Program and Abstracts

All SystemsX.ch Day 2013

Swiss-wide Systems Biology networking
and information exchange event

May 13, 2013, Zentrum Paul Klee, Bern

SystemsX.ch
The Swiss Initiative in Systems Biology

All SystemsX.ch Day 2013

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Program and Abstract Book

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Welcome Note ¦ Lucas Pelkmans

Dear Colleagues,

I am very pleased to welcome you to the *All SystemsX.ch Day 2013* in Bern.

SystemsX.ch, the Swiss initiative for Systems Biology was launched in 2007. It strives to advance the aims of Systems Biology by supporting collaborative research among nine universities and three research institute partners in Switzerland. Systems Biology research is inter- and trans-disciplinary in nature, complementing traditional biology with computational, modeling, and advanced technological approaches. SystemsX.ch supports substantial research and educational programs and technology facilities to support scientists in Switzerland to successfully carry out systems level research.

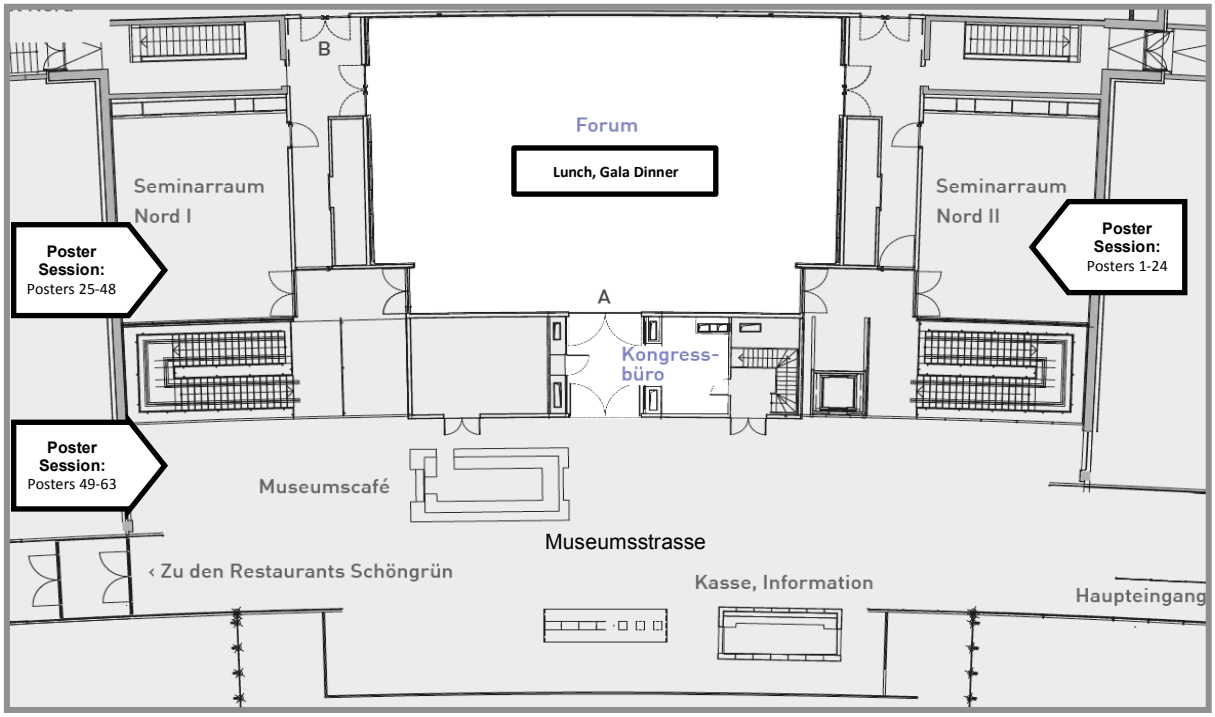
Our one-day conference is divided into the four topic areas of *Medical and Clinical Relevance, SystemsX.ch, Technology Transfer,* and *Modeling in Systems Biology.* We are very pleased to offer an exciting scientific program with presentations of selected new Research, Technology and Development (RTD) and new Transfer projects. In addition to our invited speaker program, the SystemsX.ch community will be informed on topics such as Data management, IT-support, intellectual property and entrepreneurship.
Furthermore, there will be ample time devoted to a Poster Sessions to allow interactive discussions between presenters and their audience, promoting feedback and stimulating new perspectives. The social program of the conference will include a Gala dinner to allow our participants to meet in a more relaxed environment for communication and networking.

I hope that our conference will provide not only a venue for presenting the latest advances in systems biology research, and opportunities for stimulating discussions, but also for demonstrating the collaborative synergy in systems biology research reached by the efforts of the SystemsX.ch initiative.

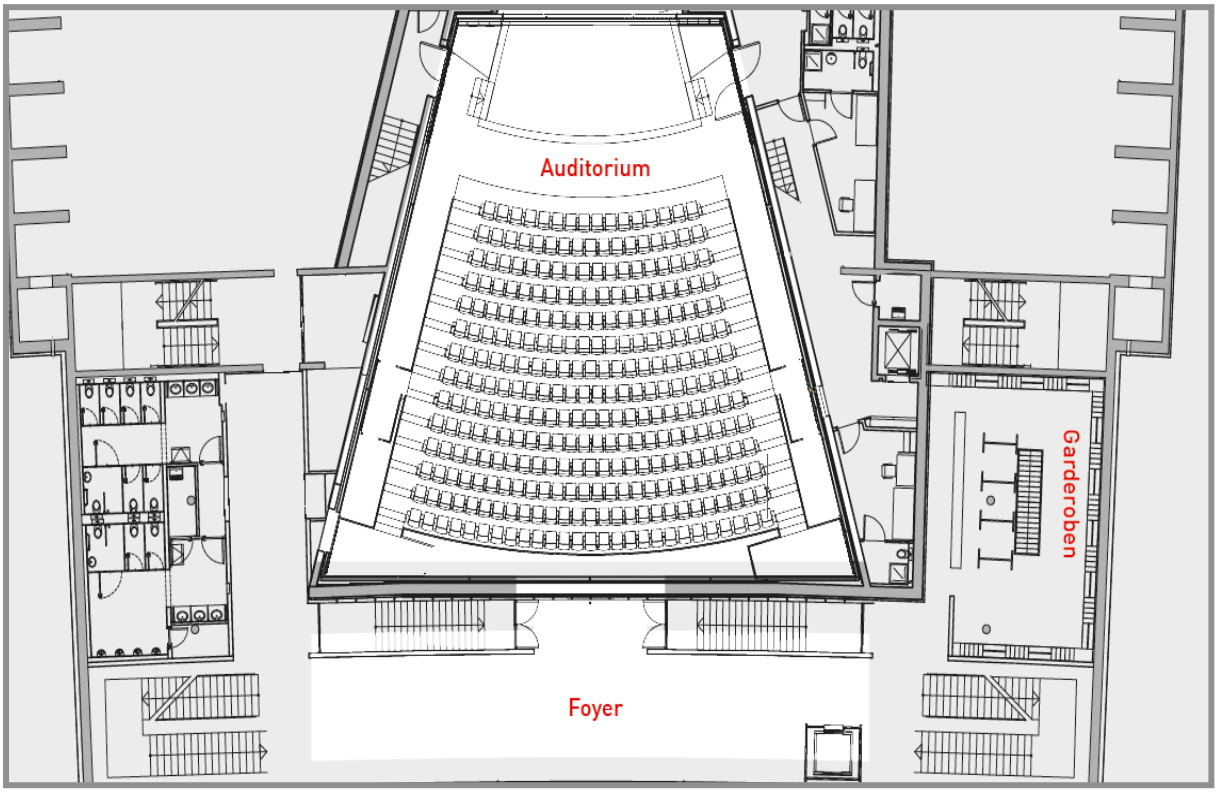
Lucas Pelkmans

Floor Plan

Ground Floor



Basement



Program

08.00 – 09.15	**Arrival and Registration**
09.15 – 09.45 Auditorium	**Opening remarks** by Lucas Pelkmans (UZH) and **Review of SystemsX.ch's 1st phase** by Ruedi Aebersold (ETHZ)
09.45 – 11.00 Auditorium	**Medical and Clinical Relevance – Chair: Ruedi Aebersold (ETHZ)** Sai Reddy, ETHZ (AntibodyX) Verdon Taylor, UniBas (NeuroStemX) Emmanouil Dermitzakis, UniGE (SysGenetiX)
11.00 – 11.20 Museumsstrasse	**Networking and Coffee**
11.20 – 12.30 Auditorium	**SystemsX.ch – Chair: Jens Selige, SystemsX.ch** Daniel Vonder Mühll, SystemsX.ch Peter Kunszt (SyBIT) Michael Dillhyon, SystemsX.ch (Entrepreneur in Residence)
12.30 – 14.15 Forum, Seminar Rooms NI and NII, Museumsstrasse	**Lunch and Poster Session**
14.15 – 15.30 Auditorium	**Technology Transfer – Chair: Daniel Vonder Mühll, SystemsX.ch** Silvio Bonacchio, ETHZ (ETH transfer) Marc Creus (UniBas/Basilea) Alex Soltermann (USZ/IBM)
15.30 – 16.00 Museumsstrasse	**Networking and Coffee**
16.00 – 17.15 Auditorium	**Modeling in Systems Biology – Chair: Lucas Pelkmans, UZH** Mihaela Zavolan, UniBas (StoNets) Michel Milinkovitch, UniGE (EpiPhysX) Ueli Grossniklaus, UZH (MecanX) **Intermezzo with Prof. Leopold Ranzenhuber**
17.30 – 19.00 Museumsstrasse	**Poster Awards / Apéro**
19.00 – 23.00 Forum	**Gala Dinner** (reservation mandatory)

Review of the first phase (2008 – 2011) of SystemsX.ch

Ruedi Aebersold

Institute of Molecular Systems Biology, Department of Biology, ETH Zurich

The overarching goal of the Swiss Initiative for Systems Biology is to establish and sustain systems biology research in Switzerland at an internationally competitive level. To achieve this ambitious goal, the first phase of SystemsX.ch was crucial and fundamental cornerstones have been placed. SystemsX.ch was formed as a “simple society” that, in turn, advances systems biology in Switzerland by (i) Supporting academic research projects, (ii) Educating the next generation systems biology scientists, (iii) Supporting private-public sector partnerships, and by (iv) Participating in international systems biology programs. SystemsX.ch consists of twelve partner institutions across Switzerland.

Now in its 6th year, the main scientific and coordinating activities of SystemsX.ch are well established and projects are productive, Until now, over 140 projects involving about 300 research groups from various disciplines have been approved for funding, after undergoing rigorous international peer reviews administered by the Swiss National Science Foundation (SNSF). The projects approved in earlier years as well as the newly approved projects contribute to achieve the diverse objectives of SystemsX.ch.

A number of measurable indicators suggest that the implemented programs are effective towards the SystemsX.ch goals: (a) About 50% of the scientists participating in the approved projects are not biologists, attesting to the high level of interdisciplinarity that has been achieved; (b) Numerous publications reporting SystemsX.ch research results in the top-ranked international journals indicate the high quality of the projects; (c) The PhD students supported by IPhD fellowships, but also those working on RTD projects, benefits from students days, summer schools and other activities. SystemsX.ch PhD students find after termination of their thesis attractive positions at renowned institutions; (d) The BIP projects have forged new interactions between the private and public sector. Transfer projects (TF) will solidify these contacts (2013) and form the foundation for an even more intensive collaborations in the future; and (e) SystemsX.ch has been co-organizing and supporting a range of international systems biology events for research and educational indicating that the program is becoming well connected and recognized also on an international level.

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Medical and Clinical Relevance

Chair: Ruedi Aebersold

Institute of Molecular Systems Biology, Department of Biology
ETH Zurich

Medical and Clinical Relevance ¦ Chair: Ruedi Aebersold

Introduction to AntibodyX

Sai Reddy

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The overall goal of AntibodyX is to optimize and use systems biology-based methods for the quantitative molecular analysis of antibody repertoires that develop in response to vaccination and pathogenic infection. We aim to characterize antibody responses at an unprecedented level of depth on molecular diversity and the evolution of humoral immunity.

An extensive amount of effort and resources is devoted to establishing and optimizing the experimental and computational methods needed for high-throughput analysis of antibody repertoires. Also, we utilize sophisticated and well-characterized mouse models of viral infection (lymphocytic choriomeningitis virus, LCMV), such an approach allows us to study the evolution of the antibody repertoire during both acute and chronic viral infections. In addition to model systems, we also track the evolution of antibody responses from human clinical samples, including patients exposed to HIV and influenza. Finally, we will be integrating genetic, protein, and cellular level data to build computational models of the contrasting and conflicting evolutionary pathways being utilized by both pathogenic viruses and host antibody responses. The generation of such precise and quantitative models will provide a tremendous amount of insight into the evolutionary arms race that takes place between pathogen and host. We believe the overall success of AntibodyX will have a substantial impact on systems immunology, specifically in areas such as therapeutic antibody discovery, vaccine development, clinical diagnosis, and prediction of immunological protection and viral escape.

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Medical and Clinical Relevance ¦ Chair: Ruedi Aebersold

NeuroStemX: Systems Analysis of Mammalian Forebrain Development

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The mammalian brain is the most complex organ in the animal kingdom. The cerebral cortex controls complex functions including cognition, motor function and memory. The increase in the complexity of the cerebral cortex has paralleled and probably driven increased cognitive function through evolution. Many diseases in humans including microcephaly, cognitive impairment and autism can be linked to disrupted formation and patterning of the cerebral cortex. Although the cerebral cortex of adult mammals contains millions of cells with hundreds of functionally distinct neurons types, it is generated from a thin sheet of neuroepithelial cells (neural stem cells). Temporal regulation of neurogenesis in the embryonic cerebral cortex is controlled by hypostable transcription factors. We have found that the RNAseIII Drosha and DGCR8/Pasha, key components of the microRNA (miRNA) microprocessor (MP), play important functions in cortical neurogenesis. Loss of MP function in forebrain results in precocious differentiation of neural stem cells through a miRNA-independent mechanism. Drosha negatively regulates expression of the transcription factors Neurogenin2 (Ngn2) and NeuroD1 by directly cleaving evolutionarily conserved hairpins in their mRNAs. Our findings implicate direct and miRNA-independent destabilization of proneural mRNAs by the MP, which facilitates neural stem cell maintenance by blocking accumulation of differentiation and determination factors.
The complexity of the cerebral cortex has been a major challenge for developmental neurobiologists. However, the wealth of data about cortical development, and the coordinated formation of neuronal layers make it a challenging but ideal system for an interdisciplinary systems biology approach to understand its formation. A detailed understanding of the dynamic interplay between transcriptional networks and their upstream regulators will help in the regeneration of defined cortical neurons and structures. The maintenance of stem cell potential and control of fate commitment during development cerebral cortex are regulated through the integration of dynamic signaling pathways organized in space and time, with transcription factor networks. Modulation of these key networks controls the regimental differentiation and fate specification of neural stem cells to form the six-layered structure of the cerebral cortex. We hypothesize that cortical neural stem cells are heterogeneous in their gene expression over time. We will address this stem cell heterogeneity through development of the mammalian cerebral cortex at the single cell level using a systems biology approach and predictively model the process of cortical development. This project could lead not only to a better understanding of brain formation but also provide a means to generate specific neuron types for drug screening and cellular therapy.

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Medical and Clinical Relevance ¦ Chair: Ruedi Aebersold

Inference of Local Regulatory Networks Employing Genetic and Cellular Variation in Human Cells

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The understanding of local regulatory interactions in the large number of genes and genomic regions is a fundamental issue, given that many of the disease and complex trait variants have been hypothesized and partly shown to be regulatory. The elucidation of the function of regulatory genetic variants will be of utmost importance for the understanding of human biology and phenotypic variability. In this project we aim to disentangle the relationships of genetic, epigenetic and transcriptional mechanisms in order to achieve two goals simultaneously: 1) derive the direct molecular functional effects of human genetic variants; 2) disentangle the mechanistic relationships and interactions of molecular effects in regulatory landscapes. We will use deep interrogation of genetic information (>7.5M SNPs) from 200 individuals for which we have obtained two cell types, fibroblasts and lymphoblastoid cell lines. For each of the cell types in all 200 individuals we will also screen 4 chromatin modifications that are known to be relevant to regulatory interactions, and gene activation or repression. We will also perform small-scale interrogation of global run-on sequencing (GRO-seq) and genome-wide methyl-sequencing to assess the effect of variation on more refined phenotypes.

The specific aims of our proposal are:

1. To derive a detailed and comprehensive map of key chromatin modifications and PolII jointly with genetic variation, gene expression and protein abundance of key TFs.
2. To integrate all these data into a meaningful and analysis friendly framework for the needs of the proposed project but also for the scientific community when these data are made available.
3. To detect and quantify the impact of genetic variation on regulatory markers such as chromatin modifications, gene expression, methylation and others and provide the raw material for regulatory network inference
4. To use genetic variation as perturbations of biological systems to infer the mechanistic and synergistic relationships of regulatory components in regional (local) networks and validate further with cross validation, external data comparison and targeted experimental efforts.

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Ingredients Needed to Write a Successful SystemsX.ch Proposal

Daniel Vonder Mühll

Managing Director SystemsX.ch

End of June 2013 is the deadline to submit proposals for a Research, Technology and Development (RTD-) Project within SystemsX.ch. This is the final call for scientists who want to join the RTD community of SystemsX.ch. Since the Swiss Initiative in Systems Biology will be terminated largely after the second phase, there will be no further call for RTD proposals. In contrast, there will be two other calls for each of the project types Transfer Project (TF), Transition Postdoc Fellowship (TPdF) and Interdisciplinary PhD-Projects (IPhD). In addition, there will be three international calls within the 7th FP ERA-Net ERASysAPP consortium.
There is one common character for all projects, which is at the same time key to implement the systems approach: interdisciplinarity. Students in an IPhD-Project have two mentors from different disciplines, postdocs in TPdFs are moving from one discipline into another, and Transfer Projects bridge the gap between academia and the private sector by applying an interdisciplinary approach. These three project types include each a couple of people and are rather small.
A consortium of a RTD project consists usually of about five research groups, hence, about 15 to 25 people. And here is one possible way to write a successful proposal:

1. Choose a life science research topic that offers the possibility to apply the systems approach. As an example for a possible project outline, you could start with existing quantitative data on which you elaborate theories etc describing processes that happen in the biological entity (cell, organ, etc). Then, the next part of the proposal could then be focused on building an in-silico model of the theory, and validation of it. Eventually, if all goes well, the model is able to predict the process (or parts of it) correctly even when boundary conditions change (be aware of the fact that this description is one of a geophysicist…).
2. Very important: the PI sets up the consortium with co-PIs contributing complementary know-how, skills and expertise. The PI can be a non-biologist!
3. Even more important, the vibes among the consortium members should turn out to be constructive. Life is too short to try doing research with people you don't like!
4. Shape your proposal by adding items such as:
   - quantitative modeling of biological processes,
   - integration of large, complementary datasets describing dynamic biological systems,
   - develop new theoretical tools,
   - intensify collaboration with the private sector,
   - systems biology approaches to clinical questions,
   - translational Systems Biology,
   - combining experimental and theoretical approaches,
   - considerable quantitative and modeling parts are a MUST.
5. Rework the proposal until the text is coherent and integrative.

Don't forget to discuss about the large cornerstones of the project first and to write the proposal reviewer-friendly. The reviewers will spend only little time reading your text, so explain them right-away what you intend to do. The better they comprehend it the higher the probability that they will fight for your proposal in the panel. Good luck!

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SystemsX.ch ¦ Chair: Jens Selige, SystemsX.ch

SyBIT - the Bioinformatics Project from SystemsX.ch

Peter Kunszt

SyBIT

SystemsX.ch has established SyBIT as the central Bioinformatics and IT support service for all its funded research projects. SyBIT supports researchers in many ways. SyBIT provides access to the necessary local and central infrastructure to the SystemsX.ch projects to store and analyze their data. SyBIT also makes the necessary bioinformatics software and tools available for data analysis, data management and data mining. Software engineering and refactoring of existing science code is also done, making it reusable and maintainable for the community in the long term. SyBIT supports all projects with collaboration services: websites, mailinglists, wikis, code repositories and other tools like macros and software packages.The talk will show highlights of SyBIT from the first period of SystemsX.ch and will elaborate on the plans for the second phase.

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SystemsX.ch ¦ Chair: Jens Selige, SystemsX.ch

Learning to be a Founder

Michael Dillhyon

Entrepreneur in Residence, SystemsX.ch

Contrary to popular opinion, successful Founders are more often made not born.
Over the years, as I have shared my entrepreneurial experiences and listened to others, much of the discussion centers on the typical media-driven subjects of funding or product development, versus the more critical task of learning to be a Founder.
Effective Founders are good students: **willing to listen and practice.** An essential in adopting a Founder mentality is the art of listening to customers, your employees and the market. Founders then utilize this information in three key areas prior to embarking on any technology roadmaps, marketing plans or revenue pro forma. This abstract will be an overview of best practices in these three areas of expertise: idea generation, culture formation and operations.

Idea Generation
Selection. Along with creativity, a trait of idea generation is frustration with the status quo. Founders (like scientists) learn to constantly evaluate the efficacy of the current solution to a perceived problem. Why does it work now and what alternatives might be a better "fit"? Learning to embrace change (by allowing it into one's life) enhances the creative mindset to present a broader range of possibilities.
Evaluate. Serial entrepreneurs (and seasoned investors) learn how to quickly test an idea's fit against the market opportunity. Many market moving ideas are stuck in traditional market paradigms. Founders must learn how to leverage new behaviors and platforms with ideas (new & old) to create marketable products. Quantify the problem. Is it a productivity, knowledge sharing, insights or truly disruptive idea?
Feedback. Building novel products and services is only commercially interesting if there is a viable market. Anecdotal evidence of buyer need is the single biggest failure point for new Founders. Experienced Founders constantly educate (and test) themselves on market perception. The end goal for any Founder is to be truly empathetic to multiple consumer viewpoints.

Culture Formation
People. Getting the balance of different personalities within the business correct can ensure years of efficient production. Getting it wrong can kill the business overnight. Founders learn to categorize behaviors without falling into the trap of stereotyping.
Values. The optimum time to define the values (and ultimately the culture) of the company is before the first employee arrives. Experienced Founders know that seeding values into the environment (and pruning as necessary) is more likely to produce positive results than relying on pure evolution. Establishing a process for when things go wrong (and they will) is paramount. Negotiating after a conflict will derail much needed energy and attention.

Operations: First-time non-Finance Founders usually learn the basics of being a COO by trial (and error). Although the role is typically surrendered in the growth phase, understanding how cash, credit, equity and assets flow in (and out) of the business is a vital Founder skill. The same can be said for HR and sales. Building the best possible product means getting in front of customers at every possible opportunity.

In conclusion, most successful Founders (like startups) are combinations of keen insight and confirmations gained through iterative learning.

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Technology Transfer

Chair: Daniel Vonder Mühll

SystemsX.ch

Technology Transfer Offices: Services and Support Provided for Researchers and Founders

Silvio Bonacchio

ETH Zürich

Besides their high intellectual importance for the scientific world, the results from academic fundamental research may also have a socio-economic value. They may therefore raise the interest from third parties such as the industry or investors. In order to help the researchers to assess, evaluate and explore the potential for further exploitation of such results and deriving inventions, many universities and research institutions have set up their own technology transfer offices (TTO).

In the presentation the various services such offices offer are explained with the example of ETH Zürich. A special focus is given to the securing of intellectual property rights (e.g. patents) and the various support programs for the foundation of start-up companies.

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Technology Transfer ¦ Chair: Daniel Vonder Mühll

EvolutionX: Analysing Evolution of Adaptation to a Novel Siderophore Antibiotic in Gram-negative Bacteria by Next Generation Sequencing

Dr. Marc Creus

Department of Chemistry, University of Basel

EvolutionX is a 2-year Transfer Project that will be carried out in collaboration between the Laboratory of Molecular Evolution (Creus group), Department of Chemistry of the University of Basel and Basilea Pharmaceutica Ltd.

The aim of EvolutionX is to investigate the evolution of adaption to antibiotics in vitro using Next Generation Sequencing technology. The drug under investigation will be the novel siderophore monosulfactam antibiotic BAL30072, currently under early-stage clinical development against multi-resistant gram-negative bacteria. Thus, EvolutionX pioneers the use of systems-biology in guiding early-stage drug-development, within an industrially-relevant pharmaceutical setting.

This talk will present the background, rationale and aims of the project, while providing some insights into the process of setting-up a new industrial collaboration in the form of a Transfer Project.

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Technology Transfer ¦ Chair: Daniel Vonder Mühll

Microfluidic Probe-based Assessment of Oncogenic Predictors at Lung Carcinoma Invasion Fronts by Topographic DNA Extraction and Micro-Immunohistochemistry

Aditya Kashyap[a], Govind Kaigala [b], Peter Schraml[a], Emmanuel Delamarche [b], **Alex Soltermann**[a]

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Vision: Strategies to perform spatial genetic profiling and visualizing multiple markers on tissue sections at high resolution will likely advance our understanding of tumor microenvironments. Our objective is to translate the Microfluidic Probe (MFP) technology invented at IBM-Zurich into the clinical molecular pathology framework of the Institute of Surgical Pathology (USZ) Zurich to enable range of investigations previously infeasible.

Background: The MFP is a non-contact, scanning technology, which spatially confines nanoliter volumes of chemicals hydro-dynamically on biological surfaces at the µm-length scale. With the MFP, we will investigate the tumor microenvironment of non-small cell lung carcinoma for molecular characterization of predictive oncogenic driver mutations and their corresponding immunoreactivity at the tumor invasion front. Among the currently targetable genomic alterations, we will focus on v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation. BRAF renders cancer cells susceptible to transforming growth factor beta (TGF-β) induced epithelial-mesenchymal transition (EMT). EMT is a TGF-β driven morphologic trans-differentiation programme, occurring at tumor invasion fronts. Thereby, tumor cells acquire an invasive fibroblastic phenotype. Understanding the signaling network at invasion fronts may provide insight into tumor progression and response to EMT-directed drugs.

Methods:
A. DNA extraction: Localized DNA extracts from tissue footprints of 100 x 100 µm over entire sections of formalin-fixed and paraffin-embedded (FFPE) tumors will be used for PCR amplification following direct sequencing of exon fragments covering major mutations such as BRAF V600E. Results will be compared with corresponding DNA extracts from punch core macrodissection and from laser capture microdissection on frozen tissue pieces from the same tumors. Extracts will also be tested for multiplexed PCR on next generation sequencers.
B. Micro-immunohistochemistry: To study the expression of BRAF proteins at tumor invasion fronts in the context of EMT, we will perform MFP-based immunohistochemistry (IHC) that we term as µ-IHC to create microscopic immunoreactivity lanes for EMT markers including BRAF.

Results: We are currently adapting and optimizing assays for the MFP technology. As a first step, we modify the DNA extraction protocols used at USZ. Two methods for DNA extraction are being evaluated: (a) column-based solid phase extraction and, (b) crude extraction by simple boiling. The DNA extracted from whole section scrapes of FFPE tissue sections, was successfully amplified for the BRAF gene. In addition, we are developing qPCR-based assays and melt-curve analyses for quantification. Towards the objective of scaling down the tissue area which can be sampled by the MFP for detectable amount of DNA, we are establishing the sensitivity of the assay for different starting amounts of extracted DNA.

Conclusion: The multi-modal techniques that will be developed in the context of molecular pathology on FFPE whole sections will likely enable a range of investigations to be performed in an interactive and precise manner. We speculate that the combination of DNA extraction and immunohistochemistry to be performed using a single device may be invaluable for the processing of small biopsies containing scarce amounts of tumor cells and may also be highly cost-effective.

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Modeling in Systems Biology

Chair: Lucas Pelkmans

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Modeling in Systems Biology ¦ Chair: Lucas Pelkmans

StoNets: Controlling and Exploiting Stochasticity in Gene Regulatory Networks

Mihaela Zavolan

Swiss Institute of Bioinformatics, University of Basel

Stochasticity pervades all levels of gene expression regulation, from the thermodynamic process of regulators binding to cognate sites in DNA, to the complex stochastic behaviors exhibited by small regulatory circuits, to the stochastic switching of cell fates that is observed during cell reprogramming. To ensure robust function, biological systems must thus have evolved mechanisms for controlling and even exploiting the inherent stochasticity of gene regulatory processes. In StoNets we will undertake a systematic investigation of the mechanisms that gene regulatory systems employ to control and exploit noise at different scales. Taking a ‘slice’ across different levels of organization of gene expression, from the gene regulatory elements in DNA to the stochastic switching of cell fate during reprogramming, we have selected an example system at each level and we will study the mechanisms with which it controls and exploits noise in its gene regulatory dynamics.

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Modeling in Systems Biology ¦ Chair: Lucas Pelkmans

EpiPhysX: The Physics of Epithelia

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A key question in systems biology is how tissues proliferate to achieve their final size and shape during development and how these systems conserve their homeostasis during physiology. The best-studied developmental proliferating systems are epithelia because, among other reasons, they reduce the biological questions of growth to a two-dimensional problem. However, epithelia live in a three dimensional world, and the landscape of mechanical tensions, as well as heterogeneity of cell densities generated by growth of a two dimensional sheet can cause the tissue to invade the third dimension through bulging. In return, it has been proposed that cell tension affects growth properties of epithelia. Both issues (how growth affects mechanics and how mechanics affect growth) are emerging as hot research topics, but progress is hampered by *(i)* the lack of a comprehensive and quantitative approach spanning several scales, from membrane mechanics to higher levels of integration (the cell, the epithelium, the organ), and *(ii)* the need for a powerful and flexible numerical model that integrates physical parameters at various scales and that is able to simulate the emerging properties of developing epithelial tissues.

Our EpiPhysX consortium synergistically integrates the expertise of four laboratories in Geneva and Zurich. Our goal is to comprehensively understand the intrinsic and extrinsic mechanisms shaping epithelia.
The cell-based numerical model of tissue growth and morphology evolution will combine physical processes (e.g., strain and stress) with biological processes such as cell growth, cell division, and signaling.

We will couple biological and physical parameters and will incorporate physical constraints mimicking the tissues in contact with the epithelium. The model will be adapted for parallelization on high-performance multiple-core computers in order to solve large-scale problems, comprising many thousands of cells with a complicated layout in 3D and sophisticated biological rules. Finally, we will make the numerical model and associated software available to the system biology community, as it will be amenable to the investigation of many additional problems.

During the final stages of the project, we will transfer the generated knowledge of tissue physics in proliferating model tissues to the clinics and explore human skin pathological and aging conditions.

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Modeling in Systems Biology ¦ Chair: Lucas Pelkmans

MecanX – Understanding the Physics of Plant Growth

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Almost all our food, feed, fuel and fibre are derived from plants. Thus, understanding growth processes in plants and how they interact with their environment during growth is of fundamental importance. To this aim, the quantitative modelling of growth at the cellular level is required. Once the principles of plant cell growth are better understood at the cellular level, they can be used as a basis for modelling the growth of tissues and organs. Cellular expansive growth is a foundation of morphogenesis and involves changes in cell size and shape. These changes require the combined action of two mechanical processes: the deformation of the existing cell wall and the secretion and deposition of new cell wall material. The dynamics of the growth process itself and the final cell size and shape are controlled by the changing mechanical behaviour of the cell wall.

The RTD project MecanX focuses on developing an accurate physics-based growth model of plant cell morphogenesis supported by mechanophysical data obtained from two recently developed force microscopy systems. As part of the previous RTD project PlantGrowth, we developed the MEMS-based Cellular Force Microscope (CFM), which we will now use to investigate the mechanophysical properties of growing pollen tubes. Pollen tubes represent a single cell system that grows rapidly but only at the tip, allowing the investigation of the effect of biochemical processes on cell wall mechanophysical properties. Furthermore, we will use multifrequency Atomic Force Microscopy (AFM) to determine mechanophysical properties of the cell wall. The cellular sensing of mechanical stress and the physiological responses to external stimuli are also essential to develop accurate quantitative models. The result of MecanX will be accurate mathematical models of plant cell morphogenesis. These models will allow researchers to derived the principles of plant growth, and will help optimize plants for future applications needed to adapt to climate change and needs for new products such as biofuels.

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Poster Abstracts

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Poster 1

Functional Identification of APIP as Human mtnB, a Key Enzyme in the Methionine Salvage Pathway

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The methionine salvage pathway is widely distributed among some eubacteria, yeast, plants and animals and recycles the sulfur-containing metabolite 5-methylthioadenosine (MTA) to methionine. In eukaryotic cells, the methionine salvage pathway takes place in the cytosol and usually involves six enzymatic activities*:* MTA phosphorylase (MTAP, EC 2.4.2.28), 5'-methylthioribose-1-phosphate isomerase (mtnA, EC 5.3.1.23), 5'-methylthioribulose-1-phosphate dehydratase (mtnB, EC: 4.2.1.109), 2,3-dioxomethiopentane-1-phosphate enolase／phosphatase (mtnC, EC 3.1.3.77), aci-reductone dioxygenase (mtnD, EC 1.13.11.54) and 4-methylthio-2-oxo-butanoate MTOB transaminase (EC 2.6.1.-). The aim of this study was to complete the available information on the methionine salvage pathway in human by identifying the enzyme responsible for the dehydratase step. Using a bioinformatics approach, we propose that a protein called APIP could perform this role. The involvement of this protein in the methionine salvage pathway was investigated directly in HeLa cells by transient and stable short hairpin RNA interference. We show that APIP depletion specifically impaired the capacity of cells to grow in media where methionine is replaced by MTA. Using a *Shigella* mutant auxotroph for methionine, we confirm that the knockdown of APIP specifically affects the recycling of methionine. We also show that mutation of three potential phosphorylation sites does not affect APIP activity whereas mutation of the potential zinc binding site completely abrogates it. Finally, we show that the N-terminal region of APIP that is missing in the short isoform is required for activity. Together, these results confirm the involvement of APIP in the methionine salvage pathway, which plays a key role in many biological functions like cancer, apoptosis, microbial proliferation and inflammation.

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Poster 2

Cortical Tension and Stiffness During Symmetric Cell Division

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Asymmetric cell division (ACD) generates cellular diversity and is as such a fundamentally important process during development. Stem cells in particular utilize ACD in order to self-renew the stem cell yet generate differentiating siblings. Some stem cells undergo both physical and molecular ACD and it is unknown how biophysical parameters, such as cortical tension, stiffness or osmotic pressure generates physical asymmetry. The molecular mechanisms governing the distribution of cortical tension and its effect on the position of the cleavage furrow remain elusive. Also, whether physical asymmetry is linked with the generation of molecular asymmetry is another pending question. We use *Drosophila* neural stem cells (neuroblasts) to study the effects of cortical tension during ACD. *Drosophila* neuroblasts provide an ideal experimental system since precise genetic manipulations are possible and superb imaging properties are available.

We are pursuing the following fundamental questions relevant to ACD and stem cell biology: (1) How is cortical tension distributed during ACD? (2) What is the contribution of cortical tension to ACD? (3) How is cortical tension correlated with the distribution of the actomyosin cytoskeleton?

In order to address these questions, we will first map cortical stiffness in asymmetrically dividing *Drosophila* neuroblasts using atomic force microscopy (AFM).
We have implemented a recently published primary neuroblast culture protocol, enabling us to obtain isolated neuroblasts from third instar larval brains to be used for AFM measurements. We show that our cultured neuroblasts can undergo multiple asymmetric cell divisions with similar spindle alignment, polarity marker localization and cell cycle timing as observed in neuroblasts in intact brains. We are combining fluorescence microscopy together with AFM to measure and monitor the localization and dynamics of the actomyosin network and cortical stiffness of cultured neuroblasts. Our preliminary measurements indicate that cortical stiffness is quite low at prophase and prometaphase and subsequently increases to the highest value in metaphase, where it distributes uniformly along the cortex. At anaphase, stiffness at the cleavage furrow remains the same while it drops to lower values at all other points except for a region on the apical side where we measure high stiffness values. Interestingly, Myosin has been depleted completely from the apical region but the apical cortex still registers a high stiffness value. In contrast, Myosin continues to accumulate at the cleavage furrow without a concomitant increase in stiffness. At telophase, we register reduced stiffness values along the cortex and at the furrow site.

Our current results suggest that cortical stiffness does not necessarily correlate with Myosin levels throughout ACD. We are currently in the process to validate these measurements on isolated wild type neuroblasts using different AFM tips. We will also measure cortical stiffness on neuroblasts with reduced levels of Myosin or altered Myosin distribution.

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Poster 3

Generation of Photoswitchable Peptide Ligands by Phage Display

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The function of many proteins is regulated by the binding of ligands. The temporal control of this interaction of protein and ligand would allow to study and elucidate biological processes with important applications in system biology (1). One strategy is to insert azobenzene-based compounds into peptidic ligands to control their structure and function. (2,3). A limitation of currently available photoswitchable cyclic peptides is the relatively small change in binding affinity between the *cis* and *trans* conformation. To overcome this limitation, we are using phage display to screen large combinatorial libraries of cyclic peptides containing a photoswitchable linker. By using different synthetic strategies (4), we have developed azobenzene-based compounds that are compatible with phage display. Currently, by affinity selection, we are enriching peptides that bind tightly in one conformation (*cis*) but not in the other one (*trans*).

(1) Ud-Dean, S.M.M., Kinetics of optical control of enzyme activity with photoswitchable inhibitors. Interdiscip Sci, ***3****, 79-90, (2011).*
(2) Beharry, A.A., Woolley, G. A. Azobenzene photoswitches for biomolecules. Chem. Soc. Rev. ***40****, 4422-4437, (2011).*
(3) Renner, C., Kusebauch, U., Löweneck, M., Milbradt, A.G, Moroder, L. Azobenzene as photoresponsive conformational switch in cyclic peptide. J. Petide Res. ***65****, 4-14, (2005).*
*(4) Bellotto, S., Reuter, R., Heinis C., H.A., Wegner. Synthesis and Photochemical Properties of Oligo-ortho-azobenzenes. J. Org. Chem****. 76****, 9826-9834, (2011).*

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Poster 4

Modeling the Cellular Phosphorylation Response to Double Strand Breaks Using Quantitative Mass Spectrometry

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The genomes of all living organisms are constantly subjected to deleterious attacks. Of the many types of DNA lesions, the most dangerous is probably the double-strand break (DSB). To induce the repair of DSBs, the DNA damage signal must be conveyed to numerous pathways across the cell, and protein phosphorylation has been demonstrated to play a central role in conveying the signal to elicit the DNA damage response (DDR). Previous studies have identified numerous protein kinases involved in DNA damage-induced protein phosphorylation. Some of these kinases play critical roles in the DDR, but they have mostly been studied as isolated functional entities. Thus, the elucidation of the functional relationships between these kinases and their roles in determining the cellular response is a critical task for the understanding of the DDR at the systems level. Given the difficulties of relating complete or extensive signaling networks to phenotypes, it is no surprise that studies focused on smaller sub-networks and selected network nodes have progressed faster. In this regard, targeted proteomics by selected reaction monitoring (SRM) is a mass spectrometric (MS) approach expected to show promise in collecting large-scale data compendiums of sub-networks, since it allows the quantitative analysis of a limited number of analytes in multiple samples at a high level of reproducibility, sensitivity, and accuracy. We propose to delineate the involvement of selected kinases, which have been previously documented to be modulated by DNA damage, by using targeted proteomics to study phosphorylations mediated by those kinases. The overarching aim of the proposed project is to develop a MS-based strategy to derive an integrative systems-level model which will shed light on the relationships between these kinases and predict the cellular response to DSBs.

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Poster 5

www.metanetx.org: A Website and Repository for Accessing, Analyzing, and Manipulating Reconciled Metabolic Networks

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A major difficulty in genome-scale metabolic networks reconstructions and comparisons is to integrate data from different resources. Actually, these may use different nomenclatures and conventions for metabolites and reactions. To address this issue, we have developed MNXref, a precompiled automatic reconciliation of many of the most commonly used metabolic resources (ChEBI, Rhea, KEGG, MetaCyc, BRENDA, BiGG, The SEED, UniPathway, BioPath, HMDB, LipidMaps) (1). Based on MNXref, we designed MetaNetX.org, a website for accessing, analyzing, and manipulating genome-scale metabolic networks (GSMs) as well as biochemical pathways (2). It consistently integrates data from various public resources and makes the data accessible in a standardized format using the MNXref reconciliation. Currently, it provides access to hundreds of GSMs and pathways that can be interactively visualized, compared (two or more), analyzed (e.g. detection of dead-end metabolites and reactions, flux balance analysis, or simulation of reaction and gene knockouts), manipulated, and exported. Users can also upload their own metabolic models, choose to automatically map them into the common MNXref namespace, and subsequently make use of the website's functionality. New methods and data developed within MetaNetX, a project supported by the SystemsX.ch initiative, are also provided through this website.

(1) Bernard, T., Bridge, A., Morgat, A., Moretti, S., Xenarios, I., and Pagni, M. (2012). Reconciliation of metabolites and biochemical reactions for metabolic networks. Briefings in Bioinformatics.
(2) Ganter, M., Bernard, T., Moretti, S., Stelling, J., and Pagni, M. (2013). MetaNetX.org: a website and repository for accessing, analysing and manipulating metabolic networks. Bioinformatics 29, 815–816.

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Poster 6

Identification and Quantification of ADP-Ribosylated Proteins as Biomarkers for Cellular Oxidative Stress

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ADP-ribosylation is a reversible post-translational modification (PTM) in which the ADP-ribose (ADPr) moiety of NAD^+ is transferred onto side chains of specific amino acids of acceptor proteins. Under steady state conditions, levels of polymers of ADP-ribose (PAR) are generally low and hardly detectable, because they are quickly degraded by cellular poly-ADP-ribose glycohydrolase (PARG). The induction of PAR synthesis occurs in response to a variety of stress conditions (oxidative stress, DNA damage, ionizing radiation) and is mainly catalyzed by the ADP-ribosyltransferse diphtheria toxin-like 1 (ARTD1) or other intracellular ADP-ribosyltransferses of the ARTD family. Up to now, more than 200 covalently modified nuclear proteins such as histones, topoisomerase I and II or CTCF have been identified. According to recent data, approximately 1% of all histones are ADP-ribosylated as part of the epigenetic code. However, despite strong efforts, a comprehensive understanding of the molecular mechanisms and cellular functions of ADP-ribosylation is still missing, which is largely due to the inability to identify the ADP-ribosylome (all modified proteins in a cell).
We hypothesize that ADP-ribosylated proteins are reliable biomarkers for the detection of stress conditions and that ADP-ribosylation of specific target sites serves as a quantitative sensor for oxidative stress. Thus, we aim to develop fast and reliable assays for the quantification of specific ADP-ribose marks using liquid chromatography coupled to tandem mass spectrometry using the selected reaction-monitoring (LC-SRM/MS) approach, which recently has been successfully applied for quantitative analysis of proteins with different posttranslational modifications like phosphorylation and acetylation in complex biological samples.
Improvement in the fragmentation techniques and introduction of electron-transfer dissociation (ETD) and high-energy C-trap dissociation (HCD) accelerated the analysis of a variety of PTM. The results showed that both of these fragmentation methods lead to comparable identifications of ADP-ribosylated peptides. Moreover, the combination of these two fragmentation techniques in one MS/MS procedure appeared to be a powerful and valuable approach since it enabled the assignment of 50-75% of the spectra and efficient identification of *in vitro* modified peptides and their ADPr acceptor sites. This could be further improved by applying a product dependent HCD-PD-ETD protocol. In this case, ETD fragmentation is triggered and performed only for the peptides that contain characteristic ADP-ribose ions in the HCD spectra.
Recent data with whole cell extracts revealed that the complexity of cell proteome does not allow the identification of ADP-ribosylated proteins. In order to identify modified proteins in the whole proteome, we additionally developed an enrichment method for ADP-ribosylated proteins. Most promising results are obtained using boronic acid beads and with protein domains that bind ADPr (macrodomains or WWE domains).

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Poster 7

Automatic Spine Segmentation for 4D 2-Photon Microscopy Data

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Fluorescence microscopy (e.g. 2-photon microscopy) is a heavily used imaging technique to image living neurons. Thanks to time series imaging of living neurons many experiments are feasible. This situation requires the automatic analysis of fluorescence data because many data exists and can be produced within short time. Furthermore, manual analysis is error-prone and results vary from human to human.

In our approach we learn a statistical dendrite intensity model and a corresponding spine probability model. This is feasible by using information from the shape of dendrites that can be (manually) reconstructed in data from Serial Block-Face Scanning Electron Microscopy (SBFS-EM). The geometrical correct shape of dendrites and spines can be segmented and labeled. This information is transformed to the fluorescence image domain by using Digitally Reconstructed Fluorescence Images (DRFI) that we introduced 2011. From this transformed information the models are learned. For a single time point thanks to the learned models spines can be predicted and segmented.

We implemented a software that uses a manual clicked backbone to initialize the first time point, aligns all time points, approximates the backbone for all time points and predicts for every time point independently the spines respectively the spine candidates. Then a second application uses this time series results and computes the most probable relation over time of the spine candidates and finds the most feasible series of spines over all time points. The number of possible related spines over different time points grows rapidly using more time points, even if only in a local neighborhood is searched. A further difficulty is that spines can move independently of the overall movement of the dendrite piece.

In first analysis of time series with more than 10 time points and about 20 spines per time point we were able to find in all time points the spines for about 80% of the expected spines (compared to an expert). Spines are sometimes missed at a single time point. This is mostly due to larger movements over time or the spines are not visible in the images.

Our approach and software demonstrates the possibility to analyze automatically time series of fluorescence data showing pieces of dendrite. Furthermore, we demonstrate the information transfer from the electron microscopy domain respectively the geometrical correct shape reconstruction to the fluorescence domain. In this approach we use the opposite way of most other approaches and do not a deconvolution but transfer (convolve) the information available in the reconstruction to the fluorescence domain. This makes our approach independent of a successful deconvolution of the test data. The information transfer and the learned statistical models for image analysis enable us to overcome the difficulties to automatically segment spines in time series of fluorescence images.

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Poster 8

Evidence for Pyruvate as an Important Metabolite at the Interface between Human Host Cells and Intracellular Shigella

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Shigella flexneri causes dysentery in humans called Shigellosis, which is a major health problem all over the world, especially in developing countries. For identification of new drug targets, a better understanding of pathogen-host cell interaction is crucial. Metabolic interactions are one important aspect. Here, *in vivo* and *in vitro* growth of *Shigella flexn*eri mutants was used as readout to discover metabolic genes important during *Shigella flexneri* infection. The in vitro growth was measured by absorbance and infection experiments were analysed with flow cytometry. A standard HeLa cell line was used to screen a large variety of metabolic mutants. The most promising mutants were then validated in intestinal epithelial Caco-2 cells, and in primary HUVEC cells.
The analysis revealed that the metabolic network of *Shigella flexneri* is very robust against perturbation; only few mutants showed severe growth defects, for example phosphotransacetylase (*pta*) and acetate Kinase mutants (*ackA*). Interestingly, a mutant lacking both pyruvate kinases (*pykA, pykF*) in which glycolysis was disrupted retained 70% of the wildtype growth rate. Inclusion of pyruvate in the external cell culture medium even restored normal wildtype growth levels. These findings suggest that the pyruvate node is crucial for *Shigella flexneri* intracellular growth independent of any *Shigella* glycolytic flux.

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Poster 9

Large-scale Dynamic Models of Metabolic Networks

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The development and application of methods quantifying the metabolic fluxes such as Flux Balance Analysis (FBA) has been one of the driving forces behind the successful growth of metabolic engineering. However, applications of FBA have been limited by the fact that FBA does not take into account kinetic properties of the network and therefore it cannot be used to identify rate-limiting steps and comprehend time course evolutions of the system. Dynamic mathematical descriptions of the metabolism offer a large advantage compared to constraint-based stoichiometric models, but unfortunately their development comes with inevitable difficulties due to:

(i) structural uncertainties, such as incomplete knowledge about stoichiometry or about kinetic laws of the enzymes, and
(ii) quantitative/parametric uncertainties such as lack of knowledge concerning kinetic parameters.

Recent developments and vast resources of curated genome scale metabolic networks address to a great extent the issues around stoichiometric uncertainty. However, the knowledge about kinetic rate laws and in particular their parameters is to these days still limited. In this contribution, starting from large-scale stoichiometric models we use the ORACLE (Optimization and Risk Analysis of Complex Living Entities) framework that integrates available information in a set of stable log-linear kinetic models sharing the same steady state. These models are used to compute kinetic parameters of the enzymatic mechanisms in the metabolic network, e.g. the maximal velocities, V_{max}, and Michaelis constants, K_m. Using these kinetic parameters, we systematically develop populations of stable dynamic models having the same steady-state as the log-linear ones. The estimated parameters are comparable to the experimental information as seen in BRENDA and other databases. These non-linear estimations about the stable state can be therefore used to analyze properties of the system upon large perturbations and investigate time course evolutions in and around this steady state.

We demonstrate the capabilities of the proposed approach by building a dynamic *E. coli* core model that includes ca. 200 metabolites and more than 400 reactions. We discuss the strengths and limitations of this approach and possible avenues for development.

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Poster 10

screeningBee Open Source Data Analysis Framework for High Content Screening

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The goal of InfectX is to comprehensively identify the components of the human infectome for a set of important bacterial and viral pathogens and to develop new mathematical and computational methods with predictive power to reconstruct key signaling pathways controlling pathogen entry into human cells.
In this poster we present a fully integrated software framework for high content screening that allows automated data analysis, statistical evaluation and management of image-based RNAi screens. The novelty is the seamless integration of free and open source tools into a robust and flexible software framework that is able to cope with the demands of large screening groups or facilities. We also present several novel or improved image analysis and data normalization methods, and show their successful application. End-to-end integration starts at the microscope, includes image analysis, feature extraction, infection scoring, supervised machine learning, data normalization, library management, off-target prediction and goes all the way to an enriched hit-list for review in Spotfire or other data mining tools. All intermediate steps are fully automated and require only a first-time setup, after which they can be applied over and over to new assays and screens. Data management and a publication portal are included via the openBIS biological information database.
Our framework has been validated in the context of the InfectX consortium for pathogen entry into human cells, to analyze more than 3.500 plates in 384WP format. Our biological findings on the Kinome subset of libraries are published alongside in a separate poster.

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Poster 11

SystemsX.ch and Systems Biology: Historical Roots and Context

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SystemsX.ch in today's form is the result of a complex historical process. As a new form of science funding it reflects changes in Swiss funding policy and the economical and social fluctuations of the last 20 years. Also Systems Biology as a new research area reflects modifications in the Life Sciences over the same historical period. One can say that in both SystemsX.ch and Systems Biology the two historical changes came together and embody the current form of both. In this sense both are new and innovative, but both phenomena are the result of historical development.

I will present this historical development from two sides: first by analyzing the political, economical and cultural background of SystemsX.ch. This includes an overview of the economical changes of the 1990s, which had serious effects on the Life Sciences. Good examples are the Sandoz- Ciba Geigy merger into Novartis in 1996, and the closing of the *Basel Institute of Immunology* (*BII)* in 2000. It also includes a shift in Swiss funding policy from micro-funding to bigger projects of national interests, such as the development of SPP-programs in the nineties to the NCCRs, and finally to the nationwide initiative SystemsX.ch. This part of the story is determined by local events, economical needs and Swiss national politics.

In the second step I will describe the changes in the Life Sciences in the post-genomic era. In the last 20 years the Life Sciences have been in a state of re-orientation and reconfiguration. The complete sequencing of the human-genome in 2001 can be described as the fulcrum of this process: The main target of molecular biology was accomplished, but it became clear that associated anticipations in genetic-drugs would not be met - knowing the genome doesn't automatically give scientists a key for understanding life and diseases. With this insight, biology lost one of its central points of reference and the mode of thinking began to change. Accordingly, certain attempts were made to restructure the entire Life Sciences under new terms and approaches. One of these, and maybe the most successful, is Systems Biology.

However, the systems approach with its holistic view, big data operations, emphasis on computing and modeling, and an understanding of biological phenomena as networks and systems, is characteristic of the 21st Century. Even though the scientific activities are highly specific, the terms to describe the systems approach are well understandable. By talking about networks, communication-pathways and 3D-systems, systems biologists activate the understanding of the public and politics - it thus seems reasonable to rethink current biology in terms of our information-society. Nevertheless, this observation matches also with SystemsX.ch: the initiative is a form of networking-agency as it has a company-like structure and a catchy appearance. Thus, SystemsX.ch is a perfect example to study the structural and conceptual change in the Life Sciences *and* in Swiss funding policy.

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Poster 12

A Kinome-wide RNAi Screen to Identify Genes Controlling Membrane Lipid Homeostasis in Human Cells

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Lipidomics is an emerging field, focused on the large-scale study of the lipids arrangement in a specific biological system. There is a great heterogeneity of lipid structures in biological systems. This diversity is present at every levels of organisms' organization: between the cell types, organelles, and between the two leaflets of the lipid bilayer.
In mammalian cells, homeostasis of membrane lipids is essential in order to conjointly maintain the structure of the system, responses to cell environment, the energy storage, membrane trafficking and signalling events. A disruption in this balance leads to physiological disorders, such as metabolism, neural or cardiovascular diseases, cancers, etc.
If more and more genes are found to be involved in homeostatic mechanisms, no systematic view of membrane lipid regulation is available yet. In order to identify molecular regulatory circuits that control lipid homeostasis, a single gene perturbation screen has been started in Human cells, focusing first on the kinome. In every conditions of a kinome-wide RNAi screen, membrane lipids were extracted from HeLa cells and hundreds of them could be identified and quantified by GC-/LC-Mass Spectrometry with techniques of Multiple Reaction Monitoring (MRM). After normalization and filtering on data quality, clustering of lipid profiles, using techniques of transcriptomics, reveals groups of genes involved in same processes, leading to the elaboration of new hypotheses about general mechanisms of membrane lipids homeostasis.

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Poster 13

Metabolic Regulation of the Human T Cell Response

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Upon antigenic stimulation, naive T cells undergo a program of cell growth, proliferation and differentiation. The resulting effector cells assist in the clearance of the antigen. At the end of the immune response, most effector cells die by apoptosis while a small subset survives as long-lived memory T cells.
To further characterize these processes, we determined the proteome of primary T cells in the resting state and after different time points of activation. Within 48 hours after activation the expression of 1005 proteins changed significantly, reflecting the extensive alterations a stimulated T cell undergoes. Within the group of proteins whose expression increased most, metabolic factors were found significantly enriched. In parallel, we followed 650 metabolites by mass-spectrometry. Integration of the proteomic and metabolomic datasets allowed us to analyze how different metabolic networks respond to the increased energy demands of activated cells. The impact of the identified metabolic networks on T cell differentiation and life span is currently tested using complex shRNA pools or shRNAs targeting individual enzymes.

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Poster 14

Stochastic Effects on Phase Regulation of Circadian Genes

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All interactions between protein transcription factors (TFs) and DNA are inherently stochastic, or under the influence of what is often called “intrinsic noise.” This noise arises from a variety of fundamental physical sources, including the random search of the TFs for their cognate sequences, as well as the irregular dynamics of binding and unbinding of the protein to the nucleic acid (c.f. *doi:10.1093/bioinformatics/bts132)*. Circadian regulation genes are an interesting case for study due to the observation of staggered phases for different groups of genes. We are interested in whether the intrinsic noise of gene regulation can have an effect on the interactions of multiple regulatory sites, which may be active at different circadian phases. In our approach, the properties of the noise are parameterized by a small number of coefficients that describe the likelihood of a promoter to bind and unbind a TF. The circadian rhythm is treated as a source of oscillation in the activity of a TF. Any significant macroscopic phase shift due to microscale noise properties can help determine bounds on the characteristics of the particular intrinsic noise at the intersection of TF and DNA.

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Poster 15

Development of Bioinformatics Tools towards the Automated Structural Elucidation of Unknowns from MS/MS Spectra

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Metabolomics rely on liquid chromatography coupled to mass spectrometry to detect and identify metabolites for two principal reasons: i) the high throughput capability of LC-MS/MS and ii) the high sensitivity of LC-MS/MS. While high resolution mass spectrometry allows the determination of elemental formulas for precursors, compound identification is generally based on library searches or the use of fragmentation rules of postulated structures and fragment ions. Here we apply bioinformatics tools to analyse the quality of high resolution MS/MS libraries and to identify MS/MS fragment substructures which can be used in combination with elemental formula assignment for the interpretation and annotation of unknown MS/MS spectra.
Two different high resolution MS/MS datasets were used in this study: i) RIKEN plant metabolites database (www.massbank.jp/download/data/) for hypothesis generation and ii) the Leibniz IPB records database (msbi.ipb-halle.de/MassBank/Download.html) for confirmation.
A MS/MS unknown spectral interpretation workflow was established based on accurate mass measurement using i) common fragment substructure information gained from high resolution database analysis, ii) elemental annotation of fragments, and iii) common neutral losses (H_2O, NH_3). MassBank provides a MySQL format relational database together with tools to import mass spectrometry data and compound structures. For the RIKEN database, 347 MS/MS spectra in positive mode of unique compounds, representing 2428 fragments, were selected. Prior to the main clustering work, a quality control check of the database was performed based on the mass accuracy of the residual precursor and the feasibility of the fragments based on the assignment of elemental formulas for the fragments, constrained to the composition of the precursor. For 205 *m/z* values (8.5%) no elemental formula could be determined within 100 mmu implying these fragments result from contaminants which have a precursor *m/z* within the 0.7 Da selection window. 1890 fragments (77.8%) had at least one elemental formula with a tolerance of 5 mmu. 2223 fragments were clustered using a threshold of 3mmu and 829 clusters were obtained. The most populous cluster had 40 *m/z* values and a median *m/z* value of 153.0184; 40 clusters had at least 10 members. From these clusters the results were grouped by elemental formulas and a substructure library generated using the Fragment Inspector tool. As an example the cluster with a median *m/z* of 121.0262 ($C_7H_6O_2$) was found to be most probably an aromatic cycle with two ester moieties. Annotation of unknown spectra based on the most frequent fragment substructures, elemental formulas and common neutral losses allowed rational spectral interpretation by annotating unknown spectra using a bottom-up approach which is independent of fragmentation rules. The IPB library was used as a quality control to evaluate the performance of the workflow.

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Poster 16

Studying the Dynamics of Protein Degradation in Arabidopsis thaliana

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Genome-wide proteomic and transcriptomic analyses have shown discrepancies between mRNA and protein dynamics in many organisms including Arabidopsis thaliana (e.g. Baerenfaller et al., Mol Sys Biol, 2012). In order to unveil the underlying regulatory mechanisms, the presented SystemsX iPhD project aims to obtain and integrate quantitative genome-wide datasets of protein synthesis and degradation rates. We will then fit statistical and mathematical models onto the data to model the dynamics of protein level regulation.
Genome-wide protein turnover rates can be measured using a ‘dynamic SILAC' approach, where the replacement rate of isotopically-labeled amino acids are quantified with mass spectrometry based proteomics. For a sensitive analysis, a high quota of labeled amino acids is required. However, direct supplementation of labeled lysine to the growth medium of A. thaliana suspension cultures has led to a low incorporation efficiency of about 10%, despite of the reported successful application of this method (Schütz et al., Plant Cell, 2011). Presumably, these cultured plant cells can still endogenously produce a considerable amount of lysine, which prevents effective uptake and incorporation of externally supplied amino acids.
Therefore, we aim to lower the level of endogenously produced lysine. The first approach is to inhibit DapL (LL-diaminopimelate aminotransferase), an essential enzyme in the lysine synthesis pathway with no isozymes. For this we apply an o-sulfonamidoarylhydrazide DapL Inhibitor, which was identified in in-vitro tests (Fan et al., Org Biomol Chem, 2012). Its efficacy of inhibition and its toxicity to A. thaliana cultured cells are currently analyzed.
In our second approach we aim to inhibit DapL transcription using amiRNAs. Two dexamethasone-induced amiRNA constructs were developed and transformed into A. thaliana suspension cultures. The efficiency of transcription inhibition as well as the reduction of DapL activity are currently assayed.
These experiments will provide the basis to enable dynamic SILAC analyses of the plant proteome. This will allow the estimation of protein turnover rates, which constitutes the first step towards achieving a system-wide understanding of protein dynamics

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Poster 17

Combining RNAi Screens and Phosphoproteomics to Study *Shigella flexneri* Pathogenesis

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More than 100 years after its discovery, *Shigella* spp. still account for an estimated 10% of all diarrheal episodes worldwide. The pathogen is transmitted via the feco-oral route and infects the intestinal epithelium in humans. The entry process into epithelial cells is highly complex and requires dynamic molecular interactions between host and bacterial proteins and the transient re-orchestration of the host cytoskeletal machinery by the bacteria.
By combining data sets from high-content image-based RNAi screens and phosphoproteomics, we have systematically identified host proteins, which are important for a successful invasion process and subsequent intracellular growth of *Shigella flexneri*. These proteins represent potential targets for the development of novel antimicrobials.
Upon successful entry into epithelial cells, intracellular bacterial pathogens are recognized by receptors of the host cell. These receptors will activate several signaling cascades that aim at triggering an inflammatory response. A hallmark of this response is the secretion of cytokines. However, many bacterial pathogens use effector proteins to modulate the host's inflammatory response. During a Shigella flexneri infection, the infected cell's ability to produce cytokines is completely abolished. In a previous study (Kasper et al., Immunity 2010), we found that infected cells propagate inflammatory signals to uninfected neighboring cells. These uninfected bystander cells are now able to produce and secrete cytokines.
The exact molecular mechanism of this cell-to-cell communication remains to be identified. The high-content RNAi screens therefore include the production of cytokines by bystander cells as an additional readout. In the context of a genome-wide screen and in combination with a phosphoproteomic approach, we expect to identify new components that are involved in the signaling cascade controlling cytokine production in uninfected bystander cells.

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Poster 18

Connectivity-based Neurofeedback: Dynamic Causal Modeling for Real-time fMRI

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Neurofeedback based on real-time fMRI is an emerging technique that can be used to train voluntary control of brain activity. Such brain training has been shown to lead to behavioral effects that are specific to the functional role of the targeted brain area. Recent studies even demonstrated therapeutic effects in specific patient populations. However, real-time fMRI-based neurofeedback so far was limited to training localized brain activity within a region of interest. Here, we overcome this limitation by presenting near real-time dynamic causal modeling in order to provide neurofeedback information based on connectivity between brain areas rather than activity within a brain area. Using a visual-spatial attention paradigm, we show that such a connectivity feedback signal can be used to train voluntary control over functional brain networks. Because most mental functions and most neurological disorders are associated with network activity rather than with activity within a single brain region, this novel method is an important methodological innovation in order to more specifically target such brain networks.

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Poster 19

Deciphering the Logic of Phase-specific Circadian Transcription in Mouse Liver with DNase I-hypersensitive and H3K27Ac Site Mapping

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Understanding the kinetics of circadian transcriptional regulation has recently advanced thanks to genome-wide dynamic mapping of RNA Polymerase II, chromatin marks such as histone 3 modifications, and several core clock regulatory factors. To obtain deeper mechanistic insights into the determinant of phase-specific transcription, we here extend our previous analyses by integrating new genome-wide datasets enabling systematic identification of active regulatory regions in the mouse liver. DNase I-hypersensitive site (DHS) mapping within nuclear chromatin is a powerful method to identify active regulatory elements in the genome. Experiments were performed throughout the diurnal cycle to obtain a temporal map of active regulatory elements. In addition, we identified H3K27ac marked regions corresponding to active regulatory regions in the same conditions using chromatin immuno-precipitation. Analysis of the regions displaying diurnal patterns revealed promoter, transcription start site (TSS)-proximal and TSS-distal elements potentially involved in the regulation of rhythmic gene transcription. Peak phases of DNase I accessibility and H3K27ac correlated well with RNA Polymerase-II loadings of nearby active genes, which prompted us to use these signals to infer regulatory relationships. Using a combination of digital DNase I footprinting with data mining approaches identified known and new potential actors in circadian gene regulation in the liver, which we are currently validating.

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Poster 20

A Microfluidics-based Pipeline for the Quantitative Analysis of Yeast Aging

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Aging is an universal process in eukaryotes, yet its mechanisms and their actual significance are still poorly understood. The budding yeast *Saccharomyces cerevisiae* serves as an important model system for cellular aging research. Budding yeast cell divides asymmetrically, producing a smaller daughter cell that emerges from the surface of its mother. While most daughters of a given mother are born with a full lifespan potential, the mother ages over time and can give birth to only a limited numbers of daughters. Current research in the field focuses on the characterization of the mechanisms leading to the formation of age in the mother, molecular nature of aging factors, and how they affect cellular viability as well as the mechanisms ensuring the rejuvenation of the newborn daughter cell.

Conventional yeast lifespan assay relies on the laborious manual micro-dissection method, which virtually excludes the possibility to use fluorescent markers to follow intracellular events. Recent efforts resulted in the development of microfluidic devices that trap yeast mother cells and perform on-chip dissection, which allows tracking the cell over its whole lifespan at high resolution. Microfluidic microscopic observation enables to study aging on both single cell level and high-throughput scale, knowing the full life history of each cell. Using fluorescently tagged protein markers, we can follow the changes in the structure of the organelles, activity of signaling pathways and behavior of single proteins, which will shed light on the molecular mechanisms of aging.

One of the particularly interesting phenomena are proposed changes in the protein homeostasis during aging, i.e. decline of the protein quality control capacity and accumulation of misfolded or aggregation prone proteins. We are looking into the contribution of protein aggregation to aging and cell diversity, by following aggregation of distinct proteins *in vivo* and investigating how they alter the phenotype or fitness of single cells. Microfluidic platform allows also a simple pedigree analysis, which will be helpful in studying the phenomenon of the daughter cell rejuvenation and testing the hypothesis that protein aggregates can act as age determinants.

We aim to introduce further developments, which will help to establish microfluidic dissection platform as a method of choice in cellular aging research. Introducing on-chip valves to control and switch the media will allow to study fitness of cells in the changing environment, while multiplexing of the chip and automated image analysis will greatly facilitate the use of genetic screening for new factors and regulators of yeast longevity.

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Poster 21

Inference Algorithm for Mutation Mapping with High-Throughput Sequencing

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The mapping of mutations and their molecular cloning is a key step in genetic studies and important for many aspects of breeding. Using high-throughput DNA sequencing, the time required for mapping and cloning can be drastically reduced by substituting the screening of individual markers with multiple analytical reactions by a single sequencing run. However, the inherent stochasticity of mutagenesis and sequencing technologies leads to noisiness in the sequencing data.

The aim of this study is to develop an inference procedure for sequencing-assisted genetic mapping and find optimal experimental setup, namely the number of plants taken for analysis and sequencing coverage. For this, we implement a continuous space hidden Markov chain model describing the inheritance of the mutations under and without selection. Further, the model is applied for inference of the position of mutation under selection.

As an advantage compared to previous models proposed, our model provides for possibility to incorporate not only the knowledge about the genetic linkage but also about the effect of the mutations on the protein sequence. Additionally, it is based on a physical description of the process. Here we apply the inference algorithm to two samples available. Additionally, we setup a stochastic simulator of the process. We discuss the inference and simulation results and propose further directions for improvement of the predictive power of the algorithm.

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Poster 22

EpiPhysX: Simulating the Process of Physical Cracking that Generates Crocodile Head Scales

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Various lineages of amniotes display keratinized skin appendages (feathers, hairs, and scales) that differentiate in the embryo from genetically-controlled developmental units whose spatial organization is patterned by a reaction-diffusion (RD) mechanism. Contrary to skin appendages in other amniotes (as well as body scales in crocodiles), crocodile's head scales are random polygonal domains of highly keratinized skin. We have shown that crocodilians' face and jaws scales do not follow a RD mechanism but instead emerge from a physical stochastic process: cracking of the developing skin in a stress field [*Milinkovitch et al. Science 339: 78 (2013)*]. We now further investigate cracking of the crocodile jaws and face skin during development with numerical simulation techniques. We start with a simple Computer Graphics model for cracking simulation, similar to [*H. Iben, J. O'Brien. Graphical Models (2009)*], and fine-tune the mathematical model on the basis of the statistical features of the scale pattern generated *in silico.* Developing meaningful (truly physical) models is highly challenging as it requires

- *(i)* the development of methods for the acquisition of embryo 3D geometry across the full range of developmental stages,
- *(ii)* inference of an accurate topological map of head growth rates across time and space,
- *(iii)* identification of the distributions of skin thickness and elasto-plastic parameters across the embryonic head, and
- *(iv)* inference of stress and elastic forces generated by the relative growth rates of skin and bones, taking into account the mechanical parameters identified above.

Furthermore, given the likely important roles of bulging and proliferation during the formation of cracks on the face and jaws of crocodiles, a challenging perspective will be to develop a numerical cell-based model incorporating physical parameters at all scales (from cells to tissue), biological parameters (such as proliferation and signaling), and the interactions between the two for investigating the dynamic of initiation and propagation of bulging [See the poster 'EpiPhysX: a numerical model of epithelium development in 3D' by Merzouki et al.].

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Poster 23

EpiPhysX: A Numerical Model of Epithelium Development in 3D

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The central goal of this project is the development of a powerful and flexible numerical model, able to simulate the emerging properties of developing epithelial tissues.
Such a model is important to describe and understand how a two-dimensional sheet can invade the third dimension through bulging, as observed in multiple stages of embryonic development, from gastrulation in multicellular animals *[C. Tamulonis et al., Developmental Biology, 351, 217-228 (2011)]* to the development of wrinkles on the human skin or of a specific type of scales on the face and jaws of crocodiles *[Milinkovitch et al., Science 339: 78 (2013)]*.
In our model, the epithelium is considered as a 2D sheet of packed cells that grows with time and deforms in a 3D space. The model will describe the evolution of the tissue directly at the level of the biological cells. It will combine physical processes such as strain and stress at various scales, with biological processes such as cell growth, cell division and cell signalling.
Our project starts from the cell-based numerical model proposed by Farhadifar *et al. [Current Biology 17 (24), 2095-2104 (2007)].* We will modify some of its aspects considering the recent model proposed in *[C. Tamulonis et al., Developmental Biology, 351, 217-228 (2011)]*, and will adapt it in the following directions:

- *(i)* Make the 2D tissue evolve in a 3D space;
- (ii) Submit cells to external mechanical constraints, which can be related to the growth of the underlying tissue to which the skin is attached;
- (iii) Include the interaction between the epithelial cells and the other layers of the skin (e.g. collagen), and add bulging into the model as a possible response to local increase of surface;
- *(iv)* Add new relevant biological rules that describe the specific features of the problem we are addressing (*e.g.* new internal rule specifying the conditions of cell division as a function of local constraints);
- *(v)* Parallelize the model to allow running simulations on a high performance computer and solve large scales problems, comprising many thousands of cells with a complicated layout in 3D and sophisticated biological rules.

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Poster 24

Understanding Mechanics of Plant Growth

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In order to understand how gene expression controls plant growth, we must first understand how gene products modify the mechanical properties of the cells. The first step in this process is quantification of cell material properties and osmotic pressure in different growth phases. Using the Arabidopsis hypocotyl as our model system, we perform force-indentation experiments with the Cellular Force Microscope. Since these measurements are a combination of geometry, turgor, and material properties, we use geometrically accurate finite element simulation models of Arabidopsis hypocotyls cells in order to interpret our data. Cells used for the experiments are digitalized via our in-house confocal imaging processing software (MorphoGraphX) and then the experimental results are compared to our simulation models. As our system has too few constraints, we use an alternate approach to investigate elastic properties: osmotic treatments. By performing both osmotic and indentation experiments on the same tissue, we are able to estimate the turgor pressure of cells and their stretch ratios. We then fit the force-indentation curves and the stretch ratios to our FEM simulation of the indentation and inflation experiment. This allows us to compare pressure and cell wall mechanical properties before and after growth.

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Poster 25

Robustness of *C.elegans* Development at Thermal Limits

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In ectotherms (cold blooded animals), robustness to temperature changes is particularly essential for survival. Although the kinetics of individual reactions are expected to vary with temperature within their thermal range (as per the Arrhenius equation), biological processes must be temperature-compensated to some extent. Hence, identifying temperature-compensated processes during development might reveal critical control points that need to be robustly achieved. Therefore, it is important to investigate how cellular processes behave over a wide range of temperatures, and in particular what happens at the thermal limits of the organism. Indeed, although it is possible to wire a network such that temperature compensation is achieved at any temperature, the existence of a thermal range for living organisms suggests that temperature compensation might only be achieved within that range, ensuring robustness while still allowing for some flexibility in the network.

In order to tackle these questions, we investigate the first embryonic cell division in *C. elegans*. Note that we are interested in the adapted response at the thermal limits, which is different from the stress response observed upon e.g. heat shock. Using DIC microscopy, which allows for high spatio-temporal resolution, we imaged several one-cell embryos over the whole thermal range of *C. elegans* and its sister-species *C. briggsae*. Detailed quantification of these movies allowed us to identify temperature-compensated as well as temperature-dependent processes. Future investigations and modeling will enable us to better understand the mechanisms underlying these observations.

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Poster 26

Consortium-based RNA Interference Data Sets for Pathogen Infection Suggest Best Practices for siRNA Screening

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Large-scale siRNA screening has become an important technique to find novel gene targets for a biological process of interest. However, siRNA off-target effects have raised concerns on the reliability of the technology. InfectX consortium has performed several kinome-wide assays to study the cellular infection process of eight pathogens using four different commercially available siRNA libraries. Our common experimental protocols and high-content data analysis workflow provide several reproducible readouts, which enable comprehensive assay comparison and the use of powerful statistical methods. We find that siRNA libraries from different vendors do not show strong differences in siRNA performance. However, siRNA pooling provides a cost advantage compared to using separate siRNAs while keeping the found candidate hit genes similar. In follow-up validation screening of candidate hits, at least 3-4 unique siRNAs are required to discover statistically significant hits. The data provides a publicly available resource for further statistical and biological analyses for the siRNA high-content high-throughput screening field.

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Poster 27

Wnt Signaling Coordinates Endosomal Cholesterol and Lipid Droplets

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In most mammalian cells, the bulk of cholesterol is derived from extracellular origins and enters the cell via low density lipoprotein receptor(LDLR)-mediated uptake by endocytosis. Upon delivery to the late endosome, cholesterol is released from the LDL particle, de-esterified and subsequently exported from the endosome by mechanisms that remain ill defined. It is clear however that disruption of this process has profound consequences for cellular sterol homeostasis and can results in debilitating human pathologies such as the neurodegenerative disease Niemen-Pick C (NPC).
To better understand the mechanisms of endosomal cholesterol export, we initiated several genome-wide, imaging-based screens to identify gene products involved in regulating endosomal lipid dynamics. These unbiased screens implicated the Wnt signaling pathway as a potent regulator of cholesterol homeostasis. While secondary experiments including quantification of sterols by mass spectrometry did confirm that treatment of cells with Wnt3a, or targeted disruption of the pathway significantly reduces cellular levels of cholesterol, we also find a striking increase in the size and number of lipid droplets both under normal culture conditions, and oleic acid-induction. Further, we find that treatments that block LDL-mediated cholesterol uptake significantly diminish the appearance of lipid droplets suggesting lipoprotein-derived cholesterol as a primary driver of lipid droplet formation. Real-time PCR and protein expression determinations reveal an upregulation of lipid droplet constituents and enzymes. Together, these results suggest Wnt3a is a potent inducer of cellular programs for lipid storage.

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Poster 28

Quantitative Dissection of Polymorphic Neuronal Receptor Families Using a Selected Reaction Monitoring Approach

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Organization and function of complex tissues relies on cellular recognition events. The selective connectivity of neurons into functional circuits represents one of the most remarkable examples of tissue organization. Cell- and synapse-specific recognition in the nervous system has long been hypothesized to rely on molecular cell surface codes. Neurexins (NRXs) are a polymorphic receptor family with over 3000 predicted isoforms that regulate neuronal connectivity and function. Dissecting NRX function is severely limited by the lack of tools to detect and quantify isoforms. Due to the particularly wide dynamic range in which NRX isoforms can be found in cells, and considering the significant complexity originated by the extensive splicing events, a highly sensitive and specific mass spectrometry method had to be envisaged. A targeted proteomics approach such as Selected reaction monitoring (SRM) has thereby permitted us to specifically confront the complexity of NRX dynamics with great reproducibility. This methodology has allowed for the generation of quantitative snapshots of highly diverse neuronal proteins (including isoforms and splice-variants) in different mouse brain compartments within a single targeted experiment, thus confirming for the first time the existence of the NRX splice-variants at the protein level. Our results show that the relative abundance of specific splice-variants varies significantly across the brain. Moreover, male and female mice show gender-specific patterns of NRX-isoform expression. A most recent variation of the above-mentioned workflow utilizes *in vitro*-produced full-length heavy labeled proteins as standards for absolute quantification, as to investigate NRX stoichiometries and assess their abundance in the different brain areas in absolute amounts. Ultimately, we are expanding our analyses to synapse-specific complexes of NRX isoforms isolated based on their association with synaptic interaction partners. These studies should provide novel insights into the contribution of polymorphic NRX proteins to synapse formation, maintenance and function.

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Poster 29

Translational Systems Biology: Understanding the Limits of Animal Models as Predictors of Human Biology

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Inferring how humans respond to external cues such as drugs, chemicals, viruses or hormones is an essential question in biomedicine. Very often, however, this question cannot be addressed due to the impossibility to perform experiments in humans. A reasonable alternative consists of generating responses in animal models and “translating” the results to humans. The limitations of such translation, however, are far from clear, and systematic assessments of its actual potential are badly needed.

We have designed a series of challenges in the context of the ‘sbv IMPROVER’ project (Systems Biology Verification, Industrial Methodology for Process Verification in Research; www.sbvimprover.com) to address the issue of translatability between humans and rodents. Our main aim is to understand the limits and opportunities of species to species translatability at different levels of biological organization: signalling, transcriptional, and release of secreted factors (such as cytokines, chemokines or growth factors).

To address this question, we selected Normal Bronchial Epithelial Cells from both human and rat origin. These cells were exposed to 50 different substances and for each stimulus, samples were collected at different time points to generate phosphoproteomic (after 5 and 25min stimulus exposure), gene expression (after 6 hours) and secreted protein (after 24 hours) data.

Our challenge will provide participants with both training and test data sets which are designed to assess the ability of methods to predict the responses in Normal Human Bronchial Epithelial cells, from the responses observed in Normal Rat Bronchial Epithelial primary cells.

The central questions that we will pose in this challenge are:
- Can the phosphoproteomic responses induced by stimuli addressing several distinct signalling pathways in human cells be predicted given the responses generated with the same stimuli in rat cells? How does the accuracy of the prediction depend on the nature of the applied perturbation?
- Which gene expression regulatory processes (biological pathways / functions) are translatable and therefore predictable across species, and which are too divergent?
Summarizing, we will present the community with questions and data aimed at assessing methodologies designed to infer human biology from non-human biology.

The sbv IMPROVER project, the website, and the Symposium are part of a collaborative project designed to enable scientists to learn about and contribute to the development of a new crowd sourcing method for verification of scientific data and results. The project team includes scientists from Philip Morris International's Research and Development department, IBM's Thomas J. Watson Research Center and IBM Global Business Services. The project is funded by PMI.

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Poster 30

The Circadian Clock Adjusts Post-transcriptional Regulation of the Transcriptome

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Background: Circadian clocks regulate many physiological processes in a cell-type specific manner. Composed of interlocked negative and positive feedback look, the core clock genetic network regulates the oscillations of a cascade of output genes. So far, rhythmic transcriptional regulation of gene expression is well characterized but little is known about post-transcriptional regulation of rhythmic transcripts. Our aim was to quantify the half-lives of rhythmically expressed mRNAs and to identify those that are post-transcriptionally regulated by the clock.

Method: We measured the temporal accumulation of pre-mRNA and mRNA in mouse liver every 2h for 2 days and combined those temporal profiles with a mathematical model expressing the balance between transcription and degradation of transcripts.

Results: We identify the transcripts that show evidence for rhythmic transcription, rhythmic degradation or both. In addition, we provide the amplitudes and the phases of maximal transcription or degradation. Among the transcripts that exhibit diurnal oscillations (~ 10% of the expressed transcriptome), less than a half is regulated at the post-transcriptional level.

Conclusions: Our results suggest that the latter mRNA are less stable during the night (activity period in rodents). We also observe that post-transcriptional regulation is mainly used to adjust the amplitude and the phase of transcripts that are rhythmically transcribed. To a lesser extend, post-transcriptional regulation generate oscillations when transcription is constitutive. Altogether, these results may help the identification of mechanisms regulating the dynamics of post-transcriptional regulation.

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Poster 31

Using Flow Cytometry for Positive Selection of *Shigella* Mutants with Intracellular Growth Defects

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Shigella infections causing dysentery still represent a severe health problem in developing countries. To identify *Shigella* genes that are specifically required for intracellular growth in human host cells, we constructed a transposon library in a GFP-expressing *Shigella* strain. We used this library to infect HeLa cells under conditions where each infected cell initally contains only a single bacterium. Within three hours, wildtype *Shigella* proliferated to some 40 – 60 bacteria, while mutants with impaired growth reached lower loads. Based on GFP fluorescence we could distinguish and sort HeLa cells with high and low *Shigella* loads. HeLa cells with low *Shigella* loads containing potentially impaired *Shigella* clones were lyzed to liberate the bacteria. After plating this sublibrary, clones with poor intracellular growth were further enriched in additional infection/sorting cycles. Individual clones recovered after four cycles were tested in single-clone infections to validate their poor intracellular growth phenotypes. As expected from the enrichment strategy, we did not uncover mutants affected in early invasion steps. Sequencing of 152 validated clones resulted in identification of 70 unique insertion sites in 30 different genes. Interestingly, almost all identified genes encoded metabolic enzymes, nutrient transporters or metabolic regulators that clustered in distinct pathways such as nucleoside metabolism or biotin biosynthesis thus highlighting the major importance of *Shigella* metabolism for intracellular growth. On the other hand, most mutants had only moderate effects suggesting large-scale resilience against perturbation, but also a high sensitivity of our approach. In conclusion, we developed a versatile, specific, and sensitive approach for identifying pathogen genes that support intracellular growth in human host cells.

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Poster 32

Proteome Analysis of *Shigella* Infection Reveals Host Cell Nutrient Supply

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Infectious diseases remain a major threat for human health. With fast emerging resistant strains new strategies to control infections are required. In the BattleX we strive to provide a system-level description of the battle between host and pathogen for metabolic resources and to identify target/target combinations to combat the infection.
Our model system is infection of human cells with bacterial pathogen *Shigella* flexneri. During its rapid growth inside host cells *Shigella* must adapt to new environment and get all nutrients required for its growth from the host. To get an overview of functionally relevant pathogen activities during intracellular growth we performed LC-MS/MS proteomic analysis of bacterial population isolated from the host cells by FACS. A large number of transport proteins as well as nucleotide, amino acid and cofactors synthesis proteins were upregulated during intracellular growth of *Shigella*. These results were coherent with findings of the independent transposon mutagenesis screen which identified several genes encoding upregulated proteins as important for *Shigella* intracellular growth. Subsequent analysis of culture conditions revealed that *Shigella* intracellular growth is highly dependent on host cell extracellular media composition. In fact, supplementation of extracellular media with “missing” compounds restored many defective phenotypes of *Shigella* mutants. Together, these data showed how human host cells modulated nutrient supply from the external medium to intracellularly growing *Shigella*. Moreover, the data revealed the crucial importance of medium formulation for infection experiments, and caveats for interpreting experiments with commonly used cell culture media with non-physiological metabolite concentrations.

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Poster 33

Mechanical Induced Reduction of Sclerostin Level in Single Osteocytes in an *In Vivo* Model of Bone Adaptation

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Mechanical loads are one major factor controlling bone mass in adults. Embedded deeply within the mineralized bone matrix, osteocytes are thought to be the key mediators of the load induced remodelling process. Owing to their inaccessible location, the molecular mechanisms by which mechanical forces are translated into metabolic signals remain poorly understood. Sclerostin (scl) has recently been identified as an anti-anabolic signal secreted by osteocytes that reduces the bone forming activity of osteoblasts[1]. The aim of the study presented here was to determine the role of scl in mechanical induced bone remodelling in trabecular bone *in vivo*. We therefore established a working protocol to i) measure the scl level of single osteocytes in histological sections, to ii) map these osteocytes into their three dimensional mechanical micro-environment and to iii) determine bone formation and resorption activity in their close neighbourhood.
The 6th caudal vertebrae of 2 adult female C57BL/6 mice were dynamically loaded[2] and scanned by *in vivo* micro computed tomography (µCT) at week 0, 2 and 4. The 3D dynamic morphometric parameters were assessed using advanced registration techniques[3]. Micro finite element (µFE) analysis was used to calculate the strain energy density (SED) on the local tissue level. Samples were harvested one day after the last loading cycle, decalcified, embedded in paraffin, sectioned (8µm) and stained for scl using immunohistochemistry[1]. To quantify scl levels, the number of positive stained pixels inside each osteocyte was counted. Osteocytes were mapped into the *in vivo* 3D µCT volumes and the corresponding µFE models. They were grouped according to regions of formation, quiescence and resorption. SED was quantified around each osteocyte location (sphere with 40µm radius) prior to both loading and sacrifice. The change in SED (ΔSED) was compared to scl level.
The results demonstrated that scl level was lower in osteocytes in regions of bone formation than in regions of bone resorption confirming our hypothesis. No correlation between scl level and SED was found. However, when comparing scl level with ΔSED, significantly decreased scl levels were found in osteocytes in volumes with increased ΔSED. Therefore osteocytes seem to respond to changes in their mechanical microenvironment rather than to the mechanical microenvironment itself. Moreover they seem to enhance bone formation locally by reducing scl concentration in areas with increasing strains. This is the first study to demonstrate that scl is locally regulated in the mechanically induced trabecular bone adaptation process.
Using the methodology developed here we will be able to identify and investigate further key molecules and thereby extend our molecular understanding of the local remodelling processes in bone.

[1] *Nakashima et al, Nat Med, 2011*
[2] *Webster et al, Comput Methods Biomech Biomed Engin, 2008*
[3] *Schulte et al, Bone, 2011*

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Poster 34

EpiPhysX: Buckling of a Physically-constrained Growing Epithelium

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In a growing tissue, coupling between cell proliferation and tensile/compressive forces generated by growth is essential for controlling shape and size of organs during development. Based on theoretical models, it has been proposed recently that geometrically constrained proliferating epithelia can accumulate enough compressive stress to buckle out of their plane (Hannezo E et al. 2011, Tamulonis C et al. 2011). Such buckling can produce different three-dimensional (3D) shapes of the epithelium depending on the initial geometry of the confinement. Therefore, it was hypothesize that the formation of the 3D epithelial structures, such as villi in intestines (Hannezo E et al. 2011) and germ layers during blastula gastrulation (Tamulonis C et al. 2011), can be a result of epithelium buckling. However, there is no experimental evidence supporting this theoretical proposition. To test this hypothesis experimentally we develop an assay to form a single-layer epithelium in a confined geometry. For this, we trap MDCK epithelial cells in a gel spheres or tubes made of alginates, a natural polymer extracted from algae. To form the alginate spheres, three co-axial micro-pipets generate a dripping flow (Alessandri K et al. 2013 submitted). The outer micro-pipet contains alginate, the middle one matrix gel (containing a mix of laminin and collagen), and the inner one contains MDCK cells. When the droplets reach the calcium bath, the alginate polymerizes instantaneously, forming a rigid shell around layer of the matrix gel, to which MDCK cells later adhere. Changing the relative flow rates of the solutions inside the micro-pipets controls the shape and the wall thickness of the capsules. This provides a convenient tool to study in 3D the effect of shape and stiffness of the alginate shell on the buckling of the confined epithelial layer. Following the epithelia growth in 3D with the fluorescent microscopy, we address the questions of how the epithelium accommodates the mechanical stress caused by cell proliferation and how the mechanical parameters of the tissue and the substrate generate changes in cell density or cause tissue buckling. Our study will either support or disprove the hypothesis of the epithelium buckling being one of the primary mechanisms of formation of 3D epithelia structures during development.

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Poster 35

BrainStemX: A Computational Analysis of Forebrain Development

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The mammalian brain is the most complex organ in the animal kingdom. The increase in the complexity of the cerebral cortex across species has paralleled and probably driven the increased cognitive function throughout evolution. The layered structure of the cerebral cortex develops sequentially from a thin sheet of neuroepithelial cells (neural stem cells) and involves the migration and differentiation of neural stem cells to become layer-specific neurons.

While some of the key regulatory factors have been defined, many regulatory components are still unknown and the regulatory network is too complex to be understandable by verbal reasoning alone.

As part of the BrainStemX project we are analyzing the dynamics of the cellular transcriptomes over developmental time using RNASeq. We aim at reconstructing the transcription networks and at developing a spatio-temporal computational model of the regulatory process during forebrain development.

A detailed understanding of the dynamic interplay between transcriptional networks and their upstream regulators will help our understanding of brain formation and may enable the generation of defined cortical neurons populations.

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Poster 36

The Host-pathogen Interaction Network of *Shigella Flexneri* Infection of Human Cells; Quantification and Post-translational Modification Studies

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Infectious diseases are a major cause of morbidity and mortality worldwide. Growing resistance to current antimicrobials and lean pipelines for novel therapeutics increasingly limit treatment options. However as infectious diseases are usually the consequence of a fight between two networks of hundreds to thousands of individual factors and not just a single mechanism, interfering with any single factor often has insufficient effects on infection outcome and an integrated system-level analysis is thus required for rational development of novel control strategies. This project focuses the metabolic pathways as a subsystem of the HeLa-*Shigella flexneri* interaction network, as metabolism is highly relevant for pathogen growth and host control. The main objective is to quantify as many and specific changes in abundance in the metabolic proteins upon various conditions and stages of infection.
The discovery studies aimed to maximize the number of confidently identified proteins and give relative quantification through label free MS intensity and spectral counting analysis. Both pure HeLA and *Shigella Flexneri*, as well as HeLA cells infected by *Shigella* at 3 separate time points were used for analysis. The optimised approach was applied to 6 biological replicates which verified high correlation between sample results. Quantification of the host-pathogen system during infection progress is sensitive to the normalisation method applied, because of the constant increase in pathogen abundance, hence, this was evaluated for valid results in protein abundance change over infection time.
Selected key metabolic proteins are studied and quantified more carefully using Selected Reaction Monitoring (SRM) for absolute quantification. In addition, siRNA treated samples, targeting a few crucial proteins have also been analysed. A more detailed view of the host-pathogen system is also addressed by analysis of differences in post translational modifications, in this case mainly phosphorylation and acetylation.

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Poster 37

A Computational Multi-scale Hybrid Framework for Modeling Virus Transmission

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Viruses spread between cells, tissues and organisms by cell-free and cell-cell transmission mechanisms. Both mechanisms enhance disease caused by different viruses, but it is difficult to distinguish between them. We have previously characterized the transmission mode of human adenovirus in monolayers of epithelial cells using experimental data from live-cell fluorescence microscopy. Employing these data as experimental parameters we have developed an in silico model using multi-scale hybrid dynamics, cellular automata and particle strength exchange (CA-PSE). Here we present a generalized open source simulation framework based on the model we have developed. Based on the inherent flexibility of the CA-PSE model this framework aims at enabling predictions of spatial spread of from different families, taking into account their different modes of spreading, in particular cell-to-cell and cell-free spreading. The frame work promises to be useful for better understanding of various parameters which modulate the viral spreading dynamics. By this, we hope to provide mechanistic insights into commonly used endpoint measurements in virology, such as plaque assays or fluorescent focus forming assays.

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Poster 38

Computational Framework for Integration of Lipid Structures and Metabolic Networks

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Lipids are hydrophobic molecules with many key biological functions in the living cells. Until recently, there were not many specialized databases on lipids analysis and classification. In 2007, Lipid Maps Structure Database (LMSD) became available. LMSD is a comprehensive database for lipid structures that currently contains over 30,000 different classified structures, with all the relevant physical and chemical information. However, having a comprehensive database of lipid structures is not enough to fully understand their multiple biological roles in cell biology and pathology. It is essential to study the lipids in the context of biological pathways to better understand their interactions with enzymes and other lipids. This shall further clarify their functions and the enzymes related to their metabolism.

We have developed a computational framework, NICELips, to enrich our knowledge of lipid metabolism by integrating all the known lipids to biological pathways. Using generalized reaction rules, which are distilled from molecular signatures of known enzymatic reactions, we were able to predict the anabolic and catabolic fate of all the LMSD molecules. Using a retrosynthesis approach, we then connected these pathways back to known metabolic pathways. In the current study, we present a case study of bis(monoacylglycero)phosphate (BMP) metabolism using NICELips. BMP is a unique lipid due to its stereochemical configuration, which is different from that of other animal glycerophospholipids. Despite numerous studies, its biosynthetic and catabolic pathways remain unknown. Our results suggest various novel synthesis and degradation pathways for this compound. Overall, we demonstrate how NICELips can provide a full overview of lipids in the context of metabolic pathways, which contain all the chemical interactions and transformations between lipid compounds and enzymes.

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Poster 39

NICEdrug as a Prominent Method for the Prediction of Metabolic Fate of Drugs: Study of Antiplatelet Drugs

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We present the development of NICEdrug (Network Integrated Explorer for Drug Metabolism), a computational framework for prediction of drug biodegradation and metabolism. In this report, we applied the NICEdrug framework on the metabolism of antiplatelet drugs (clopidrogel and prasugrel), in order to identify how differential metabolism of these drugs can affect their therapeutic efficacy. NICEdrug is based on concepts from the BNICE (Biochemical Network Integrated Computational Explorer) methodology. BNICE is a computational network generation method, based on a set of enzyme reaction rules and a set of starting compounds, to generate every possible biochemical reaction. In the NICEdrug project, we developed biochemical reaction rules based on all known enzymes, participating in Phase I and Phase II drug metabolism. By using a pathway search algorithm, we were able to derive novel metabolic routes based on the individual reactions. We also performed thermodynamic analysis to establish the feasibility of all derived pathways. The framework also identified all the possible metabolites, that are expected based on the Phase I and Phase II biotransformation reaction rules.
We applied the NICEdrug framework for the study of the clopidogrel and prasugrel metabolism in the liver. Both of them are thienopyridines and are widely used as antiplatelet drugs. Their active forms prevent ADP from binding to the P2Y12 receptor. Although prasugrel has shown a faster onset of activity and a low rate of non-responsiveness, clopidogrel has shown a variance in response, which can be attributed to genetic effects. Clopidogrel and prasugrel are pro-drugs and they have to be converted to thiol-containing active metabolites, in order to act therapeutically. Although clopidogrel is metabolized to its active thiolactones in the liver by P450, prasugrel must undergo first a hydrolysis by carboxylesterases during intestinal absorption and then followed by the P450 action in the liver NICEdrug was able to reproduce the metabolism of both antiplatelet drugs and moreover it also predicted the participation of alternative enzymes that were not part of the “traditional” hypothesis regarding their metabolism. The analysis of all possible and thermodynamically feasible derived pathways allowed us to confirm these results with novel experimental and clinical data for clopidogrel and prasugrel metabolism.
Our results highlights the potential of NICEdrug as a powerful method for the study of drug metabolism, that can have broad use for research in translational medicine and Systems Pharmacology.

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Poster 40

A Novel Method to Find Gaps and Orphans Reactions: BridgeIT

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Genome-scale metabolic reconstructions (GSMRs) are valuable resources in the analysis and understanding of cellular metabolism. They are based on genome sequence and annotation. These models are used in a wide variety of studies ranging from metabolic engineering to evolutionary studies, and they are essential to develop bottom-up mathematical models of metabolic networks. Actually, there are incomplete pathways and orphan metabolites in all GSMRs, even for the most well studied organisms. These knowledge gaps are due to the lack of experimental or homologous information. As current methods rely on a database of known reactions to generate possible pathways for bridging these gaps, they fall short when there is no sequence homology.
We present a novel computational framework called BridgeIT, that is able to generate hypothetical reactions and pathways that bridge gaps in reconstructed pathways. The novel reactions generated are based on the third level of enzyme commission classification system (EC), which is consistent with known biochemical reactions, protein structures, genomic sequences, and enzyme properties that follow the EC classification. Within the BridgeIT framework, we generate all biochemically plausible reactions and pathways, which can link two or more metabolites. These pathways are then ranked according to their length, thermodynamic feasibility, and network feasibility. We next use chemical similarity metrics to link the generated hypothetical reactions with known reactions, through their substrate and product similarity. The protein and gene sequences of the linked known reactions are used to identify possible sequences within the GSMR to further refine and improve the annotation of the existing GSMR. We demonstrate the ability of this method to identify gaps that can be easily filled by known reactions and also gaps that require novel reactions which existing methods fail to do

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Poster 41

Pollen Tube Mechanics Revealed by Cellular Force Microscopy

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Plants play an important role as food producers and suppliers of renewable resources. In addition, photosynthetic organisms have a major impact on the global climate. Therefore, it is important to increase our knowledge of how plants grow and how this growth is influenced by environmental factors. While there is a plethora of information at the genetic, biochemical and physiological level, information about the mechanical aspects of growth is scarce.
To measure the mechanical properties of living cells we developed the Cellular Force Microscope (CFM), which is based on microelectromechanical systems (MEMS) force sensors. As a model for polar growth we study pollen tubes, in which cell expansion is restricted to the tip. Cell expansion requires the deposition of new membrane and cell wall material mediated by transport vesicles. The plant cell wall is a rigid structure of cellulose, hemicellulose and a pectin matrix. In order to allow the pollen tube to grow in a directed manner, the mechanical properties of the cell wall have to be modified at the tip.
Cellular stiffness, revealed by CFM measurements, combined with geometrical parameters of the cell was used as an input for a finite element method (FEM)-based model, which allowed us to determine mechanical parameters of the pollen tube, such as turgor pressure and the Young's modulus of the cell wall. Comparison of Arabidopsis cell wall mutants showed that CFM combined with a FEM-based model allows us to quantify the relatively small effects of changes in cell wall biochemistry on the mechanical properties of pollen tubes. xxt1 xxt2 double mutants lack xyloglucane, a hemicellulose component, while xeg113 does not contain functional extensins, a family of cell wall proteins. Although both plant lines show a similar bursting phenotype, our approach revealed that the xxt1 xxt2 mutants have a more elastic cell wall, while turgor pressure is unchanged compared to wild type pollen tubes. In xeg113, however, cell wall elasticity is similar to wild type while the turgor pressure is significantly higher.
Our data show that the CFM/FEM approach is a powerful method to study the mechanical properties of individual cells. Due to its large measuring area and flexibility in the choice of the force range, the CFM is also suitable to create force maps of entire tissues. Furthermore, the CFM will be instrumental to study the effect of local mechanical stimuli on physiological parameters

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Poster 42

Growth Factor Induced Polarized Fibroblast Migration Requires a Podosome Front-back Mechanical Insulation Module

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Directional cell migration requires establishment of robust front back polarity. We find that platelet derived growth factor (PDGF) treated, pre-polarized fibroblasts (e.g. that are plated on a fibronectin line substrate), exhibit persistent cell migration, in one specific direction for hours. This exquisite cell polarization is allowed by the establishment of two spatially defined functional modules at the front and the back of the cell. At the front, formation of a podosome containing zone provides adhesion to the substrate and at the same time inhibits the formation of contractile structures such as focal adhesions (FAs) and stress fibers (SFs). This uncouples these contractile structures from the leading edge, allowing 1. highly persistent leading edge membrane protrusion, and 2. the cell front to be mechanically uncoupled from the back. Directly behind the podosome zone, a prominent myosin cluster pulls on SFs at the back of the cell and provides mechanical forces allowing tail retraction. Such podosome/myosin modules display constant size throughout the cell migration process, allowing co-ordination of front and back activities. In absence of PDGF on the line substrate, the podosome zone is lacking and stress fibers and FAs mechanically connect the front to the back of the cell, only allowing transient fibroblasts polarization episodes with non-persistent leading edge protrusion. Consistently, a FRET biosensor reveals two distinct modes of leading edge RhoA activation dynamics in presence or absence of PDGF. In non pre-polarized cells, on classic isotropic 2D substrates, PDGF also leads to the formation of adjacent podosome-myosin modules with identical size features than on the line substrate. However, these are embedded within large SF/FA arrays, precluding any stable cell polarization, explaining the requirement of a pre-polarized cell state for efficient PDGF-induction of stable polarization (e.g. how spatiality is sensed). We propose that podosomes act as a front-back mechanical insulator to allow efficient cell polarization. Thus, polarized fibroblast migration require non-canonical membrane protrusion modalities, not observed in currently studied non-polarized cell migration paradigms.

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Poster 43

Towards a Unified Portal for Bioimaging Software: The Open Bio Image Alliance

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The aim of image analysis in bioimaging is to use cutting-edge techniques from the fields of Image Processing and Computer Vision to achieve insights into biological problems through analysis of image datasets. The domain of action of the tools provided by these fields is very large. It begins during the image acquisition process, and extends until the final statistical analysis used when extracting the spatio-temporal information of the biological system. In order to properly analyze the experiments and draw conclusions from analysis results, the scientist should be aware of how these tools work. Simply pressing a button in a piece of software and interpreting the results is not good scientific practice. Open-source software provides the necessary transparency, giving scientists the opportunity to understand the algorithms and the computational methods behind their tools.

Among all open-source bioimage analysis tools, the one that has had the most impact so far is ImageJ. However, other open-source related platforms have recently emerged. Due to the possibility that all these image-processing packages diverge and interoperability becomes an issue, the Open Bio Image Alliance (OBIA) was constituted in 2012. Its primary mission is to provide biologists and researchers in life sciences with high-quality public-domain software resources and a corresponding knowledge base to analyze and quantitate their image data in a sound and reproducible fashion.

OBIA is building a web portal that centralizes the access to the open bioimaging software. It is designed to host various resources such as software, documentation, testing datasets, and references to scientific articles. Not only the system will host generic image-processing solutions like ImageJ, CellProfiler or Icy, but it will also include references to their plug-ins. Browsing and searching though all these items will be made easy thanks to an advanced search mechanism based on complementary search trees and nametags. The whole portal is designed as a community website: the content of the portal will be developed by the bioimaging community for the bioimaging community. Any user will be capable of contributing to the portal by creating new items, or by commenting and rating existing ones.

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Poster 44

A Versatile Toolkit for FRET Biosensor Generation to Monitor Cell Signaling in Time and Space

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Genetically-encoded, ratiometric biosensors based on fluorescence resonance energy transfer (FRET) are powerful tools to study spatio-temporal dynamics of cell signaling. However, many biosensors lack sensitivity and their generation remains a tedious task often involving multiple rounds of optimization. We present a library of unimolecular biosensors based on circularly-permutated mutants for both the donor fluorophore mTFP1 and the acceptor fluorophore Venus. This allows for accommodating structural constraints imposed by different signaling sensing modules. The universal design of the biosensor library can easily be modified to incorporate new sensing modules. The value of our approach is exemplified by the improvement of brightness and dynamic range of pre-existing RhoA and Erk biosensors and successful generation of novel Rac1 and Cdc42 biosensors. We also report on a high throughput microscopy-based screening platform for rapid identification of the most sensitive biosensor in the library.
Due to its improved spectral properties, the RhoA sensor revealed micrometer-sized RhoA activation zones at the tip of F-actin bundles in growth cone filopodia during neurite extension. In contrast, RhoA was activated globally in collapsing growth cones. Activated RhoA was also found in filopodia and protruding membranes at the leading edge of motile fibroblasts. These filopodia-localized RhoA activation patterns were not detectable using the 1st biosensor generation. Rac1 and Cdc42 biosensors also uncovered specific spatio-temporal activation patterns in motile fibroblasts. Our 2nd generation FRET reporters for Rho family GTPases allow for shorter excitation times and less phototoxicity and thus enable higher sampling rates necessary to measure Rho GTPase activity dynamics, which occur at a time scale of seconds. The benefits of our 2nd generation Erk biosensor consists in measuring Erk activation dynamics on population level using low numerical aperture air objectives and performing *in vivo* FRET imaging in zebrafish. Taken together, we provide a widely applicable construction toolkit consisting of a vector set, which allows straightforward generation of highly sensitive biosensors.

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Poster 45

Time Correlated Single Photon Counting Applied on FLIM

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Time-correlated single photon counting (TCSPC) is at the core of many fluorescence detection based techniques. In our work we the technique to perform Fluorescence-lifetime imaging microscopy (FLIM), an imaging technique for producing images based on the differences in the exponential decay rate of the fluorescence lifetime. We perform FLIM using a custom made two-photon excitation scanning microscope with a dedicated TCSPC board and a hybrid photo-detector that allow us to obtain multi-dimensional FLIM. This means that the acquired image contains the lifetime information for each pixel. Here we show results of FLIM as applied to the Drosophila brain. We imaged the GFP expression genetically induced in glutamatergic cells in the adult fly brain.
The analysis of the FLIM data is performed using the “phasor approach” [4]. This technique allows eliminating the computational difficulties arising from multi-exponential fits. In fluorescence experiments with biological samples, in fact, multiple lifetime components arise from different molecular species or different conformation of the same molecule, generating formidable computational problems when using exponential fitting procedures.
In parallel we investigate the influence of a calibrated complex dielectric environment on the fluorescent lifetime. We prepare dense colloidal liquids where short-range structural order induces an enhancement of the scattering strength while at the same time the total transmission shows strong wavelength dependence, reminiscent of a photonic crystal [3]. Initial results show that for non-adsorbed fluorophores the impact on the fluorescent lifetime is negligible while for fluorophores adsorbed to the dielectric particles surface a substantial change in lifetime is observed.

[1] W. Becker, Advanced time-correlated single-photon counting techniques, Springer, Berlin, Heidelberg, New York 2005.
*[2] D.V. O’Connor, D. Phillips, Time-correlated single photon counting, **Academic Press**, London 1984.*
*[3] F. Scheffold et al., Photonic properties of strongly correlated colloidal liquids, **Physical Review Letters**, Volume 90, Number 7 2004*
*[4] E. Gratton et al., The phasor approach to fluorescence lifetime imaging analysis, **Biophysical Journal**, Volume 94, Issue 2, L14-L16, 15 January 2008*

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Poster 46

Diffraction-unlimited Imaging of *Drosophila* Brains with STORM

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This project aims at imaging chemical synapses in the Drosophila adult brain. Since the global size of a synapse is typically ~ 500 nm [1] optical microscopy encounters its limitations in resolution, rendering it impossible to use traditional fluorescence microscopy techniques to observe specific populations (pre- or post-synaptic sites) of proteins expressed in sub-micrometer structures. This is unfortunate since these are often the features of primary interest in the system.
Here we've implemented the Stochastic Optical Reconstruction Microscopy (STORM [2]) technique with which shall be able to overcome the diffraction limit of resolution. Presently we have started imaging populations of pre-synaptic sites. STORM imaging makes it possible to perform a quantitative study on the number of synapses as a function of the age of the insect. With the availability of different optical switches [3] we've also started to image pre- and post-synaptic sites simultaneously, as well as the distribution of other proteins to validate the method.

[1] V. Nägerl, T. Bonhoeffer, "Imaging Living Synapses at the Nanoscale by STED Microscopy". The Journal of Neuroscience, 30(28):9341-9346 (2010)
[2] M. J. Rust, M. Bates, X. Zhuang, "Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)", ***Nature Methods 3****, 793-795 (2006)*
[3] M. Bates, B. Huang, G. T. Dempsey, X. Zhuang, "Multicolor Super-Resolution Imaging with Photo-Switchable Fluorescent Probes", ***Science 317****, 1749-1753 (2007)*

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Poster 47

Lipidomics of Host-pathogen Interactions – a Systems-based Approach from the Bench to the Field

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The cell biology of intracellular pathogens (viruses, bacteria, eukaryotic parasites) has provided us with molecular information of host–pathogen interactions. As a result it is becoming increasingly evident that lipids play important roles at various stages of this intricate interaction between the pathogens and their hosts. Being positioned at the cell surface, lipids contribute to the interplay between host and pathogen, acting in first line recognition and host cell signaling during pathogen docking, invasion and intracellular trafficking. Serving as a basic building block of membranes and as an energy source, lipids are critical for the growth and replication of a pathogen.

Despite the growing appreciation of the relevance of lipids in infectious diseases, many gaps remain to be filled. Advances in analytics, in particular mass spectrometry, have revolutionized lipid biology research and brought it into the '-Omics' field in recent years. Through the development of novel lipidomics approaches and combining with infection biology and molecular epidemiology, we aim to elucidate the functional roles of lipids during host-pathogen interactions. In addition, the systems-based approach is applied translationally for therapeutic targets discovery as well as for diagnoses of Mycobacterial and protozoan infections which represent major global health and socioeconomic concerns.

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Poster 48

UHPLC-MS-based Metabolomics Reveals Induction of New Metabolites in Fungal Co-culture: A Way to Obtain New Antimicrobial Compounds?

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In natural product research, the search of new sources of compounds is a key element. In this respect microorganisms have provided a large number of biologically active molecules [1]. Recently, the use of fungal co-culture for the induction of new natural products has emerged as a new field in drug discovery [2].
A key point for the success of such studies is the development of co-culture experiments that provide high reproducibility of metabolite induction pattern and that are compatible with high throughput analytical procedure for the screening of a large number of mono-culture and co-culture samples and further data mining.
To tackle this issue, a method based on 12-well plate miniaturized Petri dishes compatible with high throughput UHPLC-TOF-MS metabolomics [3] has been developed. Various culture condition parameters were optimized for fungal growth such as culture medium volume and culture duration. This strategy was used to screen for metabolite induction and study their dynamics in the co-cultures of a plant pathogenic fungus Aspergillus clavatus and a systemic human pathogenic fungi Fusarium sp.
This approach provided a satisfactory reproducibility and was used for the identification of induced biomarkers. This study demonstrates the consistent induction of new metabolites through co-culture. Moreover, upscaling of the co-cultures conditions to large Petri dishes shows that the main induced metabolites were also produced allowing their purification for further de novo identification and evaluation of their bioactivity.

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[1] Berdy J, J. Antibiot. ***2005****, 58, 1.*
[2] Glauser G et al., J. Agr. Food. Chem., ***2009****, 57, 1127.*
[3] Bertrand S et al., J. Chromatography A , ***2013,*** *in press*

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Poster 49

A Computer Vision Approach to Study Growth Cone Morpodynamics

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Elucidating the signaling mechanisms of neurite outgrowth is essential for understanding biological processes associated with neuronal development and the re-establishment of functional neuronal connections after injury. The neurite possesses a dynamic, actin-rich tip called the growth cone at the end of a tubulin-rich shaft. Importantly, growth cone cell edge morphodynamics (e.g. the movement of growth cone filopodia), as well as the underlying cytoskeleton fluctuate on single microns length and sub-minute time scales. Thus, the regulating signals ought to operate on identical scales, and proper understanding requires tools that can grasp this complexity. Rho GTPases are key regulators of cytoskeletal dynamics that allow neurite outgrowth. The classic model posits that Rac1 and Cdc42 regulate neurite outgrowth while RhoA controls growth cone collapse and neurite retraction. However, this simple model results from experiments that do not resolve the spatio-temporal scales mentioned above.
Here we introduce a novel computer-vision tool for automatic extraction of multiple morphodynamic features from high magnification, high temporal resolution movies of neuronal growth cones labeled with the F-actin marker Lifeact. This approach allows for the automatic extraction of parameters describing filopodia and lamellipodia dynamics, two essential growth cone functional and morphological units that are likely regulated by different spatio-temporal signaling networks. Our computer vision approach allows cell shape invariant representation of growth cone dynamics, allowing to pool noisy results from multiple cells in statistically relevant datasets.
Using this method to quantify growth cone dynamics of N1E-115 cells in which the three canonical Rho GTPases RhoA, Rac1 and Cdc42 have been perturbed using siRNA, we observe a much more complex picture than the classic model. Loss of function of each Rho GTPase leads to distinct morphodynamic and cytoskeletal phenotypes, quantified by specific parameters. This implies that the three canonical Rho GTPases collaborate to fine tune growth cone motility. We also analyzed the effects of loss of functions of different GEFs and GAPs (upstream regulators that activate and deactivate Rho GTPases) identified in a siRNA screen. We show that loss of function of different GEFs regulating distinct combinations of Rho GTPases leads to specific morphodynamic phenotypes, that are also different than those induced by loss of function of one single Rho GTPase. These observations suggest that combinations of GEFs and GAPs might serve to co-ordinate the spatio-temporal regulation of multiple Rho GTPases in the growth cone, allowing fine tuning of cytoskeletal dynamics. Our approach allows unbiased, quantitative analysis of growth cone dynamics at the relevant spatio-temporal scales, and opens new avenues to dissect spatio-temporal signaling networks regulating neuronal outgrowth.

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Poster 50

Evolutionary Genomic Studies of Self-compatibility in *Arabidopsis kamchatica*

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The genetic basis of the evolutionary transition from outcrossing to selfing has been a major focus in evolutionary biology. Selfing, most commonly, evolved through the breakdown of the self-incompatibility system that consists of the male specificity component, the *S*-locus cysteine-rich protein (SCR), the female specificity component, the *S*-locus receptor kinase (SRK) and genes that involve in downstream signaling pathway. SCR, a small cysteine-rich protein, expresses at the pollen coat and acts as the ligand of SRK, a transmembrane serine/threonine receptor kinase that expresses on the stigma. Interaction between SCR and SRK from the same S-haplogroup triggers downstream mechanism to inhibit pollen tube germination on the stigma. My PhD project focuses on the evolution of self-compatibility in *Arabidopsis kamchatica.* It is a selfing tetraploid species, originated through allopolyploidization of multiple individuals from two diploid species, *Arabidopsis halleri* and *Arabidopsis lyrata* that are predominantly outcrossing. In previous study, five *SRK* haplogroups have been identified in *Arabidopsis kamchatica*. Interpecific crosses with its parental species, *Arabidopsis halleri* showed that *SRK* and genes involved in downstream signaling pathway are still functional in some accessions, while no functional *SCR* has been identified. This suggests that mutation in the male component, SCR is responsible for the loss of SI in *Arabidopsis kamchatica*. However, the isolation of *SCR* through conventional PCR method has been difficult due to its short and highly polymorphic sequences. Therefore, next-generation sequencing will be exploited for the isolation of *SCR* to unveil the evolution of SC in *Arabidopsis kamchatica*.

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Poster 51

Label-free Image-based Screening with Digital Holographic Microscopy (DHM)

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Digital Holographic Microscopy (DHM) is an interferometric technique which generates quantitative phase images allowing to visualize transparent cells without the need of labeling or specialized well-plate; therefore making it a perfect non-invasive method to monitor real time cellular morphological/phenotypic modulation in response to the cell environment.
We first validated the use of DHM in the context of High-Content Screening (HCS) by comparing morphological outcome quantitatively measured by DHM with benchmark fluorescence microscopy methods for a cellular viability assay. For this purpose, we automatized a DHM microscope for image acquisition of living/dying cells using commercially available 96 and 384-well plates. Data generated through both label-free DHM and fluorescence-based imaging methods were in good agreement for cells viability identification and an excellent correlation was obtained between experimental cytotoxicity dose response curves and known IC_{50} values for a range of toxic compounds tested. Moreover, a Z'-factor of 0.9 was determined, validating the robustness of DHM assay for this type of viability phenotypic screening.
To demonstrate the screening capability of DHM, we subsequently tested a library of 1200 FDA-approved drugs on cells lines and validated hit compounds with time-lapse measurement to further investigate the temporal effect on various morphological parameters quantitatively measured with DHM. Interestingly, with these DHM parameters, we are able to classify cytotoxic/anti-proliferative compound effects according to their cellular dying modes of action.
The DHM appears as an interesting alternative to fluorescence microscopy for a series of image-based assays using living cells with major potential advantages for time-lapsed experiments. The advantages of this label-free microscopy technique, the complementarities with fluorescence-based methods and the domain of HCS applications will be discussed.

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Poster 52

Yeast Lipid Homeostasis – New Insights from a Lipidomic Screening of Kinases and Phosphatases

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Lipids are essential eukaryotic cellular constituents with multiple roles in cellular processes. The major lipid components in eukaryotic cells are glycerophospholipids, sphingolipids and sterols, whose molecular structures present a remarkable complexity. The cell's lipid composition is maintained by coordinated pathways between glycerophospholipids, sphingolipids and sterols. This interconnected network constitutes a dynamic biological core that allows cells not only to adapt their lipid profile to environmental challenges, but also to sense the levels of the different lipids and adjust their lipid composition to preserve cellular functions. Protein phosphorylation is a major regulatory mechanism that controls many basic signalling cellular processes in cells. Although some enzymes of lipid metabolism have been already described to be regulated by phosphorylation, a systematic analysis of how kinases and phosphatases affect lipid homeostasis was not done yet. With a few hundreds of lipid species, and sharing conserved metabolic pathways with mammals, the yeast *Saccharomyces cerevisiae* represents a useful model organism for studying lipid homeostasis. In this work, we performed a systematic and semi-quantitative lipid analysis of 130 yeast strains with gene deletions for kinases and phosphatases. By multiple-reaction-monitoring mass spectrometry (MRM-MS) we quantified lipids from the major classes in yeast, revealing lipidome-wide changes in several mutants. This work presents the lipid profiling of 130 yeast strains with gene deletions of kinases or phosphatases. We show that disturbances of distinct members of signaling pathways involved in nutrient sensing can be similarly reflected in lipid profile. Through an unbiased approach, we found new connections on nutrient sensors, reinforced previously reported findings in the regulation of lipid metabolism and also presented new candidates for regulation of lipid metabolism.

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Poster 53

Linking Single Cell Phenotypes to Single Cell Gene Expression

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Variation in single cell microenvironment and phenotype, such as cell size and shape, DNA content, and organelle activity, is an inherent characteristic of all metazoan systems. However, the extent to which such factors contribute to variability in mRNA abundance, and thus act as a source of extrinsic noise in stochastic gene expression, remains unclear. To study such contributions, we combine high-throughput image-based single cell phenotyping with automated single-molecule branched DNA fluorescent *in situ* hybridization. We find that for most genes, a large part of the mRNA abundance variability can be explained by aspects of the phenotype and microenvironment of individual cells.

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Poster 54

Characteristics of the Decay of a Type-I Membrane Protein: A Dynamic Study of Calnexin's Palmitoylation

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Calnexin is a type I membrane protein consisting of 574 residues and localized mainly in the endoplasmic reticulum (ER). It works as a chaperone and is also involved in the quality control system of the ER, helping proteins to fold correctly and retaining in the ER proteins that are improperly folded. On the cytosolic side of the protein there are 2 sites that can be reversibly palmitoylated through S-acylation, which is catalysed by a transmembrane palmitoyltransferase (DHHC6). These two modifications are involved in the control of Calnexin localization in the ER network. When Calnexin is palmitoylated, it is targeted to the central ER. Moreover S-acylation promotes the interaction of Calnexin with the ribosome-translocon complex (RTC), leading to the formation of a Calnexin-Ribosome-Traslocon supercomplex. This complex is able to translate the mRNA into the corresponding peptide that is simultaneously transferred to the ER lumen through the translocon pore. The nascent glycosylated polypeptide chain is then captured by Calnexin, which helps the peptide to assume the correct folding.
Given the importance of this post-translational modification, we developed a dynamic model describing this process of reversible palmitoylation. Our original assumption was based on a model including mass action terms and through a process of consistent reduction, we derived a model with Michaelis-Menten rate terms. Experimental results were used to calibrate the model. More specifically, we compared the model output with data coming from pulse-chase labelling experiments. In these experiments HeLa cells were labelled for 20 minutes with radioactively labelled amino acids. The labelling was then halted and the presence of labelled Calnexin inside the cell was measured at regular intervals, resulting to a fingerprint of Calnexin's decay as a function of time. We explored the parameter space of the model in order to identify parameter sets that allow us to replicate different experimental data e.g. half-life, steady state species distribution, palmitate incorporation. Sensitivity analysis was performed to study how input parameters or initial conditions influence the decay of the protein.
Our results helped us to hypothesize the decay characteristics of Calnexin that was observed experimentally. We concluded that Calnexin's degradation is influenced by its palmitoylation status and that the non-palmitoylated protein is responsible for an apparent biphasic degradation profile. Furthermore, preliminary results support the hypothesis that one palmitoylation site acts as an activator for the second palmitoylation site, revealing a potentially important kinetic aspect of the process. Finally, the resulting model is able to estimate the experimentally measured apparent half-life of the Calnexin in various overexpression and silencing experiments, as well as set the basis for designing new experimental strategies.

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Poster 55

SwissRegulon: A Repository of Bioinformatics Tools and Databases Related to Transcription Regulatory Processes

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SwissRegulon portal (www.swissregulon.unibas.ch) is a repository of databases and bioinformatics tools related to transcription regulatory processes.

It includes:
SwissRegulon: A database of genome-wide annotations of regulatory sites. We currently have annotations for 17 prokaryotes and 3 eukaryotes (including human and mouse) in our collection.
PhyloGibbs: An algorithm for inferring regulatory motifs and regulatory sites from collections of DNA sequences, including multiple alignments of orthologous sequences from related organisms.
ISMARA: Integrated System for Motif Activity Response Analysis is a free online tool that models genome-wide expression data in terms of our genome-wide annotations of regulatory sites.
TCS: A database of predicted two-component signaling interactions across bacterial genome.

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Poster 56

“Off-Target-Effect”: Modeling and Removing Using Machine Learning

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Understanding both gene interactions and functions is a major challenge in biology. RNA interference is a popular method to perturb cellular systems to phenotypically infer the function of given proteins. However, RNA interference technology is prone to a phenomenon called ``off-target-effect'', which produces additional phenotypes than the one only linked to the loss of function of a specific protein. In biology, the most popular method to deal with the ``off-target-effect'' is to test different siRNAs targeting the same protein, and retaining the most similar phenotypes indicative of function, other phenotypes being discarded. Another way consists of making an average phenotypic profile. Biologists usually perform the following protocol. The siRNA-specific phenotype obtained is first modeled by a vector. Each component of this vector is related to a given feature (morphology, dynamics, context, etc.) of the cells. A component of this vector specifies a similarity measure between the distribution of control cells and the distribution of knockdown cells. Then, the methodology to deal with ``off-target-effects'' is applied the phenotypic vectors coming from siRNA-mediated knockdown.

In this paper, new methods are proposed. We model the distribution of data directly in the cell feature space. In comparison to previous works, we do not have a first step to detect the best features. Our method is able to model the distribution of both ``on-target'' and ``off-target'' phenotypic components. Then, it is possible to go further in the analysis, by giving statistics about the components (first and second moment). After modeling, we can remove a noisy cell if it is recognized as being derived from an ``off-target'' component, and then, we can process an analysis to explain the knockdown phenotype.

We propose three models: a generative model, a bagging-based model, and a model based on vector quantization. The removing performances are evaluated using generated scenarios. More precisely, starting from cell populations that we know to be different in terms of phenotypes, we build (by random) classes that are considered as ``on-target'' or ``off-target''. We show that a combination between the generative model and the bagging-based model provide the best recognition performance.

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Poster 57

A Comprehensive Reconstruction of the Fatty Acid Biosynthesis in *Saccharomyces cerevisiae*

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The role of lipids in eukaryotic cells is of high importance. They are one of the main components of cell membranes, act as storage of high potential energy as well as being a scaffold of signaling proteins or participate in signaling themselves. Many diseases are associated with alterations in the lipid distribution in the cell and the composition of membrane domains. Metabolic syndrome, obesity, atherosclerosis, as well as Alzheimer's, Huttington diseases and cancer, all originate from alterations in some stage of lipid biosynthesis. Therefore, advancement of knowledge in the field of lipid metabolism will provide novel insights for further biomedical research and potential strategies for drug development.

Fatty acids are the backbone of the lipid metabolism. Being lipids themselves they also participate in the biosynthesis of the majority of the lipid classes. Thus, the study of their synthesis and biotransformation in a cell constitutes the basic step towards a systematic investigation of the phenomena attributed to lipids.

In the current study we reconstructed a comprehensive representation of the biosynthesis of fatty acids in a model eukaryote such as Saccharomyces cerevisiae. The model accounts for approximately 300 reactions and 230 metabolites, taking part in 3 compartments of the cell, describing the steps of synthesis, elongation and β-oxidation of fatty acids. We make use of other systems approaches in order to identify the input fluxes to the model and we explore the diverse capabilities of the network with respect to flux profiles and distribution of fatty acids.

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Poster 58

Light Intensity Modulates the Network Topology of the Shade Avoidance Response in Arabidopsis thaliana

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Plants can sense and adapt their growth to changes in their light environment. Under a canopy or in the presence of neighboring plants, Arabidopsis thaliana seedlings develop elongated stems, thus increasing their chances to outgrow competitors for light. This Shade Avoidance Response (SAR) is triggered by a drop in the red (R) to far-red (FR) light intensity ratio with (canopy) or without (neighbor detection through light scattering) a reduction in the overall light intensity. The low R:FR ratio signaling cascade involves both Phytochrome Interacting Factors (PIFs) and the auxin hormone. This hormonal signal is produced in the embryonic leaves, where sensing takes place, and sent to the embryonic stem, where elongation occurs. Here we investigate how the PIFs modulate this auxin signal using computational and experimental tools.
The regulation of the SAR was first modeled by a coarse regulatory network, which has the light properties as input and the elongation signal as output. To inform the network, we measured hypocotyl elongations in response to various light conditions. Using a novel parameter sampling method, hypothetical networks could be evaluated on their ability to accurately predict stem elongation values for the various mutants.
Several hypotheses were generated and then experimentally validated. For example we uncovered that the HFR1 gene regulates auxin production. Taken together, our results demonstrate that the auxin neighbor detection signal is modulated by the light intensity. More precisely, auxin production is enhanced in high light intensity, while auxin sensitivity in enhanced in low light intensity. As plants grown in high light intensity have more resources, this may reflect a resource dependent trade-off between the cost of the elongation signal and its robustness.

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Poster 59

The LipidX Lipidomics Analysis Platform

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In order to store and analyze consistently large quantities of lipid mass spectrometry data for different projects, we conceived and implemented a single platform integrating the most common steps of lipidomics research strategies. Notably, this platform supports both the exploratory phase, to find new lipid species of biological interest (OrbiQuant application) and the quantification phase to obtain precise profiles for lists of known lipids (MRMQuant application). A web application provides a common way to access, store, manage and analyze experimental data acquired from mass spectrometers. The usage of an underlying relational database guarantees data integrity as well as consistency across experiments and over time.
A total of about 2’000 lipids are linked in our so-called LipidX database. About 1’000 experiments studying cell extracts of ten different types of organism have been uploaded from four mass spectrometers located in different institutions (two TSQ triple quadrupole, a VARIAN 320-MS LC/ GC-MS, and an LTQ Orbitrap Fourier Transform machine). Collectively, these experiments represent about 131'000 samples (technical and biological replicates comprised). To date, more than 750 quantitative analyses of Multiple Reaction Monitoring (MRM) experiments have been performed with the preliminary version or the production version of MRMQuant. About 90 OrbiQuant analyses have been run to identify relevant changes between full scan profiles of different lipid extracts.
We have added several improvements to the MRM analysis pipeline. In particular, MRMQuant integrates now an automatic detection and analysis of +1 isotope from existing MRM lists (based on non-isotopic masses). Downstream cross-experiment analyses have been added to be able to evaluate the reproducibility over different biological replicates and to find statistically relevant changes in lipid quantities between different conditions.
The OrbiQuant and MRMQuant tools are running in a routine mode. New analysis methods are being implemented to support LC-MS and MS^2 data.

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Contact Angle at the Leading Edge Controls Cell Protrusion Rate

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Plasma membrane tension and the pressure generated by actin polymerization are two antagonistic forces believed to define protrusion rate at the leading edge of migrating cells. Membrane tension is oriented in the plane of the membrane, while actin filaments are growing generally parallel to the substrate; but it was not known if mutual configuration of the membrane and the substrate affects the force balance. To understand how the protrusion rate at the edge is controlled, we manipulate both the cell shape and substrate topography in the model system of persistently migrating fish epidermal keratocytes. We find that protrusion rate does not correlate with membrane tension, but, instead, is strongly correlated with cell roundness, and that the leading edge of the cell exhibits pinning on substrate ridges – a phenomenon characteristic of spreading of liquid drops. These results indicate that the leading edge could be considered as a triple interphase between the plasma membrane, substrate, and the extracellular medium, and that contact angle between the membrane and the substrate controls the load on actin polymerization and, therefore, edge protrusion rate. Our findings illuminate a novel relationship between the 3D shape and cell edge dynamics, which may have important implications for cell migration. Supported by SystemsX.ch.

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Image-based Transcriptomics in Thousands of Single Human Cells

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Fluorescence *in situ* hybridization is widely used to obtain information about transcript copy number and subcellular localization in single cells. However, current approaches are not suitable for high-throughput automation, and thus do not readily scale to the analysis of whole transcriptomes. We show that branched DNA technology combined with automated liquid handling, high-content imaging, and quantitative image analysis allows highly reproducible quantification of transcript abundance similar to RNA-seq in thousands of single cells at single molecule resolution. Since it can be incorporated in a wide variety of high-throughput image-based approaches, we expect it to be broadly applicable in studies that quantify phenotypes of single cells and cell populations.

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Molecular Dissection of SipA-mediated *Salmonella* Typhimurium Invasion

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The enteropathogenic bacterium *Salmonella* Typhimurium is a common cause of gastroenteritis and can trigger pronounced mucosal inflammation. Like many other pathogenic Gram-negative bacteria, it employs two type-III-secretion-system (TTSS) to inject effector proteins into host cells. The effectors SopE, SopE2 and SopB are injected by TTSS-1 to facilitate invasion into epithelial cells. These proteins stimulate actin rearrangement by interfering with RhoGTPase signaling and phospho-inositol metabolism. The resulting pronounced actin polymerization leads to the formation of characteristic membrane ruffles, enabling *Salmonella* invasion. On the contrary, the TTSS-1 effector SipA directly binds to F-actin, promoting local actin polymerization, filament stabilization and bundling.

Recent research in our group shows that SipA efficiently mediates invasion in the absence of SopE, SopE2 and SopB. Furthermore, internalization does neither require nor induce membrane ruffling and is RhoGTPase-dependent. In contrast to wild type (WT) and *Salmonella* only harboring SopE, SipA-only mutants show no ability to facilitate invasion of any other *Salmonella* bacteria into epithelial cells, adding further evidence to the hypothesis that SipA-mediated invasion is mechanistically different compared to WT and SopE-induced internalization. Scanning electron micrographs of SipA-only *Salmonella* shortly after effector protein injection into the host cell indicated two distinct entry morphologies that were phenotypically distinguishable from membrane ruffles triggered by WT *Salmonella*: (I.) bacteria are entangled by a high number of filopodia from the epithelial cell and (II.) bacteria sink into the host cell without obvious morphological changes in adjacent membrane areas. Additionally, mechanism II is distinct from the zipper-mechanism used by *Yersinia* spp..

A genome wide Qiagen unpooled siRNA screen on SipA-mediated *Salmonella* infection performed in our laboratory is currently being reanalyzed with the InfectX pipeline. Using the results of this screen and subsequent confirmatory screens in the context of the InfectX initative, we are aiming to identify cellular factors that are involved in infection events described above and to decipher on a molecular level the two entry mechanisms employed by SipA-only *S*. Typhimurium. Given the variety of screening settings (pooled/unpooled siRNA, esiRNA and miRNA screens, inhibitor screens), secondary screens with different readouts (docking, membrane closure and ruffling) and suppliers (Qiagen, Dharmacon, Ambion) we are confident to gather a substantiated dataset to be able to gain more insights into SipA-mediated *Salmonella* infection.

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Automatic Single Cell Analysis of Microscopy Images

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Single cell analysis has greatly aided our understanding of the complexity underlying biological processes, and the technological achievements of the past decades have undoubtedly catalyzed this progress. Automated microscopy constitutes a widely used measurement technology to perform single cell analysis; it offers the distinctive possibilities of (i) monitoring the time evolution of molecular quantities of individual cells, (ii) accounting for spatial characteristics of each cell or of the cell population, and (iii) capturing cell lineage information. Modern microscopes typically produce large amounts of imaging data. Thereby, the applied image analysis methods need to be automatic, fast and accurate.

Two central, inextricably linked, image analysis processes for single cell quantification are cell segmentation, i.e., the spatial identification of the cells in an image, and cell lineage construction, i.e., the tracking of cells and their offspring throughout the experiment. Yet, current automatic algorithms are designed in a way that fails to account for the possibilities that microscopy offers. As a result, they perform well for the experimental settings for which the algorithm was developed. Here, we first describe a more general segmentation method, which exploits characteristic patterns of cell membranes that can be acquired with many different microscopy techniques. We combine directional cross-correlation and graph-cuts, and demonstrate the method's applicability by analyzing images of various cell types grown in dense cultures. Second, we developed a general method for construction of cell lineages that operates on imperfect segmentation results, and images carrying information indicative of cell division. Consequently, it can be combined with any segmentation algorithm, and exploits the breadth of imaging possibilities that denote the end of a cell's cycle. We combine a series of weighted graph matching steps, and construct lineages of superior quality compared to other well-established frameworks.

As a proof of concept, we monitored the internalization of GFP-tagged plasma membrane transporters and the dynamic evolution of the cytosolic pH upon external perturbations in single yeast cells. The resulting single cell data and the subsequent computational analysis offer insight to the sources of heterogeneity present in the cell population. The image analysis methods described here are implemented in the software CellX, which has an intuitive graphical user interface and supports parallel computing for quantitative analysis of large image data sets.

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