

Helmholtz Alliance on Systems Biology

Spring School 2010 on Systems Biology

April 14 – 17, 2010

Kloster Seeon



Helmholtz Alliance on Systems Biology

Spring School 2010
on Systems Biology

Contents

Program	2
Lectures	5
Progress Reports	19
Poster	37
Address List	47

Spring School 2010 on Systems Biology

Kloster Seeon, April 14 – 17, 2010

Wednesday, April 14

15:00 – 18:00	Arrival at Kloster Seeon
19:00 – 20:30	Dinner

Thursday, April 15

07:30 – 9:00	Breakfast
09.00 - 09.10	Opening Address
09.10 - 10.10	Martin von Bergen, UFZ Leipzig: <i>Proteomics goes globally targeted and combines nicely with Metabolomics</i>
10.10 - 10.40	Hannah Schmidt-Glenewinkel, DKFZ Heidelberg: <i>Multiparametric image analysis of EGF receptor endocytosis</i>
10.40 - 11.00	Coffee Break
11.00 - 12.00	Frank Allgöwer, Universität Stuttgart: <i>Systems theoretical analysis in systems biology.</i> <i>A tutorial introduction to sensitivity analysis with applications to apoptosis</i>
12.00 - 13.30	Lunch
13.30 - 14.30	Poster Session
14.30 - 15.30	Ralf Steuer, HU Berlin: <i>A general principle for robust signal transduction</i>
15.30 - 16.00	Coffee Break
16.00 - 16.30	Michael Schwarzfischer, HMGU: <i>Single-cell analysis of multipotent hematopoietic progenitor cells</i>
16.30 - 17.00	Jenny Russ, MDC Berlin: <i>Identification of disease pathways for ALS from protein-protein interaction data</i>
17.00 - 18.00	Hans-Werner Mewes, HMGU: <i>Reflections on Systems Biology: About Reductionism and Complex Systems</i>
19.00 - 20.30	Dinner

Friday, April 16

07.30 - 09.00	Breakfast
09.00 - 10.00	Fabian Theis, HMGU: <i>Modeling based on spatial expression patterns</i>
10.00 - 10.30	Benedikt Wachinger, HMGU: <i>Mining Biomedical Literature</i>
10.30 - 11.00	Coffee Break
11.00 - 11.30	Werner Römisch-Margl, HMGU: <i>Metabolomics at metaP</i>
12.00 - 13.00	Lunch
13.00 - 14.30	Tour of the monastery
14.30 - 15.00	Daniel Ellwanger, HMGU: <i>MicroRNA mediated progression of AML</i>
15.00 - 15.30	Edna-Clarisse Cieslik, FZJ: <i>Dynamic interactions in the fronto-parietal network during a stimulus-response compatibility task</i>
15.30 - 16.00	Coffee Break
16.00 - 16.30	Poster Session
16.30 - 17.00	Mareike Clos, FZJ: <i>Role and mechanisms of predictive coding in language perception – outline of an fMRI study</i>
17.00 - 18.00	Simon Eickhoff, FZJ: <i>Meta-analytical functional connectivity mapping and functional data-mining</i>
18.00 - 18.10	Closing Remarks
19.00 - 20.30	Dinner

Saturday, April 17

07.30 - 09.00	Breakfast
09.00 - 11.00	Departure

Spring School 2010 on Systems Biology
Kloster Seeon, April 14 – 17, 2010

Lectures

Speakers:

Martin von Bergen

Frank Allgöwer

Ralf Steuer

Hans-Werner Mewes

Fabian Theis

Simon Eickhoff

Proteomics goes globally targeted and combines nicely with Metabolomics

PD Dr. Martin von Bergen

Department of Proteomics, Helmholtz Centre for Environmental Research (UFZ),
Leipzig

Franziska Herden¹, Saskia Trumpf², Jake Micholson³, Sven Baumann⁴, Tibor Kohajda⁴, Juliane Mai⁵, Saadia Faisal⁶, Kristin Schirmer⁷, Andreas Beyer³, Sabine Attinger⁵ and Irina Lehmann² and Martin von Bergen^{1,4}

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In quantitative proteomics there are two major trends, first the global approaches cover more and more proteins but stick to relative quantifications and second the targeted approaches become global since databases collecting selective reaction mode transitions grow by the minute and allow absolute quantification. Here the techniques of proteomics are reviewed and one example of a gel-based study and one application of lc-ms based proteomics will illustrate advantages and pitfalls of the different approaches.

Furthermore, the combination of proteomics and metabolomics offer the chance first to measure activity instead of protein abundance and secondly to gain an in depth understanding of molecular phenotypes. This is shown in a study combining proteomic and metabolomic assessment of sera from lean and obese patients.

For your notes:

Systems theoretical analysis in systems biology.

A tutorial introduction to sensitivity analysis with applications to apoptosis

Prof. Dr. Frank Allgöwer

Institute for Systems Theory and Automatic Control, Universität Stuttgart

In this talk it will be shown, using the example of sensitivity analysis, that systems theoretical methods can play an important role in gaining understanding of biological systems. Examples of these systems are signaling pathways, metabolic or gene regulatory networks and they can often be modeled using ordinary differential equations with variables representing the involved species and parameters describing reaction rates or environmental conditions.

Sensitivity analysis is a systems theoretical tool that allows to investigate the importance of individual model parameters on interesting quantities in a structured way by computing how much this quantity changes if the parameter is changed. The results of such an analysis bear several important advantages. On the one hand, if a parameter has a strong influence on a model output, it will be necessary to determine this parameter (for example experimentally) with higher precision while not so much effort needs to be taken to measure less important parameters. On the other hand, knowing the effect that certain parameter variations have can help to detect promising target sites for new drugs.

In the first part of this talk, local sensitivity analysis for ordinary differential equations is introduced and methods for its computation are presented in a tutorial way. It is then shown how this method is applied to a comprehensive model of an apoptosis network and which conclusions can be drawn from this analysis. However, this example also reveals the limitations of local sensitivity analysis, namely that its results are only valid in a limited region around the nominal parameter values. To overcome these limitations a method for “semi-global” sensitivity analysis is presented in the second part of the talk which has been developed recently by the authors. This method is able to capture the effects of larger parameter variations and its application to the model of the apoptosis network shows its advantages and reveals interesting biological insight.

For your notes:

A general principle for robust signal transduction

Dr. Ralf Steuer

Institute for Theoretical Biology, Humboldt Universität Berlin

Cellular signaling has to operate reliably under conditions of high uncertainty and in the face of constant perturbations. However, knowledge of the precise mechanisms that allow cells to function reliably and with high fidelity in uncertain environments is still fragmentary.

The talk will present a novel formalism that pinpoints the necessary architecture for robust signal processing in living cells. The presented mathematical framework accounts for diverse manifestations of cellular robustness and enables the predictive design of perfectly robust synthetic network topologies. We provide experimental evidence that the proposed design principle is a dominant mode of prokaryotic signaling.

For your notes:

Reflections on Systems Biology: About Reductionism and Complex Systems

Prof. Dr. Hans-Werner Mewes

Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München
German Research Centre for Environmental Health (HMGU), Munich

Systems Biology has generated a rather irritating collection of its own definitions. Some authors have delivered strong statements that others tried to avoid. I believe, some fundamental thoughts might help to understand the problems we face while investigating biological systems.

How can we deal with complexity in the life sciences and what are we looking for? While laws as concepts in physics should be eternal and without exception, biology is not in conflict with these laws, but they have not been useful to explain the observed phenomena such as the genotype/phenotype relations. While molecular biology did not need any philosophical justification due to its success, Systems Biology comes with a rather rich flavor of concepts and ideas. Molecular Biology was driven by technology first whereas Systems Biology understood itself from the beginning as a novel paradigm as stated by its protagonists (e.g. D. Noble and Hans Westerhoff).

Some topics to be discussed in my presentation are: (i) about laws and the difference between physics and biology (ii) The difference between a puzzle and a mystery: is the problem understanding solvable in a rational way, as promised by systems biology? (iii) how can the available information form millions of publications be used or do we have to restart from zero?

For your notes:

Modeling based on spatial expression patterns

Prof. Dr. Dr. Fabian Theis

Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München
German Research Centre for Environmental Health (HMGU), Munich

In systems biology, we aim at deriving models from multivariate biological observations in order to predict and establish biological knowledge and design new experiments. Ideally we want to work on quantitative data, available in a high number of homogeneous replicates. This may be possible in *E. coli* and yeast - however if we want to work on mammalian systems, we will often deal with heterogeneous, qualitative in-vivo data. Here we will derive models from data from in situ hybridization experiments. The presented work flow allows us to cope with both heterogeneity and the only qualitative nature of the data.

After reviewing qualitative modeling techniques based on boolean logic, we develop a method to derive a regulatory network describing the well-defined pattern of locally restricted gene expression domains around the mid- hindbrain boundary (MHB). This pattern is established and maintained by a regulatory network between several transcription and secreted factors that is not yet understood in full detail. We show that a Boolean analysis of the characteristic spatial gene expression patterns at the murine MHB reveals key regulatory interactions in this network. This approach allows us to predict also the interplay of the various regulatory interactions. In particular, we predict and then experimentally verify a maintaining rather than inducing effect of *Fgf8* on *Wnt1* expression.

Altogether, we will demonstrate that similar to temporal also spatial expression patterns can be used to gain information about the structure of regulatory networks.

References:

[1] D. M. Wittmann, F. Blöchl, N. Prakash, D. Trümbach, W. Wurst, and F. J. Theis. Spatial analysis of expression patterns predicts genetic interactions at the mid-hindbrain boundary. *PLoS Computational Biology*, 5(11):e1000569, 2009.

- [2] D. M. Wittmann, J. Krumsiek, J. Saez-Rodriguez, D. A. Lauffenburger, S. Klamt, and F. J. Theis. Transforming boolean models to continuous models: Methodology and application to t-cell receptor signaling. *BMC Systems Biology*, 3(98), 2009.
- [3] S. Klamt, U.-U. Haus, and F. J. Theis. Hypergraphs and cellular networks. *PLoS Computational Biology*, 5(5), 2009.

For your notes:

Meta-analytical functional connectivity mapping and functional data-mining

Prof. Dr. Simon Eickhoff

Institute of Neurosciences and Medicine (INM), Forschungszentrum Jülich

Brain connectivity has many aspects. Most commonly, three concepts are distinguished, namely structural (assessed using diffusion tensor imaging), functional (assessed by resting state correlations) and effective (models of interacting regions) connectivity. The advent of large-scale databases on neuroimaging results, however, has created the opportunity for assessing a completely different aspect of connectivity, namely task-based functional connectivity as defined by co-activation patterns. The basic idea, which will be illustrated in this talk, is to identify those regions, which co-activate above chance with a given seed-region across many hundreds of neuroimaging studies. Both theory and applications of this approach will be demonstrated, including a comparison of meta-analytical connectivity mapping to resting state fMRI analysis and probabilistic DTI tractography. The talk will close with a short overview on the possibilities for functional datamining using brain-activation databases. Using the meta-data archived with the respective activation coordinates, functional inferences on the role of a given brain area can be performed that allow the objective interpretation of, e.g., morphometric data. Moreover, this approach allows to test for functional components of co-activated networks and hence contextual-influences on co-activation probabilities.

For your notes:

Spring School 2010 on Systems Biology
Kloster Seeon, April 14 – 17, 2010

Progress Reports

Speaker:

Hannah Schmidt-Glenewinkel

Michael Schwarzfischer

Jenny Russ

Benedikt Wachinger

Werner Römisch-Margl

Daniel Ellwanger

Edna-Clarisse Cieslik

Mareike Clos

Multiparametric image analysis of EGF receptor endocytosis

Dr. Hannah Schmidt-Glenewinkel

Institute for Theoretical Bioinformatics, German Cancer Research Centre (DKFZ),
Heidelberg

Endocytosis regulates receptor tyrosine kinase signaling by controlling the spatial distribution of activated receptors in the cell. We present here a framework for analyzing endocytic trafficking using a combination of high-resolution laser-scanning microscopy, automated image processing and statistical inference. We apply our framework to analyze endocytosis of the epidermal growth factor receptor (EGFR) and investigate how trafficking of individual endosomes determines (i) EGFR degradation and (ii) endosome activity state. Further, our analysis reveals several principles of the trafficking process. These results may help to elucidate the role of endocytosis in signaling in general, and in pathologic conditions in particular. To this end, we discuss the application of the presented framework to analyze the role of the tumor suppressor Caveolin in EGFR endocytosis.

For your notes:

Single-cell analysis of multipotent hematopoietic progenitor cells

Michael Schwarzfischer

Institute of Bioinformatics and Systems Biology, German Research Centre for Environmental Health (HMGU), Munich

Innovative approaches in hematopoietic stem cell research allow for analysis of single cells rather than heterogeneous cell population. Due to long-term observation of multipotent progenitor blood cells with uorescence-labeled proteins it is now possible to correlate cell fates and molecular concentrations on a single-cell level.

In this project we analyze movies from time-lapse microscopy of common myeloid progenitor cells. Several technical problems like illumination correction, bleaching, quenching and ickering have to be dealt with. We applied new experimental set-ups and computational methods in an integrated fashion to purify the data. This allows us to track cell movement, to observe cell division or other important events during di_eren-
tiation and to measure uorescence intensities of the transcription factor PU.1.

Two distinct peaks of the uorescence levels of all cells over four days arise, representing di_erentiated cells in the granulocyte/macrophage progenitor and megakaryocyte/erythrocyte progenitor lineage respectively. Further analysis shows that PU.1 expression rises before cells become mature assessed by their morphology. We search for a speci_c single-cell expression pattern which predicts the cell fate even in early progenitors. Determining the noise in single-cell time courses reveals uctuation behavior depending on the cell fate and allows to estimate protein kinetics like protein decay or the absolute amount of protein present in the cells.

For your notes:

Identification of disease pathways for ALS from protein-protein interaction data

Jenny Russ

Institute of Neurodegenerative Diseases, Max Delbrück Centre for Molecular Medicine (MDC), Berlin

Jenny Russ, Pablo Porras-Millan, Raphaelae Foulle, Erich E. Wanker

Amyotrophic lateral sclerosis (ALS) is the most common adult onset motor neuron degeneration. Most cases are sporadic, but 10 % of them are inherited. Until today several hundred mutations in 11 genes have been identified to cause familial ALS. The most prominent protein is the cytosolic superoxidized dismutase 1 (SOD1). But the recent discovery of mutations in the DNA / RNA-binding proteins TDP-43 and FUS in familial ALS and of the cytosolic aggregation of TDP-43 occurring in sporadic ALS change the current view on ALS pathogenesis. In order to identify ALS-relevant disease pathways, we screen a large number of proteins involved in ALS for protein-protein interactions (PPI) using the yeast-two hybrid method. The screen resulted until now in a network of 676 proteins connected via 1330 interaction. Many of the identified interactors are transcription factors or cytoskeletal proteins and play a role in cell death, the cell cycle, or in cell development. To obtain a high confidence PPI network for ALS we validate the interactions in human cells using FRET and develop an interaction score based on *in silico* data such as functional annotation and domain-domain interactions. Finally, we want to identify disease-relevant pathways amongst others by integration of gene expression data from ALS patients and a network structure-based analysis.

For your notes:

Mining Biomedical Literature

Benedikt Wachinger

Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München
German Research Centre for Environmental Health (HMGU), Munich

The growing amount of data generated from scientific experiments subsequently increases the amount of available literature on particular fields of research since the knowledge gained from processing and interpreting the experimental data is published in scientific articles. The available literature on any topic, such as regulatory networks, biological processes, or complex multifactorial diseases, can run into the millions of different articles. This knowledge is rarely stored in databases where computers can have ready access to it, as this is only possible when humans manually annotate it.

The text contained in scientific literature is usually made up of sentences containing nouns, verbs, or adjectives and other grammatical structures placed in a context to each other. For a computer, however, there is no easily recognizable structure behind this text. A computer cannot read text and understand it like humans can. Therefore, the necessity arises to develop new tools and methods, called text mining systems, which are capable of structuring unstructured free text written by humans into a form that a computer can store in databases and understand.

This talk will give an overview of basic natural language processing (NLP) techniques to automatically extract the knowledge contained in scientific literature. It will cover the most important concepts and methods for structuring unstructured text. The talk will introduce some state-of-the-art text mining systems and compare them to each other. Also, our own text mining system, EXCERBT, will be presented. In the end, an outlook to future developments in the text mining field will be given.

For your notes:

Metabolomics at *metaP*

Dr. Werner Römisch-Margl

Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München
German Research Centre for Environmental Health (HMGU), Munich

Metabolomics is a fast growing research field with an unbiased approach for characterisation of biological samples. Using many different but maybe correlated parameters even silent or subsidiary phenotypes could be determined. Two main approaches in metabolomics are performed at present: targeted (quantification of a chosen set of metabolites) and non targeted metabolomics (profiling or search for biomarkers). Analysis of large sets of metabolites in a sample provides an integrated overview on biological events. Metabolomics relies on sensitive and fast analytical methods and bioinformatic processing of data.

The Metabolomic Platform (*metaP*) at the Helmholtz Zentrum München is a scientific interdisciplinary cooperation of four Institutes with the aim to mediate progress in science through development of new metabolomic methods and provision of measurement services applicable to man, animal models, plants, environmental samples and ex vivo systems. The activities include non-targeted as well as targeted analyses. Our methods include ultrahigh resolution mass spectrometry for the profiling approach, triple quadrupole LC-MS/MS combined with robotics and kit technologies for the high throughput quantification of analytes and web-server based automated data processing. We successfully performed studies in population based human cohorts and in animal models in elucidating metabolomic effects in complex diseases or drug development.

For your notes:

MicroRNA mediated progression of AML

Daniel Ellwanger

Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München
German Research Centre for Environmental Health (HMGU), Munich

MicroRNAs have emerged as central post-transcriptional regulators of gene expression in higher eukaryotes. They regulate various key biological processes, including cell growth, apoptosis, development and differentiation by intertwining with gene regulatory networks. Furthermore, microRNAs play a critical role in many malignant processes such as leukemogenesis.

Especially in complex diseases the understanding of regulatory interactions of microRNAs and their role in biological systems is still in its infancy.

However, based on the example of acute myeloid leukemia (AML) we will discuss strategies how combinations of high-throughput experiments and bioinformatics approaches (including biomedical textmining) can be utilized to unravel the systemic function of microRNAs in complex diseases.

We will cover semi-automatic approaches for mid-scale model generation as well as suitable strategies for small-scale qualitative models. Finally, we discuss the inference and explanation of disease-relevant hypotheses for microRNA-mediated acute myelogenous leukemogenesis.

For your notes:

Non-Markovian Stochastic Models for Regulatory Mechanisms

Edna C. Cieslik¹, Karl Zilles^{1, 2, 3}, Simon B. Eickhoff^{1, 3, 4}

¹Institute of Neuroscience and Medicine, INM-2, Research Centre Jülich, Germany; ²C&O. Vogt Institute of Brain Research, University of Düsseldorf, Germany; ³JARA-BRAIN Translational Brain Medicine, Jülich/AachenGermany; ⁴Dept. Psychiatry and Psychotherapy, University Hospital Aachen, Germany

Introduction: Attentional orienting can be modulated by stimulus driven bottom-up as well as task dependent top-down processes. In a recent study we investigated the interaction of both processes in a manual stimulus response compatibility task. Whereas the intraparietal sulcus (IPS) and the dorsal premotor cortex (dPMC) were involved in reflexive orienting towards the stimulus side facilitating congruent motor responses, the right temporoparietal junction (TPJ), right dorsolateral prefrontal cortex (DLPFC) as well as the medial preSMA sustained top-down control processes involved in voluntary reorienting. Here we used dynamic causal modeling to investigate the contributions and task-dependent interactions of these regions.

Methods: Thirty-six models were tested, all of which included bilateral IPS, dPMC and primary motor cortex (M1) as a network transforming visual input into motor output as well as right TPJ, DLPFC and the medial preSMA as task dependent top-down regions influencing the coupling within the network. The models differed in the way how and at which level the top-down influences on the visuo-motor transformation were modelled. All models were compared against each other by random and fixed effects analyses. In a next step we tested with a non-parametric ranksum test if the mechanisms (coupling parameters) represented in the winner model were consistently expressed across subjects.

Results: When all thirty-six models were compared by random and fixed effects analyses, congruent evidence emerged in favour of a family of three models differing only in the specific impact of the DLPFC on both dPMCs and their transcallosal coupling. At the level of the parietal cortex, the winner family showed significantly enhanced transcallosal coupling between both IPSs mediated by the right TPJ in a non-linear fashion. Furthermore, the winner family showed significant preference for bidirectional intrinsic coupling between IPS and dPMC compared to a simple feed forward mechanism. Finally, it showed influences of the preSMA on M1 rather than the dPMC. In a subsequent analysis of the derived model parameters all connection strengths

were positive with one exception. For incongruent left hand responses the preSMA showed positive coupling with right M1 and a concurrently negative coupling with left M1 and vice versa for incongruent right hand responses.

Conclusion: The data provide further evidence for context dependent top-down control of right TPJ and DLPFC as well as the medial preSMA in stimulus response compatibility. In particular, we propose the right TPJ to play a mediating role during attentional reorienting processes by modulating the inter-hemispheric balance between both IPSs. Furthermore, the IPS and dPMC seem to be coupled bidirectionally providing evidence for feedback-loops in this fronto-parietal circuit. Analysis of connection strength furthermore supported the proposed role of the preSMA in executive monitoring of motor responses promoting or suppressing activity in primary motor cortex controlling the accurate motor output. As the results did not show a clear tendency towards a role of the right DLPFC, we propose this region, against the usual interpretation of an inhibitory influence in SRC tasks, to subserve generic monitoring processes.

For your notes:

Role and mechanisms of predictive coding in language perception – outline of an fMRI study

Mareike Clos

Institute of Neurosciences and Medicine (INM), Forschungszentrum Jülich

This study represents the first part of a newly started PhD project. It is based on the predictive coding model of language perception and aims at investigating the neural mechanisms of integrating sensory data and prior information. The project involves measuring the neuronal responses by means of functional magnetic resonance imaging (fMRI), while the participants perform a delayed match-to-sample task. Specifically, normal and low-pass filtered (delex) versions of 25 sentences will be presented in a pair-wise fashion, such that half of the sentence-pairs are matches which have to be indicated by the subjects. The filtering yielding the delex-sentences preserves only the prosodic parameters of the speech signal but renders the sentence basically impossible to comprehend due to the lack of lexical and syntactic information. However, when a delex-sentence is preceded by the corresponding unfiltered version, it becomes understandable. This effect is hypothesized to be due to predictive coding, where the preceding presentation of the original sentence allows for feedback from higher areas providing priors for sensory decoding. These should facilitate inference of ambiguous stimuli such as the delex-sentences and thus sentence comprehension. It is hypothesized that, in contrast to trials in which a delex-sentence is preceded by another delex-sentence or by a non-matching normal sentence, the neuronal activation response to a delex-sentence preceded by its normal version should be comparable to that to the normal version.

For your notes:

Spring School 2010 on Systems Biology

Kloster Seeon, April 14 – 17, 2010

Poster

- Gabi Kastenmüller: *metaP-server: a web-based metabolomics data analysis tool*
- Werner Römisch-Margl: *A High Throughput Protocol for Metabolomic Analysis of Tissue Samples*
- SysMBo Consortium: *Systems Biology of Metabotypes – the BMBF-funded MedSys Project SysMBo*
- Ralf Steuer: *A general principle for robust signal transduction*
- Ruth Merkle: *Regulation of EpoR signaling by microRNAs in lung cancer*
- Michael Strasser: *A stochastic model of the PU.1/GATA-1 toggle switch*
- Marco Nici: *Differential regulation of IL-6 signaling pathway in HaCaT A5 benign tumor keratinocytes and fibroblasts*
- Benedikt Müller: *EPO-signalling in the tumor microenvironment of Lung cancer*

metaP-server:

a web-based metabolomics data analysis tool

Gabi Kastenüller¹, Werner Römisch-Margl¹, Brigitte Wägele^{1,2},
Elisabeth Altmaier¹, Karsten Suhre^{1,3}

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Due to recent technical advances, metabolomics can now be used widely as an analytical high-throughput technology in drug testing and epidemiological metabolome and genome wide association studies. Analogous to chip-based gene expression analyses, the enormous amount of data produced by modern kit-based metabolomics experiments poses new challenges regarding their biological interpretation. We developed the metaP-server to facilitate data interpretation. metaP-server provides automated and standardized data analysis for quantitative metabolomics data, covering the following steps from data acquisition to biological interpretation: (i) data quality checks for identifying outliers, (ii) estimation of reproducibility and batch effects (iii) hypothesis tests for multiple categorical phenotypes, (iii) correlation tests for metric phenotypes, (iv) optional including all possible pairs of metabolite concentration ratios as quantitative traits, (v) principal component analysis (PCA), and (vi) mapping of differentially concentrated metabolites onto colored KEGG pathway maps. All graphical output is fully clickable and cross-linked to sample and metabolite identifier. Interactive coloring of PCA and bar plots by phenotype facilitates on-line data exploration. For users of the Biocrates AbsoluteIDQ™ kit, cross references to the HMDB, LipidMaps, KEGG, PubMed, and CAS databases are provided. The server is freely accessible at <http://metabolomics.helmholtz-muenchen.de/metap2/>

A High Throughput Protocol for Metabolomic Analysis of Tissue Samples

Werner Römisch-Margl¹, Cornelia Prehn², Ralf Bogumil³,
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While biological processes in higher organisms mainly take place in specialized cells and whole tissues, the established matrices for metabolomic studies, like blood, urine, or saliva, are typically of extracellular type. Metabolite concentrations from body fluids therefore reflect the result of various simultaneously occurring processes over different places and cell types in a given organism.

Hence, accurate and reproducible quantification methods for metabolites in tissue samples are of high importance to identify metabolic changes that occur in different tissue types. Especially, animal models for specific diseases could be characterized more intrinsically, yet the preparation of metabolite extracts from tissue samples is often a critical and very labour intensive step.

We developed a high-throughput method for parallel extraction of metabolites from multiple tissue samples. The method utilizes a homogenizer with ceramic beads in individual disposable tubes in combination with a simple extraction protocol and the AbsoluteIDQ™ Kit.

The AbsoluteIDQ™ kit was originally validated for human plasma and can simultaneously quantify 163 endogenous metabolites by flow injection analysis mass spectrometry (FIA-MS). The metabolite spectrum of the kit covers molecules with significant different lipophilic and hydrophilic properties, including acylcarnitines, amino acids, hexose, glycerophospholipids, and sphingolipids. Thus, different extraction solvents were tested, and reproducibility as well as suppression effects were evaluated for several different animal tissue types including liver, kidney, muscle, brain, and fat tissue.

Systems Biology of Metabotypes - The BMBF-funded MedSys Project SysMBo

SysMBo Consortium^{1,2,3,4,5,6,7}

¹ Institute for Bioinformatics and Systems Biology, ² Institute for Human Genetics, ³ Institute for Epidemiology, ⁴ Institute for Experimental Genetics, Helmholtz Center Munich, German Center for Environmental Health

⁵ Institute for Clinical Chemistry and Laboratory Medicine, Regensburg University

⁶ Else-Kröner-Fresenius Center for Nutritional Medicine, Technical University Munich

⁷ Friedrich-Baur-Institute, Ludwig-Maximilians-University Munich

Systems Biology, in contrast to the traditional mode of examining individual pathophenotypes and their classification into phenotypic disorders, takes a fresh look at the etiology and course of complex diseases. Metabolomics provides access to a comprehensive set of intermediate endophenotypes that link genetic and life style parameters to clinical outcomes.

SysMBo aims to integrate expertise from clinical research, genetic epidemiology, animal disease models, and computational methods around a central metabolomics platform (metaP) into a combined systems-based approach. The central prerequisite for this work is the active communication and interaction between experimentally and theoretically working groups. It shall ultimately lead to the generation and validation of large-scale systems biological models.

The expected outcome of SysMBo is to uncover genotype–environment–phenotype relations with an emphasis on lipid metabolism and to make relevant contributions to the understanding of disease mechanisms in the field of diabetes, cardiovascular and other metabolic disorders.

Regulation of EpoR signaling by microRNAs in lung cancer

Ruth Merkle^{1,2}, Julie Bachmann¹, Andreas Kowarsch³, Mirco Castoldi⁴, Jie Bao⁶, Agustin Rodriguez¹, Hauke Busch⁶, Martina U. Muckenthaler⁴, Fabian Theis³, Ursula Klingmüller^{1,3}

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Lung carcinoma is the third most frequent type of cancer worldwide and the most common cause of cancer-related deaths. Depending on the stage of the tumor and the patient's state of health it often has to be treated with chemotherapy. This can cause anemia, which can be counteracted by either blood transfusion or by treatment with erythropoietin (Epo), which stimulates the synthesis of erythrocytes. However, recent studies have shown that Epo can have a tumor stimulating effect. Furthermore, the Epo receptor (EpoR) and EpoR signaling was found in some tumors and cancer cells including non-small cell lung carcinoma (NSCLC) cells, and Epo and EpoR coexpression has been associated with poor survival of non-small cell lung cancer patients. For this reason, the treatment with Epo is controversially discussed.

EpoR signaling has been studied in great detail for erythroid progenitor cells (CFU-Es, colony forming unit-erythroid), but very little is known regarding the presence of the EpoR and the regulation of signaling in NSCLCs. A recent study reported expression of the EpoR in the NSCLC cell line H838 and Epo induced phosphorylation of ERK, AKT and STAT5. To address the regulation of EpoR signal transduction through microRNAs in these NSCLCs, we generated quantitative time-resolved miRNA expression profiles of Epo-stimulated H838 cells covering a time range from 30 minutes up to 24 hours. The time course data was processed and revealed that Epo stimulation resulted in the upregulation of a specific subset of microRNAs. By employing prediction tools such as TargetScan, PITA microRNA targets and microRNA networks will be analyzed. The theoretical predictions will be validated using quantitative Real-Time PCR and

quantitative immunoblotting. In conclusion, the identification of microRNAs regulated in response to Epo stimulation might provide an important basis to identify biomarkers to estimate the risk of Epo treatment for lung cancer.

A stochastic model of the PU.1/GATA-1 toggle switch

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We present a simple stochastic model of the PU.1/GATA-1 toggle switch in hematopoiesis. The mutual inhibition of this two genes is known to determine the cell fate of common myeloid progenitors (CMPs) to either megakaryocyte/erythroid progenitors (MEPs) or granulocyte/macrophage progenitors (GMPs).

Recently, various models tried to capture the dynamics of this switch. Here we look for a model where unbiased switching occurs in a wide range of parameters, assuring equal probability for both progeny cell-types. Motivated by experimentally observed low mRNA copy numbers we implement a stochastic model of the switch and analyse its dynamic features.

Differential regulation of IL-6 signaling pathway in HaCaT A5 benign tumor keratinocytes and fibroblasts

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Tumor progression is regulated by a crosstalk between different cell types within the tumor and its surrounding tissue. The stromal microenvironment contains several cell types including fibroblasts, smooth muscle, immune and inflammatory; and endothelial cells. The activated progression promoting tumor microenvironment is initially induced by a network of tumor derived growth factors (cytokines) that induce cellular responses in tumor and stromal cells. In a tumor transplantation model of HaCaT skin squamous cell carcinomas we could demonstrate the functional contribution of an IL-6 regulated growth factor network to tumor progression. In response to ligand binding the IL-6R activates the JAK/STAT signaling pathway in stromal fibroblasts and tumor cells but pathway activation results in the induction of different target genes and triggers different cellular responses in both cell types. This differential target gene response is most likely mediated by differential kinetics of expression, phosphorylation and nuclear localization of STAT proteins (STAT1 and 3) after IL-6 stimulation in both cell types.

The present study aims to establish the model based analysis of IL-6-mediated receptor activation and signal transduction in stromal fibroblasts in comparison to tumor keratinocytes. In our current studies we could detect STAT3 as well as gp130 activation after IL-6 stimulation in MSU-1.1 monocultures by quantitative Western blotting after immunoprecipitation. We monitored the activation in a time window from 0 to 120 minutes. Also the effects of IL-6 on fibroblasts *in vitro* with respect to induction of soluble factors and gene networks will be examined. Additionally, the influence of tumor cells on signaling transduction in fibroblasts will be analyzed in 2D as well as in 3D coculture systems. IL-6 mediated receptor activation and signaling transduction (JAK-STAT, MAP-kinase pathway) in stromal fibroblasts will be analyzed by time resolved quantitative immunoblotting and mass spectrometry. The data will be used for the establishment of mathematical model of IL-6 signaling in fibroblasts in comparison to HaCaT A5 tumor cells.

EPO-signalling in the tumor microenvironment of Lung cancer

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Application of different forms of recombinant human erythropoietin (rHuEpo) as erythropoiesis-stimulating agents (ESAs) for the treatment of anemia in cancer patients is well described. However, two clinical phase III trials reported worse survival outcome in patients that received ESA treatment compared to those who received placebo. Additionally it was demonstrated recently that both Epo and Epo receptor (EpoR) are expressed in activated endothelial cells in the tumour-microenvironment. Interactions between the tumour and its activated tumour stroma, including fibroblasts and endothelial cells, contribute to tumour growth and progression. The aim of the present study is to analyze Epo-signaling in the tumour microenvironment of lung cancer. As a first step we determined the expression status for EpoR in the human microvascular endothelial cell line HMEC-1 and primary human pulmonary arterial endothelial cells (HPAEC). In HMEC-1 we could not detect the EpoR protein expression by immunoprecipitation. Also the levels of EpoR mRNA were found to be 10 fold lower than in H838 (a NSCLC cell line with high expression of EpoR) by qPCR. As a next step we examined the cellular reactions, such as proliferation, migration and apoptosis, of stromal- and cancer cells in response to Epo in monoculture as well as in a transwell-filter coculture system. However we found no effect of Epo stimulation on proliferation and migration of monocultures of HMEC-1 and H838 and in cocultures of both cell types. For further investigation we will analyze cocultures of H838 with HPAEC and lung fibroblasts. Additionally, we will focus on signalling transduction of Epo stimulation, mainly the JAK-STAT and MAP kinase pathways. Finally, Epo effects on the interaction between tumour and stromal cells in the presence or absence of chemotherapeutic drugs will finally be examined with 3D organotypic cocultures (OTCs).

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