.de

Why a workshop on Dynamic Principles of Cellular Function?

Cell function (growth, differentiation, proliferation, apoptosis etc.) are nonlinear spatio-temporal processes operating across levels of the structural and functional organisation of cells. Understanding the dynamic principles of cell functions is the basis for advances in understanding the onset of diseases.

The recent advances in cell biology research suggest a rather astounding conclusion: the more we learn about cells and complex cell populations the less we are encouraged to generalise. Apart from the inherent flexibility of living systems evolution has obviously invented a large diversity of "systems solutions", which, differently organised, produce similar "readouts". Despite the tremendous technological advances we are far away from direct, quantitative observation of living systems like we are used to in the physical and engineering sciences: We assume the functions of cells by envisioning their components and changes thereof. Instead of asking general questions about the functioning of cells, we interpret data for studying a particular signal transduction pathway, for a particular cell line, cell type generated within the limitations of the measurement devices used (microcopy, transcriptomics, proteomics etc.). Our ability to understand completely how a cell works is probably limited, partly because we may have difficulties in defining the functions being implemented and partly because we may not recognise the collection of components that implement the function. Fortunately, this does

not necessarily mean that one cannot predict the responses of a biological system by computer simulation.

However, cell functions are dynamic processes that can be understood in terms of dynamical systems theory with modelling employed to formulate and test hypotheses while supporting the design of experiments: If there are many pathways relevant to one cell function, why study pathways in isolation instead of focusing on a cell function and then hypothesize about the realisation of dynamic principles? The elucidation of the functional organisation of living systems is obviously indispensable for understanding malfunctions, i.e. disease conditions.

Adressing the implications of this approach the workshop's experts discussed the following fundamental aspects of the dynamics of cell function:

Model Integration

In some cases different parts of a model can be treated separately on the basis of their different time scales. For example, this is common in metabolic models where the reactions constituting the internal processes of the enzymes are assumed to be at steady state on the time scale of changes in cellular metabolite pools. This allows the enzymes to be treated as a module with an input-output function. However, this approach cannot be easily applied in many biological systems, for example where the transient behaviour of the fast system has consequences for the behaviour of the slow system, which then feeds back to the faster system after a delay. This may be the case with signal transduction systems, where the signal may be encoded in oscillations (Ca signalling) or transient movements between cellular compartments (NF-κB). Epidermal growth factor signalling causes transients in the MAP kinase cascade on time scales of minutes and relaxes to a new quasi-steady state within an hour. However, important down-stream consequences take much longer to emerge; entry of cells in to the cell cycle and commitment to division requires several hours sustained signalling, whilst growth factor receptor internalization and recycling and altered gene expression alter the concentrations of the components of the signalling system also on time scales of hours.

Therefore merging models of modules with different time scales leads to the introduction of new dynamic interactions that were not present in the sub-models and techniques for developing such multi-scale models need further development by biologists, computer scientists and mathematicians. One technique could be to run the detailed submodel many times to establish its range of behaviour and then to emulate this with a simpler model. However, there are disadvantages if this model reduction loses the mechanistic/physical interpretation of the system structure and parameter values, since it is then more difficult to incorporate perturbations such as the action of a drug that targets a specific component of the module.

Issues of validation also arise, since even if the models of the isolated modules have been separately validated, adjustments will certainly be needed in the merged model so some re-validation will be needed. The same module may need different adjustments when incorporated in different scenarios, so although the concept of re-usable component modules

is attractive, it may be difficult to implement in practice. Certainly issues of selection of appropriate model structures, and resolution of the conflicts between the need to use sparse data for parameterisation whilst retaining some results for model validation are areas where guidance is needed from experts, particularly statisticians.

Merging Signalling, Metabolism and Gene Expression

There is a clear distinction between the technologies which are available for experimental measurement of cell signalling, metabolic or transcriptional mechanisms and this gives rise to operational divisions amongst experimentalists. The development of technology platforms such as proteomics is providing new tools for experimentalists which provide the means for a more integrated view of these concepts to be taken. On the other hand mechanistic models of signalling, transcription and metabolic control are being developed and studied primarily in isolation and there is growing realization that principled integration of these models will be an important capability in systems biology.

The question is how can this be achieved both from a theoretical perspective as well as experimental and cultural. The levels of conceptual detail available to modellers differ across signalling, metabolism and gene expression and this is viewed as a challenge to the successful merging of these concepts. Some are particularly well developed, for example the availability of detailed molecular interaction maps and software tools such as Cell Designer, whereas notions of cross-talk are conceptually not at all well developed and should be considered in full-network behaviour. This lack of consistent conceptual granularity across these areas presents further challenges in this merging. Yet despite this there is a real need, for example, explicitly modelling gene expression mechanisms within signalling pathway models.

One way forward is to develop the idea of a canonical process model describing the very basic components of a sub-cellular process which may take the form of a consistent set of basic dynamic motifs describing e.g. degradation, transcription and translational mechanisms. The development of such canonical components and their study in both autonomous and embedded mode, in the form of libraries, will provide a valuable resource which can guide the overall modelling process. These canonical components will differ significantly based on the levels of abstraction adopted, assumptions made and approximations required.

Crossing Levels

The highly advanced multiscale heart models developed by Noble, Hunter et al probably constitute the best example to illustrate the potential applications of systems biology in medicine. It is important to explore whether some of the methodology developed specifically for heart modelling can be exploited in building integrative models of other biomedical systems. Moreover, lessons could also be learned from other fields, such as climate and weather modelling.

Cancer is a complex, multifactorial, multistep process. Cancer modelling therefore provides a good case-study for the biological, mathematical, computational and technological problems

normally encountered when building an integrative model (e.g. coupling processes across levels of organisation and exploring their interaction).

One of the most exciting/challenging aspects in multiscale modelling is the possibility to explore the impact of interactions between phenomena taking place at different time and length scales). For instance: (1) how do mutations in signalling pathways translate into changes at the tissue level; (2) how do biochemical and biomechanical alterations of the stroma (enviroment) affect intracellular networks?

Techniques are required for analysing and understanding large, complex multiscale models, and their underlying mathematical properties. It will be necessary to simplify and/or reduce models, and perhaps also to develop approximate emulators of sub-components of the full model system. Some form of modularity will need to be exploited. Model simplifications will enable analysis of model sensitivity and validity that would be otherwise computationally intractable. Similarly, fast simulations will be required for the development of effective algorithms for optimal experimental design.

There will also be a need to reconcile bottom-up mechanistic models with more phenomenenological top-down statistical models of full system behavior. Although this is generally true, it is especially important in the multiscale context. It may also be desireable to use statistical models as emulators for model components.

A proper understanding of the importance of spatial effects in models will also be required. When is it OK to ignore sub-cellular spatial effects, and when is the specific 3d arrangement of cells in a tissue non-ignorable.

Stochastic (hybrid) models are likely to be required for effective multiscale modelling. Noise and heterogeneity occurs at many levels, beginning with intrinsic intra-cellular noise at the level of gene expression, through to genetic and environmental differences associated with individual organisms (people?!) in a population. Proper incorporation and propagation of noise and heterogeneity across scales will be necessary for validation and calibration of multiscale models against available experimental data.

European-wide collaboration in multiscale modelling is currently inhibited by a lack of standard language for describing biological models at multiple scales. Although SBML has proved invaluable for the sharing of single-cell continuous deterministic models, use of SBML for stochastic models is still not widespread. More fundamentally, SBML lacks important features such as modularity and support for arrays, which are necessary for the development of large and complex models. Furthermore, SBML is essentially single-cell based, and therefore modelling cell population behaviour is not directly possible. There are plans to include such features in the future, but progress is currently very slow. CellML includes some of these features, but is not widely used within the systems biology community.

Do modules exist naturally or are they organising principles?

Due to the size and complexity of biological systems it will be necessary to construct models of parts of the system that will then be merged at a later stage. Whereas such modules are important for us to conceptualize biological systems, i.e. identify pathways such as glycolysis and PP-pathway, it is unclear as to whether such modules really "exist" in the system. Thus, we addressed the question whether modules can be identified in biological systems for which separate models can be constructed. Such a module should be separate enough from the rest of the system, where separation can be made on the basis of 1) structural organisation (e.g. organelles separate reactions in the cytosol from those in the organelle), or 2) a functional separation (e.g. reactions involved in different functions such as differentiation, growth, apoptosis, coordination of cell-cell interaction). In addition classic separation techniques such as 3) time scales can be incorporated for identification of modules (e.g. the cell cycle is affected through treatment over hours, signalling pathways are studied over minutes). One specific example of a functional separation that is often used to modularize cellular systems is between transcription/translation and metabolism. Although reactions in each of these modules are linked it is possible to separate them experimentally by adding an inhibitor of translation.

Since a system is defined as linked components, there will not be any modules that are completely separated from the system, i.e. there will always be some links. Thus, the consideration should be, is the module separated enough from the rest of the system. Effects of links between the modules and the rest of the system must be taken into account. This can be done by measuring time traces of the linking metabolites and studying the effects of such changes on the isolated module. Indeed, such an experimental validation could be used as a tool in the identification of modules, i.e. only define modules where it is possible to measure the linking metabolites.

The participants also took the opportunity to give their views on the scientific challenges, priorities, and bottlenecks of systems biology. The results are summarised below:

What is your most important/interesting problem/question?

- Integration of metabolism, signal transduction and gene expression for an experimentally accessible system and study optimisation at variable external conditions.
- Are there principles physiological evolutionary adaptation?
- Do we really understand all the requirements for a cell to be 'live', i.e. an autonomous self-generating entity?
- Through which mechanism does a cell adapt and is robust against all kinds of unforeseen perturbations?
- What general principles underlie cell differentiation and development?
- How do multicellular organisms age and what underlies the interspecies differences in metabolic rate and longevity?

- How can we build, simulate and calibrate (against data) timely multiscale, multcellular hybrid stochastic heterogeneous population models of biological systems with direct relevance to human health?
- What is the overall organising and central mechanism(s) for cellular + organ function from birth, development to death?
- How do we identify a subsystem (pathway, module) to study it in isolation and then integrate models thereof to understand a complex whole (cell, physiology)?

Has it to do with metabolism, cell signalling, gene expression?

- All, Answer not possible if only one strand followed
- All, metabolism
- All, adapt the mechanism through signalling and gene expression
- Gene expression -> cell signalling -> metabolism
- All; signalling followed by gene expression
- Doesn't matter; Cell signalling

To which cell function does it relate?

- All, growth,
- All, adaptation to new environments
- All, robustness of one function e.g. growth
- Differentiation -> cell cycle -> proliferation -> apoptosis -> growth
- All; proliferation and apoptosis key
- All; differentiation, following by cell cycle (proliferation)
- Doesn't matter; cell differentiation

Which experimental system would you choose?

- Unicellular organism; microorganism (*E.coli*), yeast, parasite, microbial ecosystems
- Lymphoid cells(T-cells); Drosophila embryo
- More than one required (e.g. Drosophila, mice) for interspecies comparisons
- Yeast / human, cell line, most relevant
- Doesn't matter, eukaryotes
- Doesn't (shouldn't) matter

Which pathway, network or cellular process would be most important?

- All
- don't know
- Aim is to understand whether there are more important components
- TCR signalling pathway -> Notch, wnt, ith
- Tissue renewal / cellular senescence

- Not clear but cell proliferation, apoptosis and necrosis pathways
- Signalling pathway, e.g. MAPK
- To be decided

What methodology/approach would you use for modelling?

- One that does the job, starting with the simplest; ODEs and up
- Simple <-> detailed model; deterministic & stochastic, all kinetic models
- This probably can't be encompassed by a single model. Parts may be stochastic, parts by ODEs; multi-scale modelling
- Possibly evolve large ODE systems and ultimately a realistic ODE model

Qualitative modelling; logical multi-leveled models, Petri nets -> stochastic extensions of

- Petri nets -> hybrid modelling (HPN, diff. eqs.)
- Stochastic, delay ODEs
- Multicell multiscale hybrid stochastic models
- All; ODE + stochastic
- Whatever (is best)

Which technology would be required?

- All; quantiative measurement of metabolites, time series
- Cell – ecosystem level: metabolomics (small and large scale), flux analysis
- All - but quantiative and time-resolved; quantitative proteomics
- All Omics first; see whether adaptions effect only small parts and then narrow it down if possible
- All; chip-on-chip, chIP on chip, and transcriptome
- Several to cover different levels of organisation; single-cell technology
- All; transcriptomics, proteomics initially, single-cell for refinement, facs etc. for calibration
- All; single cell technology -> proteomics (transcriptomis, quant. blot.)
- All; proteomics

What are the mayor hurdles in answering your question?

- Experimental data of suitable precision
- Long term endeavour that requires tools (experimental & theoretical) at all stages
- Not clear whether we have the right conceptual framework
- Money and men power, don't know where to start
- Time constraints, interdisciplinarity, money
- Cover different levels of organisation experimentally and theoretically
- Standard language for describing multscale multicell (stochastic) models
- Technology for observing biochemical system dynamics
- Complexity -> interdisciplinarity

On Research Programmes

The participants agreed on recommending the formation of a European DPCF Network which aims at understanding DPCFs. By creating a respected network the network would attract new people to the area of Medical Systems Biology. A good start of would be an internet platform supporting young investigators by awards and the publication of projects, dissertations and papers. Research Training Network grants would be helpful since current calls are too specific and do not fit the objectives of DPCF research.

SYSBIOMED Workshop on Systems Biology and Cancer

8-9 November 2007, Nice, France

Participants

Blüthgen, Nils	The University of Manchester	UK	nils.bluthgen@manchester.ac.uk
Cascante, Marta	Universitat de Barcelona	Spain	martacascante@ub.edu
Ciliberto, Andrea	IFOM	Italy	andrea.ciliberto@ifom-ieo-campus.it
Drasdo, Dirk	INRIA	France	dirk.drasdo@inria.fr
Hornberg, Jorrit	Organon/Schering-Plough	The Nederlands	jorrit.hornberg@organon.com
Jaster, Robert	University of Rostock	Germany	jaster@med.uni-rostock.de
Lenormand, Philippe	CNRS UMR6543	France	philippe.lenormand@unice.fr
Moquin-Pattey, Carole	ESF	France	cmoquin-pattey@esf.org
Schürrle, Karsten	DECHEMA	Germany	schürrle@dechema.de
Sers, Christine	Charité Berlin	Germany	christine.sers@charite.de
Swat, Maciej	Biosystems Informatics Institute	UK	maciej.swat@biiuk.com
van Leeuwen, Ingeborg	University of Dundee	UK	ingeborg@maths.dundee.ac.uk
Wolkenhauer, Olaf	University of Rostock	Germany	ow@informatik.uni-rostock.de

Despite their variety cancer diseases are systemic by nature. Accordingly, the participants expect SB to contribute valuable information required to elucidate pathological mechanisms thus providing key molecular targets and help to clinicians who have to decide on the targets (or combinations thereof) to be hit with maximum efficiency and minimal side effects. SB could possibly also shed light on fundamental problems like the notorious discrepancy between mouse and human 'model systems' with regard to therapeutic outcomes.

Defining and deciding upon experimental models in cancer systems biology projects

The discussion on which tumours to start studying by SB approaches with good prospects was guided by the following criteria:

- *High medical relevance.* (researchers have to rely on statistical data on frequency and mortality from the US National Cancer Institute since comprehensive, consistent data from Europe is not available.)
- *Well understood molecular pathology*
- *High quality experimental models available*
- *Variety of therapeutic options*

The following table, presented by Robert Jaster, gives a concise overview on the advantages and limitations of presently available tumour models:

Model	Strength	Weaknesses
Established tumour cell lines	• Unlimited availibility • Useful to generate quantitative data for modelling	• Very artificial • Inaccurate reflection of human diseases
Patient-derived tumour cells / cell lines	• reflect individual molecular pathology of individual patients/tumours	• time consuming, lower throughput • availibility
Tumour-bearing immunodeficient animals (e.g., nude or SCID mice)	• Relatively easy to establish • Generation of in vivo data	• Still, quite far away from human reality
Immunocompetent animals with hetero- or orthotopic transplants	• Increased practical relevance	• Often difficult to handle; low throughput
Genetic models	• Most accurate model of human disease	• availibility

The participants thoroughly discussed whether some comparatively rare cancer diseases (e.g. CML) could provide good opportunities for SB strategies. They concluded that such diseases, often lacking suitable model systems and a sound biomedical knowledge base, would not differ from diseases with higher incidence.
With regard to possible cancer stem cells they found that the yet not well understood nature and role of these cells does not suggest SB approaches into this special field at present.

A likely proof of principle case is **colorectal carcinoma** (CRC) which is considered a good choice since there is a high medical need, a good knowledge base from all stages of this multi-step disease and there is access to clinical material. A challenge for SB of much higher order, and probably not a good proof-of-principle case, is the difference/relationship between primary tumours and metastases, especially the investigation of the high flexibility of **metastatic cells** when evading chemotherapy (drug resistance). For example, given a limited set of 'anti-apoptotic signaling pathways', metastatic cells normally manage to activate/re-combine alternative routes whose regulation can possibly be elucidated by SB. Insights into these mechanisms should translate into unprecetented leaps in therapy since it is the formation of metastases which makes tumour diseases hard to cure and eventually lethal.

The workshop participants were aware of the fact that the above recommendations for priorities in cancer research employing SB methods are sound but incomplete. Interdisciplinary SB projects involving experts from different fields generally face the problem on how to decide upon a suitable experimental system for which data can be shared allowing comparative studies and the integration of knowledge across scales from intracellular processes to structural models of tumours and a wide range of technologies employed.
It would therefore seem appropriate to organise a meeting that could guide consortia to decide upon common cell lines, suitable mouse models, tumor types, pathologies, technologies, pathways, methodologies employed. The group is to discuss which types of cancer would provide important challenges or would be suitable for proof-of-principle studies.

On modeling
The development of computational models from signalling to tumour growth (bottom-up and top-down) shall be done on the intracellular, cellular, multicellular level. Discussing the scope and requirements of modeling of cancer diseases the participants agreed on the priority task of linking structural models with signaling and metabolic pathways and cell cycle. This would require the identification of functional units (signaling pathways: identify key targets within; metabolic networks: metabolic adaptation that support tumor proliferation; identify targets) and modeling across time scales from minutes in signalling to hours and days when studying growth. Model integration incorporating 'cross-talk' of signaling pathways is essential. Phenotypic information on cells and cell populations is required to identify possible signal transduction pathways. Clinical data from patients is considered valuable for applying SB models to translational research.

A bottleneck
Apart from the huge technological challenges, SB in biomedical research faces serious risks from insufficient (wo)manpower. Traditional career paths in the medical sciences still are incompatible with SB research: Researchers with a medical background and career ambitions are currently hard to recruit because systems biology projects are more time consuming, complex, interdisciplinary, and the bedside-benefits not around the corner.

Research presentations
The participants briefly presented their research projects and views. *Robert Jaster* provided a concise introduction to tumour biology and discussed the requirements for successful SB approaches. *Andrea Ciliberto* showed that qualtitative data was sufficient to build a comprehensive model for the cell cycle of yeast. Mammalian model systems, which are not well developed yet, are harder to study as genome duplication makes the situation complex and it is difficult to define the cell cycle engine. Current modeling attempts concentrate on key checkpoints. *Philippe Lenormand* supported this view and pleaded for chosing HeLa cells as model systems. Stem cells are probably not well suited, their G1/S transition being different from adult cells. *Jorrit Hornberg* reported on the impact SB is expected to have on pharmaceutical research and therapy. Tumours, sensitive to kinase inhibitors, should be destroyed by combinations of many drugs of this class, the therapies based on the analysis of biomarkers. SB is considered indispensable to generate the required multi-dimensional knowledge. *Marta Cascante* looked at the metabolome of tumour cells. The robustness of tumor metabolism often counteracts single hits of drugs. Unfortunately, multiple-hit strategies also fail due to unpredictable by-pass reactions of the tumour metabolism. SB is obviously urgently needed to increase the knowledge of the metablic network and to rationally design drugs. *Christine Sers* focused on cell signaling. The time scale of signaling spans from seconds to days (e.g. secondary changes like DNA methylation associated with wound healing). She pointed to the lack of technical tools to quantitatively detect the components of pathways and to correlate signals and phenotypic output. *Dirk Drasdo* presented a 3D model of tumour growth controlled by contact inhibition of cells and nutrient supply. By varying parameters (cell cycle time, adhesion strength, migration speed, elastic modules) one can adjust the virtual tumour tissue to display a growth behaviour closely resembling the 'real thing'. It is noteworthy to mention APO-SYS, a FP7 project which grew out of FP6 'European Systems Biology Initiative for combating Complex Diseases' and aims at applying 'Apoptosis Systems Biology' to cancer and AIDS.

Scientific challenges
The participants took the opportunity to concisely outline their favourite scientific goals/questions in cancer biology which they would like to see tackled by medical SB research. The following list provides brief profiles of the objectives of the proposals as communicated by the workshop members:

Proposal 1

Objective:
- Integrative spatial-temporal model of tumour growth and therapy in in-vitro setting, animal model and human.

Proposal 2

Objectives:
- Comprehensive pathophysiological understanding of the networks involved in colorectal carcinogens (e.g. oncogene/anti-oncogene, metabolic network)
- Generation of rational basis for therapeutic decisions: which drugs to choose in a given context to archieve maximum efficiency / strong therapeutic effect, low side effects
- Optimisation of therapeutic regimes (scheme, dosage, time course)

Proposal 3

Objectives:
- Build a multiscale model for normal ... dynamics and the early stages of cancer
- Couple fundamental pathways to cellular processes
- Provide a user-friendly tool which experimentalists and clinicians can use to study the system and generate new biological and testable hypotheses
- Carry out experiments in silico which provide new inight that complements and/or enforces the knowledge aquired by experimental methods

Proposal 4

Aims:
- Characterise metabolic adaptations that support colon cancer cell proliferation and metabolic changes accompanying oncogenic transformation and signal transduction activation/silencing

Objectives:
- Construct a model of dynamical metabolic fluces in tumour cancer cell lines from tracer based metabolomics and metabolic profiling data obtained by GC/MS, LC/MS and NMRA platforms and software ...
- Quantify metabolic flux changes accompanying different oncogenic transformations using tracer metabolomics
- Quantify metabolic changes accompanying drugs targeting metabolic network, signal transduction networks or cell cycle mashinery

Proposal 5

Objectives:

- Identify functional signalling pathways in colorectal cancer cells relevant for proliferation, survival, motility (MAPK, Wnt, PI3K, CDC42/Rac)
- Study the behaviour of these pathways in response to therapeutic interventions in vitro
- Identify underlying epigenetic modifications relevant for pathway function
- Identify epigenetic modifications caused by activated pathways relevant for proliferation, survival and motility

Proposal 6

Objectives:

- Role of MAPK signalling pathways to influence the balance between cell proliferation and poptosis in colon cancer model cell line: Normal versus transformed cell
- Collaboration with modeller to understand cross-talks between MAPK pathways

Proposal 7

Objectives:

- Desease models data base which will be a collection of reference models
- Standardising of models
- Cancer and acute inflammations

Proposal 8

Objectives:

- Increase the success rates of drug development by
    - Identify better targets and
    - Identify combination of targets to

  Increase efficacy and reduce toxiscity
- For this we need models that include molecular targets such that combinations can be simulated. Please confer to the last slide of my presentation: That summarises what we need to have an impact in the near future

Proposal 9

Objectives:

- Building predictive mathematical models at appropriate molecular detail to a) integrate the measured data and b) be able to predict the outcome of interventions at signalling level
- Integrate changes of gene expression caused by signalling into the model to incooperate cross-talk (mainly through negative regulators such as DUSPs)
- Investigate targets and possible strategies to interfere at multiple points in the network.
- Simulate strategies of the cancer cells to circumvent treatment and find the best way to prevent that
- Make a statistical model that links signalling state to biophysical cell parameters to fascillitate multi-scale modelling
- Make a link to the cell-cycle model

SYSBIOMED Workshop on Diabetes and Systems Biology
30-31 January 2008, Berlin, Germany

Participants

Benson, Mikael	Queen Silvia Children's Hospital,	Gothenburg, Sweden	mikael.benson@vgregion.se
Bruhn, Thomas	European Science Foundation (ESF	Strassbourg, France	tbruhn@esf.org
Cascante, Marta	Universitat de Barcelona	Spain	martacascante@ub.edu
Cottone, Rosita	BMBF	Berlin, Germany	rosita_cottone2002@yahoo.de
De Meyts, Pierre	Hagedorn Research Institute,	Copenhagen, Denmark	pdm@novonordisk.com
Groen, Bert	Academic Medical Center	Amsterdam, The Netherlands	a.k.groen@amc.uva.nl
Illig, Thomas	National Research Center for Environment and Health	Munich, Germany	illig@gsf.de
Lage, Kasper	Technical University of Denmark	Lyngby, Denmark	kasperlh@cbs.dtu.dk
Liu, Xiaohui	Brunel University	United Kingdom	XiaoHui.Liu@brunel.ac.uk
McGuire, Jim	Steno Diabetes Center	Gentofte, Denmark	JMC@novonordisk.com
Nerup, Jørn	Steno Diabetes Center,	Gentofte, Denmark	jne@steno.dk
Nimmesgern, Elmar	BMBF	Berlin, Germany	Elmar.Nimmesgern@bmbf.bund.de
Pociot, Flemming	Steno Diabetes Center	Gentofte, Denmark	FPoc@steno.dk
Schürrle, Karsten	DECHEMA	Germany	schürrle@dechema.de
Tiedge, Markus	University of Rostock	Germany	markus.tiedge@med.uni-rostock.de
van Driel, Roel	University of Amsterdam, Netherlands Inst. for Systems Biology	The Nederlands	roeld@science.uva.nl
Wolkenhauer, Olaf	University of Rostock	Germany	ow@informatik.uni-rostock.de

Roel van Driel presented "Systems Biology to combat Metabolic Syndrome (SBMS)", a EuroBioFund initiative. The EuroBioFund Programme was set up in 2006 by the European Science Foundation and the European Commission to identify future grand challenges in the life sciences which require a coordinated European approach for their financing and

implementation. SBMS was selected a hot topic for several reasons, most important is the insight that traditional approaches are obviously failing to achieve breakthroughs. Conceived for a ten years duration SBMS aims to achieve a "true understanding of MetS which allows rational and effective therapies and drugs." The programme builds on two pillars: 1. *Excellent ongoing MetS-related research* (strong research groups in Europe, large high quality data sets, national and international (EC) programs) and 2. *Rapid developments in systems biology* (data integration and system analysis; experimental design; integrate biology, physics, engineering, mathematics; national and international (EC) research programmes). Complexity of living systems, fragmented research activities, and data integration are considered essential hurdles. Driven by the huge socio-economic pressure from MetS/ diabetes funding is expected to come from many parties: EC, ESF, charities, research councils, governments, investors, banks, and industry (food, pharma, health insurances, others). SBMS is in its first phase, the outline for the whole duration is as follows:

	proposed main objectives	duration	approximate investment (M€)
Phase 1	• develop governance structure • agree on roadmap and major milestones	12 months	0.2
Phase 2	• start pilot research program	18 months	6
Phase 3	• expand research activities	30 months	60
Phase 4	• full-blast program execution	60 months	200

SBMS Cost-Benefit Analysis by Arthur D. Little

SYSBIOMED is invited to join forces with SBMS, some of the scientists involved are already members of both initiatives.

Pierre De Meyts gave an in-depth introduction into the current understanding of type 2 diabetes (T2D). He discussed the different topics of diabetes research:
- Insulin resistance vs. beta cell defect in T2D, the two fundamental reductionist hypotheses on the pathogenesis of T2D, besides an "inflammation-centric" and a "brain-centric" view, which suggest that T2D is either mainly a signal transduction defect or a gene transcription defect, or both.
- The need to develop a Systems approach to investigate insulin signal transduction defects and the beta cell defect in T2D that incorporates insights from genome-wide scans for association, epigenetic studies, tissue interactions, metabonomics and the microbiome. He

also briefly looked at diabetes type 1 (T1D) as a dynamical instability of intercellular communication.

Recent research has come across amazing puzzles, e.g. the conflicting results when comparing obesity and T2D incidences or the unexpected role of insulin as a signal molecule for beta-cells giving rise to the positive feedback of insulin secretion on beta cell function. Reversing the traditional dogma of translational medicine by quoting Sidney Brenner's motto "From bedside to bench!" Pierre DeMeyts presented the results of recent genome-wide scans: At least 10 genes/ loci have been validated as relevant for T2D. Among them were surprise candidates like SLC30A8 which codes for the zinc transporter ZnT8 or PPARG, a nuclear receptor for thiazolidinediones. Knockout studies with *C. elegans* revealed that as many as 112 genes increase fat storage when inactivated.

Flemming Pociot is investigating T1D which is an immune-mediated, multi-factorial disorder. Cytokines are specifically toxic to beta cells and may be key players in the pathogenetic processes. It is a multifactorial disease with polygenic predisposition and no disease-specific mutations exist so far. According to recent research, gene-gene interaction is important but, unfortunately, individual effects and sub-phenotypes are hard to identify. Novel analytical approaches, expression profiling and computational methods for positional candidate prioritizing are promising. A multidisciplinary approach is regarded very well suited for identification of genes and proteins involved in the disease.

Jørn Nerup reported on the epigenetic manipulation of gene expression in fetal rat islets of Langerhans. Some results from genetic research in this field are rather Lamarckian: Unfavorable intrauterine conditions (e.g. caloric or protein restriction, reduced placental blood supply) often produce a diabetes-like phenotype characterised by reduced body weight, reduced beta cell mass, reduced insulin secretion and glucose tolerance, enhanced sensitivity to cytokine toxicity and beta cell toxins. This phenotype persists for life and is transferable to subsequent generations.

Proteomic 2D-gel studies of islets from rat fetuses given a low protein (8 percent) and isocaloric diet showed statistically significant changes ($p<.01$) of expression levels of 70 proteins (total on gel 2810). They are involved in pathways which when perturbed most likely cause the enhanced sensitivity to cytokines and beta cell toxins. However, addition of taurine to the drinking water (2.5 %) of the pregnant rats throughout the gestational period normalises the diabetes-like phenotype.

One would presume that the phenotype associated with low protein diet and changed islet protein expression would result from changes in expression of genes controlling beta-cell development/differentiation or genes responsible for islet hormone production/secretion, and the taurine transporter gene. Surprisingly, genes known to be involved in beta-cell development and differentiation (Pdx1, Hnf6, Hnf1, Ngn3, Nkx6, Pax4 and 6) were not affected. Both insulin genes, the glucagon, somatostatin, glucose transporter genes, and the taurine transporter gene (slc6a6) all did not show any change, too. Instead, changes occured in genes involved in cell proliferation and cell cycle (CC), cellular defense mechanisms, and respiration (mitochondria).

Integrative approaches

The participants agreed to the conclusion that for decades reductionist approaches have failed to provide an understanding of the pathogenesis of T2D, methods to prevent or cure it, and drugs to treat it optimally. There are numerous puzzles left, e.g. the unclear correlation of diabetes and obesity. Thus, there is a high need for integrative approaches in order to understand the multi-layered complexity of diabetes mellitus. Pierre De Meyts expects that genome-wide scans for association will sooner than later establish a complete nomenclature of the common alleles that in unfavorable combination predispose to disturbed metabolism, and that these will reveal new critical nodes/pathways in metabolic regulation. Connecting these dots is regarded as the premier contribution of systems biology generating useful models.

Discussing which systems to study from a beta cell defect standpoint, beta cells appeared as a priority. Focussing on beta cells, however, carries the risk of missing important interactions, i.a. between cells of other tissues. Moreover, the quality of currently available primary beta cells is considered low. When studying the second hypothesis, insulin resistance, there is a number of cell types worth looking at: muscle cells, adipocytes, liver cells, and other tissue cells. Lines of muscle cells and adipocytes are available already. Hepatocyte cell lines have recently become accessible in course of HEPATOSYS, a German systems biology research programme dedicated to the hepatocyte system. A similar approach to study the beta cell may be worthwhile. The participants welcome the emphasis on diabetes/obesity in the current 'translating research for human health' call of FP7.

Data situation: Generating data from all levels - genes, proteins, metabolites – is considered possible, the latter type of data, however, not that well accessible. Genetic population studies are considered important, but difficult to perform. Protocols to produce standardised time rows are required when studying the pathogenesis of diabetes. Standardisation is key issue and regarded as a matter of 'mind set' of the consortia's partners.

Current **technology trends** are pushing the limits of experimental design: Parallel high throughput (HT) sequencing of DNA at low cost has become a reality like the parallel monitoring of gene activities which has been available for some time. Metabolomics technologies are already delivering reliable results, while extracting such data from single cells remains on the whish list as does the option to measure intracellular metabolite gradients. Generating proteome data still bears difficulties with respect to quantification while recent progress in the development of 'capillary western blot' systems allows to identify phospho-isoforms of phosphoproteins in HT assays. Systems to perform HT microscopy/imaging experiments are not available yet, but in development. They promise to make a difference in protein analysis, especially for monitoring the intracellular spatial dynamics of proteomes. HT data on protein-protein interactions are highly desirable, the required technological advancements are currently being made. Data from combined RNAi experiments are considered highly important and the participants expect them to be a 'commodity' in the near future. There is a strong case for concentrating such technology facilities in HT centres as this would allow for efficient standardisation and access.

Education is a key issue of interdisciplinary research. Projects in medical systems biology, in particular, would require the cooperation of trained clinicians. Since systems biology is close to the clinicians way of thinking it should not be difficult to attract physicians to this research field. However, experience shows that the heavy non-research workload on clinicians and the far time horizon of publications from integrative projects are serious obstacles when trying to attract young scientists from medical disciplines. The participants proposed to organise summer schools devoted to relevant topics from medical systems biology. Endowed with travel grant money the courses should help to ease this problem.

SYSBIOMED Workshop on 'The Ageing-Cancer Link' and Systems Biology
12 February 2008, Paris, France

Participants

Cottone	Rosita	Guest	rosita_cottone2002@yahoo.de
d'Onofrio	Alberto	European Institute of Oncology, Italy	alberto.donofrio@ieo.it
Downes	Stephen	University of Ulster, Republic of Irland	cs.downes.ac.uk
Fuellen	Georg	Ernst Moritz Arndt University, Germany	fuellen@uni-greifswald.de
Gonzalez	Julio	University of Rostock, Germany	jv030@informatik.uni-rostock.de
Irminger-Finger	Irmgard	University Hospitals Geneva, Switzerland	irmgard.irminger@medcine.unige.ch
Kooijman	Bas	Vrije Universiteit, Amsterdam, The Netherlands	bas@bio.vn.nl
Lain	Sonia	University of Dundee, UK	s.lain@dundee.ac.uk
Lunkes	Astrid	ESF, France	alunkes@esf.org
Proctor	Carole	Newcastle University, UK	c.j.proctor@ncl.ac.uk
Schürrle	Karsten	Dechema, Germany	schuerrle@dechema.de
van Leeuwen	Ingeborg	University of Dundee, UK	ingeborg@maths.dundee.ac.uk
Wolkenhauer	Olaf	University of Rostock, Germany	ow@informatik.uni-rostock.de

Ageing is not a disease. However, many serious disorders are obviously correlated with age, their incidences often being significantly higher in the elderly population. Epidemiological data suggest that the incidence of certain cancers, such as colorectal carcinoma (CRC) and prostate cancer, is age-dependent. A large variety of mechanisms, ranging from accumulated damage by radiation, oxidative stress, infections, etc., to dysfunctional DNA repair, that might underlie the links between cancer and ageing are currently under **debate**. Looking closer, one needs to distinguish three groups of cancers: tumour diseases correlated with genetic predisposition (e.g. breast cancer); cancers with likely involvement of stem cells (e.g. testicular cancers); and typical childhood cancers (e.g. some **leukemias**). Failed tumour suppression, an impaired immune system and accumulated damage are considered responsible for the emergence of age-related **cancers**; tumour suppressor proteins, ageing genes and environmental stress representing the obvious links to ageing-related processes. A review by

Toren Finkel *et al.* gives a comprehensive summary of the current knowledge of the ageing-cancer link.[1]

The participants agreed that causal mechanisms of ageing and the interplay of putative key factors are not well known today. Many different processes seem to be involved. For example, cellular senecence, i.e. the loss of the ability to divide after a certain number of cell divisions, is considered a major cause since it limits the potential of tissue regeneration. The role of stem cells is a major challenge to both ageing and cancer research. Another puzzle to be solved is the role of sirtuins, NAD-dependent histone deacetylases, found in both prokaryotes and eukaryotes. They affect cellular metabolism through selective gene expression in eukaryotes and may be able to control age-related disorders like obesity, metabolic syndrome, type II diabetes mellitus and Parkinson's disease. Comparing fruit flies which live a few days, birds species that live up to seven decades and plants that enjoy lifespans of several centuries, one finds that cell differentiation is obviously decisive: the arrested growth of the somatic cells of fruit flies is responsible for accumulating eventually lethal damage. Regeneration and maintenance of cells is essential for birds which have low reproductive rates and need to mobilise energy reserves for long-distance migration. The longevity of plants is essentially due to the ability of plant cells to reverse differentiation when regenerating tissues. Moreover, caloric restriction is generally believed to extend the life-span of many organisms. Intensive research efforts will have to be devoted to the striking difference of the susceptibilities of different organs to tumour formation (e.g. heart vs. breast tissue).

Integrative approaches

The participants are well aware of the many research projects on ageing currently funded in EC framework programmes (e.g. AGEACTION "Changing Expectations of Life" within FP6). Systems biology approaches are expected to complement these efforts and to provide deeper insights into some specific questions, such as those addressed by the following research proposals collected from the participants:

• Investigate the possible feedback loops between sirtuins and p53 by monitoring the response, in terms of tumour incidence and ageing symptoms, to sirtuin activators and inhibitors. **Suitable animal models include** mice, short-lived fish (Notochorda) and cell

[1] *The common biology of cancer and ageing*, Toren Finkel, Manuel Serrano, Maria A. Blasco, Nature 448, 767-774 (2007)

cultures expressing fluorescently labeled proteins that make it possible to study the proteins' dynamic behaviour. Ultimate aim: identify (combinations of) agents capable of preventing or curing cancer without causing premature ageing.

• *Individual level:* Study differences in nutrition, nutrition history and body size related to tumour **growth** by a model consisting of a hierarchy of allocation rules (modification/extension of the κ-rule in dynamic energy budget theory, DEB). *Cellular level:* Study and simulate population dynamics of mitochondria affected by ROS (reactive oxygen species), using data from unaffected, healthy mitochondria populations monitored under different nutritional conditions. *Ecological level:* Compare ageing in different species with respect to timing of reproduction; how is ageing connected with the genetic diversity of germ cells? (modelling context: body size scaling in DEB theory). Technologies to precisely monitor tumour growth in a non-invasive manner and quantitative biochemical indicators of ROS damage are required.

• Large scale effort to identify targets that are responsible for prolonging the *health*-span of humans. Ideal animal model system would be primates, better humans, other: mice and dogs. Data sources: SNPs, micro arrays, metabolome, proteome profiling, toponome data.
Structures:
European Ageing Research Laboratories (EARL) that spend a third of their funds to external labs (collaboration grants) with a minimum bureaucratic burden.

• Discriminate between those causes of cancer that are genuinely related to the ageing process and those that depend on *time* rather than *age* (e.g. the onset time of virus-related tumours depends on the incubation time rather than on the host's age *per se*). *In silico* multiscale mathematical models are pointless if not combined with 'wet' experiments.

• Are ageing cells (suboptimal functions, mutations etc.) more prone to develop cancer ? Studying transformed epithelial cells and the emergence of cancerous stroma cells: Is the aberrant stroma often observed surrounding tumours a cause or a consequence of the malignancies? (Special project: What is the role of the ubiquitin ligase encoded by BRCA1which affects multiple important players like cell cycle (CC) proteins and the estrogen signalling pathway?)

Approach: Prospective population studies on breast cancer in age groups 30-40 and 60-70. Comparison of premalignant-and healthy tissue in non-tumour environment with early tumour and later stage tumour tissues by studying stroma in healthy tissue, tissue with early-stage tumour and tissue with advanced tumour. Methods: Comparative genome hybridisation (CGH) studies, cytogenetic analysis, and monitoring how the level of genetic instability and length of the telomeres evolve in time course.
Consortium funded by EC: Collection of tissue samples (Austria, France), CGH (London, Sanger Institute), Cytogenetics (Athens), Arrays (Geneva, Lausanne), telomere analysis (indirect immunofluorescence lab, Athens)

- Is the observed correlation between diet and cancer/ageing due to a causal relation? *Related to ageing:* Study DNA metabolism in very long-lived, slowly evolving animals (bird species, salamanders, lampshells, chelonians). Systems: cell cultures. Methodologies: metabolic analysis, cell cycle analysis. Collaboration with relevant countries, such as the Seychelles. *Related to cancer-ageing link:* Effects of fruit/ fresh vegetables on genomic stability? Systems: Cultures of human cells, biopsies. Methodologies: analysis of gene-specific repair replication, expression, mismatch correction, and methylation. Analysis of protein methylation as function of supplementation; bioinformatics is needed, and systems biology is essential for interpretation of results.

- What is the role of cellular senescence in ageing and is there a link to between ageing and cancer? *Systems:* human fibroblasts, gut epithelium, stem cells. *Methodologies:* micro array analysis, proteomics. Modelling attempts would need to include information on oxidative stress, mitochondric telomeres, DNA damage vs. repair, signalling pathways (p53, p21 etc.).

- How do cells decide between senescence, proliferation, apoptosis? Is ageing reversible? The answers should shed light on the development of processes leading eventually to tumour formation and/or ageing. Experimental systems should be as close as possible to humans.

Generally, it was felt that modeling should be done on various levels (cells, organs, organism) independently and that the models should then be integrated at a later stage. Comments were made that the data situation with respect to the ageing-cancer problem is difficult since patient data is available but data is rarely available from the "pre-cancer" phase.

SYSBIOMED Workshop on Chronobiology, Chronotherapy and SB
13 February 2008, Paris, France

Participants

Altinok	Atilla	Université Libre de Bruxelles, Belgium	aaltinok@ulb.ac.be
Chassagnole	Christophe	Physiomics, Oxford, UK	cchassagnole@physiomics-plc.com
Clairambault	Jean	INRIA, Le Chasnay, France	jean.clairambault@inria.fr
Cottone	Rosita	Guest	rosita_cottone2002@yahoo.de
Delaunay	Franck	Université Claude Bernard, Lyon, France	delaunay@unice.fr
Goldbeter	Albert	Université Libre de Bruxelles, Belgium	agoldbet@ulb.ac.be
Herzel	Hanspeter	Humboldt Universität, Berlin, Germany	herzelha@cms.hu-berlin.de
Iacobelli	Stefano	University G d'Annunzio, Chieti, Italy	iacobell@unich.it
Lunkes	Astrid	ESF, France	alunkes@esf.org
Kramer	Achim	Charite, Berlin, Germany	achim.kramer@charite.de
Levi	Francis	INSERM U 776, Paris, France	levi-f@vjf.inserm.fr
Roenneberg	Till	Ludwig Maximilian Universität, Munich, Germany	till.roenneberg@med.uni-muenchen.de
Schibler	Ueli	University of Geneva, Switzerland	ueli.schibler@molbio.unige.ch
Schürrle	Karsten	DECHEMA, Frankfurt, Germany	schuerrle@dechema.de
Wolkenhauer	Olaf	University of Rostock, Germany	ow@informatik.uni-rostock.de

Circadian rhythms determine the sleeping and feeding patterns of most animals, including human beings. The circadian clock, one of evolution's oldest inventions, appeared as early as in cyanobacteria almost 3.5 billion years ago, where it likely is required to the synchronise metabolism and sunlight exposure. In multicellular organisms a timing-system orchestrates the activities of large numbers of cells, co-ordinating metabolism and proliferation. There are obvious links of core body temperature, brain wave activity, hormone production, cell regeneration and other biological activities to a daily cycle. The mammalian circadian system is organized in a hierarchical manner in that a central pacemaker in the suprachiasmatic nucleus (SCN) of the brain's hypothalamus synchronises cellular circadian oscillators in most peripheral body cells. Fasting-feeding cycles accompanying rest-activity rhythms are the

major timing cues in the synchronisation of many, if not most, peripheral clocks. Malfunctioning of the circadian clock is related to transient and chronic disorders of the sleep-wake cycle (jet lag, altered sleep schedule, and delayed sleep-phase syndrome, advanced sleep-phase syndrome, irregular sleep-wake cycle). Surprisingly, there still is no consistent data on the effect of shift work on health.

Chronotherapy

Although sound animal model systems are still missing there is evidence that even very serious diseases - depression, mania and cancers - are connected with dysfunctions of the circadian clock. Whether these correlations represent statistical associations or real causal links still is an open question. Anyway, medical researchers are currently trying to prey on these correlations in chronotherapy by optimising dose-time schedules of medication. Presumably due to the rhythmic control of hepatic, intestinal, and renal detoxification systems the efficacy of some cytostatic drugs depends on the time of adminstration. Moreover, the fact that normal cells have their cell cycles coupled to the circadian rhythm could allow for hitting the body system when most normal cells are out of the replicative phase and, therefore, are not that much affected as the de-synchronised dividing cancer cells.

Following this strategy, Francis Lévy presented clinical results of chronotherapy studies in the treatment of colorectal carcinoma (CRC) with oxaliplatin, a cytostatic drug whose clinical development was discontinued by Rhône-Poulenc due to its toxicity. Monitoring the 24h levels of marker drugs (e.g. theophiline) revealed that the amplitudes of drug concentrations in blood can have differences of factor five. Building on this knowledge, Lévy's group succeeded in devising complex delivery schedules, adjusted to the patient's individual rhythms, which allow to apply the drug in a cocktail of cytostatics (5FU-LV-oxaliplatin) allowing for maximun anti-tumour effect while avoiding toxic concentrations. So far, more than 2000 patients have been recruited to chronomodulated therapies. One key finding was that schedules strongly influence the therapeutic outcome. The studies also revealed that there is a clear 'gender effect' in terms of reproducible results which is most likely due to the interfering fertility cycle. It has been known for many years that gene activity patterns of liver cells from male and female mammalians display huge differences. Similar differences are observed comparing children and adults. Whether S phase shifts are really relevant for the outcome of the chronotherapy studies was debated in the discussion.

Integrative approaches

The circadian clock is a well-coordinated system, but poorly understood so far. The participants are convinced that a circadian clock and many diseases are mechanistically connected, but consider it a 'hard problem' for research. Disruptions of the circadian rhythm seem to underly phenomena like the higher rate of cardiac stroke patients in the morning half of the day due to the daily blood pressure profile. Investigating internal timing, i.e. individual chronotypes, with regard to the effects of treatment options would be of importance for clinical research, e.g. for studying the effects of pulsed vs. continous dosing of insulin with diabetics. The striking difference between children and adults in the outcome of leukemia therapies is also suspected to be related to the circadian clock. On the other side, embryogenesis, for example, is obviously de-coupled from the circadian clock. Gastro-intestinal cancers, especially CRC, are considered an expedient model for studying the influence of a circadian clock on tumours, especially valuable when looking for differences between normal and cancer cells. Other types of tumours may also be even more susceptible to biological rhythms. An important aspect of studying the temporal changes in tumours is the formation of metastases which usually tend to degenerate into entities which have lost many traits of the origininal tumour.

Fundamental questions regarding the relation of the circadian clock, cell cycle, and metabolic rhythm call for intensive research. Fortunately, oscillatory biological processes represent a clear case for systems biology, and modelling is expected to help unravel many of the still unknown mechanisms. For instance, the gating of cell cycle rhythm within the oscillations of the circadian clock is a perfect analogy to a well-known concept from control engineering. Systems biology could also help to analyse data from model systems to discriminate the effects of the circadian clock from genetic factors. Generally, time series experiments must provide quantitative data on multiple parameters ('temporal metabolome') suited to eliminate statistical effects. Different 'chronotypes', i.a. the individual phase relations of organs, are a principal problem of population studies. The integration of models from cells to organs should eventually provide characteristic 'chrono biomarkers' and is also considered essential for optimising personalised chrontherapeutic schedules or finding optimum combinations of drugs. Modeling is, no doubt, well-suited to explain a remarkable result of research in 'advanced sleep-phase syndrome' having found the phosphorylation of a single protein modified by a single mutation responsible for the phase shift.

Data situation

Efforts to build models of the temporal organisation in physiology and disease rely on measuring the abolute changes of parameters. The decay of proteins and changes in various metabolite concentrations seem to be measurable by current methods, while accurate rate coefficients of intracellular reactions are notoriously difficult to measure. Regarding the aquisition of quantitative data the experts suggest to concentrate on preferably non-invasive technologies for multi-parameter monitoring (i.e. simultaneous measurements of skin resistance, temperature, cortisol, FGF, melatonin levels) and on hightroughput data, e.g. from temporal transcriptome profiling (RNAs) employing chip technology. Such efforts could be supported by a 'chronochip', or 'chronosensor' development. Another concept to obtain temporal personal data is based on reporter cells immobilised in a microreactor which are exposed to a continous microlitre/minute flow of blood from a cannule. Suited model systems are primary cells, tumour samples and mice *in vivo* models which also would allow for studying the effects of X-chromosome inactivation, gene-knockouts, hormone cycles and gender differences. A lot of high throughput data on liver is already available.

Resources

Clinical researchers are in short supply since most of them are involved in studies within commercial drug approval procedures. Although chronotherapy is a promising field which is closely related to clinical research drawing resources to these projects is considered a hard problem, given present time and funding constraints of academic research. Discussing how to involve researchers from related initiatives and programmes the participants referred to BioSIM, a network of excellence closely cooperating with the pharmaceutical industry aims to develop *in silico* simulation models of cellular, physiological and pharmacological processes. They esteem BioSIM as a valuable measure to generate knowledge by inter-faculty and academia-industry co-operation.

Integrative approaches to chronobiology problems are also comprised by the French 'Mathematical Analysis of Complex Systems' initiative, the Swiss 'SystemsX.CH' programme and the German 'Clockwork' project which is focused on health effects of shift working. Large and smaller industrial entities are known to have launched SB projects. Recently, Pfizer opened a SB division.

SYSBIOMED Workshop on Colorectal Carcinoma and Systems Biology
10-11 April, Barcelona, Spain

Participants

Blüthgen, Nils	University of Manchester	UK	nils.bluthgen@manchester.ac.uk
Helen Byrne	University of Nottingham	UK	helen.byrne@nottingham.ac.uk
Cascante, Marta	Universitat de Barcelona	Spain	martacascante@ub.edu
Drasdo, Dirk	INRIA	France	dirk.drasdo@inria.fr
Jaster, Robert	University of Rostock	Germany	jaster@med.uni-rostock.de
Lenormand, Philippe	CNRS UMR6543	France	philippe.lenormand@unice.fr
Schürrle, Karsten	DECHEMA	Germany	schürrle@dechema.de
van Leeuwen, Ingeborg	University of Dundee	UK	ingeborg@maths.dundee.ac.uk
Wolkenhauer, Olaf	University of Rostock	Germany	ow@informatik.uni-rostock.de

The participants of the SYSBIOMED workshop on systems biology (SB) and cancer (7 Nov 2007 Nice, France) identified colorectal carcinoma (CRC) as a highly prevalent disease which likely is amenable to SB approaches: There is a good knowledge base from all stages of this multi-step disease, there is access to clinical material, and there are CRC-derived cell lines available.

The participants of the subsequent Barcelona workshop discussed a 5 years research project which is based on parameterising a model on 3-5 well-characterised, low-passage CRC-derived cell lines available from cell bank repositories and collaboration partners. The cells have defined molecular defects corresponding to the different tumour stages affecting signaling components Wnt/APC (adenomatous polyposis coli), Ras, and p53. Investigating the role of signaling pathways via Raf/Ras, extracellular signal-regulated kinase (ERK), or PI3K–Akt signalling would require systematic experiments, which rely on directed perturbations by inhibitory and cytostatic drugs as well as interference with components and feedback loops like dual-specific phosphatases (DUSPs). Mouse models were seen to be beneficial but not essential for this purpose.

Datasets and modeling

The experimental setting would compare the different cell lines and their response by thorough analysis of proteome, metabolome, transcriptome, epigenetic, and phenotypic data.

- Proteome: array-based monitoring of the activity of all kinases in the pathways, of beta-catenin, and of key transcription factors.
- Transcriptome: Genome-wide studies, 5 cell lines, 10 treatments, 5 time points
- Metabolome: Application of high throughput methods combining liquid/gas chromatography and mass spectroscopy
- Epigenetic Status: monitored e.g. by oligo nucleotide arrays
- Phenotypic status: Growth rate, percentage of apoptotic cells, percentage of S-phase cells

All data will, wherever possible, be gathered as time series in order to parameterise dynamical models. Relevant quantitative data will be generated within the consortium consisting of specialised labs. Data processing and statistical analysis will be probably trusted to a specialised bioinformatics partner or company. Experiments and technologies shall be focussed on the respective pathways. A proteomics core facility accessible to the consortium partners would be the preferred option, where special phosphoproteomics methods are being made available to the consortium.

The data essentially serve for parameterising computational dynamic models of

- signalling networks,
- transcriptional response,
- metabolic response.
- tumour growth

The project aims at generating multiscale models comprising evolved pathways and metabolic models whose outputs, responding to perturbations, serve to test the hypothesised systems structure in the usual evolutive cycle of experiment-analyis-modeling.

The participants agreed that studying the development of CRC would require an excellent genetic animal model, which is not available today. However, good access to patient-derived cell lines of all stages (adenoma biopsies and tumour surgery) is a special advantage of studying CRC. Later in the project, comparing these cells with the immortal cell lines in the above mentioned experimental settings is expected to yield valuable insights into the differences between tumour cells *in vitro* and *in situ*, and would shed light on the role of the microenvironment (stroma cells) and cytokines. This information will help improve the initial

models which are based on *in vitro* data and, in later stages of the project (after year 3), could also serve to develop models suited to simulate different treatment scenarios and to optimise drug response.

SYSBIOMED EU-US Workshop on Cancer Systems Biology[2]

7 - 11 June 2009, Rostock-Warnemünde, Germany

Participants

Auffray	Charles	CNRS, Villejuif, France	charles.auffray@vjf.cnrs.fr
Baltrusch	Simone	U Rostock, Germany	simone.baltrusch@med.uni-rostock.de
Blüthgen	Nils	Charité, Berlin, Germany	nils@sys-bio.net
Byrne	Helen M.	U Nottingham, UK	helen.byrne@nottingham.ac.uk
Cascante	Marta	U Barcelona, Spain	martacascante@ub.edu
Dale	Trevor	U Cardiff, UK	daleTC@cardiff.ac.uk
Drasdo	Dirk	INRIA, Paris, France	dirk.drasdo@inria.fr
Fell	David	Brookes U, Oxford, UK	dfell@brookes.ac.uk
Ferrell	James E.	Stanford U, USA	james.ferrell@stanford.edu
Gallagher	Richard	The Scientist, Philadelphia, USA	RGallagher@the-scientist.com
Gallahan	Daniel	NCI, Bethesda, MD, USA	gallahad@mail.nih.gov
Gatenby	Robert	Moffit Center, Tampa FL, USA	robert.gatenby@moffitt.org
Günther	Ulrich	U Birmingham, UK	u.l.gunther@bham.ac.uk
Harms	Brian	Merrimack, USA	bharms@merrimackpharma.com
Herzel	Hanspeter	Humboldt U, Berlin, Germany	h.herzel@biologie.hu-berlin.de
Jaster	Robert	U Rostock, Germany	robert.jaster@med.uni-rostock.de
Junghans	Christian	U Rostock, Germany	christian.junghanss@med.uni-rostock.de
Kunz	Manfred	Universitätsklinikum Schleswig-Holstein	manfred.kunz@uk-sh.de
Leeuwen van	Ingeborg	Karolinska I, Stockholm, Sweden	ingeborg.vanleeuwen@ki.se
Lenormand	Philippe	U Nice, France	philippe.lenormand@unice.fr
Lévi	Francis	INSERM, Paris, France	francis.levi@inserm.fr
Linnebacher	Michael	U Rostock, Germany	michael.linnebacher@uni-rostock.de
Lowengrub	John	UC Irvine, CA, USA	lowengrub@math.uci.edu
Maini	Philip K.	U Oxford, UK	maini@maths.ox.ac.uk
Malik	Arif	MicroDiscovery, Berlin, Germany	malik@microdiscovery.de
Marcus	Frederick	European Commission, Belgium	frederick.marcus@ec.europa.eu
Rateitschak	Katja	U Rostock, Germany	kr084@informatik.uni-rostock.de
Sansom	Owen	Beatson I, Glasgow, UK	o.sansom@beatson.gla.ac.uk
Schäfer	Reinhold	Charité, Berlin, Germany	reinhold.schaefer@charite.de
Schnell	Santiago	U Michigan, USA	schnells@umich.edu
Schürrle	Karsten	DECHEMA, Frankfurt, Germany	schuerrle@dechema.de
Sers	Christine	Charité, Berlin, Germany	christine.sers@charite.de
Shibata	Darryl K.	USC LA, CA, USA	dshibata@usc.edu
Tyson	John	Virginia Tech, Blacksburg, VA, USA	tyson@vt.edu
Vera	Julio	U Rostock, Germany	jv030@informatik.uni-rostock.de
White	Mike	U Liverpool, UK	m.white@liverpool.ac.uk
Wolkenhauer	Olaf	U Rostock, Germany	olaf.wolkenhauer@uni-rostock.de
Zhivotovsky	Boris	Karolinska I, Stockholm, Sweden	boris.zhivotovsky@ki.se

The workshop served to discuss and assess the requirements of successful research in medical systems biology (MSB) with regard to suitable exprimental systems, available experimental technologies, and

[2] The workshop was combined with the Trans-Atlantic Summer School on Cancer Systems Biology and supported by the EC, the NCI and German BMBF

modeling/data analysis. Before the working groups started reviewing the relevant topics in detail Robert Jaster (Department of Medicine, University of Rostock) gave a stimulating introduction titled "Tumour Diseases and Systems Biology - Facts and Personal Points of View". Table 1 summarises the moderate progress in terms of survival after decades of steadily intensified cancer research:

- Estimated 1.480 000 new cases in the USA in 2009; 562.000 cancer deaths
- Europe 2006: 2.300 000; 1.170 000 cancer deaths

Trends in Five-year Relative Survival USA

Site	1975-1977	1984-1986	1996-2004
• All sites	50	54	66
• Prostate	69	76	99
• Melanoma	82	87	92
• Breast (female)	75	79	89
• Colon	52	59	65
• Lung and bronchus	13	13	16
• Pancreas	3	3	5

2004: more than 72 Billion $ spent on cancer treatment in USA !

Sources: American Cancer Society; USA National Cancer Institute; Ferlay et al., Annals of Oncology 2007

Although modern oncology has elucidated central molecular mechanisms of tumourigenesis (oncogenes, perturbed signaling) large areas remain uncharted, e.g. important aspects of epigentic modifications and intercellular communication have not been studied systematically yet, and insufficient biomarker sensitivity and specificity require improvements. The widely appreciated success of targeted terapeutics has come with some old problems (failure, resistance, side effects) as well as novel ones (extreme costs; unclear individual treatment options). However, the immense complexity of cancer calls for SB approaches. Mathematical models describing interactions between molecules involved in tumourigenesis (e.g., ERK-, Wnt-, TGF-b signaling pathways), as well as cancer-related cellular pathways have already been presented, and computational tools for multi-scale modeling are being developed. Dr Jaster ventured to explore criteria of "model tumour diseases" which are amenable to systems biology approaches. Statistical data on prevalence and mortality clearly indicate that prostate/breast, lung and colon cancer represent a huge burden for industrial societies. Taking into account the level of pathophysiological understanding, the availibility of suitable experimental models and the potential for diagnostic/therapeutic options gastro-intestinal tumours - colorectal carcinoma in particular - appeared as appropriate candidates.

Experimental Systems

Looking upon suitable exprimental systems the workshop participants, divided into three expert groups, analysed the following aspects:

Experimental Systems – Cellular and Subcellular Level (Chair: Manfred Kunz)

James Ferrell introduced the topic. In the discussion it became clear that the rub is with the well-known fact that the most 'powerful' cell systems are not quite realistic models while the more realistic cell systems are not very 'powerful'. The experts emphasised the importance of understanding basic cellular processes to provide a foundation on which to build our understanding of tumor-specific behaviours. They consequently favour a strong track record of model organisms with particular

experimental advantages (yeast, flies, worms, Xenopus...). The value of having a "gold standard" to compare other cells to (e.g. a protein abundance catalog) would be appreciated. Not willing to compromise on the quality of the experimental data the experts would prefer highquality data from an artificial cell type to mediocre data from a more realistic cell type. Best candidates available are HEK293 and HeLa due to human origin, growth characteristics, outstanding transfectability, ease to perform RNAi experiments and homologous recombination. Unfortunately, they are aneuploid, artificial, and single cell types are not good for every type of response. They expect cells from the NCI 60 panel, cell lines from patients and differentiated iPS cells to prove superior.

Experimental Systems – Multicellular Level, Animal Models (Chair: Dirk Drasdo)

Owen Sansom gave a thorough introduction to the current state and discussed the presently available models as summarised in table 2 below.

Model	Strength	Weaknesses
Established tumour cell lines	• Unlimited availability • Good clinical predictive value • Useful to generate quantitative and dynamic data for modelling	• Very artificial • Inaccurate reflection of human diseases
Patient-derived tumour cells / cell lines	• Reflects individual molecular pathology of individual patients/tumours	• Time consuming, lower throughput • Availability
Tumour-bearing immunodeficient animals (e.g., Xenografts in nude or SCID mice)	• Relatively easy to establish • Generation of in vivo data • Very good clinical predictive value, when panels are used	• Still, quite far away from human reality
Immunocompetent animals with hetero- or orthotropic transplants	• Increased practical relevance	• Often difficult to handle; low throughput; missing clinical predictive value for murine allografts
Genetic models	• Most accurate model of human disease	• Availability
Humanized Xenograft models	• Most accurate model for interaction of human immune cells and (idealiy antilogous) tumour cells	• Very labour intensive; complex logistics

He made a strong case for genetically modified animal models based on the facts that mutations occur in their correct physiological settings and that cross talk between epithelium and stroma cells and tumour associated fibroblasts is tissue-specific. Signaling pathways and feedback loop proteins blocking these pathways are also tissue specific.

Findings: Mouse models with defined mutations in specific signaling pathways may allow for proof of concept studies that will assess if mice of different genotypes might respond differently to different drugs, e.g. will mice carrying APC PTEN mutations respond better than mice that carry APC KRAS mutation to PI3 Kinase inhibitors. Large clinical trials required for a growing number of drugs have become very difficult. Genetic mouse models should allow to test combinations of drugs and antibodies (in various treatment regimes) which may inform which combination best to trial in humans. Given the mouse models were set up with defined genetic lesions that need other stochastic events for tumour formation to occur, it was suggested that modeling cancer and cancer resistance from an evolutionary perspective would be a way of integrating the mouse model data with

mathematical modeling. Sequencing of mouse tumours of different genotypes (potentially pre and post treatment) would allow to have high resolution of the changes that occur through carcinongenesis and resistance. In humans, most therapies are based around the clinical endpoints of tumour shrinkage while most agents are used at maximum tolerated dose. In mouse studies, the maximum tolerated dose can be compared to the dose of the drug which 'hits the target' of the drug, e.g. a kinase inhibitor, to see if this gives comparable results. Also studies can be performed whether lower doses of drugs that stop tumour growth (rather than cause tumour regression) extends lifespan. Thus there is good reason to investigate whether regimes can be developed that treat cancer as a 'chronic' disease. Another suggestion was whether computational modeling of toxicity, previously used in developmental biology, could be applied to cancer.

Linking Basic Research with Clinical Research (Chair: Boris Zhivotovsky)

Charles Auffray started with some general considerations. The driver of research should be the clinical question/situation while animal and cellular models are required for validation and verification of mechanistic explanations. He emphasised the importance of host/tumor relationships vs cell lines and recommended to specify the key clinical questions not without identifying patient-related constraints and limitations. Since the biology of animal models developed for basic science may not be directly relevant to clinical situations one should develop both models, and then cross-integrate.

Findings: In practice one should take the effort to identify what is feasible vs what is desirable. Criteria are access to early stages, a blood sampling window for metabolomics/ proteomics/ transcriptomics studies and access to tissues from biospies. Data collection has to be driven by clinical/biological questions and hypotheses. A rational design of experimental follow-up studies is needed taking into account which parameters are most informative to characterise a pathway/network efficiently and at reasonable costs. This would prompt the identification of critical targets within oncogenic/signaling pathways for therapeutic intervention. Analysis and prediction of the cellular response to anti-tumour agents (cytostatic drugs, monoclonal antibodies, and novel small molecule inhibitors) could provide clues to mechanisms behind drug efficiency in experimental (cell culture) models. Genetically-engineered mouse models are considered the best option for establishing treatment schemes and the exploration of possible mechanisms of drug action. Experimental results need to be correlated with retrospective clinical data and SB approaches. The evaluation of predicted optimal therapeutic strategies can be performed in mouse models (tumour xenografts) which also supports the design of rational treatments based on model predictions.

Experimental technologies

The workshop experts reviewed the potential of major high throughput approaches to extract quantitative data from experiments with biological systems. Three working groups discussed the following technologies in detail.

Transcriptomics, Epigenomics (Chair: Nils Blüthgen)

Focusing on epigenetic modifications Christina Sers reviewed the current developments in the analysis of transcriptome information. She compared the technologies currently applied in molecular genetic research as summarised in table 3 below. Epigenetic modifications are important for cancer

development (e.g. methylation of tumor suppressor genes) and epigenetic markers are already on the market and might become important for diagnostics. Besides simulations of dynamic chromatin structures there are only very few mathematical models available yet.

Knowledge of the relation between epigenetic modification and gene expression is available for few genes. The participants saw opportunities for modeling approaches in understanding the relation between gene expression and epigentic modifications – albeit of a few candidate genes only – and for studying population dynamics. The global analysis of methylations to find patterns and informative regions appeared indispensable. Table 3 gives an overview on current technologies of genome analysis.

Table 3: technologies of genome analysis

Bisulphite sequencing	Low	Single locus if PCR-able	high	laborious; drop-out regions
COBRA	Medium	Single CpG	Low	Little information
MeDIP	High	Genome-wide	High	Expensive IP-dependent Chip-dependent Low sensitivity
MeDIP-seq	High	Genome-wide	High	Expensive IP-dependent Low sensitivity
ChIP-chip for Histones	High	Genome-wide	High	Expensive IP-dependent Chip-dependent
2nd generation Bisulphite sequencing	High	Genome-wide	High	Expensive

Proteomics (Chair: Trevor Dale)

Philippe Lenormand critically discussed the advantages and limitations of the available technologies for transcriptome and proteome analysis. Some characteristics of transcriptomics technologies are summarised in table 4.

Table 4: transcriptomics technologies

DNA arrays	**spot oligonucleotides on slides / hybridize cDNA** **very high throuput** **difficult to get absolute quantification** **not great dynamic range, limited to spotted probes**
quantitative RT-qPCR	**amplify specifically target sequences** **medium throughput : multiplex up to 5 dyes** **many samples, few targets (dry mix of primers on 384 wells)**
Xtag technology (luminex)	**multiplex PCR** **allele or target specific amplification** **binding to beads by DNA tags : 100 measures per sample** **high throuput / quantitative (? end-point)**
deep sequencing	**sequencing entire transcriptome from samples** **even small RNA can be sequenced (siRNA, miRNA...)** **can give informations on alternative transcripts** **mRNA transcript frequency = quantity ?** **allele-specific transcription** **chromatin-IP purified sequences** **medium throughput by tagging samples and parallel sequencing (labor intensive bio-informatics)** **improving fast / affordable soon**

Although not high throughput yet, deep sequencing comes with attractive advantages: there is no predetermination of targets, and when multiple samples are tagged, they are treated equally all along the procedure. Regarding quantification in deep sequencing he emphasised that it remains to be proven that the frequency of sequences relates directly with quantity (of transcripts) and that it is a relative measure due to the amplification steps.

Today, there is a variety of methods allowing for relative quantification. For biomarker proteomics and systems biology, however, absolute quantification rather than relative quantification is required. Western blotting is the most widely used method in quantitative protein analysis. Its is laborious and due to the largely variable affinities of antibodies employed there is a short linear range of detection. Recently Luminex' xmap technology was introduced in high throughput proteomics. It employs dyed polystyrene beads with antibodies fixed on the surface and target-specific labelling antibodies. The method is highly specific and allows for up to 100 measurements per sample. However, measurements are not absolute and require standardisation. A method enbaling absolute quantification involves concomitant mass spectrometric determination of signature proteotypic peptides and stable isotope-labeled analogs (QconCAT). Biological samples spiked with designed and isotopically labelled concatenated signature peptides are subjected to proteolyis and analysed. The method has interesting advantages: it is quantitative, one can measure up to 100 proteins per sample and also detect secondary modifications. However, the availability of standard labeled signature peptides in accurately known amounts is a limitation to the widespread adoption of this approach and data analysis is labor intensive. A very recent technology, nanostring, was developed at the ISB in Seattle. It essentially works by counting individual dye-tagged biomolecules and does not require any amplification steps. Current technologies of analysing proteins *in vivo*, e.g. employing fluorescence microscopy, can provide information on single cells but usually lack the option to gain quantitative data. With regard to cancer systems biology the participants are convinced of the value of proteome data and suggest to pursue some specific hypothesis-driven questions of relevance to cancer, e.g. are there checkpoints at which level apoptosis is all or none? or why do drugs fail? (isoforms / cross-talk) and 'how can these component hypotheses be linked into a coherent whole?' In summary, transcriptomics for cancer systems biology need highthrouput arrays be combined with more precise quantitative measurements, e.g. provided by quantitative reverse transcription PCR (RT-qPCR). Deep (pyro)sequencing may become the future gold standard.

Metabolomics, Fluxomics (Chair: Marta Cascante)

Ignoring the rather artificial distinction between metabolomics and metabonomics Marta Cascante introduced the metabolome of an organism as the result of the *in vivo* function of gene products closely tied to its physiology and its environment. Metabolomics offer a unique opportunity to look at relationships between genotype, phenotype and the interaction with the environment. The identification of metabolite biomarkers is key for research in cancer systems biology. Fairly advanced technologies – gas chromatography, mass spec, nuclear magnetic resonance and combinations thereof – allow for extremely sensitive analysis of metabolites. However, important questions have to be addressed before data analysis: What are the key metabolites? In principle, 'all' metabolites are considered important. Key metabolites are likely to depend on tumor type, tumor heterogeneity, cell type, and stage. Untargeted unbiased search for novel biomarkers as strategy would not rule out the hypothesis-driven analysis of samples. The analysis of metabolic fluxes, dubbed fluxomics, is hard to

perform. Not correlating with concentrations inter- and intracellular metabolite fluxes cannot be measured directly, they have to be calculated for each model. Targeted analyses of fluxes in tissue samples or biopsies would provide valuable information. In summary, metabolomic data complement genetic and proteome information, provide valuable biomakers, and - as link between genotype and phenotype - help a lot to refine models.

Mathematical Modeling, Data Analysis

John Tyson views math models as hypothesis-testing tools which, principally, are all wrong because hypotheses ultimately prove to be incorrect in one way or another. Models can be useful, provide insight, and suggest new experiments even when its consequences are counter-intuitive. Evolvable models serve to improve hypotheses. Modeling of cancer essentially serves to simulate and understand information processing in mammalian cells and tumor progression. Interesting problems amenable to modeling are (perturbed) signal transduction pathways, 'decision making' in the cell cycle, mitogenesis, apoptotic control, and stem cell maintenance. However, there are serious caveats: 'cross-talk', e.g. due to the formation of receptor complexes, has to be taken into account to and there still are huge gaps in the knowledge of signaling pathways. The surprising results from recent research on microRNAs and new insights into the dynamics of RNA polymerisation advise caution when building models. The challenges are huge since life and disease are multi-scale phenomena encompassing genes, networks, cells, tissues and more.

Modeling of Subcellular Processes (Chair: Santiago Schnell)

A distiction between modeling (M) and theory (T) appears reasonable. Advancing theory (T) requires the development of new theoretical approaches that will improve our understanding of fundamental principles that integrate phenomena inside the cells while advancing modeling (M) requires the application of theoretical approaches to improve our understanding of processes inside the cells. Models can be divided into 'reminiscence models' comparable to a clockwork toy reproducing the motions of a living dancer. This is analogously accomplished by large simulations. Mechanistic models, generally used for hypothesis-testing, are derived from mechanisms extracted from experimental data. The experts ventured on a SWOT (Strengths,Weaknesses, Opportunities, Threats) analysis of the opportunities for modeling approaches on the subcellular level. *Strengths:* There are specific biochemical pathways with substantial experimental data (cell cycle, MAP kinases, etc) and at the same time there is a substantial amount of theoretical work on these biochemical pathways (cell cycle, MAP kinases, etc) available. *Weaknesses:* Poor quality of data both quantitative (parameters) and qualitative (mechanisms) and ineffective communication between experimentalists and theoreticians. *Opportunities for modeling:*

(M) There are specific systems (cell cycle, MAP kinases, etc) in cancer research where experimentalists and theoreticians can co-operate forming research consortiums.

(T) New theories for new technologies: the nano-world and single molecule biochemistry/biophysics

(T) New theories for multiscale modeling from genes to cell activity

(T) New theories for individualised medicine: forecasting

(T) Development of identifiability and distinguishability approaches for parameters and model estimation

Threats: There are small funding opportunities for theory only projects and modeling toolboxes are not supported in the long term by funding agencies. Experimentalist often consider collaborating with modellers high-risk and communication still is difficult.
Proposal: The participants propose to focus on a specific animal and cancer model and favour the villus crypt of CRC. Essential pathways for normal cell function (Wnt, RAS, TGF, cadherins, etc.) and growth, division, differentiation, transcription should be investigated. The objective is understanding of information processing in normal and disease crypt.

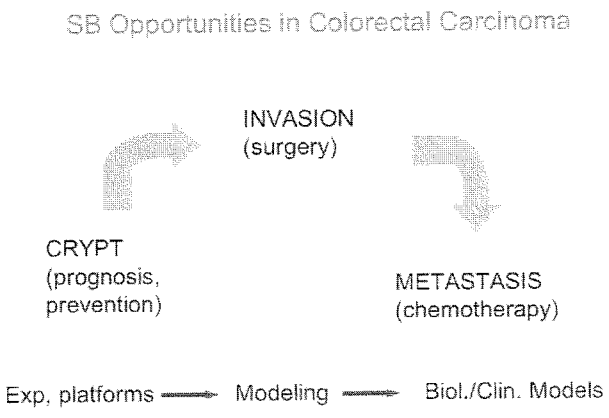
Multi-scale Modeling, Cell/Tissue Data (Chairs: Philip Maini/John Lowengrub)
Multiscale modeling is required since biological function arises across different scales which interact nontrivially. Therapies are multi-scale processes too. The participants identified the following *challenges:* We need to develop theoretical technologies to understand strengths and weaknesses of the diverse multiscale modeling methodologies. Targeted funding for such studies would advance the field. The studies would require consistent, comprehensive experimental measurements across one cell line enabling the validation and comparison among models. One could investigate the effects of variation among data for a particular cell line and compare to the *in vivo* situation.
Predictions can also be used to discriminate among the models, e.g. by parameter sensitivity analysis. The use of models to predict those parameters to which the system is "most sensitive" may also allow for model reduction and yield insight as to which additional data would be most beneficial to measure. Error propagation is a big problem of linking across scales and different model types.
Experimental data across scales may be hard to obtain, in particular with the quality required. The practical strategy of linking models of different levels of detail will produce a suite of models.

Bioinformatics, Data Management
The participants acknowledged the availability of a large number of diverse repositories from big data bases to small specialized data bases. They agreed on the need for high quality, high throughput data and for new bioinformatics and data management tools for modeling because the requirements of 'classical' bioinformatics are different. They recommend to pay attention to the 'social aspects of data sharing', i.a. the standardisation of data. Tools which enable the assessment of data quality are highly demanded. An example is the 'RNA integrity number (RIN)' which is a software tool designed to help scientists estimate the integrity of total RNA samples. Emerging new technologies are expected to come with large amounts of data and novel formats. Not only with regard to the costs of data base maintenance they support the concept of central curation of data and single platform for data exchange. System flexibility is central to data management in systems biology, i.e. the integration of content management systems with bioinformatics databases is recommended. The participants consider repositories for models especially helpful to provide access to new tools for experimentalists and modelers, e.g. tools for visualisation required for linking of pathways, for feeding networks with information from biological databases, and for mapping data onto pathway diagrammes.

General discussion

The concluding discussion served to consolidate and to critically assess the findings of the topical workshops. It was acknowledged that pharmaceutical research already benefits from systems biology approaches, e.g. 25% of Merck's current portfolio of drug candidates are said to stem from SB programmes.

Finally, the participants ventured to sketch a project which, they are convinced, deserves further scrutiny. They recommend to embark on systems biology research on colorectal carcinoma through all stages from initial cellular degeneration of crypt cells to the separation of aggressive metastatic cells. The value is obvious. Translational research, i.e. the cooperation of bioscientists, modelers and clinicians, would benefit most from the discovery of novel targets and the insights needed for improving therapeutical regimes. Improved prevention lowering the burden of the disease would be another outcome. Whithin the time horizon of 5-10 years one could expect to have successfully tackled problems from the early stages of tumour formation from crypt cells.



SYSBIOMED Workshop on Challenges for SB

2 October 2008, Costa Adeje, Tenerife (Spain)

Participants

Alves, Rui	Universidad de Lleida	Spain	ralves@cmb.udl.es
Auffray, Charles	CNRS, Villejuif	France	auffray@vjf.cnrs.fr
Cloutier, Mathieu	Hamilton Institute	Ireland	mathieu.cloutier@nuim.ie
Girolami, Mark	University of Glasgow	UK	girolami@dcs.gla.ac.uk
Eufinger, Jan	DKFZ Heidelberg	Germany	j.eufinger@dkfz-heidelberg.de
Fell, David	Oxford Brookes University	UK	dfell@brookes.ac.uk
Goryanin, Igor	University of Edingburg	UK	goryanin@inf.ed.ac.uk
Gross, Thilo	Max-Planck-Inst. Magdeburg	Germany	gross@pks.mpg.de
Hornberg, Jorrit	Schering-Plough, Oss	The Netherlands	jorrit.hornberg@spcorp.com
Mendoza, Eduardo	LMU Munich	Germany	mendoza@lmu.de
Plahte, Erik	CIGENE Aas	Norway	erik.plathe@umb.no
Torres, Nestor	Univ. de La Laguna	Spain	ntorres@ull.es
Schürrle, Karsten	DECHEMA e.V.	Germany	schürrle@dechema.de
Wellstead, Peter	Hamilton Institute	Ireland	peter.wellstead@nuim.ie
Wernisch, Lorenz	University of London	UK	lorenz.wernisch@mrc-bsu.cam.ac.uk
Wolkenhauer, Olaf	University of Rostock	Germany	ow@informatik.uni-rostock.de

The participants discussed which 'grand challenges' – be it scientific or conceptual/ organisational – they expect medical systems biology (MSB) to meet in the coming years. The underlying notion of systems biology is that it is about mathematical modelling and computer simulation –a predictive science that generates testable hypotheses. Modelling is believed to 'make a difference' analogous to 17th century science[3] when Johannes Kepler – analysing large amounts of data which Tycho Brahe had carefully measured before – built his celestial mechanics models which eventually inspired Isaac Newton to derive the general law of gravitation.

The nature of a 'grand challenge' was considered also worth discussing: Is it a 'hard' problem like pertinacious mathematical conjectures (e.g. the Riemann hypothesis) calling for novel ways of thinking? Or is it a matter of efficient, very clever combination of available technologies to achieve an ambitious goal (e.g. Apollo lunar missions)?

[3] an analogy emphasised early by Stas Swartsman and Jim Ferrel

Scientific challenges

In February 2008, researchers at a workshop held in Tokyo announced a bold project: to create, over the next 30 years, a 'virtual human'. This molecule-based computational model would describe the systems or networks of interactions between the tens of thousands of genes and proteins that underpin biological processes in both health and disease. In the workshop it was consensus that such 'virtual human' would not be a very realistic perspective. They expect that every big scientific challenge in MSB will eventually result in a diversity of models, not necessarily mathematically equivalent, but each one able to reliably predict certain parameters. A 'grand challenge' would be to push MSB beyond the achievements of the current Physiome Project with defined medical applications and diseases being addressed. All workshop participants agreed that the best (disease) model system is the human body. However, a system's behaviour can only be understood through stimulus-response experiments. This will for ethical reasons not always be possible. In many cases it is also impossible to generate a stable, reproducible experimental system from human derived samples. Despite known deficiencies of established animal models, e.g. mouse model in cancer, well defined mutations can only be studied in such model organisms.

The experts present at the workshop clearly identified cancer and neuro-degenerative diseases calling for MSB approaches in order to make significant progress. Some participants pleaded to also consider infectious diseases in addition to the disease areas looked at so far within SYSBIOMED (cf. workshop reports at www.sysbiomed.org). Infectious diseases pose a real, growing threat to public health. A lot of knowledge is being generated by current microbial SB projects and will be eventually complemented by insights from systemic studies of host-pathogen interactions and the immune system. Important funders of medical research like the Bill and Melinda Gates Foundation and the WHO identified infectious diseases as major challenges to humankind and strive to fill the gap the pharmaceutical industry created by neglecting anti-infectives research. The workshop participants welcomed the Innovative Medicines Initiative (IMI), to which industry and EC equally contribute €2bn total funding, as a good framework to implement MSB projects which address the above mentioned medical challenges.

With regard to the dramatic threat of malnutrition and famine to almost a billion humans the participants agreed that funders and researchers should let loose on the 'grand challenge' of generating C4 rice plants, an endeavor which is likely to benefit from SB projects in plant biology.

Organisational challenges

Going for 'grand challenges' carries some risks. For example, one could possibly neglect useful model organisms or important pathways. Moreover, the big money big programmes use to come with could attract the wrong people who sell conventional research under a new label, or, not unusual in medical sciences, one sees 'empires' of research activities gravitating

around key figures who jealously dominate the project while discouraging creative (younger) scientists. During the discussion it soon turned out that the management of large projects is crucial and a 'grand challenge' by its own right. Although sufficient funding is essential, 'grand scientific challenges' would require the motivation of the 'right' people to co-operate and to combine the 'right' resources. Referring to the well-managed SysMo initiative the participants considered ERA-Nets especially helpful to support integrated large-scale efforts. It was questioned whether top-down managed initiatives would be more successful than programmes run by more autonomous groups with a high degree of freedom - bearing the risk that the partners are tempted to follow their own favourite research priorities.

The workshop participants see room for improvement on the management side. In order to make sure that 'right' qualifications and expertise come on board, one could adopt a 1year grant scheme as implemented in the Swiss SystemsX.CH initiative. Such grants would allow to evaluate "newcomers" and young researchers before closing longer-term contracts. Finally, a rather radical concept was discussed: Why not trust project co-ordinators with the authority to allocate funds (not assigned to at the beginning) in due course according to the performance of the partners? A more extreme view would require the co-ordinator to solely take charge of the grant application and to build the consortium.

Education

Education was unanimously considered a 'grand challenge', too. Tight collaboration of biologists/physicians, engineers and informaticians/mathematicians requires adaption and understanding of concepts from the other disciplines and to overcome 'cultural' barriers built from conventional mindsets and deficits in familiarity with other ways of thinking. Education obviously is key to achieve optimum conditions for supplying this future technology sector with the qualified researchers it needs. Education in MSB deserves special attention. In most countries training in the medical sciences is quite different from training in biosciences and engineering, hampering the introduction of mathematical modeling and quantitative methods. Since many research institutes and labs in the medical sciences recruit doctoral and postdoctoral researchers with a medical training, it is necessary to provide more opportunities of experimentalists working in a medical environment to interact with modellers.

Views

Finally, the participants took the opportunity to give their individual views on some 40 issues listed in a questionnaire. Although having different backgrounds the participants shared similar opinions on what biological systems are, on the deficits of education in biology and engineering/ physical sciences, on experimental techniques needed, and on the main hurdles for model integration. Not surprisingly, most agreed on Dennis Noble's heart model and the Physiome project being examples of SB "success stories" already achieved whereas the participants' nominations of successful applications of mathematical modeling in the life sciences proved far more diverse. The answers to many questions, e.g. related to grand

challenges ('moonshot projects'), models, or the priorities of project preparation, showed a diversity of aspects which, in summary, represent a sound, concise plea for large-scale programmes. The complete feedback to the questionnaire is given below:

Your View on Systems Biology for Medical Applications

(Please notice: individual answers are separated by // in the text)

1. Name ---

2. Affiliation ---

3. What is your current major research topic in Systems Biology (SB)?

Modeling of GRNS, brain energy metabolism // transcription in *E. coli* // protein (interaction) networks // dynamics of biological networks // modeling of pathways involved in tumour progression // metabolic networks // Parkinson's disease // Computational SB // network reconstruction, design principles // multi-scale integration // chronobiology, systems virology, spatial and stochastic modelling // signal transduction, cancer, and autoimmune diseases //

4. What does the word "Systems" in Systems Biology refer to?

Aggregate of interacting entities of processes which produce or exhibit system properties or behaviour // systems properties that arise from the interactions of biomolecules and cannot be assessed by traditional reductionist approaches // level of description is on the scale of systems (rather than molecules or populations) // complex interactions of components coordinated in task-oriented manner // set of components that can be studied as a whole in isolation from other parts of the cell and still behave as they do in toto // given the reductionist (or "component-oriented") thrust in the past, the SB approach can also be viewed as integrative //

5. Which part of Biology do you think is most relevant to SB?

Evolution, the ultimate text of any model of living organisms // biological regulation // biophysics // quantitative questions of molecular biology // all (from cells to populations) this is largely gated by available measurement technologies // all beyond structural molecular biology // biology is an application area as are all areas where dynamics and feedback are relevant // molecular biology and physiology // molecular biology // all of biology would benefit from integrative approaches // medicine. Of course, principles have to be discovered and methods have to be developed using simple systems, but ultimately SB will only receive the attention and input it needs if it has major impact on society //

6. What is a systems approach?

analyse a problem by looking at multi-parameters instead of single cause -single effect relations // orchestration of experiment and theory // approach integrating different experimental and theoretical techniques possibly on multiple scales // studying emrgent 'systems' properties (systematic collection of quantitative information, how the system is controlled // considering a system in a holistic rather than reductionist view defining relationships between components // bridging between different levels of explanation //understanding by showing how properties at one level can be accounted for, logically and quantitatively, in terms of lower-level interacting components typically requiring a mathematical model // „The consideration of the behaviour of a process by considering the interactions and feedback that produce the

observed behaviour" (Arnold Austin) // iterative process combining question-driven inquiry, precise measurements and perturbations, modeling and generation of hypotheses // a systems approach studies a collection of components and their interactions in terms of the structure(s) and dynamics, formalizes the insights in a computational model and validates through experimental data its validity //

7. What is systems-level understanding?

modeling of partial reactions and prediction // coarse-grained understanding that describes causal mechanisms on a systems scale // recognising patterns of behaviour at the higher level that arise from specific interaction patterns between the components // Ockham's razor at dynamic behaviour level: analysis should be sufficiently detailed to expose the features of interest – but not more detailed // kinetic modeling and pathway analysis // understanding how different components interact // understanding biological processes and behaviour that cannot be derived solely from component properties or more global levels of biological organisation // understanding of a set of biological processes uncovers design and operating principles underlying the mechanisms of the processes and their function //

8. Should SB be taught as a separate undergraduate study programme?

Yes, there is a successful programme at Harvard (David Botstein)
Not necessarily, SB benefits from people with different backgrounds
No. a few courses are sufficient for students having a good background in mathematical physics //
No // No, better train specialists then let them collaborate (using a common language) // include more mathematical modeling topics in molecular biology education //
No – it could be a component of natural and pysical sciences education //
Not in detail, systems biologists need a specific area of expertise to bring to interdisciplinary collaboration //
No. We should give biologists the same mathematical and analytical training as engineers and physicists – in addition to their current sylabus // Yes // No // Yes, should be combined with at least one advanced course // No, we need courses which enhance the understanding of the different scientific and engineering disciplines for complex systems and methods to study them and which enable these disciplines to better communicate with each other, and especially with biologists and clinicians // Yes, already early on. We frequently see Systems Biologists who are unable to communicate because they came from different backgrounds, e.g. Theoretical Physics vs Molecular Biologists. The theoreticians often do not have the goal or application in mind and may focus on phenomena like robustness or oscillations as a goal. Biologists on the other hand do not understand the analyses methods, the type of data they need to produce or how to actually integrate their data. As they have different interests, they set different goals for their research. Therefore I think especially the first years of undergraduate studies should focus on different aspects of Systems Biology, after which students will have to choose their specialization direction //

9. Should Masters Programmes in SB specialise or cover a broad spectrum of topics?

Both. Specialisation is needed for research (PhD courses), broad basis is needed for collaboration // broad spectrum // both // broad education can help prevent fragmentation // broad spectrum plus engineering disciplines // broad – specialisation comes at PhD level // probably only viable if a broad spectrum of topics is covered,

specialisation best offered via summer schools // make them conservation courses in 3 classes: systems theory and maths for biologists, biology for physics, maths and biology for control engineering graduates // 2 x broad spectrum // broad, and some case studies // first year with a broad spectrum of topics and specialization in the second year // broad spectrum, but there should be research projects within research departments of at least 1 year in which students specialize on a topic //

10. State the most important change required in training //education:

a. w.r.t. the biological sciences:
basic training in math, statistics, data analysis, computing // apply this knowledge to simple models // learn to think in terms of formal models that can be proven wrong // more maths // expose biologists early to conceptual models // quantitative measuring and data processing // additional mathematical training is vital // improved mathematical and computational education (use of mathematical computational models in teaching) // informatics, bioinformatics and modeling // basic knowledge of mathematical and engineering computing formalisms // integration of math //computational aspects in teaching of all biological areas (e.g. new textbooks with equations and models instead of just pictures and "cartoons") //

b. w.r.t. to the engineering and physical sciences:
adapt curricula to the needs of biology (e.g. nonlinear systems in basic ODE courses) // introduce experimental level biology // develop critical skills in dealing with uncertain models and interpretation // more biology // (re)introduce research oriented courses of study // wet lab experience and familiarity with modern technologies in molecular biology // more biology, not just biochemistry // awareness of the richness of biological systems // biochemistry, bioinformatics and modeling // focus on uncertainty of biological circuits // awareness of biological variation and evolution // inclusion of biological system aspects in as many courses as possible, offering "bio-tracks" //

11. Name the most important quantitative methods which biologists should be familiar with:
linear algebra, simple calculus and ODEs, modern data analysis methods // mass balances and ODEs // writing and solving ODEs numerically // TIRF // FLIM // find a common language for communication with modellers // statistical interference, stochastic proceses, mathematical modeling // (any would be an improvement) sensitivity analysis // dynamical systems methods and feedback systems analysis // ODEs, PDEs, graph analysis, statistics, Baysian optimisation, parameter fitting // quantitative „omics"methods // differential and vectorial calculus // the collection in the book by Klipp *et al* is a good first approximation // should know kinetic rate equations and be able to use those to do some simple modeling, to get some kind of hands on experience. They should know the principles of control or sensitivity analysis //

12. Name the most important biological concepts which modellers should be familiar with:
basic biology, chemical and physical "reality" of the cell // evolution and that nothing is as simple as written in the textbooks // phosphorylation // First, modellers should understand the meaning of words (terms?) in biology // cellular function in general // variation, evolution and population genetics // variation and evolution // basic concepts of molecular biology // depends on the problem, a first reading of a

Molecular Biology Textbook is useful, the collection in the book by Klipp *et al* is a good first approximation // should become familiar with some cell biology (what is a cell composed of), some molecular biology (including gene expression regulation), some histology (for instance to make sure they know there are different cell types) and basics of signal transduction. Furthermore they should be aware of the pathology related to several diseases. In principle, it is most important that they will be able to understand the biological problems they are working on and also why they are working on it //

13. Considering pathway //network diagram drawn by biologists, which vocabulary do you consider most important in the diagrams description? (e.g. modulation, activation //inhibition, feedback, ...)
activation – inhibition // biologists should use standards like SB Graphical Notation, BioPAX, etc. // common vocabulary (e.g. feedback is clear for engineers, biologists use it differently) is essential // clear distinction between different interaction types (non-covalent complex formation, catalysis, covalent modification) // SBGN // interaction, activation, inhibition // develop an interactive method to capture the vocabulary the partners use and clarify them in a stepwise manner //

14. What features //functionality do you miss with existing software tools?
easy ways to export or compile results into a simulation report // ways to track changes in models, manuscripts and simulations // Nothing's missing // standard for spacial ML // I write my own software // difficult to easily construct toolboxes for a particular problem // I hardly use software tools, I am mostly developing methodology // automated network drawing from mathematical network description // Matlab is sufficient // model building // intuitive integration of bioinformatics tools // too many // in general. professional-grade (robust, well maintained) software // software tools should be accessible to people who do not know computer science or programming language. I therefore like windows-interfaced programs such as Copasi //

15. State the difference between the notion of a "networks" and a "pathway":
Patway implies a flow of material, network could be related to more functional interactions // network=multiple interacting networks // no difference // a pathway, despite possible feedbacks, implies a directionality // pathways are thought as linear branching networks with few feedbacks, networks are thought as interconnected graphs. They correspond to different strategies of exploring mechanistic systems behaviour // pathway is a linear modular diredted acyclic graph, a network is a cyclic graph composed of multiple pathways // the difference between a roadmap and a motorway: pathway is a quantitatively dominant route through the network (and hence misleading label if applied to cells other than those for which it has been demonstrated to be appropriate) // pathway is a form of network for transmission of specific signalling information // there is no definition for both in biology // pathway is a special case of a network (which integrates many pathways) including genes, proteins and metabolites that are involved in a specific cellular process or response // pathway: mostly linear set of reactions, network: linear + multiple branching sets of interactions // "pathways" tend to be sequential, i.e., there is a main line of interactions, networks are more general graphs of interaction // the difference is merely semantic //

16. State the difference between the notion of a "module" and a "motif":
module: cell subsystem producing a specific function // motif: pattern arising from many possible interactions // module is specifically characterised – motif is general //

a module is almost isolated from surrounding entities, a motif can still be identified if it is densely linked with surrounding // module represents a subnetwork with well-defined function and interface with the rest of the network, motif is an emperical observation of frequent network patterns // a motif is structural a module is functional // a motif is a recognisable pattern of component interactions which may have a functional significance, but not necessarily isolable from the system. A module is a subsystem with many more internal interactions than with the rest of the system – definable input //output characteristics // no difference // module: set of elements involved in a given process – motif: regularities found in comparable modules // module: set of components associated with physical, functional property, motif: set of interacting components with specific arrangement // "Motifs" tend to be smaller, repetitive (statistically significant) and are characterized by "information processing" functions ; "Modules" are larger, usually unique and fulfil "biological function". However, it is very difficult in several cases to really differentiate, e.g. in the bacterial chemotaxis system with only 6-7 components //

17. Define "cross talk":
necessary to introduce as pathway models tend to be too simplified // molecular signal activating many subsystems // interaction between pathways // cross-talk frequently destroys quantitative predictions // interactions normally missed when studying the pathway under a particular condition when it can be considered isolated and independent from other pathways // exogenous inputs //outputs to the system representation // a term needed by those who insist on using a pathway paradigm with reference to a network where it is inappropriate // interaction between primary transmission channels // capacity of different pathways //modules to exchange information and to regulate each other // interaction between motifs, modules or systems // a (normal) network connection between pathways (usually discovered later) // cross talk is used to indicate the connections between pathways. It is a term that remained from the time period in which biologists were discovering that pathways are not isolated and not linear //

18. Define the meaning of "cell function":
the ultimate c.f. is reproduction // evolutionary purpose of a module or processes essential for survival, fitness and reproduction // should read „cell functions" like mitosis, meiosis, stress response etc. // in multicellular organisms cells may have immediate functions (skin cells, endothelial cell), higher level functions are hypotheses about properties arising from interactions with other cells // physiological state of a cell // ultimately survival and reproduction // contribution to a biological process // function fulfilled by some processes within a cell // the process a cell performs (or a product is produces) for a larger system (e.g. organ, body, ecosystem) //

19. Name sources of complexity in SB:
the need to integrate knowledge from many diverse fields // non-linear feedback // non-linearity, multi-scale, size and identifiability problem // ... not sure whether current mathematical tools are appropriate for describing life // wide dynamic range // non-linearity, dynamics, feedback and scale // evolving self-replicating machines // environmental changes, individual variation and hierachical levels of organisation // besides the system (and its context) itself: inappropriate data, difficult communication between disciplines, inadequate computational methods // organizational complexity comes from communication problems and access to biological data. Systems complexity arises when a system is too complex to handle in your brain //

20. Give an example of a success story of mathematical modeling in the life sciences: modeling of a complete organism (genome-scale model) // experimentally confirmed prediction that one of the leu_tRNAs had to read another codon // Tyson&Novak // modeling buddying yeast cell-cycle (Chen et al) // optimisation of bioengineering reactors based on flux analysis // Hodgkin-Huxley model of transport across cell membranes, axon models of Fitzhugh-Nagumo, heart model of Dennis Noble // mathematical cell cycle models, metabolic control analysis // Hodgkin-Huxley and Physiome project (Peter Hunter) and heart model (Dennis Noble) // Hodgkin-Huxley, Watson-Crick // Demand theory and synthetic biological circuits // Eric Davidson's modeling of the development of sea urchins // "Modelling biological rhythms" paper (Mendoza *et al.*) with a whole section on predictive successes //

21. Give an example of a success story of SB in drug discovery and medicine: yet to come, however, Physiome project is a nice existing example // Gleevec // hard to tell as all projects I know about are currently ongoing and do at best look promising // 2 x heart model (Dennis Noble), Merrimack growth pathway inhibition in clinical trial // TB ARO pathways // Entelos and Merrimack Pharmaceuticals, and the work by J. Keasling on artemisinin //

22. State the most important advance necessary in SB:

a. w.r.t. drug discovery: trying to integrate the psychotropic effects of drugs (important but currently neglected) // small molecules for molecular therapy // sufficiently predictive models of disease mechanisms // sufficiently exclusive models (e.g. cell cycle + apoptosis + growth factor //cytokine signalling) with sufficient detail and molecular targets represented // working with clinicians // integration of structural biology and bioinformatics with modeling and control analysis of responses // integration of multiple types of data to predict drug efficacy and patient response // disease-focussed virtual patient models (as early testbed for drug candidates) //

b. w.r.t. technologies:
happening by advances in computing power and HT technologies // even better labelling technologies // better integration of imaging technologies // HT measurement of enzyme activities // single cell and single molecule technologies // devices with biological behaviour //

c. w.r.t. methodologies:
scale (time and space) // multi-scale models // measuring kinetic parameters in single cells // stochastic modeling and analysis // agent-based modeling //

d. w.r.t. formal concepts:
scale (time and space) // more efficient extraction of insights from diagramatic representations // biochemical formalisms are taken too literally, we miss adequate techniques as ODEs and stochastic ODEs adapted to deal with systems performing functions in highly variable conditions (robust evolvable systems), a situation not reducible to the classical chemical kinetics approach // formal framework to describe life // multi-scale modeling // understand evolution // formalism for integration of diverse types of modeling //

e. w.r.t. experimental techniques:

new technologies must yield quantitative results // single molecule technologies for eukaryotic cells // large-scale data on states and localisation of cell signalling proteins // improved temporal and spatial resolution // single cell and single molecule technologies // real-time non-invasive quantitative measurements at single-cell, single-molecule resolution //

f. w.r.t. research funding
apply SB to problems relevant for society // easy funding of small high-risk short-term projects (1y) would be great // trans-atlantic funding // cooperation between national funders // more integrated activities // decrease size (no.?) of groups that can apply for funding // support of cross-disciplinary training of researchers //

23. Which standardisation effort in SB do you consider most important (and why)?
of model formats (no one else would do it) // development of SBML // metabolic reactions // SBGN + SBML + BioPAX because we need standards for model exchange // experimental systems will continue to be too diverse // model structures and languages // SBGN, SBML2 // SBML and CellML modeling language standards // representation of experimental data suitable for modeling // ontologies for consistency in further formalisation //

24. What is in your view the main hurdle for the integration of models?
Scales (time and space), a unified framework will be required // models are built for different purposes // analysis of large models // different scales and different problems for which they are constructed // finding a formal framework for integration at different spatial and temporal scales and levels of abstraction // nomenclature of molecules, components, states of molecules // model annotation // models developed for different conditions and assumptions are hard to integrate, makes it more laborious than building new models // lack of common mathematical framework // lack of compatible concepts, initial conditions, generally insufficient information to reproduce results and validate //

25. Rank the following decisions w.r.t. to importance in preparing a project: (a) model organism (b) cell line (c) network //pathway (d) genes //proteins (e) technologies (f) data management (g) design of experiments (h)type of model
c-(afd)-h-e-g-f // a-g-d-b-c-e-h-f // h-g-e-c-a-b-f // g-a-e-c-d-h-f-b // c-a-d-b-e-g-h-f // a-f-g // e-d-a-b-g-e-f-h // (gh)-(ef)-(abcd) // when preparing an SB project, the most important point is a consensus (as clear as possible) on the question to be addressed and the solution envisioned. The problem formulation (from the experimenters viewpoint) might already point to the model organism , cell lines, genes //proteins, network //pathways to be studied. An initial design of experiment would follow to give a sense of the data that could be obtained These factors – problem and data - will then constrain the type of model to be built. Data management will help with information from literature to build the model. Depending on the insights and predictions (hopefully) produced by the model, the experiment design can be refined to validate the model predictions; i.e. follow the SB cycle à la Kitano (2002) as much as possible // a (this directly follows from your hypothesis or problem definition)-b-g-f-e-h-c-d //

26. How important do you think is the definition of "grand challenges" in SB:
'Grand challenges' are social, political, economic and scientific // important as it will have impact on society // doubt whether a project of that size can be efectively

coordinated at presen // showcase projects carrying the risk of rejection of other projects by funders // very important // driving force of community efforts // useful at certain points of the development of the discipline. Should however be from credible //accepted authorities, otherwise not effective. Examples of successful GC efforts are Hilbert's Problems (1900) and the Millenium Problems (2000) in Mathematics. Not aware of similar successes in other sciences // not important //

27. State a time frame in which a grand challenge should be solved //realised:
The time it takes. Question whether mega projects are the right answer // decades // 10years // 10-15years // 30years (scientists lifetime) // 10-20years // 10years // 30years // not feasible to give a time frame // weeks to decades and more // 15-20 years //

28. Would you prefer a "virtual cell" project over a "virtual human" project?
prefer virtual human, as the virtual cell is already there (Tomita et al 1997) // prefer virtual cell (however, it is too large, what question would it answer) // 2x yes // could be done in parallel // no // both are complementary // no preference – the question(s) to be answered and the data for validation to be generated need to be specified as clearly as possible // both are important //

29. "Moonshot projects" in SB – which would be your favourite?
"Virtual human" would have lasting impact on society // understanding of immune dynamics // model of tumour progression explaining tissue specificity // virtual yeast cell – would be more clear-cut whether you'd arrived // get beyond Physiome project with sufficient ressources // virtual human // enzynome projects to determine accurate kinetics of individual proteins // one that have impact on a broad spectrum of diseases // remedy for tuberculosis //

30. How many individuals //groups //institutes would you expect to be involved in large scale research ("moon shot") projects?
Many in many different projects // About 20 max. groups from all around the world // 42 // no. of participants according to needs, should be dynamic // 20-40 // A whole institute devoted to the project // as many as required, manageable size 20-50 groups // at least two levels – the core project and the broader research community. The core project would comprise 100-200 persons // if organised well, there can hardly be too many groups //

31. What funding volume would be required to realise moonshot projects?
€ 0.5m //year*group for 10years // funding comparable to pharmaceutical projects // > € 10bn // € 100m // several 100m € // 2 x depends // depends on expertise required, probably 10-20 times the usual grants // make sure that the researches will do what they promise. Then fund based on what the goals, milestone and deliverables are //

32. Which existing large-scale research project would you consider a success story?
CERN, Physiome project // Human Genome Project // Heart project or the Flagellum project // Physiome project has that potential // both whole genome metabolic reconstruction and Physiome project are partial successes // Physiome project // sequencing of the human genome (and other species) //

33. If you could award a prize to a living scientists for the greatest contribution to your work //field, who would that be and what should be prize be awarded for?

René Thomas for his investigations in the role of feed-back in biological systems // Otto Berg for stochastic gene expression, facilitised diffusion, diffusion limited dissociation etc. // Alfred Wittinghofer // Mark Newman&John Tyson // Spiegelhalter (statistics) Sejnowski (neurobiology computation) // Hans Westerhoff for tirelessly motivating and integrating European efforts in SB // 2 x Dennis Noble for the virtual heart // Michael Savaggan for contribution to biochemical systems theory and studies in design principles // Leroy Hood for enabling technologies and education in SB //

34. What would you consider is the role of a (mathematical //statistical //computational) model?

repositoryy of knowledge and hypotheses of the biological system it emulates // to clarify thinking and deduction // to quickly rule out wrong hypotheses and to extract functions from static //structural data in order to generate new hypotheses // unambigous description of hypotheses and mechanisms, predictive, intelligent guess when developing new concepts and ideas // testing verbal //qualitative explanations // strategic as framework for analysis and repository of quantitative knowledge // generation of plausible hypotheses // allows verification of concepts, suggest alternatives, prioritise wet lab experiments, and to test hypotheses that cannot be studied experimentally (thought experiment) // integrating multiple types of data, predict systems response to perturbations // role in communication with experimentalists particularly in a stepwise process of formalizing ideas, hypotheses and moving the iterative SB cycle forward // help integrate the available information and to identify general principles //

35. What is a "phenomenological model"?

All models in biology are phenomenological // model that describes experimentally observed phenomena // non-mechanistic model focused on describing observations // model describing a phenomenon far from ,the first principles' using ad hoc hypotheses and generic functions fitted to observations // which represents observable properties // reproduces input-output relationships with functions //parameters that do not relate to physical characteristics of the components of the system // no particular details // model that uses kinetic functions chosen to fit the data // phenomenological models take an external view of a system, in particular with respect to the dynamics of its behaviour //

36. What is a "mechanistic model"?

A reductionist model // model that targets a simple mechanism // quantitative model including interacting physical objects // constructs a function which mimics biochemical mechanisms known about the system // which represents underlying mechanisms // a model whose functional form and parameters are derived from physical properties of the components // mechanistic models (as usually used by biologists) reflect molecular and some systemic properties of the system //

37. What is a "physical model"?

Based on explicitly formulated physical laws, questionable, whether this is sufficient // model that describes a known physical phenomenon with very few hypotheses // same as mechanistic model // includes physical mechanical processes // constructed from the very first principles (atoms and molecules) // mathematical equations model // model where individual steps of the process are known and kinetically characterised // take into consideration physical properties of the system (somewhat more detailed than mechanistic models) //

38. What is a "large-scale model"?
A genome-scale model of a cell // a model containing >100 different non-identical components and cannot be easily treated by standard methods // simply that they are large and highly detailed // large no. of components spans temporal and //or spatial scales // >100 elements // large number of dependent variables // 38. "Large-scale" is relative – one needs to state "large relative to what". Examples: large relative to what can be dynamically simulated with ODEs (say 1700 ODE's used in a Hepatitis C infection model of Vertex Pharmaceuticals vs. the 1-2 hundreds usually considered; or large relative to the relevant part of the genome e.g. genome-scale metabolic network meaning that the majority of the genes encoding enzymes are included in the network ... //

39. What is a "useful", "good", "practical", "predictive" model?
models that answer questions, predict the outcome of new experiments (within a certain range) // descriptive models require further analysis to achieve explanantions // predictive model is tautological // one that produces results that are contrary to expectations // predictive, otherwise no good // providing insights and suggesting experiments // usefulness has several levels: show consistency of information, explain available data, show new relationships, make predictions, suggest new experiments to validate,... //

40. What is a "descriptive" vs. "explanatory" model?
descr. fits the data – expl. makes you understand why a systems operates as it does // recapitulates existing facts in mathematical equations, expl m explains s.th. that cannot be explained from already known facts // descriptive=phenomenological explanatory=mechanistic // descriptive: how things work, explanatory: why things work

41. What distinguishes a biological system from a physical system?
evolves and eventually dies // level of knowledge we have on both // purpose in the context of evolution // complexity // p.s. contain fundamental interactions (gravitation, electrodynamics ...) and b.s. contain emergent interactions (activation, phosphorylation, predation, ...) // evolution // alive vs. not alive // autonomous freedom of new entities // evolution // a biological system is a particular case of a physical system // all biological systems are physical // a physical system can be everything including a biological system. A biological system is necessary (or at least has a function, cf 18) for a living system //

42. What distinguishes a cell from a computer?
Evolution // cell is probably more intelligent // complexity // comp. are designed to solve huge range of problems, cells have evolved to solve the problem of survival // totally different design: von Neumann architecture vs. undirected evolution process // a cell has life // cells haven't got keyboards // self-replication // number of components and interactions, chemical composition, self-replication // lipid membrane, polynucleic acids as information storage devices, chemical energy carrier ATP // serious question? A cell is (part of) a living entity, a computer is not. A cell is far too complex to be understood by the human brain. A computer is thought up by human brains //