Clonal evolution versus cancer stem cell theory

Homo sapiens
We would like to thank the following sponsors for generously supporting the 2nd International SystemsX.ch Conference on Systems Biology:

Poster Awards
Abstract Book

2nd International SystemsX.ch Conference on Systems Biology

October 20 – 23, 2014
Swiss Tech Convention Center, Lausanne, Switzerland
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welcome Note</td>
<td>1</td>
</tr>
<tr>
<td>Scientific Program</td>
<td>3 - 8</td>
</tr>
<tr>
<td>Abstracts</td>
<td>Keynote Speakers</td>
</tr>
<tr>
<td>Abstracts</td>
<td>Theory and Biophysical Modeling</td>
</tr>
<tr>
<td>Abstracts</td>
<td>Quantitative Cell and Developmental Biology</td>
</tr>
<tr>
<td>Abstracts</td>
<td>Functional Genomics and Gene Regulation</td>
</tr>
<tr>
<td>Abstracts</td>
<td>Single-Cell Biology</td>
</tr>
<tr>
<td>Abstracts</td>
<td>Systems Genetics and Medicine</td>
</tr>
<tr>
<td>Author Index</td>
<td>203</td>
</tr>
<tr>
<td>Notes</td>
<td>223</td>
</tr>
</tbody>
</table>
Dear Colleagues,

We are very pleased to welcome you to the 2nd International SystemsX.ch Conference on Systems Biology 2014 in Lausanne.

Over the past three years since our first conference, SystemsX.ch has grown considerably. The Swiss Initiative in Systems Biology currently supports over 100 projects involving hundreds of scientists from 13 universities and research institutions in all corners of Switzerland. This second conference comes just over halfway through the lifetime of the Initiative, and serves both as a roundup of the first phase as well as an ushering in of the second phase with its new projects, research leaders and students.

Over the four-day conference, we will enjoy two keynote lectures, talks from leading international researchers as well as presentations from our own SystemsX.ch project leaders. In addition to the programme of invited speakers, each session will feature short talks selected from the abstract submissions, and there will be poster sessions, giving young scientists a platform for showing and discussing their work. The conference is divided into the five sessions Theory and Biophysical Modeling, Quantitative Cell and Developmental Biology, Functional Genomics and Gene Regulation, Single-Cell Biology and Systems Genetics and Medicine.

We hope that this conference encourages lively scientific exchange between participants, and provides an opportunity for presenting the latest international advances in systems biology research. We also aim to showcase SystemsX.ch’s success over the past few years and to demonstrate the depth of the collaborative synergy of the initiative.

Félix Naef  
(Chair of Scientific Committee)  
Lucas Pelkmans  
(Scientific Committee)
Monday, October 20, 2014

12:00  Vineyard excursion (reservation necessary)

16:00  Arrival and registration

18:00  Welcome reception and opening remarks
       Rez de Jardin

18:30  Introduction to Micronaut Exhibition
       Henning Stahlberg, University of Basel

19:00  Keynote lecture: Light-based systems biology
       Gene Myers, Max Planck Institute for Molecular Cell Biology and Genetics (Dresden, Germany)
Tuesday, October 21, 2014 a.m.

Theory and Biophysical Modeling
Chair: Félix Naef, EPF Lausanne

09:00 Gradient scaling and growth control in Epithelia
Frank Jülicher, Max Planck Institute for Physics of Complex Systems (Dresden, Germany)

09:50 Quantifying Nature's appearance: combining high-resolution, coloured 3D reconstruction and mathematical tools to analyse skin patterns in Pantherophis guttatus
Antonio Martins, University of Geneva

10:10 Automated reconstruction of genome-scale metabolic network models
Jörg Stelling, ETH Zurich

10:35 Break
Rez de Jardin

11:00 What is the dimension of molecular recognition?
Tsvi Tlusty, Institute for Advanced Study (Princeton, USA)

11:30 Robust Pom1 gradient formation through intermolecular phosphorylation in fission yeast
Micha Hersch, University of Lausanne

11:50 Computational dissection of phenotypic and functional heterogeneity in cancer
Dana Pe’er, Columbia University (USA)

12:35 Lunch and poster session
Rez de Jardin
Tuesday, October 21, 2014 p.m.

Quantitative Cell and Developmental Biology
Chair: Marcos Gonzalez-Gaitan, University of Geneva

14:15  A Doppler effect in embryonic patterning
Andrew Oates, National Institute for Medical Research (London, UK)

15:05  The in silico limb: a dynamic spatial model for the morpho-regulatory signaling during vertebrate organogenesis
Erkan Unal, University of Basel (CH)

15:25  Compression of ESCRT spiral springs induces lipid membrane buckling
Aurélien Roux, University of Geneva (CH)

15:50  Break
Rez de Jardin

16:30  Genetic and neuronal networks mediating active forgetting
Simon Sprecher, University of Fribourg (CH)

17:00  Automated quantitative histology – a machine learning approach to quantify large-scale growth processes
Christian Hartke, University of Lausanne (CH)

17:20  Cortical contractility triggers a motile switch to fast amoeboid migration in 3D environments
Carl-Philipp Heisenberg, IST (Austria)

18:05  Drinks and poster session
Rez de Jardin
Wednesday, October 22, 2014 a.m.

Functional Genomics and Gene Regulation
Chair: Bart Deplancke, EPF Lausanne

09:00  How functional genomics elucidates global transcription mechanisms
Patrick Cramer, Max Planck Institute for Biophysical Chemistry
(Göttingen, Germany)

09:50  Massive parallel genome engineering identifies transcription factor binding as a principal mechanism controlling DNA methylation states of CpG islands
Arnaud Krebs, Friedrich Miescher Institute (CH)

10:10  Expression noise facilitates the evolution of gene regulation
Erik van Nimwegen, University of Basel (CH)

10:35  Break
Rez de Jardin

11:00  Recasting the net for miRNA targets and functions
Mihaela Zavolan, University of Basel (CH)

11:30  Systematic characterization of the KRAB zinc finger proteins family using high-resolution ChIP-Seq
Michael Imbeault, EPF Lausanne (CH)
and
MITOMI-seq: a novel tool enabling the parallel characterization of DNA binding specificities of TF monomers and heterodimers
Alina Isakova, EPF Lausanne (CH)

11:50  C2H2 zinc finger proteins greatly expand the human regulatory lexicon
Tim Hughes, University of Toronto (Canada)

12:35  Lunch and poster session
Rez de Jardin
Wednesday, October 22, 2014 p.m.

Single-Cell Biology
Chair: Lucas Pelkmans, University of Zurich

14:15  Force scaling in stress fibers
Manuel Théry, iRTSV (Grenoble, France)

15:05  The Legionella pneumophila Lqs quorum sensing system –
Phosphorylation signaling and intra-amoeba single cell gene
regulation
Hubert Hilbi, University of Zurich (CH)

15:20  Break
Rez de Jardin

16:00  A structural systems-biology approach to cell biology by electron
microscopy: Multiscale structural analysis from bulk tissue to the
full proteome of a single cell
Henning Stahlberg, University of Basel (CH)

16:30  Noise facilitates NF-kappaB entrainment and transcriptional
control under complex signalling environments
Savas Tay, ETH Zurich (CH)

16:50  It’s Too Late to Apoptize: A time-dependent threshold for p53-
mediated apoptosis
Galit Lahav, Harvard Medical School (Cambridge, USA)

19:00  Conference dinner (reservation necessary)
Boat from Ouchy – make your own way there!
### Thursday, October 23, 2014

**Systems Genetics and Medicine**  
Chair: Olivier Michielin, *University of Lausanne and Swiss Institute of Bioinformatics*

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
</table>
| 09:00  | **Identifying therapeutic intervention points through systems genetics**  
        Lars Steinmetz, *EMBL (Heidelberg, Germany)*                      |
| 09:50  | **Translating cancer genomes into effective personalized immunotherapies**  
        Etienne Caron, *ETH Zurich (CH)*                                |
| 10:10  | **Act Locally, Think Globally: Pathogen behavior in complex host environments**  
        Dirk Bumann, *University of Basel (CH)*                        |
| 10:35  | Break                                                                  |
| 11:00  | **Variation and genetic control of chromatin architecture in humans**  
        Bart Deplancke, *EPF Lausanne (CH)*                            |
| 11:30  | **Influence of L-arginine on metabolic networks and the lifespan of activated T cells**  
        Roger Geiger, *Università della Svizzera italiana (CH)*         |
| 11:50  | Poster awards                                                          |
| 12:30  | **Keynote lecture: Architecture and dynamics of the Notch signaling pathway**  
        Michael Elowitz, *CALTECH (USA)*                                |
| 13:30  | Lunch and departure                                                   |

Rez de Jardin
Keynote Speakers
We are now at a time when we can systematically alter animals genetically so that any given protein or its expression can be observed in a targeted set of cells. Combined with new modalities of light microscopy, this allows us to observe molecular mechanisms within the cell, observe the developmental trajectory of growing organs, and to map the cellular anatomy of organisms and organs such as a fly brain. Several brief examples from our work will be presented: on the biophysics of cell division, on C.elegans lineage tracking, and on the reconstruction of every neuron in a fly brain. To end, we give a vision of microscopes and software that we hope will lead to an understanding of how complex tissues and shapes develop in cellular terms.
Cells use circuits of interacting genes and proteins to control their behaviors, but the design principles that govern genetic circuit architecture usually remain mysterious. My lab seeks to identify and understand such design principles. To do so, we combine three synergistic approaches: First, we apply time-lapse movies and quantitative image analysis to follow the dynamics of genetic circuits in individual cells. Second, we develop synthetic biology approaches in which we design and construct new genetic circuits (or re-wire natural ones) to implement specific functions in cells. Finally, we use mathematical models to explore the dynamics of actual and potential genetic circuit architectures. We work in bacteria, yeast, and mammalian cells, model systems that enable precise genetic manipulation and measurement of the dynamics of cellular gene circuits. In this talk, I will discuss recent work in mammalian cells in which dynamic, single cell analysis provides insight into design principles of the Notch signaling pathway.
Theory and Biophysical Modeling

Chair: 
Félix Naef
Computational Systems Biology Lab
EPF Lausanne
Gradient scaling and growth control in Epithelia

Frank Jülicher  
*Max Planck Institute for the Physics of Complex Systems (Dresden, Germany)*

During the development of an organism from a fertilized egg, cells organize collectively in space to generate complex patterns and morphologies. This collective dynamics of cells involves cell division and apoptosis as well as the communication of cells via signaling systems. An important model system for the study of the dynamics of two-dimensional tissues, so called epithelia, is the developing wing of the fruit fly Drosophila. We combine theoretical approaches with quantitative experiments to study key mechanisms that underlie the dynamics of growth and morphogeneisis. Key players in epithelial morphogenesis are morphogens which establish graded concentration profiles in the tissue and guide patterning and tissue growth. A very interesting property of such profiles is that they adjust to the growing tissue by scaling. We propose that the time dependence of morphogen levels provides a key stimulus for cell proliferation. Homogeneous growth can emerge by a temporal growth rule from graded morphogen profiles that scale.
Computational dissection of phenotypic and functional heterogeneity in cancer

Dana Pe’er
Columbia University (USA)

Cells within a single tumor are known to display extensive phenotypic and functional heterogeneity. Many life-threatening features of cancer, including drug resistance, metastasis and relapse, are facets of intratumor heterogeneity. With emerging single-cell measurement technologies, the field is poised to make important strides in understanding and controlling this heterogeneity. However, these technologies require coordinated advances in analytical methods to interpret the complex data they produce.

Acute myeloid leukemia (AML) is an aggressive bone marrow malignancy in which the importance of cellular heterogeneity has been well characterized. However, previous studies have only scraped the surface of the heterogeneity in this disease. Using mass cytometry, which measures single cells in ~40 simultaneous proteomic features, we developed novel methods for analyzing phenotypic heterogeneity in cancer. Our approach provides an extensive compendium of surface-marker and signaling phenotypes in AML that extends current boundaries of knowledge.
Automated reconstruction of genome-scale metabolic network models

Jörg Stelling
Department of Biosystems Science & Engineering (D-BSSE) ETH Zurich (CH)

Predictive genome-scale metabolic models (GSMs) have become standard tools for systems-level analysis of metabolism in contexts ranging from biotechnology to cancer. However, current GSMs insufficiently cover existing biochemical and metabolic pathway knowledge, are not very species-specific, and focus on a few phylogenetic groups of organisms because of expensive manual curation and relatively few experimental data. Here, we present automated methods for network construction that integrate diverse information: a conceptual extension of network motifs decodes the implicit information in the networks’ metabolite patterns to predict metabolic functions, and the concept of network consistency solely based on the network structure drives the automated expansion and de novo generation of GSMs. As one application, we show that AtCell, a GSM of Arabidopsis thaliana based on novel methods for automated eukaryotic model construction, predicts plant growth in a wide range of genetic and environmental conditions. Integration of model predictions and experimental in vivo data revealed diurnal growth patterns that plant metabolism analysis needs to account for, emphasized metabolic adaptations to explain growth phenomena after genetic or environmental perturbations, and identified previously unknown structural limitations of plant growth that are particularly relevant for assessing the impact of climate change on C3 plant growth.
What is the dimension of molecular recognition?

Tsvi Tlusty
Institute for Advanced Study, Princeton (USA)

When an enzyme binds to a substrate, many of its atoms move around to accommodate the bound substrate. Such large-scale conformational make molecular recognition appear as an inherently multidimensional problem, where each dimension corresponds to one degree of motion freedom. This raises several basic questions: What is the dimension of the molecular phenotype space? What is the structural resolution required to understand the function of biomolecules? What is the role of conformational changes?

We will discuss these questions in the context of molecular information processing systems. Our prime example will be the ribosome, which has to select the right tRNA among numerous competitors during translation. Simple analysis of the ribosome function suggests a generic mechanism of enhancing molecular recognition. It also suggests an intriguing answer to the questions raised above.
Robust Pom1 gradient formation through intermolecular phosphorylation in fission yeast

Micha Hersch$^{1,2}$, Olivier Hachet$^1$, Sascha Dalessi$^{1,2}$, Sophie Martin$^1$, Sven Bergmann$^{1,2}$

$^1$University of Lausanne (CH); $^2$Swiss Institute of Bioinformatics (CH)

Concentration gradients provide spatial information for tissue patterning and cell organization. Robustness under natural fluctuations is an evolutionary advantage, yet the mechanisms conferring robustness are only beginning to be understood. In rod-shaped Schizosaccharomyces pombe cells, the DYRK-family kinase Pom1 controls cell division timing and placement by forming membrane-associated gradients from cell poles. Pom1 associates with the plasma membrane at cell poles upon dephosphorylation by a Tea4-phosphatase complex. Auto-phosphorylation then promotes membrane detachment and gradient decay. Here, we show theoretically and experimentally that inter-molecular auto-phosphorylation of Pom1 provides an efficient mechanism to buffer the gradients against fluctuations. Quantitative imaging reveals Pom1 gradient robustness through two system’s properties: the Pom1 gradient amplitude is inversely correlated with its decay length, and is buffered against fluctuations in Tea4 levels. Our theoretical model of Pom1 dynamics predicts both properties with a high degree of precision. We validate our model by experimentally showing that Pom1 auto-phosphorylation occurs inter-molecularily, both in vitro and in vivo. Thus, inter-molecular auto-phosphorylation coupled with phosphorylation-dependent membrane detachment provides a built-in buffering mechanism for forming a Pom1 gradient that is robust against fluctuations. In particular, it sets an upper bound on the imprecision of the positional information conferred by the gradient.
Quantifying Nature's appearance: combining high-resolution, coloured 3D reconstruction and mathematical tools to analyse skin patterns in Pantherophis guttatus

**Antonio Martins**, Michel Milinkovitch  
*Laboratory of Artificial & Natural Evolution, Dept of Genetics & Evolution, University of Geneva (CH)*

An expanding number of studies investigate the biophysical mechanisms generating intra- and inter-specific complexity and diversity of morphological traits. However, classification of morphologic characters is often limited to qualitative descriptions or simple quantitative analysis. To address this issue, we have combined state-of-the-art robotics, high-resolution digital cameras and image-based 3D reconstruction algorithms to build a scanning system capturing geometry and colour texture details down to 20 microns. This system is (1) greatly flexible, allowing a scanning range that extends from above the meter to sub-millimeter details; (2) highly repeatable, making it suitable for a systematic approach; (3) fast, with scanning times below 5 minutes, a critical feature when animals under anaesthesia are used. These fully-coloured 3D virtual mesh models are accurate representations of the real animal and its external morphological traits. This allows for the systematic and rigorous quantification of features such as colour variations and gradients, repetitive patterns, shapes, areas, or correlation among specific traits. Moreover, they can be used to perform numerical simulations of biophysical processes on realistic geometries. By using the colour patterns of corn snakes (Pantherophis guttatus) as an example, we argue that this approach has practical importance for innovative biophysical analyses of phenotypes in 3D.
System modeling of intra-tumor heterogeneity for the development of novel combination therapies

Jean-Paul Abbuehl
ISREC, EPF Lausanne (CH)

Tumor relapse is a major complication after treatment of cancer-patients, often relying on an outgrowth of therapy-resistant cells. Many studies have investigated molecular mechanisms responsible for resistance by high-throughput technologies with cancer cell lines exposed to clinical drugs. Integrating biological knowledge with the cellular response to each compound might be useful as a preclinical model providing the basis for personalized therapies by targeting critical soft spots. Genomic instability fuels the evolution of subpopulations consisting of a distinct biology and drug sensitivity. Within a hierarchically organized tumor, a single drug can spare a clonal population bearing intrinsic resistance, and therefore favoring the acquisition of the resistance mechanism while aiming only at poorly-tumorigenic subpopulations. Here, a computational model is built on the population dynamics of cancer cells, instead of the traditional molecular approach. The strategy aims at 1) delineating subpopulations by clustering, 2) inferring the cellular spectrum of each drug, 3) defining the hierarchical organization by relating the population content of the system over time and 4) predicting optimal drug combinations that are complementary in terms of subpopulation targeting. By using NCI-DREAM7 dataset, predictions of synergistic combinations are validated on a lymphoma cell line. System modeling of tumor-heterogeneity allows anticipating escape mechanisms, subsequently extracting ideal drug combinations and therefore improving patient survival.
Linking genetic variation to structural biology: How distance to the active site, solvent exposure and physicochemical properties of the amino acids shape sequence constraints in a functional enzyme

**Luciano Andres Abriata¹, Timothy Palzkill², Matteo Dal Peraro¹**

¹EPF LAUSANNE (Lausanne, CH); ²Baylor College of Medicine (USA)

We present a structure-based analysis of the only available high-resolution map describing the tolerance to all substitutions in all positions of a functional enzyme, namely a TEM lactamase previously studied through deep sequencing of mutants growing in competition experiments. Substitutions are rarely observed within 7 Å of the active site, a stringency that is relaxed slowly and extends up to 15-20 Å, with buried residues being especially sensitive. Substitution patterns in over one third of the residues can be quantitatively modeled by simple dependencies on amino acid descriptors and predictions of changes in folding stability. Amino acid volume and steric hindrance shape constraints on the protein core; hydrophobicity and solubility shape constraints on hydrophobic clusters underneath the surface, and on salt bridges and polar networks at the protein surface together with charge and hydrogen bonding capacity. Amino acid solubility, flexibility and conformational descriptors also provide additional constraints at many locations. These findings provide fundamental insights into the chemistry underlying protein evolution and design by quantitatively highlighting links between sequence and many protein traits, illuminating subtle and unexpected sequence-trait relationships, and pinpointing what traits are sacrificed upon gain-of-function mutation.
Learning the structure of signaling networks via ensemble-of-forests models

Eirini Arvaniti, Manfred Claassen
ETH Zurich (CH)

Intracellular signaling pathways typically feature complex, dynamically varying interaction patterns. The formal representation and inference of these relationships has to account for possibly disconnected patterns as well as possibly non-Gaussian dependencies. The ensemble-of-forests model (1) meets these requirements, as it enables structure learning of Markov random fields (MRF) with multiple connected components and diverse potentials. Benchmark results on synthetic data suggest that the ensemble-of-forests approach can accurately recover sparse, possibly disconnected MRF topologies, even in presence of non-Gaussian dependencies and/or low sample size.

We apply this model to learn the structure of perturbed signaling networks in various contexts and find that these frequently exhibit non-Gaussian dependencies with disconnected MRF topologies. Our preliminary results suggest that the ensemble-of-forests model could be used to generate new hypotheses on the interplay of regulatory pathways in a variety of biological processes.

Modeling mechanically stressed epithelial tissues and its application to the simulation of Acomys Dimidiatus mice spines development

Aziza Merzouki, Antonio Martins, Athanasia Tzika, Bastien Chopard, Michel Milinkovitch
University of Geneva (Grand-Lancy, CH)

Epithelial tissues are one of the four types of tissues building all organs. They cover external and internal body surfaces and separate underlying connective tissues from their environment.

In this work, we simulate the epithelium growth process using our own implementation of the model proposed by Farhadifar et al. [Current Biology 17 (24), 2095-2104 (2007)]. This model is based on a polygonal representation of cells and the minimization of an energy function, which depends on cells’ area elasticity, cells’ perimeter contractility and line tensions along the junctions between neighbor cells. In our implementation, the forces acting on polygon vertices are derived from the energy function and the dynamics of the model is driven by Newton dynamics. We also developed non-periodic boundary condition to be able to describe fragment of tissue, such as those obtained when cells grow in a petri dish.

Based on this implementation, we submit epithelial tissues to external isotropic and anisotropic forces, and we analyze its effect on tissue growth and cell organization. We also study the relation between the mechanical properties of cells defined by the model parameters and the resulting elastic properties of tissues. Finally, we are currently using and adapting this model in order to simulate the development of spines on the back of Acomys Dimidiatus mice.
Total quasi-steady state approximation and time scale separation in enzyme kinetics

Alberto Bersani
Department SBAI - Sapienza University of Rome (Italy)

Michaelis-Menten kinetics is often handled by means of the so-called quasi-steady state approximation (QSSA), based on the hypothesis that the intermediate complex, formed by the enzyme and the substrate, can rapidly reach a quasi-equilibrium, after a short transient phase. This approximation is valid under strict conditions and not always is true, in vivo as well in vitro.

Starting from the pioneering papers by Laidler, in 1955, in the Nineties of the last century a new type of quasi-steady state approximation, called total (tQSSA), has been proposed by Borghans, deBoer and Segel; it is valid in a very large range of parameters and initial conditions, much larger with respect to the standard QSSA. Several reactions have been treated by means of the tQSSA, giving much better approximations than the standard QSSA.

Starting from some papers by Palsson and coauthors in Eighties, we link the tQSSA to the normal modes of the system of nonlinear EDOs governing the reactions, aiming at determining a general rule allowing the detection of sufficient conditions guaranteeing the separation of time scales in more general reactions and, consequently, the determination of the appropriate parameters for the corresponding asymptotic expansions.
A kinetic model describes the metabolic response of E. coli to oxidative stress

Dimitrios Christodoulou\textsuperscript{1,2}, Hannes Link\textsuperscript{1}, Luca Gerosa\textsuperscript{1}, Uwe Sauer\textsuperscript{1}
\textsuperscript{1}Institute of Molecular Systems Biology, ETH Zurich, Switzerland (CH); \textsuperscript{2}PhD program Systems Biology, Life Science Zurich Graduate School (CH)

To counteract oxidative stress and build-up of reactive oxygen species (ROS), bacteria such as Escherichia coli evolved various defense mechanisms. A rapid defense mechanism is reduction of ROS through antioxidant systems that are regenerated through NADPH. NADPH replenishing metabolic pathways such as the pentose phosphate (PP) pathway are therefore upregulated, but have so far been studied primarily at the slow transcriptional level that is not able to react at the necessary time-scale of seconds. Here we asked whether faster acting allosteric regulation is important for the initial increase in NADPH supply. Therefore we exposed growing E. coli to hydrogen peroxide and quantified the dynamic metabolite response over a few minutes. To systematically infer active regulatory interactions, we developed an ensemble of approximately 10000 kinetic models for glycolysis and PP pathway with different regulation mechanisms and ranked them by their ability to describe the metabolite dynamics. From the different regulatory interactions tested, our results indicate that in addition to the known inactivation of the GAP dehydrogenase by ROS, the previously overlooked allosteric inhibition of the first PP pathway enzyme by NADPH and a feedback inhibition from glycolysis to the PP pathway are necessary to quantitatively explain the metabolite dynamics.
Bacterial population growth and variability in cell size and division process

Pablo Crotti, Vahid Shahrezaei
Department of Mathematics, Imperial College London (UK)

Recent single cell imaging studies suggest that there is cell-to-cell variability in bacterial elongation rate and division. Therefore, the size and mass of the population evolve stochastically, and modelling of the growth and division processes of bacteria henceforth requires using stochastic analysis. In this study, we analyse the growth of bacterial populations by investigating variability in the bacterial cell cycle as noise around the birth and division lengths of bacteria. We use both agent-based simulations and a Population Balance Equation (PBE) type model to describe the system. The PBE indicates that the growth rate of the population depends on both variability around the division length and the initial cell distribution. Furthermore, we show that the growth rate is also time-dependent and not constant. By running the agent-based model on different noise and cell density settings, we give an overview of the advantages and disadvantages of variability in the bacterial cell cycle. Finally, we use recent microfluidic data on E. coli single cell growth (Wang et al., Current Biology 2010) to illustrate the expected effects based on observed experimental parameters.
A model of the impact of the cell volume cycle on intracellular pH regulation

Jorgelindo da Veiga Moreira$^{1,2}$, Jean-Marc Steyaert$^2$, Loïc Paulevé$^3$, Laurent Schwartz$^2$, Erwan Bigan$^2$

$^1$INRIA (Palaiseau Cedex, France); $^2$Ecole Polytechnique (France); $^3$CNRS/LRI (France)

The cell cycle is characterized by intertwined physical, electrical, biochemical and genetic phenomena. The intracellular pH (pHi) has been shown to have a pivotal role in cell division since small variations can lead to major changes, including cell cycle arrest. Here we propose a mathematical model of pHi oscillation occurring through cell cycle. The model encompasses the dynamics of key ion concentrations (Na+, K+, H+, Cl−, and HCO3−) by taking into account transmembrane ion pumps, transmembrane potential (TMP), charge neutrality, buffering reactions and cell volume variation from interphase to mitosis. pHi oscillation is found to be mainly driven by Na+/H+ Exchanger (NHE-1) activity whose efficiency is modulated by the volume and membrane surface change during the cell cycle. Full NHE-1 efficiency, characterized by a plateaued function, allows rapid change in cytoplasmic pHi from acidic to alkaline first, followed by linear drop to its initial acidic value. This result is consistent with experimental data showing a transient pHi increase during cell cycle interphase before a decrease during mitosis. Refinements are currently brought to the model to include coupling between pH and histone decompaction, transcription rate, and metabolism, so as to obtain a self-consistent whole-cell model. Such a model is key to fully understanding certain pathologies for which the pHi is significantly modified, such as cancer.
Solution of Master Equations for time course analysis of gene transcription

Justine Dattani¹, Martin Hemberg², Mauricio Barahona¹
¹Imperial College London (UK); ²Boston Children’s Hospital

Gene expression is a highly stochastic process, requiring a complex sequence of biological steps and potentially involving a very low number of biomolecules. Mathematically, gene transcription is described using the Master Equation (ME), for which few analytical solutions exist. Common approaches simplify the analysis so that the resulting distribution is constant between cells and over time. However, further to taking snapshots of cell populations, recent experimental advances allow us to follow single-cell time histories of gene transcription. Such experiments move the focus towards analyzing time-dependent single-cell data to understand fluctuating mRNA numbers and stochastic gene regulation at the systems level.

To this end, we present the full time-dependent analytical solution for a class of MEs relevant to gene expression, where the transcription and degradation rates are arbitrary functions of time. We show how this time course single-cell solution encompasses and differs from existent solutions based on a population snapshot viewpoint. As an application, we analyze the widely used random telegraph model and characterize regimes where the interplay of time scales leads to transient-dominated behavior where the stationary solution is never reached. Analysis of experimental data suggests that this is a common scenario, implying that assumptions about stationarity may be misleading.
Hydration and diffusion dynamics shape microbial community composition and function in soil aggregates

Ali Ebrahimi, Dani Or
Soil and Terrestrial Environmental Physics, ETH Zurich (CH)

Natural variations in soil hydration conditions (rainfall, evaporation, root water uptake) affect gas and nutrient diffusion and soil microbial community composition and function. The conditions in soil aggregates are of particular interest due to limitations to oxygen diffusion into the core often containing organic carbon (as aggregation agent). The constantly varying soil hydration conditions affect the spatial extent of anoxic conditions in aggregates and thus the sized and self-organization of aerobic and anaerobic microbial communities. We developed an artificial soil aggregate composed of 3-D angular pore network combined with individual based models of motile microbial cells that grow, move, intercept nutrients and are inhibited by presence or absence of oxygen. The hydration conditions in the model aggregate affected the community size, spatial segregation, and growth rates. The opposing diffusion directions of oxygen and carbon were essential to maintenance of aerobic and anaerobic communities in an aggregate (anaerobes become extinct when carbon sources are external). Model predictions of CO2 and N2O production rates were in good agreement with experimental data. Results illustrate how aerobic and anaerobic microbial communities are activated by certain hydration conditions that enhance either nitrogen losses or decomposition of organic matter both contributing to GHG emissions.
From single to multi-cell analysis of overshoot in escherichia coli chemotaxis

Matthew Edgington, Marcus Tindall

*University of Reading (UK)*

One aspect of the response observed in chemotactic Escherichia coli is that of overshoot – a transient period during which the motor regulating protein exceeds its pre-stimulus concentration before returning to it. We investigated this phenomenon using a nonlinear ordinary differential equation (ODE) model of the chemotaxis signalling cascade in E. coli. Model reduction combined with stability analysis of the model show that the signalling protein concentrations are important in controlling overshoot and in particular the cellular adaptation time. Our understanding of parameter and solution space at the single cell level has allowed us to test the effects this has at the multi-cell level; here we have incorporated the ODE model into a multi-scale agent based framework. We observe that predictions made using single cell behaviour do not necessarily correlate with the observed population behaviour. Our results show that it is important to consider both single and multi-cell behaviour in order to obtain a more complete understanding of the overall system dynamics.
Speed of evolution in large asexual populations with diminishing returns

Maria Rita Fumagalli\textsuperscript{1,2}, Matteo Osella\textsuperscript{1,2}, Philippe Thomen\textsuperscript{3}, Francois Heslot\textsuperscript{3}, Andrea Cavallone\textsuperscript{1}, Marco Cosentino Lagomarsino\textsuperscript{3}
\textsuperscript{1}University of Turin (Italy); \textsuperscript{2}INFN Turin (Italy); \textsuperscript{3}Université Pierre et Marie Curie - CNRS (France)

The adaptive evolution of large asexual populations is generally characterized by competition between clones carrying different beneficial mutations, that makes the theoretical description of the dynamics more complex with respect to the successional occurrence and fixation of beneficial mutations typical of small populations. Different laboratory evolution experiments show a decrease of the fitness advantage of new mutations and a decrease of the speed of evolution. We propose phenomenological models that generalize the fixed-advantage framework to include this effect in a simple way. We evaluate analytically as well as with direct simulations the consequences of decreasing advantage and decreasing mutation rate on the evolutionary dynamics. In the first case the speed of adaptation decreases in time and reaches a limit value corresponding to neutral evolution. This corresponds to an increase of the diversity in terms of "classes of mutation" in the population. For the second model the phenomenology is quite different, and the total number of "classes of mutation" decreases in time. Finally, we show how the model can be compared with data from laboratory evolution experiments, obtaining an estimate of the order of magnitude of the beneficial mutation rate.
Bayesian parameter learning from single-cell data for biochemical reaction networks

Stefan Ganscha, Manfred Claassen

ETH Zurich (CH)

The simultaneous quantification of multiple biochemical species in high-throughput, single-cell experiments yields samples of a time-variant, multivariate distribution of markers. This distribution’s empirical moments (e.g. covariance between two markers) can be employed for parameter inference in stochastic biochemical reaction networks. Moment approximation allows the description of such networks’ time course of moments by ordinary differential equations systems. Thus, the inference of stochastic reaction parameters is cast as parameter estimation of a deterministic ODE system, whose size is driven by the number of chemical species, their utilized moments and the number of reactions. The iterative re-integration of such large ODE systems – a computational bottleneck in Bayesian inference – can be avoided by expressing the likelihood as function of gradients instead of absolute values.

We propose a Bayesian inference framework based on the Riemann manifold Metropolis adjusted Langevin algorithm (MMALA), parallel tempering and gradient-matching. Our approach yields significant speed improvements by taking advantage of the biochemical network’s inherent sparsity, and automatically generates statistical models for any order of moments via the general moment expansion method. We present results for synthetic single-cell data of differently sized cell-signaling test systems and illustrate the effect of single-cell data’s statistics on parameter inference.
Self-organized microbial patterns in glass pore network models

Olga Ilie, Dani Or
Soil and Terrestrial Environmental Physics, ETH Zurich (CH)

Understanding the basic principles that promote and control spatial organization and function of multispecies microbial communities is important for ecological and engineering manipulation of such assemblies to fulfill or restore highly complex functions (e.g., bioremediation or restoration of impaired natural communities). The project objectives are to: (i) quantify mechanisms giving rise to stable spatial patterns of microbial consortia and link these to ecological function, and (ii) control of environmental conditions that promote desired configurations and select for target consortia members. We conducted experiments in glass channel networks with different degrees of heterogeneity that were partially saturated with nutrient solutions and inoculated with labelled and trophically-interacting microbial communities. The experiments were guided by insights from a biophysical model of individual based microbial communities interacting in analogue pore networks endowed with known physiological parameters. Modeling results show spontaneous emergence of patterns governed by the nature of trophic interactions. Results from ongoing growth experiments in micromodels using bacterial strains that require collaboration to carry out degradation of carbon source will be presented.
Breast cancer is a heterogeneous disease manifesting in different breast cancer subtypes that vary from patient to patient and even within the same tumor. The HER2+ subtype, characterized by overexpression of the HER2 receptor, is efficiently targeted by the clinically approved drugs trastuzumab and pertuzumab. However, drug resistance has been reported for some patients that apparently correlated with high level EGF receptor expression.

For the present study, we treated the EGFR-high/HER2+ model cell line SKBR3 for one hour with trastuzumab or pertuzumab and subsequently stimulated the cells with EGF for a further two hours. The phosphorylation of the EGF and HER2 receptors as well as phosphorylation dynamics of downstream targets of the MAPK and PI3K pathways was determined using reverse phase protein arrays. The resulting time-course data was employed for dynamic modeling of the drug-receptor interactions and the signaling pathways by ordinary differential equations. Whereas the model structure is literature-based, the model parameters were determined specifically for the SKBR3 cell line by parameter estimation. Thereby, we identified dimer formation between HER2 and its derived receptor fragment, the p95 receptor, as key to explain the effects provoked by trastuzumab and pertuzumab, and gained a deeper understanding of EGFR and HER2 cooperation on a mechanistic level.
Effects of wetting-drying cycles on soil microbial diversity - model and observations

Minsu Kim¹, Adam Šťovíček², Osnat Gillor², Dani Or¹
¹Soil and Terrestrial Environmental Physic, ETH Zurich (CH); ²Ben-Gurion University, Sde Boqer Campus (Israel)

Dynamic changes in soil water content greatly affect microbial life in soil. Aqueous phase connectivity within soil pore spaces affects ranges of microbial dispersion and shapes nutrient diffusion fields. We developed a biophysical model for systematic study of effects of hydration cycles on relative abundance and species distribution. We represent soil surface heterogeneity as roughness patches that retain different amounts of water at given matric potential values. The model considers local growth rates of motile individual microbial cells, whose growth is based on nutrient interception and on physiological parameters drawn from an initial prescribed distribution. The surface patch model was subjected to cycles of wetting and drying events (mimicking observations). The relative abundance as well as other diversity metrics of the evolving microbial community was obtained from the local interactions. Results show a decrease in overall microbial diversity following wetting events and recovery after drainage and drying. The results were in qualitative agreement with observations in desert soil following rainfall events.
Selection of a synthetic soil microbial community for quantifying effects of hydration dynamics on community structure and diversity

Hannah Kleyer, Robin Tecon, Dani Or
Soil and Terrestrial Environmental Physics Group (STEP), ETH Zurich (CH)

Soil bacteria play a central role in many ecosystem services such as formation of rhizosphere communities, nutrient transformation and global biogeochemical cycles. Molecular techniques based on rRNA genes analysis have uncovered the tremendous bacterial diversity in soil, but the mechanisms that control the assembly, functioning and maintenance of complex microbial communities remain largely unknown. The study aims at linking dynamics of soil hydration conditions and related biophysical factors with variations in microbial composition and ecological functioning. To assist with definitive community level observations we designed a synthetic microbial community comprised of 10 well-characterized bacterial species spanning a wide range of soil phyla to be inoculated onto model porous surfaces mimicking soil habitats. The experimental system consists of sand layer placed on porous ceramic surface connected to nutrient reservoir. Fluctuations in hydration conditions are induced by changing the reference nutrient reservoir level to simulate wetting-drying cycles. The activity and composition of the bacterial community is studied using 16S fingerprinting and quantitative PCR. Subjecting the synthetic community to a range of controlled physico-chemical conditions allow for systematic evaluation of the role of environmental fluctuations on changes in community composition.
Structure Learning for Stochastic Reaction Networks

Anna Klimovskaia, Manfred Claassen

ETH Zurich (CH)

Development of new deep high-throughput technologies for single cell measurements enable new approaches to modelling biochemical systems, such as signalling cascades, by means of stochastic reaction networks. We use statistical information in single-cell data sets for parameter estimation, and ab initio structure learning of mechanistic models. We propose a convex relaxation of the model selection problem over all possible topologies. The proposed method allows to the simultaneous inference of both structure and fit parameters of a biological system, described by a stochastic reaction network with unknown topology. We assume the system is described by mass action kinetics, and consider its moment equations to relate moments in the data to the kinetic parameters. This formulation allows for parameter estimation by means of linear regression. We apply this formulation to estimate the parameters of the reaction network, which includes all possible binary reactions among the system species. To select for reactions with non-zero rate constants, we introduce sparsity-inducing priors. We evaluated our method on synthetic data for different scale systems. We aim to apply this method to learn the reaction network structure of cancer related signalling pathways, such as TRAIL induced apoptosis.
Identification of sites and timing of metabolic regulation from metabolite profiling data using probabilistic graphical modelling

Andreas Kühne\textsuperscript{1,2}, Manfred Claassen\textsuperscript{1}, Nicola Zamboni\textsuperscript{1}

\textsuperscript{1}Institute of Molecular Systems Biology, ETH Zurich (CH); \textsuperscript{2}PhD program Systems Biology, Life Science Zurich Graduate School, Zurich (CH)

Crosstalk of metabolism and signaling pathways was shown to play a crucial role in cellular decision-making. This motivated several metabolomics studies and lead to a vast availability of metabolomics datasets. However, one of the major challenges in metabolomics remains the interpretation of metabolite profiling datasets. Here we present a novel probabilistic graphical modelling approach that aims at identifying metabolic adaptations from noisy and incomplete metabolite profiling data independent of artificial pathway definitions. We use Markov random fields with hidden states representing metabolites and observables that integrate measured metabolite levels. Hidden states emit a metabolic observation with a label specific probability and hidden state labels are dependent on neighboring states defined by the underlying reaction network. Optimizing the hidden state label distribution to fit measured metabolite profiles enables to identify modules of neighboring states with the same label. Transitions between two different modules indicate possible sites of metabolic regulation. Furthermore, extensions of the model for temporal metabolite measurements allow investigating also timing of metabolic adaptations. Preliminary analysis indicates that this approach can identify sites of metabolic regulation and to improve classification of temporal data. Thus, it represents a powerful method enabling the extraction of non-trivial regulation motifs from metabolomics data.
The aim of understanding the metabolism of cells has led to a wide variety of methods and approaches for the quantification of flux rates in metabolic networks. Different experimental methods like metabolic flux analysis (MFA) and isotopomer labeling as well as theoretical approaches like flux balance analysis are used to infer flux distributions. However, experimental methods allow only for resolution of few (often: exchange) fluxes, and predictions by constraint-based models do not incorporate the available experimental data systematically.

We present a new approach for the integration of different kinds of probabilistic information, where we use all information to build a single joint probability distribution over the cell’s space of feasible flux distributions. At the core of this approach are new MCMC methods, which enable the sampling from the resulting probability distributions. We present applications of this approach, e.g. for the correct handling of uncertainty in the analysis of MFA experiments or for experimental design.
Head scales on crocodiles do not form from genetically-determined units as in other amniote skin appendages like scales, feathers and hair. Indeed, evidence suggests that a physical process of cracking occurs in the developing tissue (Milinkovitch et al., 2013). These cracks propagate to form an interconnected network, generating random polygonal domains that become cranial scales.

The 3D Crocs Project’s main objective is to study cranial head scale development and evolution. Using high-resolution photographs and a reconstruction software pipeline, we generate 3D geometry and colour texture cranial reconstructions of various crocodiles, gavials, alligators and caimans. Network features such as edges, nodes and integumentary sensory organs were marked on 3D mesh models in order to conduct quantitative analyses. This study further supports that crocodile head scales develop from a stochastic process of cracking similar to fracture mechanics. Comparisons among 12 species, representing 3 families of Crocodylians, indicate that there is a wide diversity of cranial scale patterning. Lastly, we investigate potential causes of inter-specific scale pattern diversity within Crocodylia.
A tool for bio-mechanical simulations in plants

**Gabriella Mosca**¹, Richard Smith², George Bassel³, Hagen Reinhardt², Soeren Strauss², AnneLise Routier²

¹University of Bern (CH); ²Max Planck Institute for Plant Breeding Research (Germany); ³University of Birmingham (UK)

Plant growth is controlled at the cellular level by interactions between gene products and mechanical properties. In the past few years, micro-indentation methods like Cellular Force Microscopy (CFM), have been developed to measure rigidity at the cellular and sub-cellular scale. However, forces measured by micro-indentation give only an indirect measurement of the cell physical properties and are influenced by cell geometry and boundary conditions. In order to extrapolate turgor pressure and cell wall stiffness from the measurements, the indentation experiments are simulated on a realistic template, which represent a computational challenge. We are developing a tool able to handle continuum mechanics simulations on complex geometry, such as 3D plant tissues. These simulations are quite versatile and can reproduce, for example, inflation and indentation experiments so to allow us understanding what is the major factor (geometry, mechanical properties, turgor pressure) affecting the measured result with CFM. These simulations can also include growth and we are working so to link genetic signalling, growth and mechanics in a unique framework. This has been partially done while characterising the root embryo growth for example and the tool is proving to be robust and versatile for the study of different plant organs.
Distance-dependent protrusion/retraction transitions generate cell polarity, shape and motion

Franck Raynaud, Mark Ambühl, Alexander B. Verhovsky
EPF Lausanne (CH)

The ability to spontaneously break symmetry and move directionally is an essential property of most eukaryotic cells. It is believed that a directional mechanism at a scale of the whole cell, e.g. a global gradient of cytoskeletal and/or signaling components, orchestrates cell edge dynamics according to the overall motion direction. This concept is limited, however, in that external directional stimuli in combination with internal diffusible signals interacting through feedback loops, or a feedback from the motion itself, have to be considered to establish polarity axis. Here we examine the edge dynamics of polarizing and persistently migrating fish epidermal keratocytes, and propose a novel and simple principle of self-organization of cell activity in which local cell edge dynamics depends on the distance from the cell center, but not on the position with respect to a global polarity axis. We validate this principle in experiments with confined and micro-surgically manipulated cells and implement it in a stochastic model that faithfully reproduces cell migration behavior. Our findings indicate that spontaneous polarization, persistent motion, and cell shape are emergent properties of the local cell edge dynamics controlled by distance from the cell center. This work lays a foundation for a novel paradigm of cell polarization and opens the way for the exploration of distance-sensing mechanisms.
Deconvoluting off-target confounded RNA interference screens

Fabian Schmich$^{1,4}$, Ewa Szczurek$^{1,4}$, Saskia Kreibich$^1$, Sabrina Dilling$^1$, Daniel Andritschke$^1$, Alain Casanova$^2$, Shyan Huey Low$^2$, Simone Eicher$^2$, Simone Muntwiler, Mario Emmenlauer, Pauli Rämö, Christian von Mering, Wolf-Dietrich Hardt$^1$, Christoph Dehio$^2$, Niko Beerenwinkel$^{1,4}$

$^1$ETH Zurich (CH); $^2$University of Basel (CH); $^3$University of Zurich (CH); $^4$SIB Swiss Institute of Bioinformatics (CH)

RNA interference (RNAi) is a popular technology for genome-wide perturbation experiments. However, most short-interfering RNAs (siRNAs), designed to knockdown specific genes, in practice, exhibit strong off-target effects, which confound the interpretation of RNAi screens and limit their utility for functional genomics studies. Here, we present gespeR, a computational model for reconstructing individual gene-specific phenotype (GSP) contributions from observed, off-target-confounded RNAi knockdown phenotypes, modeling siRNA-specific phenotypes as a combinatorial effect of on- and off-targeted genes.

Deconvolution of image-based pathogen infection screens using 166,444 siRNAs from three companies for three different pathogens resulted in GSPs with significantly increased concordance (up to five fold) between independent siRNA sets targeting the same genes. Hit genes identified by gespeR were validated both in-silico and experimentally and were shown to be biologically significantly more relevant than observed SSP phenotypes and estimates from competing methods. gespeR can be applied to pooled- or single-siRNA screens from different companies as well as to simple or complex phenotypes, and will thus help to restore the utility of genome wide RNAi screening.
In virtually all ecosystems, microbial communities sustain recycling organic matter and thus play an important role in global carbon cycle. This biogeochemical activity often relies on complex multispecies consortia that are spatially self organized. The basic rules governing the assembly of such consortia remain poorly understood and understudied. This study focuses on bacterial trophic interactions as a driving force in shaping community spatial patterns. We designed synthetic bacterial systems whose assembly and functioning is quantitatively studied in the laboratory. We use Pseudomonas putida F1, which is a natural degrader of toluene (an aromatic hydrocarbon commonly used as organic solvent). While various F1 mutants cannot fully metabolize toluene, we have identified mutualistic interactions between F1 mutants that restore their ability to grow on toluene as a sole carbon and energy source. These synthetic consortia were grown on a Porous Surface Model that mimicks the hydration conditions found in soil, and cell-tagging with autofluorescent proteins permit direct observation of interacting subpopulations in space and help resolve emerging bacterial community patterns. Systematic control of boundary conditions (hydration conditions, supply location) enables direct evaluation of controlling variables that shape the interactions.
Pattern modulation produces a highly-regular grid of defensive hairs in the spiny mouse (Acomys dimidiatus)

Athanasia Tzika, Sophie Montandon, Liana Manukyan, Michel Milinkovitch

University of Geneva (CH)

The mammalian skin and its appendages exhibit a remarkable variety of phenotypes, with interspecies studies now required to uncover the developmental mechanisms generating this diversity. We investigated the development of spines in Acomys dimidiatus, a lineage that diverged 25 million years ago from that of the laboratory mouse. We show that the spine-forming region of the Acomys pelage organizes as a highly regular grid of follicle triplets generated in two steps of secondary placode induction. We use whole-mount in situ hybridizations and 3D reconstructions to investigate the processes that establish this spectacular micro-pattern. Topology/geometry statistics are employed to assess the regularity of the pattern. The impact of cell migration and proliferation inhibition is analysed on ex vivo skin cultures.
Folding of growing epithelial tissues in a stochastic cell-based model

**Severine Urdy**<sup>1,3</sup>, Diederik Laman-Trip<sup>2</sup>, Roeland Merks<sup>2,3</sup>

<sup>1</sup>University of Zurich (CH); <sup>2</sup>University of Leiden (NL); <sup>3</sup>Centrum Wiskunde & Informatica (NL)

Stochastic cell-based models are useful to investigate how cell behaviors translate into whole tissue geometries. Apart from cell proliferation and apoptosis, the homeostasis and folding of growing epithelial monolayers depends on mechanical constraints such as cell-cell cohesion, cell-matrix adhesion and the basement membrane stiffness.

To get more insights into these constraints, we investigate the basic properties of a 2D stochastic cell-based model that approximates the cross-section of a hypothetical three-layered tissue, where the epithelial cells, the basement membrane and the underlying stroma are represented by collections of lattice sites, interacting mechanically via their interfaces.

We show that tissue folding can be driven by stochastic variation in growth rate even when all layers grow at the same rate in average, at the condition that the surrounding medium resists cell migration to some extent. We study how cohesion within layers and adhesion between layers influence the final shape of the folded tissue. This model extends previous stochastic cell-based models and shows that differential growth may be an unnecessary assumption in certain conditions. Extensions of this model could help to distinguish whether changes in tissue geometry are related to changes in cell-matrix adhesion or in membrane stiffness in developmental and disease contexts.
Integrative studies of allosteric regulation in signal transduction networks using biophysical modeling and computational systems biology approaches

Gennady Verkhivker¹, Krisin Blacklock²
¹Chapman University (Orange, US); ²Rutgers University

Allosteric regulation of molecular chaperones and protein kinases allow for molecular communication in signal transduction networks. We report the results of integrative systems biology studies of the Hsp90 chaperone and protein kinases with an atomic level analysis of the communication pathways regulating conformational equilibrium of these protein systems in signalling networks. The results of biophysical and computational systems biology analyses combined with proteomics experiments have been integrated into a graph-based network model of allosteric regulation. Among our primary findings is the emerging evidence that a small number of functional motifs may be utilized by the chaperone and protein kinases to act collectively as central regulators of the intermolecular communications, ATP hydrolysis, and protein client binding in signaling networks. The diversity of allosteric communication mechanisms could ensure a proper balance of the network efficiency and functional redundancy required to maintain resilience against random attacks in the fluctuating protein environment. The additional layers of protection in regulatory mechanisms can be provided through recruitment of cochaperones and posttranslational modifications. Integration of biophysical and systems biology models of the Hsp90 interactions with oncogenic kinase mutants is used to develop mechanistic understanding of regulation of oncogenic mutants in molecular chaperones and protein kinases families.
Spatiotemporal, stochastic modeling of microtubule dynamics in Saccharomyces cerevisiae

Lukas A. Widmer\textsuperscript{1,2}, Jörg Stelling\textsuperscript{1,2}

\textsuperscript{1}Department of Biosystems Science and Engineering (D-BSSE), ETH Zurich (CH); \textsuperscript{2}Swiss Institute of Bioinformatics (CH)

As a part of the TubeX project, we aim to integrate both local regulation and signaling in Saccharomyces cerevisiae cells with microtubule dynamics during spindle pole body segregation. Specifically, we aim to address the spatiotemporal interaction of bud-directed astral microtubules with their environment, e.g., the spindle pole body they are emanating from, plus-tip proteins, kinesin and myosin motor proteins, or actin cables emanating from the bud. This involves modeling stochastic microtubule dynamics such as catastrophes and rescues locally – especially at the microtubule tip – as well as integrating reaction-diffusion dynamics on a cellular scale, resulting in a stochastic, multi-scale spatiotemporal problem.

In order to address this, we are constructing a stochastic reaction-diffusion model of the budding yeast cell with embedded microtubules in the reaction-diffusion master equation (RDME) framework, which is a coarse-grained approximation of microscopic Smoluchowski dynamics. To enable simulation of such models, we implemented a modular, high-performance C++ RDME solver framework which initially supports the next subvolume method (NSM), and may be extended by various multi-scale approaches. Initial tests show comparable to superior performance to published NSM solvers. Finally, we discuss preliminary results from the microtubule model.
Quantitative Cell and Developmental Biology

Chair: 
**Marcos Gonzalez-Gaitan**
Department of Biochemistry
*University of Geneva*
Genetic and neuronal networks mediating active forgetting

Simon Sprecher
University of Fribourg (CH)

The brain is without any doubt the most complex existing organ. One of the most amazing features is its capability to be plastic to learn and store information as memories. The basic mechanisms of how memories are formed have largely been identified. However it remains much less understood why and how we also forget acquired memories and learned information. Exploring theoretical hypotheses with a genetically tractable animal model allows the direct assessment of the neuronal, cellular and molecular basis of forgetting. The fruit fly Drosophila melanogaster is widely used as model to study the formation of memories. Recent findings show that in the fly forgetting is not a passive, but rather an active, tightly regulated process. Two molecular pathways have recently been implicated in the forgetting of short-term memories: The Rac-pathway and Dopamine signaling. In a Systems Biology collaborative effort we explore the theoretical basis of the biological forgetting process, the transcriptomic responses during long-term memory formation, the structural adaptations of synaptic plasticity using STORM super-resolution microscopy as well as the genetic behavioral features underlying forgetting.
Compression of ESCRT spiral springs induces lipid membrane buckling

Aurélien Roux
University of Geneva (CH)

ESCRT-III was genetically and biochemically implicated in membrane deformation and scission of membrane in many membrane remodeling events such as intra-lumenal vesicle formation in multi-vesicular bodies, abscission in cytokinesis and virion budding from the plasma membrane. However, how it breaks and deforms the membrane are still under debate, as the molecular structure of the proteins in the complex do not suggest a mechanism. Here we show that the yeast ESCRT-III protein Snf7 when reconstituted onto supported bilayer forms circular patches that extend by a growing front wave at their rim. The center of the patch is saturated with Snf7 that cannot polymerize anymore. By using High Speed AFM and electron microscopy, we show that these patches are formed of Snf7 spirals that grow into a tight hexagonal array. By using physical modeling, we predict the stop of the growth to be connected to lateral compression of the spirals. Indeed, when grown on free lipid membrane such as giant unilamellar vesicles (GUVs), Snf7 coat can provoke membrane deformations compatible with a model where Snf7 spirals act as 2D spiral springs loaded by polymerization and which release by buckling can cause membrane budding.
Cortical contractility triggers a motile switch to fast amoeboid migration in 3D

Carl-Philipp Heisenberg, Verena Ruprecht
IST Austria

Amoeboid cell migration is central to many developmental and disease-related processes, such as immune cell motility and tumor cell invasion. Despite its biological importance the specific molecular and physical mechanisms underlying amoeboid cell migration have only begun to be elucidated. Using a combination of theory and experiments, we identified a unique prototypic amoeboid cell migration mode in the early zebrafish embryo, termed stable-bleb migration. Stable-bleb cells display an invariant polarized balloon-like shape and migrate faster and more directionally than any other cell within the gastrula. Strikingly, progenitor cells can be transformed into stable-bleb cells irrespective of their primary cell fate just by increasing myosin II activity indicative for a simple motile switch mediated by cortical contractility. To understand stable-bleb cell transformation and migration, we developed a mechanistic model showing that variations in cortical contractility can trigger spontaneous cell polarization and that cortical flows drive migration of stable-bleb cells in 3D environments. Stable-bleb cells appear at places within the embryo where myosin II activity is locally up-regulated followed by fast dispersal, suggesting an effective mechanism of rapid cell extrusion.
A Doppler effect in embryonic patterning

Andrew Oates
*National Institute for Medical Research (London, UK)*

During embryonic development, temporal and spatial cues are coordinated to generate a segmented body axis. In sequentially segmenting animals, the rhythm of segmentation is reported to be controlled by the time-scale of genetic oscillations that periodically trigger new segment formation. However, we present real-time measurements of genetic oscillations in zebrafish embryos showing that their time-scale is not sufficient to explain the temporal period of segmentation. A second time-scale, the rate of tissue shortening, contributes to the period of segmentation through a Doppler effect. This contribution is modulated by a gradual change in the oscillation profile across the tissue. We conclude that the rhythm of segmentation is an emergent property controlled by the time-scale of genetic oscillations, the change of oscillation profile, and tissue shortening.
Automated quantitative histology - a machine learning approach to quantify large scale growth processes

Christian Hardtke¹, Martial Sankar¹, Ioannis Xenarios²
¹University of Lausanne (CH); ²SIB Swiss Institute of Informatics (CH)

Among various advantages, their small size makes model organisms preferred subjects of investigation. Yet, even in model systems detailed analysis of numerous developmental processes at cellular level is severely hampered by their scale. For instance, secondary growth of Arabidopsis hypocotyls creates a radial pattern of highly specialized tissues that comprises several thousand cells starting from a few dozen. This dynamic process is difficult to follow because of its scale and because it can only be investigated invasively, precluding comprehensive understanding of the cell proliferation, differentiation, and patterning events involved. To overcome such limitation, we established an automated quantitative histology approach. We acquired hypocotyl cross-sections from tiled high-resolution images and extracted their information content using custom high-throughput image processing and segmentation. Coupled with automated cell type recognition through machine learning, we could establish a cellular resolution atlas that reveals vascular morphodynamics during secondary growth, for example equidistant phloem pole formation.
The In Silico Limb: A dynamic spatial model for morpho-regulatory signaling interactions during vertebrate organogenesis

Erkan Unal\textsuperscript{1,2}, Zahra Karimaddini\textsuperscript{2}, Dagmar Iber\textsuperscript{1,2,3}, Rolf Zeller\textsuperscript{1}

\textsuperscript{1}DBM, University of Basel (CH); \textsuperscript{2}D-BSSE, ETH Zurich (CH); \textsuperscript{3}SIB Swiss Institute of Bioinformatics (CH)

Vertebrate limb development is an excellent model to study the complex genetic networks that regulate vertebrate organogenesis. We sought to gain an integrative understanding of the spatio-temporal kinetics of the molecular interactions that control limb bud development and to define the mechanism that permits pattern to scale in differently sized embryos such as those of mice and chicken. To this end, we have created an in silico model and solved the model on the physiological, growing limb bud domain, that we obtained by collecting 4D gene expression data in mouse and chicken limb buds using RNA in situ hybridization and Optical Projection Tomography between embryonic day 9 (E9) and E12. The model addresses key issues of limb bud development and explains the behaviors even of at first sight counterintuitive mutant phenotypes. We find, that the majority of the simulated patterns scale on differently sized domains. Accordingly, the model that was created for mouse limb bud development also reproduces the patterning process on the larger chicken limb buds. The modelling effort thus resolves a long-standing problem in developmental biology, and we expect that the model will provide a valuable resource to address further questions in limb bud development in the future.
The influence of pH signaling on the regulation of brain energy metabolism

Guillaume Azarias¹, Philipp Mäcler¹, Matthias Wyss¹, Marc Zünd¹, Bruno Weber¹
¹Institute of Pharmacology and Toxicology (CH)

Glucose is the unique source of energy in the brain, but controversy remains about its metabolism. In vitro experiments showed that astrocytes metabolize glucose into lactate upon glutamatergic stimulation, whereas lactate can be efficiently used as an energy substrate by neurons. Paradoxically, astrocytes also possess a substantial amount of mitochondria and the reason why astrocytes would preferentially use glycolysis for ATP production is unclear. We recently provided in vitro evidence for a pH-mediated regulation of energy metabolism pathways in astrocytes, but the physiological relevance of pH signaling remains to be validated in vivo.

We are investigating the relevance of pH signaling in the regulation of energy metabolism in the somatosensory cortex of anesthetized mice. For this purpose, we are developing cell-specific, genetically encoded, fluorescent probes sensitive to mitochondrial pH, mitochondrial Reactive Oxygen Species or lactate in the somatosensory cortex of mice. The regions expressing the sensors will be electrically stimulated and the kinetic of metabolic parameters will be recorded at cellular resolution using two-photon microscopy. The kinetics of responses will be computed and compared to an ODE-based mathematical model. Therefore, this project aims to develop a computational-experimental approach to understand the physiological cellular basis of brain energy metabolism regulation.
The DNA damage-induced phosphoproteome is modulated by inhibition of the receptor tyrosine kinase MET

Michaela Medová\textsuperscript{1,4}, Ariel Bensimon\textsuperscript{2,4}, Paola Francica\textsuperscript{1}, Astrid A. Glück\textsuperscript{1}, Matúš Medo\textsuperscript{3}, Daniel M. Aebersold\textsuperscript{1}, Ruedi Aebersold\textsuperscript{2}, Yitzhak Zimmer\textsuperscript{1}

\textsuperscript{1}Department of Radiation Oncology, Bern University Hospital (CH); \textsuperscript{2}Institute of Molecular Systems Biology, ETH Zurich (CH); \textsuperscript{3}Department of Physics, University of Fribourg (CH); \textsuperscript{4}equal contribution

Ionizing radiation (IR), is frequently used in the treatment of a variety of malignant tumors of different origins and stages. In recent years, numerous studies have demonstrated that interfering with signaling via growth factor receptor tyrosine kinases (RTKs), such as the RTK for hepatocyte growth factor, known as MET, can increase the sensitivity of certain tumors to IR. In this study, we have aimed to explore how is the cellular response to ionizing radiation modulated by MET inhibition. We have conducted a phosphoproteomics survey study to explore the cellular phosphoproteome following exposure of MET-addicted cancer cells to METi alone and in combination with IR. Analysis of the survey data has identified more than 300 phosphopeptides which have changed in one experimental condition or more. Several of these phosphorylation changes have been confirmed and further investigated by targeted proteomics. These results have pointed to a sub-network of the DNA damage response (DDR) that is activated in MET-addicted cancer cells upon DNA damage and MET inhibition, and could be responsible for synergism between these two modalities. We hope that this work will aid in understanding how treatment with such combinations could improve the clinical outcomes of patients.
 Trafficking and signaling interplay after serotonin receptor activation

Aurélien Rizk, Mauno Schelb, Maysam Mansouri, Milica Bugarski, Gebhard Schertler, Philipp Berger
Paul Scherrer Institut (Villigen, CH)

Despite the physiological and pharmacological importance of G protein-coupled receptors (GPCRs), receptor activation and its translation into cytoplasmic trafficking and cellular response remain elusive. In this project, we study the interplay between signaling and trafficking of serotonin receptors 5-HT2c after stimulation. We use RAB GTPases as markers of intracellular compartments to monitor the dynamic distribution of receptors after stimulation and ERK phosphorylation to monitor signaling output. In order to obtain statistically significant trafficking data and high temporal resolution we developed the "Squash" image analysis software for automatic vesicles segmentation, counting, and colocalization computation [Rizk et al., Nature Protocols 2014]. Based on the receptor localization data, signaling data and previous work on the modeling of GPCR activated signaling pathways [Heitzler et al., MSB 2012] we developed an ordinary differential equation model combining signaling with receptor internalization and transport to early, recycling and late endosomes. This is to our knowledge the first attempt in developing a dynamic trafficking model for a GPCR. We evaluate trafficking influence on signaling by conducting global sensitivity analysis and use the model to test hypotheses on receptor constitutive internalization, trafficking regulation and signaling from endosomes.
4D Analysis of Neural Stem Cell Commitment in the Developing Telencephalon

Marion Betizeau¹, Tanzila Mukhtar², Jannik Vollmer¹, Verdon Taylor², Dagmar Iber¹
¹Department of Biosystems Science and Engineering (D-BSSE), ETH Zurich (CH);
²Embryology and Stem Cell Biology, Department of Biomedicine, University of Basel (CH)

During brain development, neural stem cells (NSCs) follow an intrinsic differentiation program and integrate external cues to generate the proper number of neurons at the right time and localization. Given the complexity of the process, an integrated understanding of the many interacting processes has not been achieved so far. By combining state-of-the-art quantitative biology and mathematical modeling techniques we seek to gain a deeper insight into the regulatory mechanisms that control NSC fate. The Notch pathway is pivotal in NSC fate regulation as it mediates communication between neighboring cells, integrates extracellular cues and regulates intrinsic genetic regulation. Notch is a trans-membrane protein and signals through proteolytic release of the Notch intracellular domain (NICD), which diffuses to the nucleus and enhances transcription of target genes including Hes5. Hes5 expression is oscillatory due to negative feedback regulation. We constructed a mathematical model of Notch signaling in NSCs based on published reaction kinetics and measured NICD half-life and diffusion coefficient. This model enables a better and deeper understanding of the Notch signaling pathway in NSCs and will be a powerful tool to study cell fate commitment during brain development.
Systems analysis of cellular cholesterol regulation using mass spectrometry-based targeted proteomics and network modeling

Peter Blattmann¹, Michael Zimmermann¹, Johan Auwerx², Julio Saez-Rodriguez³, Ruedi Aebersold¹,⁴
¹Institute of Molecular Systems Biology, ETH Zurich (CH); ²Laboratory of Integrative and Systems Physiology, EPF Lausanne (CH); ³European Molecular Biology Laboratory, European Bioinformatics Institute (Germany); ⁴University of Zurich (CH)

Cellular cholesterol regulation is a complex biological process underlying non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease. Different pathways and transcription factors regulating cellular cholesterol synthesis and uptake are known. However, an established quantitative and mechanistic model describing how different genetic or pharmacological perturbations affect this clinical relevant biological process is missing. Furthermore, to our knowledge no study systematically analyzed this process on a proteomic level.

Using a network-based approach supported by precise quantitative proteomic data, we generated a model for cellular cholesterol regulation. Several different perturbations were applied to four different human cell lines and the response at the proteomic level was measured. The abundance of >2000 proteins was quantified using advanced targeted proteomics (SWATH-MS). Based on this high-content data, showing both differential and common responses among the cell lines, a logic network model was generated explaining mechanistically the observed changes in the proteome.

The different parameters of the models from four cell lines can be compared and correlated to the abundance of different metabolites. This shows how perturbations affect the proteome and result in perturbed metabolic profiles. A quantitatively and mechanistic understanding of cellular cholesterol regulation is crucial to understand pathogenic mechanisms resulting in NAFLD or cardiovascular disease.
A sentinel protein assay for the quantification of cellular process activities using PRM and DIA

Paul Boersema, Martin Soste, Rita Hrabakova, Paola Picotti
ETH, Zurich (CH)

We developed a novel proteomic screening approach that is based on the concept of sentinels which are biological markers whose change in abundance characterizes the activation state of a given pathway or functional module in a cell. Sentinels can be specific proteins, phosphorylation sites or degradation products and an assembled panel of these sentinels can be targeted in a single LC-MS run.

Yeast cells were sampled under various conditions. Extracted protein lysates were digested and analyzed by MS using Parallel Reaction Monitoring (PRM) and Data Independent Acquisition (DIA).

Based on literature evidence and computational prediction, we selected a panel of 309 sentinels that covers 182 different cellular processes to probe the physiology of yeast cells under different conditions. Here, we tested different PRM and DIA acquisition methods for the measurement of the sentinel assay.

The sentinel assay precisely quantified the activity of many different cellular processes in a single LC-MS run, thereby providing a rapid, system-wide snapshot of the physiology if yeast cells across the different conditions. Rather than obtaining a large amount of complex and redundant data as when striving for the highest numbers of identifications, the sentinel assay provides a condensed and information-rich snapshot.
Heterotrophic bacteria can use a wide variety of carbohydrates as the sole source for carbon and energy generation. To optimize growth, bacteria continuously have to adapt their metabolism to the availability of nutrients in their environment. However, a clear picture on the ability to catabolize carbon sources upon instant switches is still missing. Therefore, we need to elucidate how the different regulatory mechanisms are coordinated and how they control metabolic fluxes directly after the switch.

Here we ask to which extend Escherichia coli and Bacillus subtilis can instantaneously take up and catabolize a glycolitic or gluconeogentic carbon source. Notably, we exchanged one carbon source in excess with another 13C-labeled carbon source and thereby allowing us to follow label integration or dilution as a direct readout for flux reversal. Moreover, we switched carbon sources at a sufficiently short time scale (<1min) to exclude regulation at the level of enzyme expression. A glycolytic flux reversion at this time scale can be assigned to allosteric regulation, while other regulatory mechanisms would require more time.

We found that flux reversal from glycolysis to gluconeogenesis is as well in E.coli as in B.subtilis primarily regulated by transcription. From gluconeogenesis to glycolysis E.coli reverses its flux on the allosteric time scale, whereas B.subtilis controls flux reversal transcriptionally.
Quantitative proteomics and phospho-proteomics of human liver cancer biopsies to study signaling pathways operating in a tumor during cancer therapy

Eva Dazert\textsuperscript{1}, Zuzanna Makowska\textsuperscript{2}, Marco Colombi\textsuperscript{1}, Suzette Moes\textsuperscript{1}, Fabiana Lüönd\textsuperscript{1}, David Adametz\textsuperscript{3}, Luigi Terracciano\textsuperscript{5}, Volker Roth\textsuperscript{3}, Markus Heim\textsuperscript{2,4}, Paul Jenö\textsuperscript{1}, Michael N. Hall\textsuperscript{1}

\textsuperscript{1}Biozentrum, University of Basel (CH); \textsuperscript{2}Department of Biomedicine, University Hospital Basel (CH); \textsuperscript{3}Department of Mathematics and Computer Science, University of Basel (CH); \textsuperscript{4}Division of Gastroenterology and Hepatology, University Hospital Basel (CH); \textsuperscript{5}Molecular Pathology Division, University Hospital Basel (CH)

Cancer treatment is still hindered by aberrant activity of proliferative pathways in tumors and high rate of resistance against treatment. We developed a workflow to perform quantitative proteomics and phospho-proteomics from liver cancer (hepatocellular carcinoma, HCC) patient biopsies of usually very low protein amount (1mg) based on the Super-SILAC method to elucidate molecular mechanisms of resistance. Applying our workflow to a hemochromatosis patient biopsy we could quantify 5000 proteins and 8000 phospho-sites. Finally, we used our workflow to study control and tumor biopsies taken from a HCC patient before and during treatment with the Raf & angiogenesis inhibitor Sorafenib. Prominent carcinogenic pathways were covered and true biological variation over a background of experimental variation detected. Well-characterized HCC biomarkers, e.g. glutamine synthetase were elevated in the tumor. Generating a MAPK pathway map, we provide data that some targets of Sorafenib, e.g. Rsk were inhibited, while further downstream targets, e.g. FilaminA were not. Hierarchical clustering identified similarly regulated protein groups as potential resistance factors. Enrichment analysis indicated that pathways involved in resistance, e.g. down-regulation of cell adhesion, were enriched after treatment. Our results thus prove the power of our workflow for quantitative analysis of molecular changes in patients during cancer treatment.
Role of KAP1 during primordial germ cell reprogramming

Alberto De Iaco, Didier Trono
EPF Lausanne (CH)

The genome of higher vertebrates encodes for around 400 KRAB-ZFPs. These proteins can bind DNA sequences and recruit the transcriptional repressor KRAB-associated protein 1 (KAP1 or TRIM28) that induces heterochromatin formation by activating factors able to promote repressive histone modifications or DNA methylation. For example, KAP1 was shown to be involved in maintaining repressive epigenetic marks on imprinting control regions (ICRs) and some endogenous retroviruses (ERVs) during genome wide epigenetic reprogramming in mouse pre-implantation development. Mouse primordial germ cells (PGCs) also go through a genome wide reprogramming where CpG methylations are maintained just in proximity of ERV sequences. Since KAP1 is highly expressed at these stages of PGC development, we suspect that the repressor might be involved in the maintenance of the repressive marks on ERVs. We intend to address the role of KAP1 during mouse PGC reprogramming by conditionally knocking-out the repressor with an inducible system. We will then study the effect of KAP1 depletion on expression level and epigenetic state of ERVs.
Mechanisms of centrosome separation in C. elegans

Alessandro De Simone, Pierre Gönczy
ISREC - EPF Lausanne (CH)

The centrosome is the major microtubule-organizing center of animal cells and is crucial notably in spindle assembly, thus contributing to chromosome segregation. A key step in spindle assembly is centrosome separation, which occurs during prophase as the two centrosomes separate along the nucleus. In several systems, the plus-end directed microtubule-associated motor kinesin-5 drives centrosome separation. However, in some organisms, including C. elegans and Dycostelium, kinesin-5 depletion does not prevent centrosome separation. Instead, other mechanisms can promote centrosome separation, including ones that rely on the minus-end directed microtubule-associated motor dynein, although where and how dynein powers centrosome separation is not known.

We pursue a combined experimental and modeling approach to decipher the mechanisms governing centrosome separation in C. elegans embryos. We developed a 3D fluorescence time-lapse microscopy and image processing pipeline to track centrosome separation with high temporal and spatial resolution. By comparing wild-type and mutant/RNAi conditions, we identified dynein located at the cell cortex and on the nuclear envelope as the two key drivers of centrosome separation. Moreover, we revealed a critical role for contractions of the cortical actomyosin network in centrosome separation. Importantly, we show also through computer simulations that our model can quantitatively explain centrosome separation.
Cancer cachexia is a multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass. Currently there is no treatment for this condition. Cachexia affects the majority of patients with advanced cancer and is associated with a reduction in survival, treatment tolerance, response to therapy and quality of life. One major aspect of the disease is a long-term activation of inflammation pathways affecting skeletal muscle by induction of muscle atrophy and impairment of muscle regeneration.

In our SystemsX Transfer Project, we characterized myogenesis of primary human skeletal myoblasts under normal and disease mimicking conditions with the goal to elucidate which stage of myogenesis is impaired in cachexia. Our experiments were based on comparing myogenesis of untreated to cytokine treated primary human skeletal myoblasts from fetal, 17 and 83 year old donors.

Global proteome changes of myogenesis were quantified by SWATH-MS and selected proteins quantified between biological replicas using an orthogonal targeted proteomics approach (SRM-MS). Protein arrays were used for in-depth analysis of selected phospho-epitopes. Proteomics data was further confirmed using immunostaining and phenotypic characterization of established myogenesis markers.

Meta-analysis of these datasets clearly demonstrated impairment of myogenesis and uncovered novel markers for precise classification of myogenesis and cachexia.
Investigating the role of calcium in pollen tube growth and reaction to mechanical stresses with Cellular Force Microscopy

Christina Maria Franck\(^1\), Hannes Vogler\(^1\), Dimitris Felekis\(^2\), Aurélien Boisson-Dernier\(^3\), Bradley Nelson\(^2\), Ueli Grossniklaus\(^1\)

\(^1\)Institute of Plant Biology, University of Zurich (CH); \(^2\)Institute of Robotics and Intelligent Systems, ETH Zurich (CH); \(^3\)Botanical Institute, University of Cologne (Germany)

Pollen tubes (PT) are among the fastest polarised growing plant cells. This renders them ideal to study growth dynamics in plants. The coordination of cellular expansion and cell wall (CW) integrity at the pollen tube tip is mediated via the NADPH oxidases Respiratory Burst Oxidase Homologues H and J (AtRbohH, AtRbohJ). Double mutants affecting these two homologues are characterized by abolished calcium homeostasis at the PT tip, growth oscillations, and premature PT rupturing. Using cellular force microscopy (CFM) in conjunction with fluorescence microscopy, we conducted stiffness measurements on rboh mutants and evaluated mechanical stress effects on calcium dynamics. The apparent stiffness of rbohJ and rbohH rbohJ PTs is decreased compared to the wild type, revealing RBOHJ as the main contributor to apparent stiffness. Increasing calcium concentration in the growth medium greatly diminished the rbohH rbohJ mutant phenotype. Applying a mechanical stimulus on pollen tubes expressing the calcium sensor YC3.60, we observed a strong calcium flush at the PT tip upon stimulus retraction. This suggests the presence of yet unknown mechanosensitive channels. Our results demonstrate that biochemical and biophysical CW properties in Arabidopsis pollen tubes are tightly linked.
Analysis of Ski as a regulator of neural stem cell transcriptome dynamics

**Alice Grison**¹, Tanzila Mukhtar¹, Philipp Berninger², Katja Eschbach³, Erik van Nimwegen², Christian Beisel³, Verdon Taylor¹, Suzana Atanasoski¹

¹Department of Biomedicine, University of Basel (CH); ²Biozentrum, University of Basel; ³Quantitative Genomics Unit, D-BSSE, ETH Zurich (CH)

During cortical development many genes and signaling pathways are active but their functions and interconnections are poorly understood. The protooncogene Ski is a central integrator of signal and transcriptional functions through interactions with different partners.

Ski is present in proliferating progenitors cells in the ventricular zone and in subtypes of differentiated neurons of the cortical plate. Deletion of Ski results in precocious differentiation of neuronal stem cells leading to a reduced progenitor pool at early stages of cortical development. In addition, loss of Ski leads to a precocious exit of neural stem cells from the cell cycle disturbing the timing of their differentiation.

The aim of the project is to use systems biology to dissect the signaling pathways and transcriptional networks regulated by Ski during early cortical development. To compare the dynamics of various pathways in the presence and absence of Ski, we use in vivo transgenic marking of Ski-deficient neural stem cells (Hes5::GFP; Ski-/-) and committed progenitors (Tbr2::GFP; Ski-/-) to isolate and analyze the cells by RNA-seq. Using the ISMARA (Integrated System for Motif Activity Response Analysis), we are scanning the changes in the transcriptional profiles of neuronal and committed progenitors comparing Ski wt and KO samples through development.
Characterization of the hepatocellular metabolome using an integrated sample preparation and analysis platform with SWATH-MS detection

Sandra Jahn, Emmanuel Varesio, Gérard Hopfgartner
University of Geneva (CH)

In metabolomics targeted and non-targeted liquid chromatography (LC) mass spectrometry (MS)-based techniques are commonly applied to plasma, serum or urine samples for identification and quantification of up- or down-regulated compounds. However, the potential biomarkers sought-after and discovered by such approaches may differ from those in the target organ (e.g. liver) if remaining instead of being excreted. Hence, a LC/MS method was developed to characterize the metabolome of hepatocyte cells in combination with primaquine exposure. Rat or human hepatocytes were analyzed with and without different concentrations of primaquine, an anti-malarial drug. A CTC robot was used for the integration of sample preparation and sample analysis based on the Bligh & Dyer approach and a parallel multiple column setting. Aqueous and organic fractions were analyzed via LC/MS. The resulting high resolution SWATH MS2 spectra were submitted to a library search using in-house build libraries to characterize the metabolome of hepatocytes detectable by LC-MS. Additional MS tools (elemental formula, MS fragmentation prediction) were also applied to characterize analytes not identified in the library. In parallel, the metabolism of primaquine in hepatocytes was investigated as well as the effects of the drug and its metabolites on the expression change of the hepatocellular metabolome.
A passive mutualistic interaction promotes the evolution of spatial structure within microbial populations

Marie Marchal, Selina Derksen, David R Johnson, Martin Ackermann
ETH Zurich (CH)

The evolutionary origin of mutually beneficial interactions between species is unclear. The problem is that for mutualistic interactions to emerge an investment into the partner must pay off: individuals of one species that invest resources into the growth of another species must receive a benefit that is not accessible to individuals that do not invest. One way for exclusive benefits to emerge is through spatial structure; that is, through physical barriers to the movement of organisms and resources. However, mutualistic interactions are also observed in the absence of extensive spatial structure, for example between microorganisms in aquatic environments. As a solution to this puzzle, we hypothesize that organisms evolve their own spatial structure based on physical attachment between organisms: and that attachment evolves when proximity to individuals of another species is advantageous. We tested this hypothesis using experimental evolution with consortia of E. coli mutants that depend on each other to grow. We found that attachment between cells readily evolved and that many different mutations potentially contributed to increased attachment. We postulate a general principle by which passive mutualistic interactions between organisms select for attachment, which then provides spatial structure that is conducive for the evolution of active mutualistic interactions.
The role of mechanical stress during pectoral fin formation in Zebrafish

Elena Kardash\textsuperscript{1}, Laurent Holtzer\textsuperscript{1}, Susanne Borgers\textsuperscript{1}, Martin Behrndt\textsuperscript{2}, Carl-Philipp Heisenberg\textsuperscript{2}, Marcos Gonzalez-Gaitan\textsuperscript{1}

\textsuperscript{1}University of Geneva, Biochemistry Department (CH); \textsuperscript{2}Institute of Science and Technology (Austria)

Pectoral fin development in zebrafish is an excellent in vivo model for limb formation in vertebrates owing to the conservation of signaling cascades at work during morphogenesis of these structures. The aim of this work is to elucidate the role of mechanical stress during fin formation. To this end, we study fin growth at the early stages of its formation between 26 and 50 hours post fertilization (hpf).

Between 35 and 45 hpf, the fin shape can be described with a semi-ellipsoid geometry. At 35 hpf, we observe a dense actin structure, which we name “actin belt”, forming at the distal rim of the fin bud along its anterior-posterior axis. Laser ablation experiments showed revealed the tension within actin belt oriented along the anterior-posterior axis. FRET measurements showed elevated Rac activity within actin belt suggesting the role of Rac in the formation of this structure.

Interfering with Rac function led to the loss of actin belt and fin deformation causing the fin to assume an approximate of a hemisphere shape. We propose a model, in which fin shape is determined by the contractile actin structure positioned across a growing mass of cells shaping the fin into a semi-ellipsoid.
Estimating metabolic fluxes in bacteria growing in complex nutritional environments using a machine learning approach

Maria Kogadeeva¹, Uwe Sauer¹, Nicola Zamboni¹

¹Institute of Molecular Systems Biology, ETH Zurich (CH)

Metabolic reaction rates, also called metabolic fluxes, mirror the physiology of a cell and its adaptations to changing environments, such as nutrient availability or exposure to stress. Quantifying metabolic fluxes is key to understand behavioral modes of cells and control bacterial infection.

Intracellular reaction rates are however barely accessible through experimental measurements and hence, modeling approaches are crucial for their estimation. State-of-the-art methods exploit isotopic labeling of substrates to track the label progression through the metabolic network by extensive measurements of metabolites and physiological parameters. We aim at reducing the required information and measurements by using machine learning algorithm random forest to capture the relationship between local fluxes and metabolite labeling patterns. The algorithm is trained on an in silico dataset simulating possible flux distributions and labeling measurements to predict the flux ratio of interest from experimental data.

Modeling different experimental setups allows to optimize experimental design and select the most informative measurements to resolve the flux ratios. We demonstrate the performance of the method on published studies on Escherichia coli and Bacillus subtilis grown on a single carbon source and challenge it to resolve fluxes in Mycobacterium smegmatis co-metabolizing multiple substrates.
Baculovirus-based multi-biosensor expression for quantitative single cell analysis

Maysam Mansouri1, Aurélien Rizk1, Nagjie Aziraj1, Imre Berger2, Kurt Ballmer-Hofer1, Philipp Berger1
1Paul Scherrer Institute (CH); 2EMBL, Grenoble, (France)

Multigene expression systems are key technologies for many applications in biology. Examples include reprogramming of somatic to stem cells in regenerative medicine, constructing complex gene circuits in synthetic biology or monitoring different parameters in cell biology. We previously developed a cre/loxP-based system called MultiLabel, allowing simultaneous expression of several genes from a single plasmid in mammalian cells. Although useful for homogenous co-expression of genes, MultiLabel suffers from low efficiency of transfection. Consequently, we developed a baculovirus-based MultiLabel system which can be used for the infection of primary cells, infection of cell lines that are difficult to transfect, or for in vivo studies. Here, we show that it is possible to express up to five genes with a modified baculovirus in various mammalian cells with high efficiency. Also, we developed different intracellular biosensors which can be expressed simultaneously and allow single cell analysis. For instance, we apply our system for trafficking studies of VEGF receptor and show different fates of receptor inside the cell when stimulated with different ligands through snapshot and live cell imaging. Taken together, baculovirus is an efficient vehicle to deliver multigene plasmids to mammalian cells and we show applications of our system in cell biology
Descriptive and statistical analysis of colour pattern evolution in two species of lizards

Liana Manukyan, Sophie A. Montandon, Michel C. Milinkovitch
University of Geneva (CH)

Squamates (lizards and snakes) exhibit spectacular inter- and intra-specific colour variation generated by pigments (black/brown melanins, yellow and red pigments) and structural elements (guanine nanocrystals causing interference of light waves) incorporated into various types of chromatophores and iridophores, respectively (see e.g., Saenko et al., BMC Biology 2013, 11: 105; and refs therein). The adaptive value of colour variation in animals has been associated with thermoregulation, camouflage, predator avoidance, sexual selection, and speciation, although intra-specific polymorphism in colour traits can also involve pleiotropy, epistatic interactions and stochastic processes. Here, we investigate the shift in colour patterns occurring between juvenile and adult forms of two species of lizards (from the genera Eublepharis and Lacerta). For each individual investigated, we perform high-resolution 3D geometry and colour texture reconstructions at different time points during development from the juvenile to the adult stage. The different time points are non-rigidly aligned after semi-automated scale detection. The time evolution of colour patterns is then recapitulated on the 3D geometry. These analyses form the basis for characterisation and numerical simulation of pattern evolution in squamate reptiles.
How the dragon got its frill: development of a hypertrophied skin fold in Chlamydosaurus.

Sophie A. Montandon, Michel C. Milinkovitch
Department of Genetics and Evolution, University of Geneva (CH)

A variety of skin appendages are found in vertebrates. In addition to a diversity of scale morphologies, squamate reptiles can exhibit skin folds at various locations of their body. For example, some representatives of the Anolis genus possess a colorful skin dewlap used during intraspecific communication. Here, we focus on the evolutionary developmental mechanisms at the origin of a complex, spectacular, and unique trait observed in Chlamydosaurus kingii: a large folded erectile frill. This evolutionary innovation is made of a folded piece of skin supported both by the ceratobranchial I of the hyoid apparatus and by dense connective tissues situated above the tympanic membrane. In order to better understand the mechanisms that lead to the formation of the frill, we investigated, on whole embryos and histological sections, the distribution of cell proliferation, the organization of the cytoskeleton and the potential involvement of mechanical processes at the origin of the formation of the frill and its folds. Furthermore, we use micro-CT scans to correlate skin and hyoid bones development.
Systems analysis of mammalian forebrain development

Tanzila Mukhtar¹, Alice Grison¹, Zahra Ehsaei¹, Philipp Berninger⁴, Zahra Karimadini³, Katja Eschbach², Suzana Atanasoski¹, Christian Beisel, Patrick Fried³, Dagmar Iber³, Savas Tay³, Erik van Nimwegen⁴, Verdon Taylor¹

¹Department of Biomedicine, University of Basel (CH); ²Quantitative Genomics Unit, D-BSSE, ETH Zurich (CH); ³D-BSSE, ETH Zurich (CH); ⁴Biozentrum, University of Basel (CH)

The cerebral cortex of mammals is composed of millions of neurons organized into functionally distinct layers. These different types of neurons originate from what has been proposed to be a homogeneous pool of neural stem cells. We have undertaken a systems biology approach to understand development of the mammalian cerebral cortex, to elucidate the mechanisms controlling neuronal fate and differentiation, and to examine the homogeneity of the telencephalic neural stem cell population (NeuroStemX, SystemsX.ch). Through collaborative wet biology and computational modeling approaches we are deciphering the signaling and transcriptional networks that regulate the formation of cerebral cortical neurons. The control of these networks modulates the regimental differentiation and characterization of the neural stem cells, to pattern the complex six-layered structure of the cerebral cortex. Our hypothesis and preliminary data suggest that neural stem cells, rather than being homogeneous, are a heterogeneous population which vary in their transcriptional output over time and this renders them sensitive to extrinsic and intrinsic cues. The integration of the intrinsic and extrinsic signals controls neuron production and fate. A comprehensive understanding of transcriptional regulation and its interplay with an ensemble of upstream factors will pave the way for regeneration of cortical neurons and structures following disease and could have implications for cellular therapy and drug screening.
Mammalian genomes encode thousands of long noncoding RNAs (IncRNAs), but very few of these have been functionally characterized. Large-scale evolutionary analyses of IncRNAs can provide important insights into their functionality. However, evolutionary analyses of IncRNAs (and of gene expression in general) have so far been restricted to comparisons of adult transcriptomes, although most phenotypically-relevant expression patterns are likely associated with embryonic development. Here, we investigate the functionality of IncRNAs in mammalian development and aging by performing a comprehensive, multi-dimensional comparative transcriptomics study. We explore the transcriptomes of two model organisms (mouse and rat), across four major organs (brain, kidney, liver and testes), and five developmental stages (including two embryonic stages, newborn, adult and aged individuals). We show that, although both coding and noncoding transcriptomes are strongly developmentally-regulated, IncRNAs are more often subject to developmental regulation than protein-coding genes. In particular, evolutionarily conserved IncRNAs are preferentially expressed in embryonic and newborn stages. Furthermore, the extent of evolutionary conservation of coding and noncoding transcription varies among organs and developmental stages, with most selective constraint acting during early brain organogenesis. Our results indicate that IncRNAs may function predominantly during early development, suggesting an important new direction in the search for IncRNA functionality.
Cellular hallmarks reveal critical contribution of aerobic metabolism at the thermal limits

Aitana Neves, Pierre Gönczy
EPF Lausanne (CH)

All organisms live within a given thermal range (TR) delimited by lower and upper thermal limits (TLs), but little is known about the underlying mechanisms. Here, we set out to uncover such mechanisms by identifying cellular processes affected at TLs. Using quantitative time-lapse microscopy of C. elegans embryos, we discovered that mitosis duration, cell division asymmetry, and embryo surface to volume ratio (SVR) are cellular hallmarks whose thermal response changed beyond the TLs. We found these changes to be also present beyond the TLs in C. briggsae, indicating evolutionary conservation. We hypothesized that the observed increase in SVR beyond TLs reflected the need to increase aerobic metabolism via oxygen diffusion. To address this possibility, we used atp-2(RNAi) to deplete ATP levels. Remarkably, we found that the resulting embryos exhibited increased SVR already within the TR. We conclude that restricted aerobic metabolism contributes to setting the TLs in a metazoan organism.
Wireless Magnetic Nanoprobes as Tools for Characterizing Pollen Tube Biomechanics

Ulrike Nienhaus\textsuperscript{1}, André Lindo\textsuperscript{2}, Bumjin Jang\textsuperscript{2}, Bradley J. Nelson\textsuperscript{2}, Ueli Grossniklaus\textsuperscript{1}

\textsuperscript{1}Institute of Plant Biology, University of Zurich (CH); \textsuperscript{2}Institute of Robotics and Intelligent Systems, ETH Zurich (CH)

Mechanical forces have been shown to play a role in cellular processes but the exact mechanisms have not yet been elucidated. In order to better understand the influence of said forces on the development as well as the function of cells and tissues, the mechanical and physical properties of such systems need to be known. The aim of this project lies in the development of a new method with which to measure and manipulate the mechanophysical properties of a model system, namely the pollen tube, internally. To this effect, a fluorescent magnetic nanoprobe will be introduced into the pollen tube via microinjection. A magnetic manipulation system will then be used to maneuver the nanoprobe within the pollen tube, giving insight into the viscoelastic properties of, e.g., the cytoplasm. This approach also allows for the assessment of the effects of certain cellular processes on pollen tube growth via the possibility of the targeted manipulation of the processes. For example, cytoplasmic streaming within the pollen tube may be disrupted using rotating nanowires. The results gained using this approach will be invaluable to the formulation of mathematical models describing the biophysical behavior of pollen tubes.
TubeX: multiscale network control of microtubule organization and dynamics

Yves Barral¹, Mathias Bayer¹, Xiuzhen Chen¹, Ana-Maria Farcas¹, Anil Kumar⁴, Jette Lengefeld¹, Natacha Olieric⁴, Grégory Paul³, Andrea Prota⁴, Denis K. Samuylov³, Michel Steinmetz⁴, Jörg Steinmetz², Oliwia Szklarczyk³, Gábor Székely³, Lukas Widmer²
¹Department of Biology, ETH Zurich (CH); ²D-BSSE, ETH Zurich (CH); ³D-ITET, ETH Zurich (CH); ⁴Paul Scherrer Institute (CH)

Microtubule networks play a crucial role in organizing the cytoplasm, in positioning organelles relative to each other, and in guiding cellular polarity. A current challenge is to understand and model how the nature and dynamics of cytoskeletal networks, which takes place at the molecular scale, are controlled at the cellular scale. The goal of TubeX is to develop and use sophisticated modeling tools to investigate and describe the molecular principles underlying the multiscale organization and dynamics of microtubule networks, using yeast as a model system.

TubeX is divided into three subprojects involving four teams, to tackle in an interdisciplinary manner how microtubule networks function at the system level, and how regulation takes place to coordinate events throughout the different scales of the network.

While the microscopic and atomic scales, i.e., the structural and cellular levels, are amenable to observation, using crystallography and microscopy, respectively, the nanoscopic scale, i.e., the molecular events taking place in the cell, remains largely out of reach. The three subprojects together aim at understanding how molecular networks coordinate events between these different scales. Based on extensive in silico modeling, data analysis, and quantitative experimental approaches each subproject addresses one aspect of this problem.
Cortical tension and stiffness during asymmetric cell division

Tri Pham¹, Jonne Helenius², Daniel Müller², Clemens Cabernard¹
¹Biozentrum, University of Basel (CH); ²D-BSSE, ETH Zurich (CH)

Asymmetric cell division (ACD) generates cellular diversity and is an 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 generate physical asymmetry. We use Drosophila neural stem cells (neuroblasts) to study the contribution of biophysical parameters on ACD. We are combining fluorescence microscopy with atomic force microscopy (AFM) to measure the dynamics of the actomyosin network and cortical stiffness of cultured neuroblasts. Our measurements indicate that cortical stiffness gradually increases during metaphase before it suddenly drops at early anaphase and then quickly increases to a maximum value at mid anaphase. Interestingly, we detect high stiffness values on the apical cortex although Myosin is barely detectable in this region. Since our results suggest that cortical stiffness does not necessarily correlate with Myosin levels, we are currently using Particle image velocimetry (PIV) to measure cytoplasmic streaming. The combination of these measurements will allow us to propose a model, explaining how changes in physical parameters contribute to the establishment of sibling cell size differences during mitosis.
How to design reusable bioimaging pipelines we can trust? Lessons from microtubule dynamics tracking in Saccharomyces cerevisiae

Denis K. Samuylov, Xiuzhen Chen, Yves Barral, Gábor Székely, Grégory Paul
ETH Zurich (CH)

Reconstructing biological objects from microscopy data is an ill-posed problem due to limits in the spatiotemporal resolution of imaging devices. Nonetheless, reconstructing large, immobile structures is nowadays a problem with a wealth of satisfactory solutions. On the contrary, estimating the dynamics of objects with a spatiotemporal resolution below the imaging setup accuracy remains challenging. We report the first insights we gained from the reconstruction of in vivo microtubule dynamics in the budding yeast. First, the details of the image formation model and of the object model matter in order to disentangle the relevant biological signal from imaging artifacts. Second, a simulation engine for generating realistic artificial image data is extremely helpful in understanding imaging artifacts, in assessing existing reconstruction pipelines, and in optimizing imaging setup. Finally, designing and releasing efficient, modular and reusable software is an essential part of the development of a bioimage analysis pipeline. We demonstrate the importance of these best software engineering practices in developing a virtual microscope and a microtubule tracking plugin for FIJI with reusable components.
Cell-type specific transcriptome analysis identifies genes and regulatory pathways differentially expressed during sexual and apomictic reproduction

Anja Schmidt¹, Marc W. Schmid¹, Ulrich C. Klostermeier², Weihong Qi³, Daniela Guthörl¹, Christian Sailer¹, Manuel Waller¹, Philip Rosenstiel², Ueli Grossniklaus¹

¹Institute of Plant Biology & Plant Science Centre, University of Zurich (CH); ²Institute of Clinical Molecular Biology, Christian-Albrechts University, Kiel (Germany); ³Functional Genomics Center Zurich, UZH/ETH Zurich (CH)

In higher plants, the female reproductive lineage (germline) is formed only late during development in specialized reproductive flower tissues, the ovules. Typically, a single sporophytic cell of the ovule gets selected for reproductive fate, marking a key step of the plant life cycle. During sexual reproduction, this megaspore mother cell is committed to meiosis, while it omits or aborts meiosis during apomixis (asexual reproduction). Subsequently, the apomictic egg cell develops parthenogenetically (without fertilization) into an embryo. Thus, apomixis leads to the fixation of the maternal genotype in the offspring and therefore has a great potential for agricultural use. However, knowledge about the genetic basis underlying sexual and apomictic reproduction remains limiting, hampering targeted manipulation of plant reproduction for crop improvement.

To gain insights into the transcriptional basis underlying plant germline formation, we recently performed cell type specific transcriptome analyses in sexual Arabidopsis thaliana and in Boechera gunnisoniana, a closely related apomictic species. In a comparative data analysis we identified a number of genes and regulatory pathways differentially expressed during sexual and apomictic reproduction, including hormonal pathways, cell-cycle regulation, and epigenetic regulatory pathways. Our dataset provides a useful tool to identify candidate genes of potential importance for apomixis.
Wnt directs the endosomal flux of LDL-derived cholesterol and lipid droplet homeostasis

Cameron Scott¹, Stefania Vossio¹, Fabrizio Vacca¹, Berend Snijder², Jorge Larios¹, Olivier Schaad¹, Nicolas Guex³, Dimitri Kuznetsov³, Olivier Martin³, Marc Chambon⁴, Gerardo Turcatti⁴, Lucas Pelkmans², Jean Gruenberg¹

¹University of Geneva (CH); ²University of Zurich (CH); ³Vital-IT group, SIB Swiss Institute of Bioinformatics (CH); ⁴Biomolecular Screening Facility, EPF Lausanne (CH)

The Wnt pathway, which controls crucial steps of the development and differentiation programs, has been proposed to influence lipid storage and homeostasis. Using an unbiased strategy based on high-content genome-wide RNAi screens that monitored cellular lipid distribution and amounts, we find that Wnt3a regulates cellular cholesterol. Using quantitative microscopy, biochemical and mass spectrometry techniques, we find that Wnt3a stimulates the production of lipid droplets, and that this stimulation strictly depends on endocytosed, LDL-derived cholesterol and on functional early and late endosomes. Further, by using a transcriptome analysis coupled with cell biological approaches we find that Wnt signaling itself controls cholesterol endocytosis and flux along the endosomal pathway, which in turn modulates cellular lipid homeostasis. We also find this Wnt response present in a hepatocyte cell model highlighting the importance of this response in controlling lipid physiology in agreement with previously published animal studies. Together, these results underscore the importance of endosome functions for lipid droplet formation and reveal a previously unknown cellular program controlling lipid storage and endosome transport under the control of Wnt signaling.
In-depth quantitative analysis of 3D human liver proteome for drug toxicity investigation

Nathalie Selevsek¹, Jonas Grossmann¹, Paolo Nanni¹, Claudia Fortes¹, Patrina Gunness², Jens Kelm², Ralph Schlapbach¹
¹Functional Genomics Center Zurich (CH); ²InSphero AG, Schlieren (CH)

Drug-induced hepatotoxicity is currently one of the main reasons for market withdrawal of drugs and exclusion of drugs in clinical phases. Recently, 3D microtissues have shown to be suitable for testing chronic exposure toxicity compared to 2D cells, due to their longer lifespans and greater stability.

To demonstrate the relevance of the spheroids for investigating toxicity of drug compounds at the proteome level, we analyzed by shotgun proteomics in depth-fractionated protein digests isolated from 3D human liver microtissues to deliver a first view in sample complexity and protein dynamic range of the human liver proteome. In parallel, we applied label-free quantitative approach for the profiling of protein abundances from 3D liver spheroids treated with different concentrations of acetaminophen. We obtained high reproducibility in protein abundances between biochemical replicates and across all tested acetaminophen concentrations. Thus we generated quantitative profiles for more than four thousands of proteins in response to acetaminophen treatment, revealing dozens of proteins affected by that drug. Several protein pathways were identified, providing a better understanding in the mechanism of drug toxicity.

In conclusion, the results demonstrated that proteomic analysis of 3D human liver microtissues are suitable for investigating drug toxicity and can be applied to other 3D tissue models.
Super-resolution imaging of synapses involved in learning and forgetting

Isabelle Spühler, Gaurasundar Conley, Ricardo Armenta, Frank Scheffold, Simon Sprecher
University of Fribourg (CH)

Is it possible to image learning and forgetting? Formation of memories during the learning process implies modifications in synapses, due to the plasticity of the nervous system. Still unclear is whether analogous changes underlie the process of memory loss. We want to study structural changes, at the synaptic level, related to forgetting. As a model system we chose the olfactory memory system of the Drosophila brain. The fly brain has well defined regions involved in learning and associative memory, such as the neuropil of the mushroom bodies as well as the neurons connected to them; moreover it provides great advantages in manipulating the expression of genes in specific cells. These advantages, as well as the resolution required to observe changes within synapses, make single molecule localization microscopy the ideal technique for our study. We are working on the implementation of 3D Multi Color Stochastic Optical Reconstruction Microscopy (STORM) to image structures and processes within the neural tissue.
Development of microfluidic devices for high-throughput functional cell assay

Zuzana Tatarova, Sebastian Maerkl, Joerg Huelsken
EPF Lausanne (CH)

The cellular processes of migration and chemoattraction comprise key mechanisms contributing to pathological phenomena such as wound healing and tumor metastasis. Currently, macroscale and on-chip migration assays allow quantification of chemotaxis in response to an individual protein, however, they are not adopted for large screening purpose. We aim to integrate protein production from a library for all secreted factors available in the mammalian genome together with a migration assay on a microfluidic device. On this biochip, massive parallel “reverse transfection” of cDNA clones into producer cells will allow to assay hundreds of candidates simultaneously. Moreover, the small scale will enable us to perform chemotaxis assays with populations of rare cells such as stem cells. We developed microfluidic devices enabling co-culture experiments of primary mesenchymal stem cells with one or two producer cell lines respectively. Production of a test protein that approximates the protein secretion and diffusion showed uniform protein gradient formation on-chip allowing this system to be used for further reverse transfection experiments. Current designs can test up to 20 conditions with the aim to be scaled up as mentioned already. Taken together, we foresee the discovery of relevant secreted factors that are essential for the formation of tumor stroma or the establishment of fibrosis.
Effect of S-palmitoylation on HeLa cells lipidome

María Eugenia Zaballa¹, Sylvia Ho¹, Jonathan Paz Montoya², Aline Santos³, Marc Moniatte², F. Gisou van der Goot¹

¹Global Health Institute, EPF Lausanne (CH); ²Proteomics Core Facility, EPF Lausanne (CH); ³Biochemistry Department, University of Geneva (CH)

S-palmitoylation is the only known reversible post-translational lipid modification and implies the addition of a C16 acyl chain to specific cysteines in the target protein. The incorporation of the acyl chain is mediated by a family of enzymes called Protein Acyl Transferases (PATs) whereas the depalmitoylation is mediated by Acyl Protein Thioesterases (APTs). S-palmitoylation is involved in signaling, protein localization and ubiquitination, synaptic activity, lipid homeostasis and membrane trafficking, among other critical processes. Particularly, many lipid-related enzymes and regulatory proteins have been shown or suggested to be palmitoylated.

In this project we have evaluated the effect of S-palmitoylation on the lipid composition of HeLa cells. As a first screening, we have individually silenced each of the 23 PAT-coding genes of the human genome by using shRNA or siRNA and we have performed mass spectrometry-based lipidomics analysis on them. In particular, we have applied a multiple reaction monitoring (MRM) scheme using a list of around 1000 polar lipid species. Our preliminary results show that for most of the lipid classes under analysis, although a few significant differences can be detected, the lipid composition of the whole cell is tolerant to individual PAT silencing.
Functional Genomics and Gene Regulation

Chair:
Bart Deplancke
Laboratory of Systems Biology and Genetics
EPF Lausanne
Our laboratory uses both integrated structural biology methods and functional genomics to elucidate the mechanisms of genome transcription and its regulation in eukaryotic cells. Recent structural work from the laboratory includes the structures of the Mediator head module (Lariviere et al., Nature 2012), RNA polymerase II in form of an initially transcribing complex with the initiation factors TFIIB (Sainsbury et al., Nature 2013), and of the complete 14-subunit RNA polymerase I enzyme (Engel et al. Nature 2013). Recent results from functional genomics include the description of a cellular mechanism that buffers mRNA levels by compensating changes in mRNA synthesis and degradation rates (Sun et al., Genome Research 2012) and the discovery of the Xrn1 nuclease as a key enzyme in this homeostatic control (Sun et al., Molecular Cell 2013) as well as the genome-wide description of specific termination of non-coding RNA synthesis that underlies transcriptome surveillance (Schulz et al. Cell 2013). In my presentation I will concentrate on unpublished data that provide our most recent insights into the recognition of pre-mRNA during transcription and its consequences for mRNA biogenesis.
C2H2 zinc finger proteins greatly expand the human regulatory lexicon

Tim Hughes
University of Toronto (Canada)

C2H2-ZFs represent the largest class of putative transcription factors (TFs) in human and most other vertebrates, but it is unknown whether most of the ~700 human C2H2-ZFs even bind DNA, what sequences they bind, or what genes they regulate. We find that most natural C2H2-ZFs bind DNA both in vitro and in vivo, and infer a new DNA recognition code using DNA-binding motifs for thousands of natural C2H2-ZFs. In vivo binding data, obtained by ChIP-seq, is consistent with our recognition code. The high diversity of motifs obtained indicates that C2H2-ZF proteins encode the majority of motifs among human TFs. For the first time, we provide direct evidence that most of the ~350 C2H2-ZF proteins that also contain a repressive KRAB domain bind predominantly to specific classes of endogenous retroelements (EREs). Curiously, the EREs bound encompass both currently active and ancient families, suggesting that their function may go beyond transcriptional silencing. The majority of C2H2-ZF proteins, including many KRAB proteins, show widespread binding to regulatory regions, and the genes bound represent a wide variety of physiological functions. Thus, vertebrates contain an extensive and largely unstudied adaptive C2H2-ZF regulatory network that targets a diverse range of genes and pathways.
Expression noise facilitates the evolution of gene regulation

Erik van Nimwegen
University of Basel (CH)

In studies of gene regulation, it is often tacitly assumed that the interactions between transcriptional regulators and their target promoters are finely tuned to ensure condition-appropriate gene expression of the targets. However, how natural selection might evolve such precise regulation from an initial state without regulation, is rarely discussed. Using an combined experimental and theoretical approach, which included evolving synthetic E. coli promoters in the lab and comparing these with native E. coli promoters, we show that the transmission of noise from regulators to their targets is often beneficial and may even constitute the main function of coupling a promoter to a regulator. Our theory provides a novel framework for understanding the evolution of gene regulation, demonstrating that in many situations expression noise is not the mere unwanted side-effect of regulatory interactions, but a beneficial function that is key to the evolvability of regulatory interactions.
MiRNAs are 21-22 nucleotides long RNA regulators of gene expression that are necessary for the proper unfolding of processes ranging from cell differentiation and development to the response of tissues to stimuli. At the molecular level, miRNAs guide ribonucleoprotein complexes that also contain a member of the Argonaute protein family to target mRNAs, to repress their expression. Initial studies suggested that miRNA reduce the translation rate of their targets, but more recently, it has become apparent that miRNAs increase the rate of target degradation. This in turn can have various consequences. For instance, it has been proposed that miRNAs reduce the 'noise' in target expression, establish thresholds and induce correlations in the expression of their various targets. Computational models have been instrumental in unraveling the principles of miRNA-target interaction from the very beginning.

Recently, we have used large sets of experimentally identified miRNA-guided Argonaute interaction sites to infer a biophysical model of miRNA-target interaction. Building on this model, we have further developed a method for accurate, genome-wide prediction of binding sites not only of miRNAs, but also of small interfering RNAs. These molecules are broadly used to repress gene expression but have important side effects via the miRNA pathway. Combining single cell and population level measurements with computational modeling we investigate the aspects of gene expression that are most crucially regulated by miRNAs.
Systematic characterization of the KRAB zinc finger proteins family using high-resolution ChIP-Seq

Michael Imbeault, Pierre-Yves Helleboid, Didier Trono
EPF Lausanne (CH)

With about 350 members in human, the tetrapod-restricted KRAB zinc finger (KRABs-ZNFs) proteins family constitutes the largest group of transcription factors encoded by higher vertebrates. In spite of their numerical importance, these proteins remain largely uncharacterized, although cumulated evidence indicates that they can induce the formation of heterochromatin by recruiting the KAP1 corepressor to specific genomic loci. This process has been shown to play a major role in the silencing of endogenous retroelements in embryonic stem cells. Here, we present results from the first phase of a large scale effort to delineate binding sites of human KRAB-ZNFs using ChIP-exo through transduction of a library of HA-tagged factors in 293T. Interestingly, we show that ChIP-exo of the corepressor KAP1 in various cell types yields the basepair level signatures of the underlying KRAB-ZNFs. The overlap of results from overexpressed HA-tagged factors to those of endogenous KRAB-ZNFs obtained via KAP1 ChIP-exo in relevant cellular contexts, such as embryonic stem cells, completely eliminates the caveats commonly brought by overexpression of DNA transcription factors. Overall, results accumulated so far significantly improve our understanding of the KRAB-ZNFs family and yield novel insights in the function of the KRAB-KAP1 system consistent with its evolutionary dynamics.
MITOMI-seq: a novel tool enabling the parallel characterization of DNA binding specificities of TF monomers and heterodimers

Alina Isakova, Philipp Bucher, Bart Deplancke
EPF Lausanne (CH)

Understanding the DNA binding properties of transcription factors (TFs) is crucial for the reverse engineering of gene regulatory networks from genomic data. Here we present a novel technology – MITOMI-seq – that allows us to determine the DNA binding specificities of TF monomers, homo- and heterodimers in a fast, robust and a cost-effective manner. The core of this technology is a microfluidic platform that performs selection of DNA specifically bound by TFs from a pool of randomized sequences. Coupled to high-throughput sequencing, this platform allows the characterization of TF DNA binding preferences at an unprecedented resolution. Unlike other, already established in vitro technologies that also aim to determine TF binding specificities, MITOMI-seq operates at micro scale and requires minute amounts of biological material, but produces specificity models that characterize even low-affinity and transient molecular interactions. Performing de novo motif discovery on MITOMI-seq data, we derived binding models for a number of TF monomers and heterodimers that generally agree with the TF binding models identified by ChIP-seq or by other comparable in vitro methods. In addition, for a number of factors, we uncovered binding motifs that were so far never reported thus expanding the repertoire of quantitative specificity models for TFs originating from various model organisms and humans.
Massive parallel genome engineering identifies transcription factor binding as a principal mechanism controlling DNA methylation states of CpG islands

**Arnaud Krebs**, Sophie Dessus-Babus, Lukas Burger, Dirk Schuebeler
*Friedrich Miescher Institute, Basel (CH)*

The majority of mammalian promoters are CpG islands; regions of high CG density that require protection from DNA methylation in order to be functional. Importantly, how sequence architecture mediates this unmethylated state remains unclear. To address this question in a comprehensive manner, we developed a method to interrogate methylation state at hundreds of sequence variants inserted at the same genomic site in mouse embryonic stem cells. Using this assay, we were able to quantify the contribution of various sequence motifs towards the resulting DNA methylation state. Surprisingly, modeling of this comprehensive dataset revealed that CG density alone is a minor determinant of their unmethylated state. Instead, these data argue for a principal role for transcription factors, a prediction confirmed by testing synthetic mutant libraries. Taken together, these findings establish the hierarchy between the two cis-encoded mechanisms that define the DNA methylation state and thus the transcriptional competence of CpG islands.
KAP1/TRIM28 is essential for the homing and homeostatic maintenance of hematopoietic stem cells

Isabelle Barde¹, Benjamin Yazdanpanah¹, Elisa Laurenti², Aline Roch¹, Andrea Corsinotti¹, Adamandia Kapopoulou¹, Sonia Verp¹, Sandra Offner¹, Michael Imbeault¹, Matthias Lutolf¹, Didier Trono¹

¹EPF Lausanne (CH); ²University Health Network, Toronto (Canada)

KRAB-containing zinc finger proteins (KRAB-ZFPs) constitute the largest group of transcriptional repressors encoded by the genomes of higher vertebrates, and together with their cofactor TRIM28/KAP1 have been implicated in the early embryonic silencing of endogenous retroelements. Here, we reveal that many KRAB-ZFP are expressed in the human and murine hematopoietic systems, often displaying discrete stage- and lineage-specific expression patterns. Interestingly, we found no strict parallel between the expression patterns of mouse and human KRAB-ZFP orthologues, consistent with the primary targeting of transposon-based, hence species-specific cis-regulatory sequences. To explore the impact of KRAB/KAP1 on the earliest steps of hematopoiesis, we ablated KAP1 in the mouse hematopoietic system. This initially triggered a marked expansion of hematopoietic stem cells (HSC), which was rapidly followed by their exhaustion from the bone marrow. Correlating this phenotype, Kap1-deleted HSC were more prone than their wild type counterparts to enter cell cycle, and displayed a defect in homing to the bone marrow. Correspondingly, their transcriptome was characterized by decreased levels of mRNAs encoding for factors implicated in the interaction of HSC with the stem cell niche, such as Tgm2, TEK and Esam. The KRAB/KAP1 system is thus central to the homeostasis of the hematopoietic stem cell compartment.
DNA methylation: genomic and epigenomic determinants of pattern generation

Tuncay Baubec, Dirk Schübeler
Friedrich Miescher Institute, Basel (CH)

DNA methylation is a prevalent epigenetic modification of mammalian genomes involved in transcriptional repression and essential for mammalian development. Although this epigenetic modification has been profiled to unprecedented detail in the genomes of various model organisms and cell types, the principles that generate and interpret the observed patterns have not been fully explored.

Towards this, we have already profiled genomic targeting of the entire methyl-CpG-binding domain (MBD) family of proteins involved in readout of DNA methylation (Baubec et al., Cell 2013). In recent, unpublished work we now reveal the genomic binding preferences and site-specific activity of the de novo DNA methyltransferases (DNMT3A/B), the writers of this epigenetic mark. By combining computational and functional analysis we were further able to identify and quantify the contribution of DNA sequence composition, transcriptional activity or nucleosome positioning in regulating de novo methylation. Interestingly, we observe a tissue-specific recruitment of DNMTs to transcribed sequences that is mediated by readout of a co-transcriptionally deposited histone modification, H3K36me3. This recruitment of de novo methylation to active genes establishes a feedback loop required for maintenance of DNA methylation at transcribed gene bodies in mouse ES cells.
Quantification of synergy in the splicing of pre-mRNAs with multiple introns

Marie Mi Bond¹, Sylvia Voegeli¹, Antoine Baudrimont¹, Bertrand Séraphin², Attila Becskei¹
¹Biozentrum, University of Basel (CH); ²Institut de Génétique et de Biologie Moléculaire et Cellulaire (France)

Splicing reactions combine high speed with accuracy. However, some of the pre-mRNA molecules escape the nucleus without further chance of being spliced. When an mRNA contains multiple introns, the prediction of the outcome of splicing becomes even more difficult since not only the combined effect of escape rates associated with each introns have to be taken into account but also the cooperative splicing of interacting introns.

Simple measures, like the Hill coefficient, have been playing an important role in various fields by enabling the consistent comparison of experimental data and model building. The mature-to-precursor (M/P) ratio was introduced to estimate the splicing rate in steady-state conditions.

In this study, we defined new measures to quantitate synergy in splicing of mRNAs with multiple introns. Furthermore, we show that the M/P ratios of appropriate series of genetic constructs can be utilized to assess whether post-transcriptional splicing is dominant or the co- and post-transcriptional splicing occur jointly.
Detecting mitochondrial genomic variation in Drosophila melanogaster using multiplexed single molecule real-time (SMRT) sequencing

**Roel Bevers**, Antonio Meireles Filho, Maroun Bou Sleiman, Bart Deplancke
*Laboratory of Systems Biology and Genetics, EPF Lausanne (CH)*

Mitochondrial dysfunctioning is increasingly linked to neurodegenerative and metabolic diseases that emerge during aging. Although numerous genetic mutations are known to affect aging, the complex molecular, metabolic and mitochondrial networks involved are poorly understood. Here, we will use natural variation in Drosophila melanogaster as a model system to characterize mitochondrial networks and study how these influence aging. Using the Drosophila Genetic Reference Panel (DGRP), containing 140 sequenced inbred fly lines, we specifically aim to quantitatively map genetic determinants of phenotypes relevant to the process of aging.

Although preliminary data show that the DGRP lines display about 20 mitochondrial genetic variants per line, substantial variation in read coverage exists making the overall confidence in the current variant calls low. To improve this, we are enriching the 19.5 kb mitochondrial genome of Drosophila melanogaster by isolating mitochondria prior to DNA extraction to reduce ‘contamination’ from nuclear DNA. Currently we are in the process of streamlining a multiplexed SMRT sequencing protocol to sequence multiple (>100) large insert libraries of mitochondrial DNA. With this approach, we aim to derive long mitochondrial haplotypes and to study whether mitochondrial genomic variation itself, mutation rate in the mitochondrial genome, or heteroplasmy are implicated in aging.
Cys2-His2 zinc finger protein (ZFP) domains provide a convenient tool for tuning natural and artificial gene networks by functioning as programmable, DNA-targeting units. ZFP domains are among the most common transcription factors found in eukaryotes, yet only a subset of their DNA-binding specificities, and thus their roles in transcriptional regulatory networks, have been characterized. Even with existing models, it is still challenging to infer DNA binding specificities for a given ZFP, particularly in polydactyl ZFP arrays. To complement the few existing parameters guiding DNA-specificity of ZFPs, we developed a high-throughput microfluidic platform to test sets of synthetic ZFP with DNA targets.

Using a rapid gene-synthesis technique, providing a low-cost method for building a library of ZFP variants, this platform employs MITOMI to detect even weak ZFP-DNA interactions. By shuffling ZFP modules within a three-finger array, as well as making point changes in the amino acid sequence of the DNA contact positions, our platform can rapidly provide quantitative specificity data for developing a more comprehensive recognition model of ZFP binding.

This microfluidic-based functional assay, coupled with the gene-synthesis pipeline, will reduce the lag time between design and characterization of engineered proteins and improve our understanding of their activity within genetic networks.
The dynamics of RNA polymerase III occupancy during the circadian and nutrient response cycles

Cristian Carmeli\textsuperscript{1,2}, François Mange\textsuperscript{2}, Donatella Canella\textsuperscript{2}, Viviane Praz\textsuperscript{2}, Mauro Delorenzi\textsuperscript{1,3}, Nouria Hernandez\textsuperscript{2}, The CycliX Consortium

\textsuperscript{1}SIB Swiss Institute of Bioinformatics (CH); \textsuperscript{2}Center for Integrative Genomics, University of Lausanne (CH); \textsuperscript{3}Département de formation et de recherche, CHUV, Lausanne (CH)

RNA polymerase III (RNAP-III) is involved in essential cellular processes such as protein synthesis, RNA maturation, and transcriptional control. Although the genomic loci occupied by RNAP-III in mouse liver have been described, the dynamics of RNAP-III occupancy during the circadian cycle is unknown. To examine whether how RNAP-III occupancy might vary during the day, we collected liver samples from both wild-type and Bmal1 knock-out mice submitted to a 12-hour light 12-hour dark regimen, with access to food during the dark period, and performed ChIP-seq experiments with antibodies directed against a RNAP-III subunit. The Bmal1 gene encodes an essential core clock component, and Bmal1 knock-out mice are arrhythmic. Sample-to-sample normalization was performed with a recently described spiking method providing an internal reference (Bonhoure et al., 2014, Genome Res 24:1157-1168). We find that RNAP-III occupancy indeed varies during the circadian cycle, with peaks of occupancy around ZT06 (ZeitGeber) as well as ZT18, and a marked minimum around ZT10. These changes were still visible in the Bmal1 knock-out mice, although the amplitudes were generally diminished. The latter observation suggests that RNAP-III occupancy is regulated in part by the nutrient response cycle, a hypothesis we are in the process of testing.
Cross-organ analysis of circadian translation

**Violeta Castelo-Szekely**, Peggy Janich, Bulak Arpat, David Gatfield
*Center for Integrative Genomics, University of Lausanne (CH)*

Circadian clocks allow organisms to anticipate daily changes in their environment. Molecularly, circadian rhythms are generated by transcriptional feedback, typically affecting around 10% of all gene expression. With the exception of the core clock genes that are cyclic in all organs, the set of rhythmic mRNAs and proteins differs substantially between tissues often reflecting the specific rhythmic function of the organ. Furthermore, recent studies suggested that a significant proportion of rhythmic mRNAs are not generated by rhythmic transcription and that many of the observed rhythmic proteins do not cycle at the mRNA level. These discrepancies suggest the involvement of post-transcriptional mechanisms in setting the organ-specific circadian output.

In this study, we use ribosome profiling to measure translation rates across-the-day in mouse liver and kidney, and assess the contribution of protein synthesis to organ-specific circadian output. Liver is well-established in circadian research, but comparatively little is known about kidney despite important circadian renal functions. We wish to understand whether differences in rhythmic mRNA abundance between tissues are conserved, amplified or suppressed at the protein production level. Moreover this study will allow us to identify tissue-specific versus general mechanisms of translation regulation transcriptome-wide.
Enhancer repertoires are reshaped independently of early priming and heterochromatin dynamics during B-cell differentiation from multipotent progenitors

Mohamed-Amin Choukrallah\textsuperscript{1}, Lukas Burger\textsuperscript{1}, Antonius G. Rolink\textsuperscript{2}, Patrick Matthias\textsuperscript{1} \\
\textsuperscript{1}Friedrich Miescher Institute, Basel (CH); \textsuperscript{2}Department of Biomedicine, University of Basel (CH)

According to the prevalent model, a substantial fraction of the enhancers used in differentiated hematopoietic cells are primed prior to lineage commitment. To dissect the chronology of enhancer repertoire establishment during hematopoiesis, we monitored epigenome reprogramming during three developmental stages that represent HSCs, Pro-B and mature B-cells. While we find the enhancer repertoire to be dynamically reshaped during hematopoiesis, surprisingly only a small fraction of primed enhancers in HSCs or progenitors become activated in subsequent stages. In turn, the majority of active enhancers in differentiated cells were not primed in earlier stages. Collectively, our investigations indicate that early priming has a minor contribution to enhancer establishment in differentiated cells. Furthermore, our data revealed that the main chromatin associated repressive mechanisms that are heterochromatin and polycomb machinery play minor roles in enhancer dynamics. Overall, our data revisit the prevalent model about epigenome plasticity in stem cells and epigenetic reprogramming during hematopoiesis.
The role of KRAB-ZFPs/KAP1 complex in the maintenance of imprinting

**Andrea Coluccio**, Alberto De Iaco, Annamaria Kauzlaric, Gabriela Ecco, Jang Suk Min, Priscilla Turelli, Didier Trono  
*EPF Lausanne* (CH)

Imprinted genes constitute a subset of mammalian genes characterized by parent-of-origin-specific expression. Mono-allelic expression is regulated by differential DNA methylation at imprinting control regions (ICRs) which is established in the germ line and preserved from the wave of genome-wide de-methylation that takes place after fertilization. Recent studies have shown that the complex formed between ZFP57 and KAP1 plays an important role in protection against de-methylation at ICRs although little is known about the underlying molecular mechanisms. We investigated the role of different Kap1 domains in the maintenance of DNA methylation at ICRs. We generated a library of Kap1 mutants deleted for one of the functionally characterized domains: the RBCC domain, the HP1 box and the PHD-Bromodomain. Preliminary results suggest that the RBCC domain is required for Kap1 binding at ICRs, while the HP1 and PHD-Bromodomain are necessary for heterochromatin deposition and maintenance of DNA methylation. We are currently performing knockdown experiments to address the role of the different histone methyltransferases recruited by Kap1 in maintenance of imprinting. Shedding light on the molecular players involved in maintenance of DNA methylation at ICRs and understanding the mechanisms regulating genomic imprinting would provide useful insights in the process of trans-generational inheritance of epigenetic marks.
Quantifying ChIP-seq data: a spiking method providing an internal reference for sample-to-sample normalization

Mauro Delorenzi
University of Lausanne (CH)

ChIP-seq experiments are widely used to determine, the occupancy sites of any protein of interest. Comparison of occupancy maps in various cell types, tissues, and conditions require, however, that samples be normalized. Widely used normalization methods that include a quantile (rank) normalization step perform well when factor occupancy varies at a subset of sites, but may miss uniform genome-wide increases or decreases in site occupancy.

We describe a spike adjustment method that, unlike commonly used normalization methods intervening at the analysis stage, entails an experimental step prior to immunoprecipitation in which a constant, low amount from a single batch of chromatin of a foreign genome is added to the experimental chromatin. The “spike” chromatin then serves as an internal control to which the experimental signals can be adjusted.

We show that the method reliably improves similarity between replicates and reveals genuine biological differences.
Transcription factor binding and nucleosome positioning are alternative pathways for transcription start site selection in eukaryotic promoters

René Dreos\textsuperscript{1,2}, Givanna Ambrosini\textsuperscript{1,2}, Philipp Bucher\textsuperscript{1,2}  
\textsuperscript{1}SIB Swiss Institute of Bioinformatics (CH); \textsuperscript{2}EPF Lausanne (CH)

The DNA sequence determinants which direct RNA Pol-II to the correct transcription start site (TSS) are only partly understood. Conserved DNA motifs (core promoter elements, CPEs) or a conserved nucleosome architecture may play a role in TSS selection. A complicating factor is that promoters are quite variable in many respects. Some have very focused while others have highly dispersed initiation site patterns. Promoters also differ by the presence or absence of CPEs. Here we show that promoters without CPEs have a strong sequence-intrinsic nucleosome-positioning signal in the +1 nucleosome region, in both vertebrates and flies. The strength of the signal is inversely proportional to the degree of TSS dispersion. Interestingly, this promoter class also shows a 10bp periodicity in CAGE tags around it. Instead, CPEs containing promoters do not show any nucleosome-positioning signal as well as CAGE periodicity. Together, these findings suggest that transcription factor binding to CPEs and DNA sequence—induced nucleosome positioning are two mutually exclusive pathways of Pol-II recruitment to TSSs in eukaryotic promoters.
Simultaneous analysis of large-scale RNAi screens for pathogen entry

Anna Drewek  
*ETH Zurich (CH)*

Large-scale RNAi screening has become an important technology for identifying genes involved in biological processes of interest. We present the Parallel Mixed Model (PMM) approach that simultaneously analyzes several parallel RNAi screens performed under non-identical conditions like different pathogens or cell lines. We show that PMM gains statistical power for hit detection due to parallel screening. By fitting a PMM model to RNAi screening data of eight different pathogens, we found several novel hit genes.
A functional screen identifies KRAB-containing zinc finger proteins controlling endogenous retroelements in murine embryonic stem cells

Gabriela Ecco¹, Helen M. Rowe², Julien Duc¹, Sandra Offner¹, Adamandia Kapopoulou¹, Charlène Raclot¹, Carine Delattre-Gubelmann¹, Michael Imbeault¹, Bart Deplancke¹, Priscilla Turelli¹, Didier Trono¹

¹EPF Lausanne (CH); ²University College London (UK)

KRAB-ZFPs are a large family of transcriptional regulators that, together with their corepressor KAP1, participate in embryonic development, pluripotency, and control of endogenous retroelements (EREs). EREs represent about 40% of mammalian genomes, and many of their sequences were co-opted to function as transcriptional repressors or enhancers. The aim of this study was to develop a screen to identify KRAB-ZFPs recognizing specific DNA targets and to study their function. KAP1-binding sequences were selected from mouse ES cells ChIP-seq data. Out of 20 selected sites, 10 led to repression of a GFP reporter cassette in ES cells in a KAP1-dependent fashion. We then developed a screen to match KRAB-ZFPs with these sequences, using 280 KRAB-ZFP and monitoring GFP expression of a PGK-GFP cassette containing the sequence of interest. This screen confidently identified ZFP809 as the ligand of its previously mapped target and identified novel KRAB-ZFP-DNA partners. ChIP-seq of candidate KRAB-ZFPs revealed binding to LTR and LINE1 retroelements, and knock-down experiments showed upregulation of these retroelements, suggesting a role in the control of these potential regulatory sequences. We thus have developed an efficient method for the identification of KRAB-ZFP DNA targets, opening the way to functional analyses of these developmentally important transcriptional repressors.
**scsR - Towards a better understanding of genome-wide siRNA screens reproducibility**

**Andrea Franceschini**$^{1-3}$, Neha Daga$^{1-3}$, Roger Meier$^2$, Christoph Dehio$^4$, Christian Von Mering$^{1-3}$

$^1$University of Zurich (CH); $^2$ETH Zurich Scientific Center for Optical and Electron Microscopy; $^3$SIB Swiss Institute of Bioinformatics (CH); $^4$Biozentrum, University of Basel (CH)

The main source of noise in siRNA screen data is due to off-target effects that occur when an siRNA down-regulate unintended transcripts. Because siRNAs can act in a miRNA-like fashion, most of the off-target effects are mediated by the seed sequence (nucleotide 2-8) of each siRNA oligo.

We developed scsR (seed correction for siRNA), a bioinformatics method to correct genome-wide siRNA screen data for seed mediated off-target effects. We show that scsR analysis results enhance the validation rate in siRNA screens.

Besides, we provide substantial evidence that pooling is indeed a good strategy to reduce the off-target effects and that a genome-wide library composed by 3 pools x gene, each one composed by at least 4 oligo sequences (i.e. $\geq$ than 12 sequences in total) would increase dramatically the reliability of the genome-wide screen with respect to what can be achieved using the currently available libraries on the market.
Loss of transcriptional control over endogenous retroelements during reprogramming to pluripotency

Marc Friedli¹, Priscilla Turelli¹, Adamandia Kapopoulou¹, Benjamin Rauwel¹, Nathaly Castro-Díaz¹, Helen Rowe¹,⁵, Gabriela Ecco¹, Carmen Unzu², Evarist Planet¹, Angelo Lombardo³, Bastien Mangeat⁴, Barbara Wildhaber², Luigi Naldini³, Didier Trono¹

¹EPF Lausanne (CH); ²Pediatric Surgery Laboratory, Faculty of Medicine, University of Geneva (CH); ³San Raffaele Telethon Institute for Gene Therapy and Vita Salute San Raffaele University, Milan (Italy); ⁴Department of Pathology and Immunology, Faculty of Medicine, University of Geneva (CH); ⁵Centre for Medical Molecular Virology, University College London (UK)

Endogenous retroelements (EREs) account for about half of the mouse or human genome, and their potential as insertional mutagens and transcriptional perturbators is suppressed by early embryonic epigenetic silencing. Here, we asked how ERE control is maintained during the generation of induced pluripotent stem cells (iPSCs), as this procedure involves profound epigenetic remodeling. We found that all EREs tested were markedly upregulated during the reprogramming of either mouse embryonic fibroblasts, human CD34+ cells or human primary hepatocytes. At the iPSC stage, EREs of some classes were repressed whereas others remained highly expressed, yielding a pattern somewhat reminiscent of that recorded in embryonic stem cells. However, variability persisted between individual iPSC clones in the control of specific ERE integrants. Both during reprogramming and in iPS cells, the upregulation of specific EREs significantly impacted on the transcription of nearby cellular genes. While transcription triggered by specific ERE integrants at highly precise developmental stages may be an essential step towards obtaining pluripotent cells, the broad and unspecified unleashing of the repetitive genome observed here may contribute to the inefficiency of the reprogramming process and to the phenotypic heterogeneity of iPSCs.
Characterization of the gene regulatory networks mediating the immune response in Drosophila

Michael Frochaux
EPF LAUSANNE (Lausanne, CH)

The gastrointestinal tract of higher eukaryotes digests nutrients from the environment in an extracellular fashion. This allowed infectious agents to exploit this intestinal ecosystem for their own transmission. Therefore, gut-bearing organisms evolved gut-specific immune responses that accommodate the endogenous microbiota, while retaining the capability to fight pathogenic bacteria, rendering gut immunocompetence a complex trait with a large genetic component.

In this project, we aim to combine regulatory genomics and bioinformatics to elucidate the genetic and molecular mechanisms underlying gut immunocompetence and its variation in the model species Drosophila melanogaster. When infected with Pseudomonas entomophila, we observed that individual Drosophila Genetic Reference Panel (DGRP) inbred lines differ substantially in their resistance to the pathogen, yet the underlying mechanisms remain poorly understood. To elucidate these, we are in the process of profiling RNA polymerase II and various chromatin marks by ChIP-seq in the gut of control and infected flies to monitor chromatin landscape differences between resistant and susceptible lines.

We expect that our results will contribute to elucidate the regulatory mechanisms mediating high, naturally occurring variation in gut immunocompetence in Drosophila.
Hi-C analysis in arabidopsis identifies the KNOT, a structure with similarities to the flamenco locus of drosophila

Stefan Grob
Institute of Plant Biology, University of Zurich (CH)

Chromosomes are folded, spatially organized, and regulated by epigenetic marks. How chromosomal architecture is connected to the epigenome is not well understood. We show that chromosomal architecture of Arabidopsis is tightly linked to the epigenetic state. Furthermore, we show how physical constraints such as nuclear size correlate with the folding principles of chromatin. We also describe a nuclear structure, termed KNOT, in which genomic regions of all five Arabidopsis chromosomes interact. These KNOT ENGAGED ELEMENT (KEE) regions represent heterochromatic islands within euchromatin. Similar to PIWI-interacting RNA clusters such as flamenco in Drosophila, KEEs represent preferred landing sites for transposable elements, which may be part of a transposon defense mechanism in the Arabidopsis nucleus.
Circadian genome topology in mammalian tissues

Kyle Gustafson\textsuperscript{1}, Jérôme Mermet\textsuperscript{1}, Céline Jouffe\textsuperscript{2}, Frédéric Gachon\textsuperscript{2}, Félix Naef\textsuperscript{1}

\textsuperscript{1}EPF Lausanne (CH); \textsuperscript{2}Nestle Institute of Health Science (CH)

Circadian transcription-translation regulation now appears to be fundamentally linked with cell cycle control and metabolic rhythms. At the same time, genome-wide chromatin conformation capture (3C) is mapping the three-dimensional topology of diverse genomes. Functions for long-range chromatin contacts are known for the beta-globin locus control region specific to erythroid cells and for the Hox cluster in mammalian limb differentiation. We are studying circadian rhythms in genome topology using 3C with deep sequencing in murine fibroblast cell cultures as well as adult mouse liver and kidney tissue. Clock genes are used as 4C viewpoints at the known peak and trough of circadian transcription factor activity. Preliminary results show a population of static chromatin domains broadly consistent with the literature. In combination with time-resolved RNA sequencing, DNase hypersensitive sites and ChIP-seq for epigenetic histone marks, we are finding specific physical connections mediating circadian clock-regulated transcription.
Molecular basis of drug resistance-related fitness costs in Mycobacterium tuberculosis

Sebastian Gygli\textsuperscript{1,2}, Olga Schubert\textsuperscript{3,4}, Sonia Borrell\textsuperscript{1,2}, Andrej Trauner\textsuperscript{1,2}, Julia Feldmann\textsuperscript{1,2}, Xueli Guan\textsuperscript{1,2}, Mireia Coscollà\textsuperscript{1,2}, Ruedi Aebersold\textsuperscript{3}, Sébastien Gagneux\textsuperscript{1,2}

\textsuperscript{1}Swiss Tropical and Public Health Institute, Basel (CH); \textsuperscript{2}University of Basel (CH); \textsuperscript{3}Institute of Molecular Systems Biology, ETH Zurich (CH); \textsuperscript{4}Systems Biology Graduate School, Zurich (CH)

The antibiotic rifampicin (RIF) targets the bacterial DNA-dependent RNA polymerase. RIF resistance mutations often carry a fitness cost in Mycobacterium tuberculosis (Mtb). Recently, secondary, so-called compensatory mutations have been described, which restore the fitness of RIF resistant Mtb mutants. The molecular basis of resistance-associated fitness costs and their compensation is poorly understood. As RIF targets the information pathway (DNA -> mRNA -> proteins), resistance and compensatory mutations will likely influence gene/protein expression profiles of Mtb. By analysing gene and protein expression profiles of RIF resistant and susceptible Mtb strains we are aiming to better understand the molecular basis of drug resistance-related fitness costs. For this, we are comparing the gene/protein expression profiles of a pan-susceptible Mtb strain to that of a RIF resistant strain derived from it. Furthermore, we analyse the effect of a putative compensatory mutation on gene/protein expression patterns of the RIF resistant strain. We anticipate that the analysis of gene/protein expression patterns of drug resistant Mtb strains will lead to a better understanding of how drug resistance and compensatory mutations impact the fitness, and therefore the transmission potential of drug-resistant Mtb.
Pareto archetype analysis of high dimensional biological data

Jean Hausser, Yuval Hart, Pablo Szekely, Hila Sheftel, Noa Bossel Ben-Moshe, Avichai Tendler, Yael Korem, Avi Mayo, Uri Alon
Weizmann Institute of Science (Israel)

Biological research increasingly depends on interpreting large datasets in high dimensional space. Analyzing such data main relies on dimensionality reduction techniques such as principal component analysis and clustering. A recent theoretical advance suggested a complementary way to understand large biological datasets based on Pareto optimality of organisms with respect to multiple evolutionary tasks. It predicts that cells or organisms that need to perform multiple tasks have phenotypes that fall on low dimensional polytopes such as lines, triangles, tetrahedrons; phenotypes optimal for each task –– called archetypes –– are at the vertices of these polytopes.

The Pareto archetype analysis (PAA) method analyzes data in light of this theory. PAA best fits polytopes to find the corresponding archetypes, and indicates which features of the data are enriched near each archetype. We find that a 2000 tumor breast cancer gene expression dataset is well described by a tetrahedron whose vertices correspond to major tumor subtypes. Similarly, 63 mouse tissues fall in expression space within a tetrahedron, suggesting tradeoffs between four major tasks including cell division, metabolism and secretion. The present approach is less sensitive to the density of sampling of the data space than clustering approaches, and enrichment of relevant biological features is higher near the archetypes than near the centers of clusters obtained by standard methods.
Functional characterization of human KRAB zinc fingers

Pierre-Yves Helleboid, Michaël Imbeault, Didier Trono
EPF Lausanne (CH)

Krüppel associated box zinc fingers (KRAB-ZNF) represent the largest family of transcription factors with approximately 400 genes in tetrapods. They are known to interact with KAP1, which in turn induces heterochromatin formation by recruiting the histone methylase SetDB1 enzyme and the histone deacetylase NuRD complex. Through their binding to specific sequences they are thought to take a major part in retroelements silencing in embryonic stem cells - apart from that, very little is known about their function. Thus we launched a large-scale effort to determine binding sites of human KRAB ZNFs. Specifically, we are performing ChIP-exo on a library of HA-tagged KRAB ZNFs transduced in 293T cells. Here we present the logistic aspects of this project, notably quality control of the generated KRAB ZNF overexpressing cell line through western blot and immunofluorescence. Interestingly, post-translational modification patterns can be observed on some of them. We also show preliminary results, including a comparison between ChIP-seq and ChIP-exo for hZFP57, a KRAB zinc finger known for its role in imprinting.
Determinants and the cellular memory in transcriptional positive feedback systems

Chieh Hsu, Vincent Jaquet, Attila Becskei
Biozentrum, University of Basel (CH)

In a system where a transcription activator (TA) activates its own expression, if binding of TAs to the binding sites in the promoter is cooperative, the system becomes nonlinear and can display bistability. This will result in cellular memory i.e. the state of a system under certain parameter condition depends on the system’s previous state. Yeast GAL genes enhance their own transcription via the TA, Gal4p. During evolution, the Gal4p target promoters change both the number of binding sites and the core sequence. Focusing on the evolutionary divergence of the GAL1 and GAL3 promoters, we have shown that memory is strongly affected by the core promoter sequence, independent of the number of binding sites. These changes in evolution favor fast stochastic expression burst, resulting in weak memory, i.e. fast response to environmental carbohydrates.

Interestingly, we detected residual cellular memory even in such feedback system, where cooperative binding to the promoter is absent. We are establishing now a synthetic experimental system to detect hidden nonlinearities. We aim to reconstruct the feedback behavior by uncoupling the effect of TA binding strength and its amount as well as to incorporate the stochastic factors in modeling to match the experimental observation in the feedback system.
Modelling dynamics of protein synthesis and degradation in Arabidopsis

Phillip Ihmor\(^1\), Wilhelm Gruissem\(^1\), Mark Robinson\(^2\), Katja Baerenfaller\(^1\)

\(^1\)ETH Zurich (CH); \(^2\)Institute of Molecular Life Sciences, University of Zurich (CH)

Time-resolved transcriptomic and proteomic studies have shown that a substantial part of the proteome of higher eukaryotes displays dynamics that are decoupled from their corresponding transcript dynamics. Protein levels are regulated by several processes and currently it is unknown how they combine to the final protein amounts. We are approaching this question by quantifying transcription, translation, protein quantities and protein degradation rates in Arabidopsis cell cultures. We employ polysome profiling and dynamic SILAC experiments together with protein and transcript quantifications. These genome-wide assessments allow us to retrieve mRNA translation and protein degradation rates, which we will use to develop statistical ODEs to model protein level dynamics.

Currently, we have completed establishing the required methods for plant suspension cultures. SILAC experiments require high incorporation rates of supplied, labeled amino acids, which were yet not obtainable in autotrophic plant systems. We manage to reach labeling rates of over 80% and preliminary protein turnover assessments show that the rates determined for housekeeping genes correspond with those reported in small-scale studies employing \(^{15}\)N metabolic labeling and DIGE. With the methodology set, we aim to assess shortly the impact of protein degradation and translational efficiencies on protein dynamics after stimulation by the pathogen-derived flagellin peptide.
Role of KAP1 during DNA replication

Suk Min Jang, Annamaria Kauzlaric, Benjamin Rauwel, Trono Didier
EPF Lausanne (CH)

Chromatin structure plays an important role in the stability and function of eukaryotic genomes. During the cell cycle, the genome must be properly duplicated, with its chromatin features faithfully reinstated on newly synthetized DNA. If constitutive heterochromatin is lost during this process it can lead to genome instability, notably because repeated elements present within these regions can then recombine and promote chromosomal rearrangements. Constitutive heterochromatin is highly enriched in histone H3 trimethylated on lysine 9, yet how this histone modification is maintained during DNA replication is still remains poorly understood. KAP1 is a transcriptional corepressor that induces the formation of heterochromatin by acting as a scaffold complex notably comprising SetDB1 and Nurd. Based on these premises, we have investigated a possible role for KAP1 in the maintenance of constitutive heterochromatin during DNA replication. We first found that KAP1 interacts with several DNA replication factors. We then observed that these interactions are modulated by KAP1 phosphorylation. We finally demonstrate that this post-translational modification is rhythmmed by the cell cycle and that it allows KAP1 to promote the maintenance of H3K9me3 during DNA replication. We are currently identifying the partners of KAP1 in this process to maintain genome integrity in higher organisms.
Comprehensive identification of rhythmic protein synthesis using ribosome profiling in mouse liver

Peggy Janich, Bulak Arpat, David Gatfield
University of Lausanne (CH)

Rhythmic gene expression provides the basis of circadian clock controlled systems coordinating physiological and behavioral processes along the day. Over the past years, the complexity of rhythmic gene expression in individual tissues has been studied extensively, thereby mainly focusing on the level of transcription. In mouse liver around 10% of all transcripts show rhythmic expression throughout the day. However, recent proteomic approaches have pointed out that the rhythmic accumulation of proteins does not necessarily follow the cyclic pattern of the underlying transcripts. Furthermore, rhythmic transcription is not a prerequisite for rhythmic protein abundance, since several rhythmic proteins are translated from non-rhythmic transcripts. Thus, widespread post-transcriptional mechanisms, including the regulation of translation, seem to contribute to a large extent to shaping rhythmic gene output. We have used the technique ribosome profiling to study translational regulation in mouse liver on a global scale around-the-clock. Generally, translation scales well with mRNA abundance, indicating that mRNA levels are overall a good proxy for protein synthesis. However, we identified several hundred transcripts, including ribosomal and mitochondrial mRNAs, with changes in ribosome occupancy over the day. Overall, our data show that translational control adds another important layer of regulation to circadian gene expression in liver.
Circadian clock-dependent and -independent rhythmic proteomes implement distinct diurnal functions in mouse liver

Daniel Mauvoisin\textsuperscript{1,2}, Jingkui Wang\textsuperscript{3}, Celine Jouffe\textsuperscript{1,2}, Eva Martin\textsuperscript{1,2}, Florian Atger\textsuperscript{1,2}, Patrice Waridel\textsuperscript{4}, Manfredo Quadroni\textsuperscript{4}, Frederic Gachon\textsuperscript{1,2}, Felix Naef\textsuperscript{3}

\textsuperscript{1}Department of Pharmacology and Toxicology, University of Lausanne (CH); \textsuperscript{2}Diabetes and Circadian Rhythms Department, Nestlé Institute of Health Sciences (CH); \textsuperscript{3}Institute of Bioengineering, EPF Lausanne (CH); \textsuperscript{4}Protein Analysis Facility, University of Lausanne (CH)

Diurnal oscillations of gene expression controlled by the circadian clock underlie rhythmic physiology across most living organisms. Although such rhythms have been extensively studied at the level of transcription and mRNA accumulation, little is known about the accumulation patterns of proteins. Here, we quantified temporal profiles in the murine hepatic proteome under physiological light–dark conditions using stable isotope labeling by amino acids quantitative MS. Our analysis identified over 5,000 proteins, of which several hundred showed robust diurnal oscillations with peak phases enriched in the morning and during the night and related to core hepatic physiological functions. Combined mathematical modeling of temporal protein and mRNA profiles indicated that proteins accumulate with reduced amplitudes and significant delays, consistent with protein half-life data. Moreover, a group comprising about 50% of the rhythmic proteins showed no corresponding rhythmic mRNAs, indicating significant translational or posttranslational diurnal control. Such rhythms were highly enriched in secreted proteins accumulating tightly during the night. Also, these rhythms persisted in clock-deficient animals subjected to rhythmic feeding, suggesting that food-related entrainment signals influence rhythms in circulating plasma factors.
Small nucleolar RNAs are a subclass of non-coding RNAs known to have a major role in post-transcriptional processing of other non-coding RNAs mostly ribosomal RNAs. Recently, these noncoding RNAs have been found to be involved in several other biochemical pathways ranging from microRNA-like activity to involvement in alternative splicing. A crucial prerequisite for gaining a deeper understanding of these processes is a comprehensive map of snoRNA gene loci. In this work we describe an up-to-date map of human snoRNA gene loci combining data from various database sources, de novo prediction and extensive literature review. Moreover, we provide curated genomic coordinates of currently annotated snoRNAs and give insights into the plasticity of snoRNA gene expression by analysing small RNA-seq data from the ENCODE project.
Detection of single-methylation polymorphisms (SMPs) and differentially methylated regions (DMRs) using the Jensen-Shannon divergence

Önder Kartal, Ueli Grossniklaus

University of Zurich (CH)

The Jensen-Shannon Divergence is a powerful information-theoretic measure that quantifies the difference between two or more probability distributions. It has been successfully applied to the analysis of symbolic sequences. We study the application of this measure to epigenetic variation in populations. In particular, we show that for sequenced reads from bisulfite-treated DNA, it is consistent and reliable in detecting SMPs and DMRs between individuals in a population. This are important epigenetic signatures for differences in gene regulation.
Weak conservation of pathways in mouse and human aging tissues

Andrea Komljenovic\textsuperscript{1,2}, Marc Robinson-Rechavi\textsuperscript{1,2}
\textsuperscript{1}Department of Ecology and Evolution, University of Lausanne (CH); \textsuperscript{2}SIB Swiss Institute of Bioinformatics (CH)

Despite abundant experiments and diverse data available to study aging, the mechanisms of aging are still poorly understood. To tackle this, we are interested in evolutionarily conserved marks associated with aging, from short lived model organisms to long lived species such as human. We present the analysis of publicly available aging datasets from human and mouse tissues to analyze gene expression changes during aging. We characterized co-modules showing the level of gene expression conservation between homologous tissues. Meta-analysis across different tissues in mouse and human shows overall down-regulation of age-related gene expression profiles. We identified the biological processes using gene set enrichment analysis for these tissues, and found that changes associated with age-related gene expression in skeletal muscle and brain are involved in the mitochondrion pathways and inflammatory response, respectively. These tissues are known to be important to changes in aging. However, there is only a weak positive correlation between aging effects in the human and mouse homologous tissues. The co-module identification showed connection to immune response process in brain tissue between human and mouse. Our study provides a framework for further comparative analysis in aging across different species.
The weak interplay of expression, positive selection, and purifying selection in adult human and mouse

Nadja Kryuchkova1-2, Marc Robinson-Rechavi1-2
1University of Lausanne (CH); 2SIB Swiss Institute of Bioinformatics (CH)

The influence of different parameters, from gene size to expression levels, on the evolution of proteins has been previously studied in yeast, Drosophila and mammals. Here we investigate these relations further, especially taking in account gene expression in different organs. For expression we used the RNA-seq data from two large projects, covering 22 mouse tissues and 27 human tissues. Over all tissues, evolutionary rate shows no notable correlation with median expression level in mouse and human if corrected for other parameters. Expression in brain tissues does explain weakly variance in evolutionary rate.
Identifying variants affecting transcription factor-target site interactions using DNaseI hypersensitivity data

Sunil Kumar$^{1,2}$, Philipp Bocher$^{1,2}$

$^{1}$Swiss Institute for Experimental Cancer Research, EPF Lausanne (CH); $^{2}$SIB Swiss Institute of Bioinformatics (CH)

Identification of SNPs interfering with transcription factor (TF)-target site interactions is important for understanding genome regulation. Here, we define a discovery pipeline for such SNPs exploiting the fact that TF binding is partly predictable from DNaseI hypersensitivity (DGF) assays. We first generate a list of genome-wide PWM matches, which along with ChIP-seq data is used to train a TF occupancy predictor from DGF data. Subsequently, we use predictor trained on one sample to predict TFBS occupancy in all other individuals. We then compile a candidate list of SNPs that lie within a predicted TFBS and cause significant difference in PWM score. Finally, we carry out genotype-phenotype correlation tests for each SNP-TFBS pair using the predicted TF occupancy scores as phenotypes. Data used in the study includes, genotypes for 69 Yoruba individuals (HapMap Phasell) and 49 individuals (1000 genome project), DGF profiles for same individuals from Degner et al. 2012 (GEO/GSE31388), CTCF ChIP-Seq data from ENCODE (GEO/GSE33213), and a CTCF PWM from JASPAR database. We obtained a good prediction ($R=0.78$) of CTCF binding with Multivariate Adaptive Regression Splines (MARS). Using this predictor values, we identified 84 SNPs (commonly identified in HapMap and 1000 genome data) associated with differential binding (FDR$\leq10\%$).
Spatial Features for Escherichia coli Genome Organization

Bin-Guang Ma, Ting Xie, Hong-Yu Zhang
Agricultural Bioinformatics Key Laboratory of Hubei Province, Huazhong Agricultural University (China)

In bacterial genomes, the compactly encoded genes and operons are well organized, with genes in the same biological pathway or operons in the same regulon close to each other on the genome sequence. Besides, the linearly close genes have higher probability of co-expression and their protein products tend to form protein-protein interactions. However, the organization features of bacterial genomes in 3D space remain elusive. The DNA interaction data of Escherichia coli measured by the genome conformation capture (GCC) technique have recently become available, which allowed us to investigate the spatial features for bacterial genome organization. Our results show that arrangements of operons in E. coli genome tend to minimize the spatial distance between operons in the same regulon. A similar global organization feature exists for genes in biological pathways of E. coli. Besides, the genes close to each other in space (even far from each other on the genome sequence) tend to be co-expressed and form protein-protein interactions. These results provided new insights into the organization principles of bacterial genomes in 3D space and furthered our understanding of the link between the three dimensional structure of chromosome and biological function.
The circadian clock is an important endogenous time-keeping mechanism that is used by virtually all organisms to anticipate daily changes in living conditions that occur due to the 24 hour rotation of the earth. The defining characteristics of biological circadian clocks are 1) a free running period in the order of 24 hours in the absence of periodic stimuli, 2) entrainment by periodic signals (typically light-dark cycles) and 3) temperature compensation of the free-running period. In mammals, most cells contain cell-autonomous circadian clocks.

We have observed that mouse fibroblasts devoid of miRNAs (Dicer knockout) display a short period phenotype. Surprisingly this phenotype is temperature-dependent. Our aim is to investigate the molecular mechanisms underlying temperature sensitivity of miRNA-mediated post-transcriptional regulation, and the resulting observed temperature compensation in the circadian clock. For this purpose we have designed a screening method to identify 3'UTR sequences that can mediate regulation in response to a specific growth condition (in this case the cellular growth temperature). In the long term, we wish to analyse computationally and validate experimentally how specific 3'UTR sequences confer temperature sensitivity of the reporter genes, and identify genes that are regulated by this mechanism in vivo.
KRAB/KAP1-mediated epigenetic regulation of CD4+ T cells activation and function

**Flavia Marzetta**, Francesca Santoni de Sio, Evarist Planet, Julien Duc, Adamandia Kapopoulou, Priscilla Turelli, Didier Trono

1Global Health Institute, EPF Lausanne (CH); 2San Raffaele Telethon Institute for Gene Therapy, Milan (Italy)

Epigenetic mechanisms that regulate gene expression are key determinants in T cell activation and fate decision. Here, we investigated the role played by KAP1 (KRAB-associated protein 1), a transcriptional corepressor for KRAB-zinc finger proteins (KRAB-ZFPs), in the chromatin dynamics leading to these processes. We showed that KAP1 activity in CD4+ T cells is regulated by T-cell receptor (TCR) signals, via changes in expression levels, post-translational modifications, diversity and abundance of expressed KRAB-ZFPs. We also studied the genomic distribution of KAP1 binding sites in resting and activated cells, as well as the relative enrichment for its associated repressive histone mark H3K9me3, by performing chromatin immunoprecipitation sequencing. We observed that TCR-activation produced different patterns of distribution of KAP1 peaks relative to genes transcriptional start sites and to H3K9me3 heterochromatic regions. We hypothesize that alternative protein-protein interactions controlled by transient post-translational modifications of KAP1 could in part account for its differential genomic recruitment and therefore influence its repressive activity. Finally, we found that KAP1 down-modulation in CD4+ T cells induced functional impairment and significative transcriptional changes of distinct gene expression pathways. Overall, our results indicate that KAP1-mediated epigenetic repression is an important contributing factor in maintaining T cell homeostasis and function.
Chromosome conformation of circadian genes

Jerome Mermet\(^1\), Kyle Gustafson\(^1\), Céline Jouffe\(^2\), Frédéric Gachon\(^2\), Félix Naef\(^1\)

\(^1\)EPF Lausanne (CH); \(^2\)Nestlé Institute of Health Sciences (CH)

In eukaryotes, the regulation of gene expression is highly complex and the topological organization of the genome in interphase nuclei plays an important role. In mammals, the circadian molecular clock controls the temporal expression of thousands of genes, and consists of transcriptional-translational feedback loops in which BMAL1/CLOCK transcription factor heterodimer is the main activator. Overall, around 10% of genes in mouse liver are rhythmically expressed during the day. Because the circadian oscillator provides a unique dynamic model for gene expression regulation, we propose to explore the topological organization of the chromatin and its dynamic over circadian cycles. For this, we used Chromosome Conformation Capture (3C) assays and derived (4C-sequencing) approaches in mouse cells and tissues to investigate the chromatin organization surrounding key circadian gene promoters. Furthermore, we integrate temporal genomic data revealing the chromatin activity over the clock, such as RNA polymerase II loadings, histone modifications and DNase1 hypersensitivity, and published topological organization data of the chromatin. Combining those multiple genomic data with computational analyzes, we aim at inferring transcriptional regulation functions involving chromatin loops in the context of the mammalian molecular clock.
Epigenetics impact on the early aging phase of white adipose tissue

Greta Giordano Attianese, Aurélien Naldi, Barbara Toffoli, Carine Winkler, Michael Baruchet, Béatrice Desvergne, Federica Gilardi

*University of Lausanne (CH)*

Epigenetic mechanisms are key regulators of transcriptional changes upon aging in several mammalian tissues. White adipose tissue (WAT), which changes heavily throughout life, is an interesting model of aging and age-related disorders. However, knowledge of the physiological role of epigenetics during WAT aging is still lacking.

Here, we investigate chromatin remodeling events in WAT during the early aging phase, when the age-related systemic body decline is negligible. We collected WAT from 3 and 12 months old mice, representing young- and middle-adulthood. Several histone marks and RNA Polymerase II occupancy were evaluated by ChIP-seq analysis, relying on a new ChIP method for WAT. Gene expression was measured by RNA-seq. Combining ChIP-seq and RNA-seq data from the same experimental conditions allowed to assess the actual impact of epigenetic changes on mRNA levels.

Preliminary results showed that 12 months old mice are characterized by a mild hypertrophy, downregulation of genes involved in DNA repair and angiogenesis, but no inflammation. They can therefore be considered as a good model of early aging. Our results will improve the knowledge on the molecular mechanisms underlying early aging and will contribute to the potential identification of new therapeutic targets for fat related age-associated metabolic diseases.
In vitro repressilations

Henrike Niederholtmeyer¹, Zachary Sun², Sebastian Maerkl¹
¹EPF Lausanne (CH); ²Caltech (USA)

Transcription and translation can be performed in vitro, outside of cells, allowing the assembly and analysis of genetic networks. This approach to engineering biological networks in a less complex and more controllable environment could one day allow rapid prototyping of network designs. It is however still challenging to implement complex genetic networks, such as oscillators, in vitro because the reactions are usually performed in a batch format, where reaction products accumulate and synthesis rates decline over time.

To address this problem we developed a microfluidic, nano-reactor device to perform in vitro transcription and translation (TX-TL) reactions in continuous mode keeping synthesis rates at a constant steady state level. This allows us to run dynamic genetic oscillator networks such as the repressilator using an Escherichia coli lysate-based TX-TL mix. We observed long-term sustained oscillations monitoring all promoters in the network simultaneously. Close control over the reaction conditions enabled us to characterize dependence of oscillations on dilution rate and show that the repressilator is invariant to initial conditions.

This reactor-based in vitro approach will allow testing and troubleshooting novel genetic networks before implementing them in cells to speed up the design-prototype-test cycle of synthetic biology projects.
Epistasis in transcription factor binding sites

José Aguilar-Rodríguez\textsuperscript{1,2,4}, Joshua L. Payne\textsuperscript{1,2,4}, Andreas Wagner\textsuperscript{1,2,3}
\textsuperscript{1}University of Zurich (CH); \textsuperscript{2}SIB Swiss Institute of Bioinformatics (CH); \textsuperscript{3}The Santa Fe Institute (USA); \textsuperscript{4}Contributed equally

Gene expression patterns are orchestrated by large regulatory networks, which include proteins known as transcription factors (TFs) that control the expression of their target genes by binding to short sequences on DNA called TF binding sites. Despite the involvement of these sites in many evolutionary innovations, much remains unknown about how epistasis – non-additive interactions among loci - affects the evolution of TF binding sites. Epistasis can severely constrain molecular evolution because the mutations that are beneficial in one genetic background may be deleterious in another. While epistasis has been repeatedly observed in RNA and proteins, its presence in TF binding sites remains highly controversial. Here, we comprehensively quantify the extent and form of epistasis affecting TF binding. To accomplish this, we use high-throughput measurements of the in vitro binding affinities of hundreds of TFs to all possible sequences of length eight, complemented with in vivo measurements of TF-DNA binding events, made using genome-wide DNase I footprinting. Our results suggest that epistasis is pervasive in TF binding sites and has constrained the evolution of high-affinity sites. Moreover, our analysis provides the first comprehensive empirical test of a long-standing body of theoretical work on epistasis.
Identification of the gene regulatory networks underlying brown fat cell differentiation

Rachana Pradhan¹, Petra Schwalie¹, Monica Albarca², Sunil Raghav³, Carine Delattre-Gubelmann¹, Bart Deplancke¹
¹EPF Lausanne (CH); ²Univerity Hospital Geneva (CH); ³ILS, Bhubaneswar (India)

The adipose organ maintains mammalian homeostasis by regulating energy storage & expenditure via white & brown adipocytes, respectively. This thermogenic ability of the brown adipose tissue makes it an attractive therapeutic target in the context of metabolic diseases such as obesity. However, very little is known about the transcriptional regulatory mechanisms mediating brown fat cell (BFC) differentiation.

To address this, we investigated changes in gene expression & chromatin state using RNA-seq & H3K27ac ChIP-seq, respectively, across differentiation of a murine-derived brown preadipocyte cell line. We found that ~3000 genes, representing ~30% of the total probed transcriptome significantly change during differentiation. The majority of these differentially expressed genes (DEG) also show significant changes in H3K27ac, suggesting that the chromatin state is actively remodeled during differentiation. We classified DEGs using a fuzzy clustering approach & focused on genes induced during differentiation as potential positive regulators of adipogenesis. Indeed adipogenic regulators such as PPARG & BFC markers such as UCP1 showed an increase in expression from the preadipocyte to the adipocyte state. We hypothesized that important transcriptional regulators would show both a transcriptional induction & higher expression in brown versus white fat cells, & used these criteria to select the TFs BCL6 & SOX18 for follow-up. We present the first genome-wide characterization of transcriptional changes that arise during BFC differentiation & identify novel transcriptional regulators controlling this process.
Relevance of Systems Biology in Exploring Molecular Interactions Associated with Doping in Sports.

P. K. Ragunath
Sri Ramachandra University, Chennai (India)

Athletic performance is a heritable trait influenced by both environmental and genetic factors. The physical performance phenotypes for which a genetic basis can be suspected include endurance capacity, muscle performance, physiological attitude. Our study mainly focused on the PPI involved in PEP and doping drugs using systems biology tools. Logical mind map was employed to illustrate & understand the role of each gene associated with PEP in each category individually and their overlap with other categories and their combined effects. The increasing number of athletes testing positive for antidoping controls, proves that current strategies might be inadequate to prevent athletes from doping. Recognizing phenotypes of clinical relevance which arise as a result of doping and the molecular interactions leading to CP is unclear and provides research scope. For each class of doping drugs, PPI networks were built to gain insight on the interaction of the proteins which constitute target binding site of doping drugs and subsequently genes which makeup the top ranked statistically significant dense cliques were recognized. PSMA1 protein was the pivotal molecular hub for anabolic steroids drugs and SP1 and EP300 constitute the most interconnected node for Diuretics & Beta-2 agonists respectively. Gene enrichment analysis was performed to map the recognized key genes for each category of doping drugs to clinically significant phenotypes associated with them.
Unraveling the responses of RNA Polymerase II transcription to mouse liver regeneration

Leonor Rib¹, Dominic Villeneuve¹, Viviane Praz¹-², Olivier Martin², Nouria Hernandez¹, Nicolas Guex², Winship Herr¹
¹University of Lausanne (CH); ²Vital-IT, SIB Swiss Institute of Bioinformatics (CH)

The CycliX project is based on genome-wide analyses of gene transcription states in three interconnected cycles –circadian, nutrient and cell-division– via chromatin immunoprecipitation and high-throughput sequencing (ChIP-seq) data analysis. Here, we focus on the study of the cell-division cycle.

We performed partial hepatectomies on mice, thus inducing liver regeneration and consequently provoking cells to start proliferating synchronously. We identified profiles of RNA Polymerase II (Pol2) and chromatin modification by ChIP-seq at different time-points of liver regeneration. For chromatin modification marks, we chose histone modifications associated with active transcription: H3K4me3 and H3K36me3. To complement this, we analyzed chromatin association of the transcriptional co-regulator HCF-1, known to regulate cell-cycle progression in humans. Furthermore, RNA-seq analyses were done to probe mRNA levels and patterns of pre-mRNA splicing.

The results show a very good cell-cycle synchronization and robust transcriptional dynamics linked to mouse liver regeneration. We find (i) that Pol2, H3K4me3 and H3K36me3 distributions reveal coordinate gene transcription patterns; (ii) that unsupervised clustering of Pol2 transcription patterns identifies groups of genes that inform the process of liver regeneration; and (iii) that HCF-1, upon hepatectomy, is rapidly mobilized to a large number of promoters, many of which were, curiously, already transcriptionally active.
GWAS next generation: identifying mechanisms of action in association studies.

Maria Rodriguez Martinez¹, Paola Nicoletti², Damien Arnol¹, Andrea Califano²

¹IBM Zurich Research Laboratory (CH); ²Columbia University (USA)

In recent years, genome wide association studies (GWAS) have identified a plethora of genetic variants associated with complex phenotypes and disease. However, many of the identified variants map to intergenic regions or lie close to genes with unknown biological connection to the disease, and thus, interpreting their functional role remains a daunting task. To tackle this problem, we have designed gVITaMIN (Genetic Variability IdenTifies Missing INteractions), an algorithm that examines the molecular mechanisms underlying the association between genetic variants and complex phenotypes. Specifically, the algorithm tests whether a genetic variant modulates the expression level of a gene or the transcriptional activity of a transcription factor, by altering the relationship with its targets.

We have applied gVITaMIN to the study of breast cancer, a common complex disease with incompletely characterized genetic predisposition architecture. We have selected 50 SNPs previously associated to breast cancer susceptibility, run gVITaMIN using two different breast cancer expression datasets (TCGA and METABRIC), and compared the results obtained from both cohorts. Interestingly, gVITaMIN links the cancer susceptibility conferred by rs1876206 to dysregulation of TGFβ signaling, a potent growth inhibitor with tumor-suppressing activity.
Evidence of evolutionarily conserved tissue-specific repression of gene expression, mediated by miRNAs

Marta Rosikiewicz¹, Marc Robinson-Rechavi²

¹University of Lausanne (CH); ²SIB Swiss Institute of Bioinformatics (CH)

Gene expression links cell and tissue identity to gene function. Understanding the specificity of gene expression is thus key to understanding cells and genes. We searched whether specific patterns of gene down-regulation can be identified, and whether they are evolutionarily conserved and biologically meaningful. We performed a meta-analysis of 400 microarrays annotated to 10 homologous organs from mouse and human, finding both over-expressed and under-expressed genes in each organ, enriched in orthologs and confirmed with RNA-seq data. While the over-expressed genes are related to the physiological role of each organ, the under-expressed genes are enriched in general pathological conditions such as necrosis, infection, inflammation or cancer. Moreover, the under-expressed genes are enriched in miRNA targets, with a broad range of miRNAs for testis down-regulated genes, and tissue-specific miRNAs in other organs (e.g., MIR-1 and MIR-206 for muscle). Our results provide evidence for tissue-specific down-regulation mechanisms for genes whose uncontrolled expression might be especially harmful in that tissue. The genes involved in this down-regulation (miRNAs and others) are probably important to understanding tissue-related diseases.
A genomic study of the contribution of DNA methylation to regulatory evolution in primates

Julien Roux¹, Irene Hernando-Herraez², Nicholas Banovich³, Jonathan Pritchard³, Tomas Marques-Bonet², Yoav Gilad³
¹University of Lausanne, Switzerland (Lausanne, CH); ²Institute of Evolutionary Biology, PRBB, Barcelona (Spain); ³University of Chicago, (USA)

A long-standing hypothesis is that changes in gene regulation play an important role in adaptive evolution, notably in primates. Yet, in spite of the evidence accumulated in the past decade that regulatory changes contribute to many species-specific adaptations, we still know remarkably little about the mechanisms of regulatory evolution.

In this study we focused on DNA methylation, an epigenetic mechanism whose contribution to the evolution of gene expression remains unclear. To interrogate the methylation status of cytosines on a genome-wide scale, we collected bisulfite-sequencing data across 4 tissues (heart, kidney, liver and lung) in 3 primate species (human, chimpanzee and macaque). Because the 4 tissues are from the same individuals, we are able to monitor methylation differences between individuals, tissues and species. In parallel, we collected gene expression profiles using RNA-seq from the same samples, allowing us to perform a high resolution scan for genes and pathways whose regulation evolved under natural selection.

We integrated these datasets to characterize better the genome features whose methylation status leads to expression changes, and we quantified the proportion of variation in gene expression levels across tissues and species which can be explained by changes in methylation. Globally, our study leads to a better understanding of the basis for regulatory changes and adaptations in primates.
Inferring gene-specific contributions to the phenotypes in a Genome-wide siRNA screening using a biophysical prediction scheme for miRNA-target interactions

Shantanu Roy, Rafal Gumienny, Mihaela Zavolan

*University of Basel (CH)*

The current study aims at using the phenotypic data extracted from a genome-wide siRNA screening to build a quantitative model of phenotypically relevant off-target effects mediated by the siRNA seed sequences. To account for narrowing the knowledge gap between the interactions at the molecular-level and the phenotypic changes at the system-level, the goal was to model the gene regulatory cascades which connect the miRNA(siRNA)-target binding to the observed phenotypes and reconstruct gene-phenotype mappings and functional pathways of genes in the human genome. This was done in combination with an improved version of MIRZA model for predicting miRNA-mRNA interactions, which was used for a genome-wide prediction of the siRNA-mRNA interaction repertoire. To estimate the gene-level contributions to the phenotypes, we employed a linear regression model with regularization to solve the relation between the phenotypic data and our predictions of siRNA-target binding.
A unified design space of synthetic stripe-forming networks

Yolanda Schaerli\textsuperscript{1,2}, Andreea Munteanu\textsuperscript{1}, Magüi Gili\textsuperscript{1}, James Cotterell\textsuperscript{1}, James Sharpe\textsuperscript{1}, Mark Isalan\textsuperscript{1,3}

\textsuperscript{1}EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation, Barcelona (Spain); \textsuperscript{2}Institute of Evolutionary Biology and Environmental Sciences, University of Zurich (CH); \textsuperscript{3}Imperial College London (UK)

To study the function and properties of gene regulatory networks synthetic biology is a promising tool. Gene circuits with predefined behaviors have been successfully built and modeled, but largely on a case-by-case basis. Here we go beyond individual networks and explore both computationally and synthetically the design space of possible dynamical mechanisms for 3-node stripe-forming networks. First, we computationally test every possible 3-node network for stripe formation in a morphogen gradient. We discover four different dynamical mechanisms to form a stripe and identify the minimal network of each group. Next, with the help of newly established engineering criteria we build these four networks synthetically and show that they indeed operate with four fundamental distinct mechanisms. Finally, this close match between theory and experiments allows us to infer and subsequently build a 2-node network that represents the archetype of the explored design space.
A powerful method for transcriptional profiling of specific cell types in eukaryotes: laser-assisted microdissection and RNA sequencing

Marc Schmid¹, Anja Schmidt¹, Ulrich Klostermeier², Matthias Barann², Philip Rosenstiel², Ueli Grossniklaus¹
¹University of Zurich (CH); ²Christian Albrechts University, Kiel (Germany)

The acquisition of distinct cell fates is central to the development of multicellular organisms and is largely mediated by gene expression patterns specific to individual cells and tissues. A spatially and temporally resolved analysis of gene expression facilitates the elucidation of transcriptional networks linked to cellular identity and function. We present an approach that allows cell type-specific transcriptional profiling of distinct target cells, which are rare and difficult to access, with unprecedented sensitivity and resolution. We combined laser-assisted microdissection (LAM), linear amplification starting from < 1 ng of total RNA, and RNA-sequencing (RNA-Seq). Analysis of differential gene expression by RNA-Seq is frequently done using feature counts, i.e. the number of reads mapping to a gene. However, commonly used count algorithms (e.g. HTSeq) do not address the problem of reads aligning with multiple locations in the genome (multireads) or reads aligning with positions where two or more genes overlap (ambiguous reads). We developed Rcount, a software which specifically addresses these issues. Furthermore, Rcount allows the user to assign priorities to certain feature types or to add flanking regions. It also provides a fast and easy-to-use graphical user interface requiring no command-line or programming skills.
Eukaryotic pre-mRNAs undergo several maturation steps including splicing, 5’-end capping, and 3’-end cleavage and polyadenylation. In recent years, several studies demonstrated the impact of cleavage and polyadenylation on alternate 3’-end sites leading to transcript isoforms with changed rates in mRNA decay and translation. Moreover, proliferating cells and cancer cells have been shown to systematically favour cleavage and polyadenylation at 5’ proximal sites. Gaining a deeper understanding of the extent of alternative polyadenylation (APA) in different cell types will help to unravel the complex regulation of mRNA transcripts.

Recently, efforts to study APA have been propelled by the use of dedicated 3’-end sequencing protocols. However, the bulk of today’s transcriptome sequencing data is generated by mRNA-seq protocols. Recent studies demonstrated that the drop in read density after a poly(A) site can be used to infer the relative abundance of 3’-end isoforms. In this study we used data generated by mRNA-seq and A-seq, our in-house developed 3’-end sequencing protocol. In particular, we obtained paired mRNA-seq and A-seq libraries. Using these data sets we explore the detection limits and overall performance of indirect poly(A) site quantification from mRNA-seq data. Our results provide important guidelines for future analyses of mRNA-seq data regarding 3’-end site quantification.
Chromatin accessibility around the clock

Jonathan Sobel¹, Irina Krier¹, Teemu Andersin², Sunil Raghav¹, Alexandra Stylian Kalantzi¹, Matteo Dal Peraro¹, Bart Deplanke¹, Jacques Rougemont¹, Ueli Schibler², Felix Naef¹, CycliX Consortium
¹EPF Lausanne (CH); ²University of Geneva (CH)

In mammals, the circadian clock is controlling behavioural, physiological and cellular rhythms. On the other hand, daily oscillations of a multitude of genes are related to nutrient-response cycles. Time-resolved mapping of DNAse I hypersensitive sites (DHS) can identify details of the regulatory interplay between metabolism and the internal clock at a molecular level.

Here, we report a genome-wide analysis DHS using high-throughput sequencing of DNase tags from mouse liver with a resolution of 4 h (12 h light/12 h dark). Using PolII and H3K27ac ChIP-seq, we characterize the DHS and study the dynamics of each mark in wild-type and Bmal1-KO mice. We use the sequence motif content of each DHS and a linear regression model to explain DHS temporal behaviour. We monitor the temporal expression of target genes in order to understand the phase-specific regulation in the circadian context.

An important proportion of DHS showing oscillations are distal, suggesting a fine regulation of oscillating genes by enhancers, which we seek to understand better. Interestingly, DHS associated with circadian genes tend to be time-correlated with expression, Pol II and H3K27ac marks. The Bmal1-KO phenotype in light-dark conditions show a proportion of DHS with circadian rhythms associated with transcription factors related to metabolism, due to food entrainment and systemic cues.
TbX: Systems Biology of Drug-resistant Tuberculosis in the Field

Andrei Trauner¹, Sonia Borrell¹, Ben Collins², Sebastian Gygli¹, Olga Schubert², Mattia Zampieri², Michael Zimmerman², Ruedi Aebersold, Christian Beisel³, Marc Gitzinger⁴, Xueli Guan¹, Uwe Sauer², Tanja Stadler³, Joerg Stelling³, Sebastien Gagneux¹

¹Swiss Tropical and Public Health Institute (CH); ²Institute of Molecular Systems Biology, ETH Zurich; ³D-BSSE, ETH Zurich (CH); ⁴Bioversys AG (CH)

Sampling the global diversity of Mycobacterium tuberculosis complex (MTBC) revealed the existence of 7 different genetic lineages. The underlying differences (approximately 1000 single nucleotide polymorphisms between lineages) carry consequences for MTBC biology, including virulence and development of drug resistance (DR). Nonetheless, the relationship between genotype and phenotype, especially considering clinical strains of MTBC, remains poorly understood. With TbX we aim to:

1. Define and model the molecular phenotypic space across the phylogenomic diversity of the human-associated MTBC, irrespective of drug resistance.
2. Measure the impact of DR and compensatory mutations on the MTBC within the context of (1).
3. Measure the effect of exposure to anti-TB drugs (existing and novel) on the MTBC phenotype in presence or absence of DR mutations.
4. Develop a phylodynamic model of transmission to differentiate between highly transmissible Multidrug resistant (MDR) and unsuccessful MDR strains.
5. Define a metabolic profile predictive of high in clinico fitness in MDR MTBC.

The combined objective of TbX is to build on knowledge gained in the laboratory and from the field (strains collected in the Republic of Georgia, a country with a high burden of MDR-TB) to understand what defines a successful MTBC strain in the clinic.
Expression of Mechanically Controlled Genes in Osteocytes in their Native Environment

Andreas Trüssel, Robin Wilson, Gisela Kuhn, Duncan Webster, Ralph Müller
Institute of Biomechanics, ETH Zurich (CH)

Osteocytes are the main sensors of mechanical stimuli in bone and coordinate local bone formation and resorption activity to adapt the bone to mechanical loads. To understand such signaling cascades it is important to quantify key signals together with their local mechanical microenvironment. We have developed a framework to isolate small osteocyte subpopulations from bone tissue, analyze their gene expression in a multiplex fashion and correlate this to sites of high and low mechanical stimuli. To do so in-vivo micro-computed tomography images of the sixth tail vertebrae of three mice (female C57BL/6) were taken. Based on the microCT data, micro-finite element analysis was performed to calculate the local stain energy density (SED). The vertebrae were harvested, snap-frozen in liquid nitrogen, cryosectioned (9 µm) and stained with Hoechst 33342. A total of 172 osteocytes were mapped into the 3D microCT data, grouped according to their local SED, isolated using a laser capture microdissection system (Carl Zeiss, Switzerland) and processed for gene expression analysis. The bone resorption activator RANK-ligand was found to be upregulated, whereas the resorption blocker OPG tended to be downregulated in low strained environments. We conclude that gene expression is locally coupled with the mechanical microenvironment in bone tissue.
The Reptilian Transcriptomes v2.0: an extensive resource for Sauropsida genomics and transcriptomics

Asier Ullate Agote, Athanasia Tzika, Djordje Grbic, Michel C. Milinkovitch
University of Geneva (CH)

Despite its remarkable diversity, the Class Reptilia remains largely under-represented in major sequence databases and comparative genomic/transcriptomic studies. Here, we use a single pipeline (LANE runner v2) to annotate new transcriptomic and genomic data, as well as multiple published datasets, and build an integrated single resource: The Reptilian Transcriptomes Database v2.0 (http://www.reptilian-transcriptomes.org). This resource includes representatives of each of the four extant reptilian orders: (i) six Squamata, including three snake and three lizard species; (ii) the Sphenodon; (iii) three Crocodylia species; and (iv) one turtle (Testudines). LANE runner v2 integrates an improved annotation pipeline based on iterative BLAST+ searches and Reciprocal Best Hit (RBH) identification. This approach allows us to annotate a higher percentage of sequences than in previous studies. We also built the so-far largest protein alignments (above 500,000 amino acids per species) for reptiles resolving the position of turtles and the tuatara. The Reptilian Transcriptomes Database v2.0 is a new resource that can serve as a reference for expression analyses, as well as linkage mapping, comparative genomics and phylogenomics. The LANE runner v2 software pipeline can easily be used for the annotation of any transcriptomic dataset.
Method for combined analysis of RNA-Seq and Ribo-Seq datasets

Anze Zupanic\textsuperscript{1,2}, Cathy Meplan\textsuperscript{2}, Sushma Grellscheid\textsuperscript{2}, John Hesketh\textsuperscript{2}, Daryl Shanley\textsuperscript{2}
\textsuperscript{1}EAWAG (CH); \textsuperscript{2}Newcastle University (UK)

Ribo-Seq maps the location of translating ribosomes on mature mRNA transcripts. We developed a method to detect translational regulation of individual mRNAs from their ribosome profiles, utilizing changes in ribosome density. We avoided false positive predictions by normalizing the Ribo-Seq datasets with its RNA-Seq counterpart, thereby eliminating artefacts stemming from bias of short-fragment alignment to the genome. Among the mRNA transcripts found, there were several known targets of translational, such as selenoproteins, and Atf4, but also several hitherto unrecognized ones, such as Tmem55b. Additionally, our approach proved useful for identification of new transcript isoforms.
Single-Cell Biology

Chair:

Lucas Pelkmans
Institute of Molecular Life Sciences
University of Zurich
It’s Too Late to Apoptize: A time-dependent threshold for p53-mediated apoptosis

Andrew Paek, Galit Lahav
Harvard Medical School (USA)

One of the main challenges in cancer treatment is called fractional killing, a phenomenon in which a small subset of cancer cells escape the treatment. We developed a system to dissect fractional killing in response to chemotherapy at the single cell level. Our goal was to understand why a sub-population of cancer cells evades death when treated with cisplatin, a commonly used chemotherapeutic drug. Cisplatin induces cell death of cancer cells predominantly by activating the tumor suppressor protein p53. We quantified p53 dynamics and cell fate (survival or apoptosis) of individual cells treated with cisplatin and determined what aspects of p53 dynamics are correlated with each fate. Our work suggests a time-dependent threshold for p53 mediated apoptosis in which a cell’s probability to die depends on both the level of p53 and the time of induction. I will discuss the molecular mechanism protecting cells from apoptosis if p53 is induced too little or too late, and the potential clinical applications for accelerating p53 and increasing the efficacy of anti-cancer drugs.
A structural systems-biology approach to cell biology by electron microscopy: Multiscale structural analysis from bulk

Henning Stahlberg
Center for Cellular Imaging and NanoAnalytics (C-CINA), Biozentrum, University of Basel (CH)

Structural Systems Biology studies the entirety of a biological system, by characterizing multicellular tissues or also the entire proteome of one single cell. Here, the individuality of biological cells needs to be taken into account, as stochastic processes often govern cellular behavior, leading to significant differences between one cell and another.

Electron microscopy (EM) is a versatile tool for structural studies of biological samples at various resolution scales, ranging from cellular tissue to macromolecular complexes and down to the single molecule.

In this presentation, several approaches of EM to systems biology will be presented. These include multi-cellular tissue characterization by Serial Block-Face Scanning Electron Microscopy, Electron Tomography, or a newly developed single cell structural proteomics approach, termed “Visual Proteomics”. In the latter, we have developed methods to extract the entire proteome from one single, hand-picked cell, which is then analyzed by transmission electron microscopy for establishing a structural inventory of the protein complexes present in that cell. An application to model cells for Parkinson’s disease is discussed.
Cells have the remarkable ability to sense geometrical and physical cues from their environment and adapt their architecture accordingly. This process requires a tight regulation of the permanent remodeling of the acto-myosin network, that can both transmit and generate intra-cellular forces. Despite numerous works on the molecular composition of stress fibers, little is known about the mechanism determining the magnitude of force production in these structures. Here we studied the scaling of contractile force magnitude in stress fibers and investigated the role of actin network dynamics and architecture in this process.

We used micropatterned substrates to control the length and spatial organization of stress fibers in adherent cells and measured the traction forces they produced on deformable substrates. Thereby, we demonstrated that forces scaling exhibit a biphasic behavior. Force magnitude first increased with the length of the stress fibers and then dropped above a critical length. Strikingly, very long cells appeared capable to produce only weak forces.
The Legionella pneumophila Lqs quorum sensing system – Phosphorylation signaling and intra-amoeba single cell gene regulation

Ursula Schell\textsuperscript{1}, Christian Manske\textsuperscript{1}, Aline Kessler\textsuperscript{1}, Hubert Hilbi\textsuperscript{1,2}  
\textsuperscript{1}Ludwig-Maximilians University, Munich (Germany); \textsuperscript{2}University of Zurich (CH)

Legionella pneumophila, the causative agent of Legionnaires’ disease, replicates in environmental and immune phagocytes within a distinct compartment, the “Legionella-containing-vacuole” (LCV). The facultative intracellular bacterium adopts a biphasic life cycle, oscillating between a replicative/non-motile and a virulent/transmissive phase. For cell-to-cell communication L. pneumophila employs the autoinducer LAI-1 (3-hydroxy-pentadecane-4-one), which is produced and detected by the Lqs (Legionella quorum sensing) system. The system comprises the autoinducer synthase LqsA, the homologous sensor kinases LqsS and LqsT, and the prototypic response regulator LqsR, whereupon the kinase-mediated phosphorylation signal converges. Lqs-regulated processes comprise pathogen-phagocyte interactions, natural competence and the expression of a genomic “fitness island”.

The production of flagella and the expression of virulence features are closely linked transmissive traits of L. pneumophila. The Lqs system regulates flagellar gene expression in stationary growth phase, as indicated by PflaA-gfp reporter experiments. Flow cytometry analysis revealed that the lqs genes control the mean PflaA expression but not its heterogeneity. Interestingly, compared to extracellular growth in broth, the heterogeneity of PflaA expression in Acanthamoeba castellanii increased substantially at the end of the bacterial replication cycle. Current experiments aim at an in-depth characterization of bacterial subpopulations differing in PflaA expression levels and the cues triggering heterogeneity.
Noise facilitates NF-kappaB entrainment and transcriptional control under complex signalling environments

Savas Tay, Ryan Kellog
ETH Zurich (CH)

Biological systems use oscillations for time-keeping and transcriptional regulation, with examples in circadian rhythms and developmental patterning. NF-κB transcription factors are central to immunity and many diseases, and show oscillations even under constant inputs (Tay et al. Nature 2010). Pulsatile secretion of cytokines or signalling waves in tissue result in periodic inputs to cells that can lead to entrainment, where oscillators phase-lock to the input and become synchronized. We used high-throughput microfluidic live-cell imaging, gene expression analysis and mathematical modelling to characterize the frequency response of NF-κB at the single-cell level, and found that periodic TNF stimulation leads to entrainment, causing significantly reduced NF-κB and mRNA variability between cells. We measure a much broader entrainment frequency range (Arnold Tongues) than predicted by deterministic calculations, and stochastic simulations show that intrinsic fluctuations in the transcription of negative-feedback genes IκBα and A20 cause this enhanced bandwidth. NF-κB entrainment results in much increased mRNA production compared to chaotic responses under constant TNF inputs, indicating an advantage for synchronous NF-κB oscillations for efficient gene expression. Our results shed light on how cell populations may operate synchronously despite significant variability in isolate cell responses, and suggest a surprising role for molecular noise dynamical signal processing.
Involvement of the actin-polymerising factor SPIRE in Salmonella Typhimurium infection

Daniel Andritschke¹, Sabrina Dilling¹, Mario Emmenlauer², Pauli Rämö², Tobias Welz³, Eugen Kerkhoff³, Wolf-Dietrich Hardt¹

¹Institute of Microbiology, ETH Zurich (CH); ²Biozentrum, University of Basel (CH); ³Department of Neurology, Molecular Cell Biology Laboratory, University Hospital Regensburg (Germany)

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 a type III secretion system (TTSS) to inject effector proteins into host cells. The effectors SopE, SopE2 and SopB are injected by TTSS 1. These proteins stimulate actin rearrangement by interfering with Rho GTPase signaling. The resulting pronounced actin polymerization leads to the formation of characteristic membrane ruffles, facilitating Salmonella invasion. On the contrary, 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 seems to be Rho-GTPase-dependent. These results suggest a different strategy for SipA-mediated internalization compared to other effector proteins. Interestingly, in vivo infection in a mouse model of Salmonella infection seems to be mainly driven by SipA rather than SopE, suggesting that the invasion phenotype described above more likely resembles the course of infection in the mouse intestine. Here we show that the actin-polymerising factors SPIRE1 and SPIRE2 seem to be involved in invasion and vesicle trafficking during the course of Salmonella infection.
Real-time MAP kinase-induced expression studies with dynamic single cell reporters

Delphine Aymoz, Eric Durandau, Serge Pelet
Department of Fundamental Microbiology, University of Lausanne (CH)

Fluorescent proteins (FPs) have been used successfully to study gene expression, but their slow maturation and high stability hinders the quantification of fast expression kinetics.

We have designed a new kind of expression reporters, which are able to reflect dynamic changes in gene expression in single cells. The reporter is composed of two subsystems that can bind together with high affinity. One subsystem includes a fluorescent protein and is expressed constitutively in the cytoplasm of the cell. Its binding partner, which carries a nuclear localization sequence, is under the control of the promoter of interest, and triggers the relocation of the FP in the nucleus This construct also bears a destabilization sequence, which allows the quantification of the OFF-rate of expression.

MAPK pathways are signaling cascades activated by various extra-cellular signals. They promote the expression of wide arrays of genes, with different kinetics (5 to 45 minutes after stimulation), that allow the adequate response to the stimulus. With our new reporter, the expression dynamics induced by a MAPK can be monitored with high accuracy. We can use this tool to investigate the mechanisms regulating the transcription dynamics of genes responding to the activation of a pathway.
Synchronization of circadian and cell cycle oscillators

Rosamaria Cannavo¹, Jonathan Bieler¹, Kyle Gustafson¹, Cedric Gobet¹, David Gatfield², Felix Naef¹
¹EPF Lausanne (CH); ²University of Lausanne (CH)

Circadian oscillators and cell cycles are two fundamental periodic processes with a period in the range of one day. Consequently, when they run in parallel in the same cell, their coupling may lead to synchronization. Observations of circadian variations in mitotic indices in mammalian cells and studies on the daytime-dependence of cell division in mouse liver and cultured fibroblasts have led to the intriguing hypothesis that the circadian cycle might gate cell-cycle progression. Here, we performed a large-scale time-lapse imaging of single mammalian NIH3T3 fibroblasts during several days to further analyze the mutual interactions between the two oscillators and their dynamical consequences. The analysis of over 10’000 single cell traces showing circadian cycles in dividing cells indicated that both oscillators tick in a synchronized state, with cell divisions occurring tightly five hours before the peak in circadian Rev-Erbα-YFP reporter expression. Such synchrony may be caused by coupling in either direction, or both. While gating of cell division by the circadian cycle has been most studied, our data combined with stochastic modeling show that reverse coupling is predominant in NIH3T3 cells and that the two cellular oscillators adopt a synchronized state that is highly robust over a wide range of parameters.
Single-cell dynamics of host-pathogen interactions

Matthieu Delince\textsuperscript{1}, Jean-Baptiste Bureau\textsuperscript{1}, Giulia Manina\textsuperscript{1}, Pierre Cosson\textsuperscript{2}, Thierry Soldati\textsuperscript{2}, John D. McKinney\textsuperscript{1}

\textsuperscript{1}EPF Lausanne (CH); \textsuperscript{2}University of Geneva (CH)

Host-pathogen interactions have been extensively studied over the past decades, leading to a good understanding of how intracellular pathogens infect host cells. However it is still challenging to assess the heterogeneity during infection in terms of outcome of infection and timing (i.e. who dies and after what time) and cellular processes (e.g. do bacteria replicate and where). This is because most studies rely on indirect population averaged data, hiding the cell-to-cell phenotypic variations.

In this work we present a microfluidic device that enables to trap a single host cell and a pathogen for an extended period of time at a single cell resolution and with many chambers in parallel. We combine it to timelapse microscopy to study the interactions of the host Dictyostelium discoideum with Mycobacterium marinum or Klebsiella pneumoniae. We quantify the fraction of infections that lead to the death of the host cell or of the pathogen. This analysis allows us to have a quantitative measure of the pathogenicity of a bacterium and to study it under different conditions (e.g. knock-out of a gene). It is also very interesting to quantitatively compare different pathogens in terms of outcome of infection and heterogeneity of outcome.
Absolute quantification of proteins and protein modifications on the single-cell level by combining mass cytometry and targeted proteomics

Serena Di Palma¹, Paul Boersema², Andrea Jacobs¹, Paola Picotti², Bernd Bodenmiller¹
¹University of Zurich (CH); ²ETH Zurich (CH)

Single-cell biology plays important roles in systems biology, supporting our understanding of regulatory networks. The complexity of signaling pathways and their interplays requires high-throughput, quantitative and multiparameter single-cell technologies. Mass cytometry (MC), based on atomic mass spectrometry, allows for single-cell network analysis using antibodies. However, due to unknown antigen-binding site occupancies, molecules absolute copy numbers cannot be directly determined, losing information on networks structure, reaction rates and signaling thresholds that commit cells into defined disease states.

To enable the absolute quantification of proteins and their post-translational modifications on the single-cell level, we combine MC with targeted proteomics, aiming to study in a quantitative and temporal fashion signaling pathways wired in human cells, including MAPK, AKT and JAK/STAT.

After selecting specific biomarkers (proteins and phosphorylation sites) covering interplaying signaling pathways, a panel of antibodies is designed for MC analysis. Together, targeted proteomics assays are performed to determine the average absolute copy numbers per cell population of the same biomarkers. This value is then computed on MC data to extrapolate copy numbers per single cells. We propose this new integrative approach for quantitative single-cell biology and we employ it to study regulatory signaling networks in cancer.
Tracking of migrating leukocytes in video microscopy.

Diego Ulisse Pizzagalli\textsuperscript{1,2}, Marcus Thelen\textsuperscript{1}, Rolf Krause\textsuperscript{2}, Fernandez Santiago Gonzalez\textsuperscript{1}
\textsuperscript{1}Institute for Research in Biomedicine, Bellinzona (CH); \textsuperscript{2}Institute of Computational Science, Faculty of Informatics, Universita della Svizzera Italiana (CH)

Recent advances in state-of-the-art imaging have allowed the development of powerful techniques such as 2-photon intravital microscopy (2PM) that permits the visualization of cell interactions in vivo, opening the possibility to observe the dynamics of the immune system. As an example, tracking of specific cells might help to unveil the function of proteins or pathways involved in cell migration or the regulation of cytoskeleton remodeling.

The analysis of the data generated by 2PM is a challenging task. First, the design of proper algorithms that can faithfully track the migration patterns of single cells is far from trivial. Second, large amounts of data need to be processed within an acceptable timeframe.

We propose to identify a behavioral cell model from a complex and dynamic biological system such as time-lapse microscopy of mammalian tissues.

The identified model should describe the status and the position of single cells over time and may also be used to perform higher-level tasks like feature extraction for automated cell classification.

To achieve our goal we are going to develop new algorithms that exploit spatio-temporal features, implement computer vision techniques and are executed on massive parallel computing architectures.
Crosslink of cell cycle and cell migration

Hsiao-Chun Huang
National Taiwan University, Taipei (Taiwan)

Metastasis is the cause of 90% death from cancer, yet little is known about it relates to other cellular processes. A recent dynamic investigation revealed that the metastasis promoter, Slug, is under temporal regulations during the course of cell cycle. This inspired the question of whether cell migration or invasion, and ultimately cancer metastasis, could also display a cell-cycle dependent pattern. With FUCCI (two-colored Fluorescent Ubiquitination-based Cell Cycle Indicator) and single-cell fluorescent microscopy, we were able to monitor cell cycle progression in individual cells while they migrate. We found that cells appeared to travel with highest speed in G1, as compared to the rest of the cell cycle. More specifically, cells tend to jump apart after cytokinesis. We hypothesize that the cortical pulling forces from the previous cell division could aid in cell migration. When we blocked spindle bipolarity with Kinesin-5 inhibitor (an antimitotic drug), leaving cells with monopolar spindles, the high speed that peaked right after cell division was lost. Our results suggest that even if cancer cells resist killing from Kinesin-5 inhibitor, failed mitosis induced by the drug still have the benefit of preventing them to metastasize.
Identification of a new EB-binding motif in Kar9

Cristina Manatschal\textsuperscript{1}, Ana-Maria Farcas\textsuperscript{2}, Anil Kumar\textsuperscript{1}, Ilya Grigoriev\textsuperscript{3}, Ines Kretzschmar\textsuperscript{4}, Miriam Steiner Degen\textsuperscript{1}, Natacha Olieric\textsuperscript{1}, Mathias Bayer\textsuperscript{2}, Rudolf Volkmer\textsuperscript{4}, Anna Akhmanova\textsuperscript{3}, Yves Barra\textsuperscript{2}, Michel O. Steinmetz\textsuperscript{1}

\textsuperscript{1}Paul Scherrer Institute (CH); \textsuperscript{2}ETH Zurich (CH); \textsuperscript{3}Utrecht University (NL); \textsuperscript{4}Charité-Universitätsmedizin Berlin (Germany)

Saccharomyces cerevisiae undergoes asymmetric cell division. During mitosis, the protein Kar9 (APC homolog) co-localizes with Bim1 (EB homolog) at the plus ends of the mitotic spindle microtubules. Unlike Bim1, Kar9 localizes exclusively on the microtubules emanating from the mother SPB. This asymmetric localization of Kar9 is necessary for spindle positioning. We identified that a C-terminal SxIP motif mediates the binding of Kar9 to the EB homology domain of Bim1. Using SPOT assay, we also identified a novel motif downstream of the SxIP. The two motifs of Kar9 compete for binding to Bim1, suggesting common/overlapping binding sites. The presence of more than one Bim1 binding motif is conserved among Kar9 homologues, suggesting its evolution towards high affinity to Bim1. The tight linkage between Kar9 and Bim1 is functionally important to maintain the high fidelity of spindle positioning process. We are testing this hypothesis by observing the in vivo phenotype of Kar9 alleles with mutations in the two Bim1-binding motifs. The interaction of the identified second motif is not restricted to Bim1; it also binds to human EB1. The GFP tagged stretch of Kar9 encompassing the new motif tracks MT plus-ends in COS-7 cells. The new motif is a new microtubule tip localization signal (MtLS) and we are validating this hypothesis in other Kar9-unrelated +TIPs.
treeSNE utilizes tree metrics to identify and characterize cell subtypes in high-dimensional single cell data

Will Macnair, Laura D.V. Roditi, Manfred Claassen
Institute of Molecular Systems Biology, ETH Zurich (CH)

Tumor growth is posited to be driven by a subpopulation carrying cancer-driving mutations (a ‘cancer stem cell’ population). However, sequencing has revealed widespread genetic heterogeneity among tumor cells. Identifying their distinct phenotypic profiles allows for further characterization of tumor heterogeneity, with the potential for therapeutic contributions such as improving cancer subtype classification.

Single-cell readouts resolved for multiple simultaneous parameters (e.g. from mass cytometry) can allow data-driven cell type definition in such heterogeneous populations. However, visualizing and interpreting such data requires specialized computational tools, which to date have incorporated few biological constraints.

We present treeSNE, which separates cell types based on the topology of single-cell measurements in high-dimensional space, allowing cell-type definition to be shaped by potential differentiation mechanisms. treeSNE learns a robust tree metric from such measurements and visualizes them via a neighborhood relation-preserving projection technique (t-SNE). Cell-type composition of poorly characterized heterogeneous cell populations, such as primary tumors, can be defined automatically. Applied to synthetic data, peripheral mononuclear blood cell and tumor samples, treeSNE recapitulates previously identified cell subpopulations. Iterative application of treeSNE resolves fine-grained subpopulation structure, describing rare subpopulations, while the tree structure of the data reveals ‘central’ cell types, such as stem or progenitor cells.
Stochasticity and negative feedback leads to oscillations and distinct gene activity patterns

Samuel Zambrano¹, Nacho Molina²
¹San Raffaele University and Scientific Institute, Division of Genetics and Cell Biology, Milan (Italy); ²University of Edinburgh, SynthSys Centre, Edinburgh (UK)

Gene expression is an inherently stochastic process which is often regulated through negative feedback loops. However, the interplay between the stochastic dynamics of gene activation/inactivation and the biochemical network in which the gene is embedded is not fully understood. Here we use a hybrid model to study a stochastic switching gene coupled with a simple negative feedback regulatory network. We find that oscillations are induced by the stochasticity in gene activation/inactivation. Furthermore, our simple model is able to reproduce peaked distributions of active and inactive times that have been observed experimentally.
Transcriptional bursting dynamics of circadian genes around the clock

Damien Nicolas, Benjamin Zoller, David Suter, Felix Naef
EPF Lausanne (CH)

Transcription of individual mammalian genes frequently occurs discontinuously in the form of short active periods termed bursts followed by inactive windows. Such bursting can be accurately described by a stochastic model of gene expression in which the gene promoter switches between inactive and active states. Moreover, bursting displays stereotyped variability between mammalian genes. Here, we are using promoters of genes belonging to the circadian oscillator to drive the expression of a short-lived luciferase reporter. Since both the mRNA and the protein of the reporter are unstable, fluctuations in protein expression reflect the transcriptional activity of the gene. Thus, by monitoring luminescence in real-time with single-cell resolution, we are able to infer the transcriptional bursting kinetics of genes of interest. Using this approach, we study how the transcriptional bursting of the circadian Bmal1 gene changes during the 24h circadian cycle. In particular, we are interested in estimating how burst size and burst frequency temporally vary at different intervals relative to maximal Bmal1 expression. Since the dynamic bursting pattern of Bmal1 likely reflects phase-specific recruitment of transcription factors and chromatin modifiers to the promoter, we also monitor transcriptional bursting in conditions where the activity of Bmal1 key regulators have been modulated.
Mechanisms promoting spatial self-organization of microbial consortia - The MicroScapesX project

Jan Roelof van der Meer¹, David Johnson², Yok-Ai Que³, Meric Ataman⁴, Alexandros Kiparissides⁴, Vassily Hatzimanikatis⁴, Olga Ilie⁵, Robin Tecon⁵, Dani Or⁵

¹University of Lausanne (CH); ²EAWAG (CH); ³CHUV, Lausanne (CH); ⁴Laboratory of Computational Systems Biology, EPF Lausanne (CH); ⁵Soil and Terrestrial Environmental Physics, ETH Zurich (CH)

Microbial multispecies assemblies shape the ecological functioning of nearly all habitats in the biosphere. Astonishingly, the basic principles that give rise to and sustain the spatial organization of multispecies microbial assemblies still remain largely unknown. The MicroScapesX project provides an interdisciplinary platform for training and development of new approaches to understanding and manipulation of microbial consortia inhabiting natural and engineered surfaces. The project is organised in four axes of research: (1) characterization of spatial patterns of synthetic multispecies assemblies as a function of environmental conditions, species functionalities and metabolic interactions. (2) developing mathematical models and predictive methods for the dynamic formation of multispecies assemblies on surfaces using genome-wide metabolic models in individual (agent)-based population models. Application of these tools to (3) controlled microbial deposition on skin surfaces in burn-patients to inhibit colonization by opportunistic pathogens, and (4) improved bioremediation of contaminated environments and restoration of impaired natural communities.
Synthetic kinase activity relocation sensors for quantitative single cell measurements of MAPK pathways

Eric Durandau, Delphine Aymoz, Serge Pelet
University of Lausanne (CH)

Microscopy in combination with microfluidic devices offers unprecedented ways to monitor the response of single cells to complex excitation patterns. However, to quantify the dynamic response of cells to these stimuli, specific sensors are needed. We have engineered synthetic proteins that specifically relocate upon phosphorylation by a mitogen activated protein kinase (MAPK). Our biosensors are based on a modular architecture which consists in three parts: a fluorescent protein to track the location of the sensor, a nuclear localization signal whose efficiency is modulated by phosphorylation and a docking sequence which controls the specificity of the kinase. As proof of principle, we targeted the sensor to the yeast homologue of ERK1: Fus3 in the mating pathway. Two minutes after stimulating the cells with pheromone, an export of the sensor from the nucleus is observed which is dependent on MAPK activity. We further demonstrate the modularity of these sensors by exchanging the docking site to target other yeast MAPKs. Moreover, multiple sensors bearing fluorescent protein variants can be combined in the same cell to correlate directly the activity of different MAPK pathways in single cells. These sensors allow to characterize the cross-activation and cross-inhibition mechanisms present in the yeast MAPK network.
In silico analysis of DNA re-replication across a complete genome points to genome plasticity in vivo

Maria-Anna Rapsomaniki¹, Konstantinos Koutroumpas², Manuel Ramirez¹, Stavros Taraviras¹, John Lygeros², Zoi Lygerou¹

¹School of Medicine, University of Patras, (Greece); ²Automatic Control Laboratory, ETH Zurich (CH)

DNA replication in eukaryotes is a complex and uncertain process: it initiates from multiple putative origins along the genome and its progression is unique in every cell. DNA replication is also remarkably robust: it is tightly regulated by multiple control mechanisms that ensure its completion in an accurate and timely manner. Aberrations in these mechanisms lead to re-replication and genomic instability, closely connected to oncogenesis and cancer. We have developed a stochastic hybrid model of DNA re-replication that accurately portrays the interplay between discrete, continuous dynamics and uncertainty. Using experimental data for the fission yeast genome, model simulations allow us to visualize how different genomic regions respond to re-replication and permit insight into the key mechanisms affecting re-replication dynamics. Comparison of simulated and experimental population-level profiles shows a good correlation along the genome, validating our approach. At a single-cell level, great copy number variations of individual loci within a population are revealed, affected by intrinsic properties of each locus, cis effects from adjoining loci and trans effects from distant loci. Our analysis shows that, although system properties are robust, cell-to-cell heterogeneity is inherent in re-replication and can lead to a high degree of genome plasticity.
Single-cell kinetics of a single-copy genetic Repressilator

Samuel Ribeiro, Jerome Chandraseelan, Antti Häkkinen, Olli Yli-Harja, Andre Ribeiro
Tampere University of Technology (Finland)

Little is known about the effects of intracellular coupling on the dynamics of synthetic genetic clocks. To investigate this, we implemented the Elowitz-Leibler Repressilator on a single-copy F-plasmid (SCR) and compared it with the original low-copy-number Repressilator (LCR). We compared period of oscillations, functionality (fraction of cells exhibiting oscillations) and robustness (fraction of expected oscillations that occur) as a function of temperature. Finally, we compared their robustness to external perturbations. While both systems respond similarly to temperature changes, mean and variability of the period are higher in the SCR, particularly beyond optimal temperatures, in agreement with having lower RNA copy numbers, which we verified by qPCR. Interestingly, the SCR exhibits higher functionality at optimal temperatures. Next, we show that the SCR is more robust to some external perturbations, by adding IPTG in the media during the microscopy sessions. This robustness decreases for increasing perturbation strength. In the end, we discuss how the SCR, the first functional, synthetic, single-copy, ring-type genetic clock, complements the array of tasks that the LCR can perform. Also, we discuss how these circuits, provided more extensive characterization of their dynamics, can be of use in the study of other synthetic constructs, such as synthetic promoter regions.
MicroRNAs and the control of intrinsic noise in gene expression

Andrzej Rzepiela, Aaron Grandy, Andreas J. Gruber, Afzal P. Syed, Nitish Mittal, Mihaela Zavolan
Biozentrum, University of Basel (CH)

MicroRNAs are small non-protein coding RNAs which, according to the current state of knowledge, can negatively regulate gene expression by promoting mRNA decay. It is believed that effectively miRNAs also modulate a gene expression noise. Taking advantage of single cell sequencing technology, we directly studied the effect of miR-199a induction on noise reduction of predicted mRNA targets. To this aim, we constructed an inducible miR-199a over-expression system in HEK293 cells. Following this we used the Fluidigm C1™ Single-Cell Auto Prep system to isolate single HEK293 cells that express miR-199a, as well as control non-expressing cells. Performing single cell mRNA sequencing on these cells, we analysed miR-199a target gene candidates. Thus far we have not found significant decrease of mRNA expression noise in the induced cells. However, our results are likely biased due to the technical noise of single cell sequencing. Therefore, we performed 'in silico' simulation of the experiment. In this analysis we consider problems arising due to a high amplitude of the technical noise and limited number of studied cells. We estimate detection limits and discuss whether subtle regulation of miRNAs could be detected with current single cell approaches.
A microfluidic platform for quantitative single-cell analysis of antibiotic pharmacokinetics (PK) and pharmacodynamics (PD)

Katrin Schneider, Neeraj Dhar, Sebastian Maerkl, John McKinney

EPF Lausanne (CH)

A crucial step in the evaluation of a new compound proposed as a candidate antibiotic is the relationship between its pharmacokinetics (PK) and pharmacodynamics (PD), i.e. the impact that the time-concentration pattern of drug exposure has on the microorganism. There are several in vitro assay systems used for studying PK/PD relationships of antibiotics, but they all share the main disadvantages that the obtained measurements are static, they represent only a finite moment or "snapshot" in time, measured as an average of the whole population. No information about the dynamics of single-cell behavior, in terms of the killing and division rates, is obtainable using current. Understanding these dynamics is crucial since mutation rates are directly proportional to division rates. Thus, every division event represents a possible step towards resistance.

We propose to design, construct, and apply a novel microfluidic platform for the quantitative study of PK/PD relationships. Using computer-controlled microvalves, time-dependent antibiotic concentration gradients can be created to mimic concentration profiles that are expected in intermittently dosed patients. These gradients are administrated to the cells, which can be simultaneously imaged by time-lapse microscopy, enabling a rigorously quantitative analysis of the rates of cell division and death under fluctuating antibiotic concentrations.
Quantitative analysis of mouse ESCs phenotypes using single-cell live imaging

Stavroula Skylaki, Simon Hastreiter, Oliver Hilsenbeck, Timm Schroeder
D-BSSE, ETH Zurich (CH)

Embryonic stem cells (ESCs) have the unique abilities to self-renew and give rise to derivatives of all three primary germ layers. However, even though ESCs consist of a genetically homogeneous cell population, a number of studies have reported heterogeneities in ESCs cultures as a result of the fluctuation of the expression of key regulators responsible for maintaining the embryonic stem cell identity. In addition, even under defined self-renewing conditions, ESCs display a range of different morphologies that are rarely observed or reported in literature, mainly due to technical challenges. Here we combine time-resolved, single-cell live imaging of high spatiotemporal resolution with automated computational approaches to enable the quantitative analysis of the phenotypic characteristics of mouse ESCs in culture. Using our method, we quantify features related to morphology (area, volume, texture, orientation etc.), behavior (motion patterns, speed, etc.) and gene expression (expression of pluripotency and lineage markers). Finally, we use the extracted features to develop computational models for the prediction of morphologically distinct ESCs subpopulations and to analyze the molecular basis of ESCs fate control.
Microscopy-based single cell screen to identify genes involved in mycobacterial persistence

Amanda Verpoorte¹, Meltem Elitas, Nicolas Denervaud, Neeraj Dhar, Sebastian Maerkl, John McKinney
¹EPF Lausanne (CH); Yale University (USA)

Persistent cells refer to the small fraction of cells of a bacterial population that survive antibiotic exposure. It is a transient phenotype that differs from resistance, the latter being acquired by genetic mutations. The underlying mechanisms of persistence remain largely unknown. We seek to identify the genes implicated in mycobacterial mechanisms of persistence. Our goal is to identify genes involved in persistence by performing a genetic screen. Although persistence is not due to stable genetic changes, we reason that this phenotype must nonetheless have a genetic basis and it should therefore be possible to identify mutants with altered rates of persistence. We performed a microscopy based single cell screen by exposing a mutant library of Mycobacterium smegmatis, a model for Mycobacterium tuberculosis, to the Isoniazid (INH) antibiotic and selecting mutants with an altered persistence rate. We developed a microfluidic device with integrated micromechanical valves that allow a precise control over bacterial environment and simultaneous imaging of thousands of M. smegmatis mutant strains in parallel with a single cell resolution. Eleven mutants have already been identified and characterized from a preliminary screen. The final objective is to identify the "complete" set of genes that are involved in mycobacterial persistence.
Microfluidic transfection for parallel protein production and cell-based assays

Kristina Woodruff, Sebastian Maerkl  
EPF Lausanne (CH)

The genome-wide characterization of protein function requires methods that can cost-effectively produce proteins at high throughput. One such technique is reverse transfection, in which more than 5,000 DNA samples can be printed on a glass microscope slide and subsequently expressed as proteins by mammalian cells. We have expanded the utility of this technique by integrating it with microfluidics. The physical separation of the reactions into chambers decreases cross-contamination, and fluidic manipulation allows complex downstream assays to be performed. This technology is especially promising for secreted proteins because they can be spatially manipulated within the device. In addition to developing microfluidic protein arrays, we have used contact spotting to generate arrays of live mammalian cells. The automated cell handling and simplicity of this approach enable high-throughput assays to be performed on rare cell types and large libraries of cell lines.
Dynamic measurement of the transcription and translation from a single DNA locus in living cells

Victoria Wosika, Delphine Aymoz, Serge Pelet
Department of Fundamental Microbiology, Université de Lausanne (Lausanne, CH)

Transcription is the first step in gene expression and is a highly regulated process. Despite decades of intensive study, its regulation remains poorly understood from a quantitative perspective. A reporter system to monitor fluctuations of expression over time is now available with the PP7 system. This bacteriophage coat protein binds to specific mRNA hairpins. Placing these secondary structures under an inducible promoter in a strain expressing a fluorescently tagged PP7, allows a direct visualization of mRNA, and the localisation of the transcription site inside the nucleus upon activation of the promoter. Because the level of transcription of a gene is linked to the number of RNA polymerase on a single DNA locus, the dynamics of gene transcription can be extrapolated from the amount of PP7-fluorescence aggregating in the nucleus at the transcription site. In this project, we optimized this in vivo mRNA quantification system to obtain single-cell transcription dynamics from a population of cells. To verify the quantitative nature of the mRNA measurements, we have added a fluorescent translational reporter in the same ORF. By coupling the transcription site and the translational reporter measurements, a direct correlation between the mRNA level and protein level can be obtained.
Systems Genetics and Medicine

Chair:

**Olivier Michielin**
Faculty of Biology and Medicine
*University of Lausanne and Swiss Institute of Bioinformatics*
Act Locally, Think Globally: Pathogen behavior in complex host environments

Dirk Bumann
University of Basel (CH)

Infectious diseases are a major health problem worldwide. Infections usually start with just a few pathogen cells penetrating body surfaces. However, disease signs appear only later when pathogens exploitation of host nutrients enables growth to high tissue loads. Pathogen growth and counteracting attempts of the host immune system occur in complex tissues with multiple diverse microenvironments, but this complexity is often ignored in studies relying on bulk average measurements. We have developed a panel of single-cell approaches to analyze host-Salmonella encounters in situ in tissues. Our results demonstrate that Salmonella reside in at least 9 distinct microenvironments within the same mouse spleen. In some microenvironments, the host effectively eradicates local Salmonella subsets, while in other Salmonella successfully defends itself and grows at various rates. Well-nourished, fast-growing Salmonella subsets largely drive disease progression, but can be readily killed by antibiotics. In contrast, abundant moderately growing Salmonella subpopulations contribute little to disease progression, but partially tolerate antimicrobial treatment. This host-induced phenotypic variation delays treatment success posing a risk for failure in critically ill patients, relapses, and resistance development. Together, these data reveal that disease progression and control is the net result of many disparate Salmonella-host encounters that involve strikingly different sets of molecules, cell types, and tissue architectures.
Variation and genetic control of chromatin architecture in humans

Bart Deplancke  
EPF Lausanne (CH)

Non-coding genetic variants have been associated with gene expression traits, yet the precise molecular basis by which they act remains elusive. Here, we measured genome-wide gene expression, transcription-factor (TF)-DNA occupancy, and histone modification levels in 47 human lymphoblastic cell lines. We show that inter-individual variation in chromatin activity is highly abundant and coordinated, revealing the existence of variable chromatin modules (VCMs) often encompassing distinct non-coding regions. VCMs containing both putative promoters and enhancers were typically associated with gene expression changes.

We mapped thousands of cis-acting chromatin quantitative trait loci (QTL), demonstrating that a substantial proportion of chromatin activity is driven by common genetic variation. Moreover, we found that VCM QTLs tended to be enriched within TF-occupied regions, to simultaneously perturb several layers of chromatin, and to be causal to changes in gene expression. Together, this study provides a unique framework for the interpretation of cis-regulatory variation underlying complex molecular traits.
Identifying therapeutic intervention points through systems genetics

Lars Steinmetz

EMBL, Heidelberg (Germany)

Universal therapies for disease are increasingly difficult to find, motivating the search for personal treatments that are tailored to the genetic constitution and environmental exposures of the patient. To achieve this goal, it is important to identify and intervene in the precise molecular pathways that are affected in a given patient and cause disease. Given the wealth of genetic and molecular profiling data being generated, the key challenge resides in developing suitable analytical strategies to predict causal molecular regulators that relay genetic and environmental signals to disease phenotypes. We use multiple model organisms as platforms for developing technologies that enable us to dissect the genetics underlying complex traits, aiming at a systematic understanding of the molecular events that lead from genotype to phenotype. I will discuss our work to identify new molecular signatures that are predictive of genes with causal roles in phenotype, and demonstrate their effectiveness for identifying functional molecular targets for intervention. I will describe work with patient-derived cells and model systems in order to profile the underlying networks and predict effective therapeutic strategies. Beyond proposing a new route towards identifying personal molecular targets from high-throughput omics data, our results contribute to the wider understanding of molecular systems and their role in mediating condition-specific genetic effects to cause complex traits and disease.
Translating cancer genomes into effective personalized immunotherapies

Etienne Caron¹, Heiko Schuster², Matthew Gubin³, Hans-Georg Rammensee², Robert Schreiber³, Ruedi Aebersold¹

¹ETH Zurich (CH); ²University of Tübingen (Germany); ³Washington University School of Medicine (USA)

Science recently highlighted cancer immunotherapy as the “2013 Breakthrough of the Year”. In fact, compelling clinical results have shown that antibody-based checkpoint blockade therapy can restore the function of T lymphocytes to eradicate tumor cells. Although such antibodies stimulate therapeutic, T cell-dependent anti-tumor activity, the tumor antigens recognized by this type of immunotherapy remain undefined. Innovative systems-level methodologies are needed to define such rejection tumor antigens. Here, we have developed a groundbreaking approach integrating cancer genomics, prediction algorithms and targeted mass spectrometry to identify such rejection tumor antigens. By using this novel integrative approach, we have shown for the first time that somatic mutations in the genome of tumor cells translate into tumor-specific mutant proteins that function as rejection antigens for T cells following antibody-based checkpoint blockade immunotherapy. Thus, tumor-specific mutant antigens are important targets of checkpoint blockade therapy. Our systems-level approach will now be tested from a large cohort of human cancer genomes. Once validated, this strategy will be used to rapidly identify tumor-specific mutant antigens and to improve stratification of cancer patients who might best benefit from this form of immunotherapy.
Influence of L-arginine on metabolic networks and the lifespan of activated T cells

Roger Geiger¹, Jan Rieckmann², Camilla Basso¹, Tobias Wolf¹, Maria Kogadeeva³, Felix Meissner², Matthias Mann², Federica Sallusto¹, Nicola Zamboni³, Antonio Lanzavecchia¹

¹ Institute for Research in Biomedicine (CH); ² Max Planck Institute of Biochemistry, Department of Proteomics and Signal Transduction (Germany); ³ Institute of Molecular Systems Biology, ETH Zurich (CH)

T cells play a crucial role in the defence against pathogens and malignant cells. In response to antigenic stimulation, they proliferate and traffic to sites of antigen exposure. Many aspects of the T cell response are metabolically controlled but temporal alterations in metabolic pathways have not yet been systematically analyzed. Here we use high-resolution mass spectrometry to follow 429 metabolites and 7,816 proteins throughout activation of primary human T cells. Several compounds of the arginine metabolism decreased following activation, despite greatly enhanced uptake of L-arginine. Increasing intracellular L-arginine levels changed metabolism globally, including a shift from glycolysis towards oxidative phosphorylation, up-regulation of the serine biosynthesis pathway, increased fatty acid oxidation and a higher ATP content. Interestingly, these cells displayed features of longevity such as increased sirtuin-1 expression and a higher spare respiratory capacity. Consistently, T cells with increased L-arginine levels survived longer after growth factor withdrawal and, when transferred into mice, exhibited improved anti-tumor activity. Our results provide a comprehensive resource for protein copy numbers and metabolite abundances in primary T cells and reveal that the common amino acid L-arginine is a key metabolic regulator.
Photoswitchable ligands represent a potent tool to control and investigate biological processes in a spatial and temporal fashion. The rational design of such ligand is not trivial and as of now they are available only for a limited number of targets. Their main shortcomings are low affinity and small change in binding affinity between the cis and trans conformation. Thus, we have developed a robust strategy for in vitro generation of high-affinity light-activatable peptide ligands to potentially any target. Random phage-encoded peptides were cyclized with an azobenzene linker 3,3′-bis(sulfonato)-4,4′-bis(bromoacetamido) azobenzene (BSBBA), irradiated with UV-light in situ and panned against streptavidin, used as model target. After only two rounds of selection, strong consensus sequences were found and the peptides presented high affinity when cyclized with BSBBA. The binding of peptides isolated from two consensus sequences could be modulated upon UV-light irradiation. The cis exhibited a binding affinity up to 3-fold higher than the trans conformer.
Minor genetic and transcriptional differences mediate high levels of variation in gut immunocompetence in Drosophila melanogaster

Maroun Bou Sleiman¹, Dani Osman¹, Andreas Massouras², Ary Hoffmann³, Bruno Lemaitre¹, Bart Deplancke²
¹Global Health Institute, EPF Lausanne (CH); ²Institute of Bioengineering, EPF Lausanne; ³Bio21 Institute, University of Melbourne (Australia)

Gut immunocompetence involves immune and regenerative processes. To investigate the determinants underlying inter-individual variation in gut immunocompetence, we performed enteric infection of 140 Drosophila lines with the entomopathogenic bacterium Pseudomonas entomophila. We observed extensive variation in survival. Using genome-wide association analysis, we identified novel immune modulators. Transcriptional profiling further showed that the intestinal molecular states of resistant and susceptible lines differ, already pre-infection, with one transcriptional module involving genes linked to reactive oxygen species (ROS) metabolism driving this difference. We found that this genetic and molecular variation is physiologically manifested through lower intestinal ROS activity and susceptibility to ROS-inducing agent next to faster pathogen clearance and higher stem cell activity in resistant versus susceptible lines. Together, this study provides novel insights into the determinants underlying population-level variability in gut immunocompetence, revealing how relatively minor, but systematic genetic and transcriptional variation can mediate overt physiological differences that determine enteric infection susceptibility.
Analysing Evolution of Adaptation to a Novel Siderophore Antibiotic in Gram-negative Bacteria by Next Generation Sequencing

Stefan Nicolet, Marc Creus
University of Basel (CH)

We are investigating the evolution of adaption to antibiotics in vitro using Next Generation Sequencing technology. The drug under investigation is the novel siderophore monosulfactam antibiotic BAL30072, currently under early-stage clinical development against multi-resistant gram-negative bacteria by our research partner, Basilea Pharmaceutic International Ltd. Thus, EvolutionX pioneers the use of systems-biology in guiding early-stage drug-development, within an industrially-relevant pharmaceutical setting.
Disease gene prioritization by scale-aware protein interaction network measures

Christos Dimitrakopoulos\textsuperscript{1,2}, Niko Beerenwinkel\textsuperscript{1,2}
\textsuperscript{1}D-BSSE, ETH Zurich (CH); \textsuperscript{2}SIB Swiss Institute of Bioinformatics (CH)

The network architecture of physical protein interactions is an important determinant for the relations between molecules inside a cell. Network architecture can be characterized by graph-topological characteristics such as shortest paths and network hubs. These characteristics have an important shortcoming: they do not take into account that interactions occur across different scales. On the other hand, kernel diffusion can efficiently characterize interactions across scales but it is not a descriptive measure but a measure of proximity. In this work, we derive generalized, scale-aware versions of known graph-topological measures based on diffusion kernels in order to capture more complex topological properties between genes. We apply these to the problem of disease-gene prioritization by capturing the topology between the test gene (candidate disease gene) and the training genes (known-related disease genes). We define 110 disease-gene families comprising genetically heterogeneous disorders, cancer syndromes, and complex (polygenic) diseases, and we construct an interaction network based on a total of 10,195 experimentally verified or predicted protein-protein interactions. In order to measure the AUC (Area Under Roc Curve) performance of the whole optimization and training procedure, leave-one-out cross-validation has been used for each disease-gene family. Scale-aware measures outperformed current state-of-the-art gene prioritization methods.
The human infectome of Bartonella henselae approached via a genome-wide siRNA screen

Simone Eicher, Simone Muntwiler, Pauli Rämö, Mario Emmenlauer, Damian Murezzan, Gabriel Studer, Houchaima Ben-Tekaya, Christoph Dehio
Biozentrum, University of Basel (CH)

Entry routes of human pathogens into host cells are of major interest in infection biology. Central in their exploration are systematic siRNA-based screens, which, however, generally suffer from poor comparability due to variable experimental setups. To minimise such operational differences, the InfectX consortium established standardised protocols for microscopy-based siRNA-screens on HeLa cells and identified the human infectome of a series of bacterial and viral pathogens.

We applied this workflow to infection with the Gram-negative zoonotic bacterium Bartonella henselae. Its infection phenotype is characterised by the formation of invasomes, internalised bacterial clusters tightly surrounded by actin. siRNA and miRNA screens confirmed the importance of ITGB1-TLN1 signalling and revealed novel factors which decrease invasome formation. In particular, there is a potential feedback loop between the lack of ITGB1-interacting proteins TLN1 and FERMT2, and the presence of two miRNAs, miR-29 and miR-200, which all decrease invasome formation. The mutual regulation of these factors is currently probed.

The stepwise disentanglement of unexplored genes involved in Bartonella infection will improve the description of its infection strategy and allow for a comparison to the infection route of other pathogens within InfectX. Ultimately, such studies will reveal infection pathways putatively exploitable in therapeutic targeting during disease treatment.
Development of a rapid fluorescent assay to study time-kill kinetics of antimicrobials in Neisseria gonorrhoeae

Sunniva Förster¹, Lucy Hathaway², Magnus Unemo³, Nicola Low¹, Christian Althaus¹

¹Institute of Social and Preventive Medicine, University of Bern (CH); ²Institute for Infectious Diseases, University of Bern (CH); ³WHO Collaborating Centre for Gonorrhoea and Other Sexually Transmitted Infections, Örebro (Sweden)

Emerging resistance to antimicrobials is a major problem for Neisseria gonorrhoeae. Clinical resistance to the last option for antimicrobial monotherapy, ceftriaxone, has recently emerged. Antibiotic combination regimens are now recommended but their pharmacodynamic effects and impact on N. gonorrhoeae growth are not well understood. We address this challenge using a systems biology approach combining microbiological experiments and mathematical modelling. Classical time-kill experiments, based on counting colony forming units, are very laborious and time-consuming. We have developed an assay that provides kinetic data in time and concentration space at a high resolution. Furthermore, combinations of antimicrobials can be screened with higher throughput. Using a semi-automated protocol, the time-dependent killing of N. gonorrhoeae is monitored during the first two hours and at 24 hours after the addition of antimicrobials. We then fit the pharmacodynamic function to the data, which results in a detailed description of the time-kill kinetics of an antibiotic. The results from this assay can be used to apply response-surface models, making predictions about the interaction effects of antibiotic combinations. This interdisciplinary approach will facilitate the development of novel treatment options and recommendations to tackle the increasing numbers of multidrug resistant and extensively drug resistant N. gonorrhoeae strains.
Comparative sequencing of renal cancer biopsy pairs reveals co-existing subclones

Ariane Hofmann\textsuperscript{1,2}, Christian Beisel\textsuperscript{1}, Jonas Behr\textsuperscript{1,2}, Peter Schraml\textsuperscript{3}, Holger Moch\textsuperscript{3}, Niko Beerenkwinkel\textsuperscript{1,2}

\textsuperscript{1}D-BSSE, ETH Zurich (CH); \textsuperscript{2}SIB Swiss Institute of Bioinformatics (CH); \textsuperscript{3}Institute for Surgical Pathology, University Hospital Zurich (CH)

Due to the process of mutation and selection, a tumor is composed of various subclones with different genotypes and phenotypes. It is crucial to investigate the subclonal structure and to understand the dynamics of their interplay for improving treatment success. We analyzed two biopsies of each renal cell carcinoma (RCC) together with a matched normal sample from the same individual. Our analysis is based on next-generation sequencing data of the exomes of 16 RCCs from 16 patients. We performed variant calling and pairwise comparison of the variations found in the two tumor biopsies. On average 44.5\% of the mutations were shared between the two samples. The mutations detected in only one sample were oftentimes present with weak evidence in the other sample. Counting these low-evidence mutations as shared left on average 4.9\% private mutations. Pairwise comparison of the frequencies of the SNVs showed that some of them differed remarkably. The results showed that most ancestor clones exist at varying frequencies in the two samples. The private mutations represent new clones that emerged in some samples. Ultra-deep sequencing of those genes harboring mutations with different frequencies in the two samples will enable a more detailed investigation of the subclonal tumor structure.
Assembly and curation of a genome-wide metabolic model of a gut commensal bacterial community

Julien Limenitakis, A. Macpherson, K. McCoy
University of Bern (CH)

The propagation of a particular bacteria within a complex environment like the mammalian gut is central in disrupting immune homeostasis, leading to various pathologies. Commensal bacteria rely on their ecological niche but also on community member metabolic processes for growth. In this gut community context, the degree of resilience of the metabolic network remains to be studied, especially in the context of a new bacterial colonization.

In this work we have assembled the genome-scale metabolic model of a reduced but well-defined and tractable gut microbial community. The community consists of four of the most abundant and relevant murine gut bacterial species of a well-established defined microbial flora, the altered Schaedler flora. We are performing a set of experiments to measure in vivo growth, production/consumption of metabolites, and in vivo gene expression. These data will be used as constraints for the model. The latter is being analyzed using flux balance analysis.

This study aims to determine the constraints applied by the different members on the community metabolic network. The identified key nodes of the metabolic interface between the members of the community will be explored as unique and/or essential metabolic activities potentially explains metabolic dependencies between species.
Genome-wide RNAi screen reveals host factors involved in Brucella infection

Shyan Huey Low¹, Alain Casanova¹, Raquel Conde-Alvarez², Houchaima Ben-Tekaya¹, Mario Emmenlauer¹, Pauli Rämo¹, Simone Muntwiler¹, Suzana Salcedo³, Jean Pierre Gorvel³, Christoph Dehio¹

¹Biozentrum, University of Basel (CH); ²Department of Microbiology, University of Navarra (Spain); ³Centre d’Immunologie de Marseille-Luminy, INSERM-CNRS (France)

Brucella is an intracellular zoonotic pathogen that causes animal and human brucellosis worldwide. In animals, Brucella causes abortion and birth of weak offspring while in humans, a febrile disease known as Malta fever could lead to chronic infections of endocarditis or meningitis. Therefore, brucellosis is a significant threat to the economy and human health in endemic areas. Brucella invades phagocytic and non-phagocytic cells and replicates in an intracellular compartment known as the Brucella-containing vacuole (BCV). Following entry, Brucella traffics along the endocytic pathway and proliferates in an endoplasmic reticulum (ER) derived compartment. The molecular mechanisms on how Brucella interacts with its host remain largely unknown.

To study Brucella entry and replication in human cells, we performed a genome-wide, high-content microscopy-based RNA interference (RNAi) screen in HeLa cells. This allowed us to unravel host cellular pathways involved in Brucella infection, which includes actin-remodeling, transforming growth factor (TGF-β) or fibroblast growth factor (FGF) signaling, ER-Golgi bidirectional transport, and some components of the endocytic pathway. We also showed that TGF-β and FGF signaling pathways are involved in Brucella entry into non-phagocytic cells. Furthermore, we identified Vps35 - a component of the retromer complex involved in endosome to Golgi transport - as novel host factor involved in a post-entry step of Brucella infection.
Rapid optimisation of drug combinations for angiostatic cancer therapy

Patrycja Nowak-Sliwinska¹, Andrea Weiss¹, Xianting Ding², Judy van Beijnum³, Ieong Wong², Tse Wong³, Robert Berndsen³, Olivier Dormond⁴, Chih-Ming Ho², Paul Dyson¹, Hubert van den Bergh¹, Arjan Griffioen³
¹EPF Lausanne (CH); ²University of California, Los Angeles (USA); ³VU University Medical Center (NL); ⁴CHUV, Lausanne (CH)

The prolongation of cancer patient survival by angiostatic targeted agents has been disappointing, due to issues including tumor and patient heterogeneity, drug resistance and adverse effects. Drug combinations may improve efficacy, and/or enable the reduction of side effects and drug resistance. Decisions on which drugs at what doses to combine are difficult due to the large number of available options. Here, we utilize a feedback system control (FSC) technique with a population-based stochastic search algorithm to navigate through the large parametric space of nine angiostatic drugs to identify an optimal, low-dose drug combination with significantly improved efficacy. This was performed using an iterative approach of in vitro testing on endothelial cell viability and algorithm-based analysis. We found an optimal drug combination composed of three drugs whose individual drug doses were significantly reduced as compared to the corresponding single drugs at their optimal doses. Successful translation of the optimal combination into two preclinical in vivo tumor models showed a synergistic tumor growth inhibition. Our results suggest that the FSC approach allows for the rapid identification of effective, synergistic, low-dose drug combinations.
Release of Human Cytomegalovirus from latency by a KAP1/TRIM28-targeting mTOR-mediated phosphorylation switch

Benjamin Rauwel, Marco Cassano, Suk Min Jang, Adamandia Kapopoulou, Isabelle Barde, Didier Trono
EPF Lausanne (CH)

Human cytomegalovirus (HCMV) is a highly prevalent pathogen that induces life-long infections notably by establishing latency in hematopoietic stem cells (HSC). Bouts of reactivation are normally controlled by the immune system, but can be fatal in immuno-compromised individuals such as organ transplant recipients. Here, we reveal that HCMV latency in human CD34+ HSC reflects the recruitment on the viral genome of KAP1, a master co-repressor, together with the associated HP1 and SETDB1 histone methyltransferase, which results in transcriptional silencing. During lytic infection, KAP1 is still associated with the viral genome, but its heterochromatin-inducing activity is suppressed by mTOR-mediated phosphorylation. Correspondingly, HCMV can be forced out of latency in HSC by KAP1 knockdown and in HSC and monocytes by pharmacological induction of KAP1 phosphorylation. These results suggest new approaches not only to curtail CMV infection but also to purge the virus from organ transplants.
Accurate profiling of immune repertoires by molecular transcript barcoding and next-generation sequencing

Sai Reddy, Tarik Khan
D-BSSE, ETH Zurich (CH)

Recent advances in Next-generation DNA sequencing (NGS) technology have enabled high-throughput systems analysis of immunological responses. NGS of antibody repertoires has emerged as a method to support or augment existing immunological technologies. One potential pitfall of antibody NGS is the lack of uncertainty when quantifying the relative abundance between antibody clones, which compromises the quantitative accuracy of repertoire data. This uncertainty stems from systematic and stochastic biases introduced during sample preparation (i.e. PCR). Therefore, we have developed an experimental-bioinformatic approach to ensure accurate quantitative assessment of clonal frequencies based on molecular transcript barcoding (MTB). MTB relies on generating cDNA with unique identifiers, incorporated by gene specific primers in the form of oligonucleotides with specified degenerate regions, to enable bias calibration data. Identified biases are then able to be corrected using bioinformatics approaches to produce more accurate information about clonal relative abundance. In addition to correction of clonal frequencies, MTB enables removal of sequencing error and PCR error, thus ensuring high quality data and distinction of synthetic mutations from true biological mutations (somatic hypermutations). We are now applying these tools to profile the immune responses from HIV-infected individuals and mouse models of viral infection.
Drug sensitivity profiles of ABC transporter deletion strains in S. cerevisiae

Nil Sahin¹, Frederick P. Roth², Murat Cokol¹

¹Sabanci University Istanbul (Turkey); ²University of Toronto (Canada)

ATP-binding cassette (ABC) transporters are proteins that mediate transmembrane export of chemically diverse compounds in an ATP-dependent manner. ABC transporter involvement in drug metabolism is conserved from prokaryotes to humans, making their functional knowledge of immediate interest to understanding the genetic determinants of drug resistance in any organism. Here, we conducted a screen to identify the relationship between ABC transporters and drug sensitivity. We used 16 S. cerevisiae strains in which one of 16 ABC transporters was deleted. In addition, we used strains in which either all or none 16 ABC transporters were deleted. We measured the sensitivity of these strains to 28 antifungal compounds with various mechanisms of action. We generated highly reproducible sensitivity profiles for each drug and strain. Clustering of these profiles indicated groups of compound profiles that were almost identical. The strain lacking all 16 ABC transporters was the most sensitive strain against the majority of drugs. However, we found that this strain had increased resistance to rapamycin, tunicamycin, and valinomycin. We present this small-scale chemogenetic screen as a high-quality data set for studying the relationship between ABC transporters and drug sensitivity.
Absolute quantification of the entire Mycobacterium tuberculosis proteome across perturbed states by SWATH MS

Olga T. Schubert¹, Christina Ludwig¹, George Rosenberger¹, Hannes L. Röst¹, Michael Zimmermann¹, Martin Gengenbacher², Stefan H. E. Kaufmann², Uwe Sauer¹, Ruedi Aebersold¹,³
¹ETH Zurich (CH); ²Max Planck Institute for Infection Biology, Berlin (Germany); ³University of Zurich (CH)

There is great hope that systems biology-based strategies will significantly contribute to the knowledge-guided development of more effective vaccines and drugs to prevent and cure infectious diseases, such as tuberculosis. A key element of such strategies is the mechanistic understanding of the dynamic adaptation of the cell to adverse environmental conditions.

Here we describe on the example of Mycobacterium tuberculosis (Mtb) the development of a generic proteomic technology that has the ability to accurately quantify essentially all proteins expressed in a microbial cell at high sample throughput. First, we developed a library of mass spectrometric assays covering 97% of all Mtb proteins. This unique resource enabled us to quantify in a targeted fashion the entire expressed proteome of Mtb reproducibly over many samples by SWATH MS. Second, we implemented a label-free strategy to estimate cellular concentration of >2000 Mtb proteins in a single mass spectrometric run.

We applied these methods to query the dynamics of the proteomic adaptations of Mtb in response to clinically relevant stress conditions. The resulting quantitative proteomic data, together with high-quality metabolomic data, provides insights into dynamic cellular processes and helps to shed light on adaptation and persistence of Mtb during infection of the human lungs.
Topographic DNA extraction from tissue sections using the Microfluidic Probe for mutational profiling of tumors

Aditya Kashyap1, Emmanuel Delamarche2, Peter Schraml1, Govind Kaigala2, Alex Soltermann1

1Institute of Surgical Pathology, University Hospital Zurich (CH); 2IBM Zurich Research Laboratory (CH)

Background: Strategies to perform spatial genetic profiling in combination with multiple biomarker visualization on tissue sections may be crucial for understanding tumor microenvironments. We aim for transferring IBM’s Microfluidic Probe (MFP) technology into clinical molecular pathology by developing a topographic DNA extraction protocol.

Methods: The MFP is a non-contact scanning technology which spatially confines nanoliter volumes of chemicals hydrodynamically on biological surfaces at the micrometer-length scale in an aqueous environment. Cut sections of a formalin-fixed paraffin-embedded cell block from a melanoma cell line carrying the predictive oncogenic mutation BRAF V600E were locally profiled using decellularization and DNA extraction buffer, following PCR and Sanger sequencing.

Results: By means of developing chemical, fluidic and thermal systems in concert with the MFP, decellularization was achieved in a footprint area of 100 x 100 micrometer within 1 min of confinement using 5 micrometer thick cuts on Superfrost-Plus glass slides. This footprint size corresponded to 150 tumor cells. The extracted DNA was amplifiable by both quantitative and non-quantitative PCR. The BRAF V600E mutation was identified by Sanger sequencing.

Conclusions: Due to the aqueous nature of the MFP buffer system, mutational profiling may be done on the very same cells pre-stained by non-covalent immunohistochemistry.
Metabolism as a source of novel drug targets: focus on Toxoplasma gondii

Stepan Tymoshenko1,2,3, Rebecca Oppenheim2, Rasmus Agren4, Jens Nielsen4, Dominique Soldati-Favre2, Vassily Hatzimanikatis1,3

1Laboratory of Computational Systems Biotechnology, EPF Lausanne (CH); 2Department of Microbiology and Molecular Medicine, University of Geneva (CH); 3SIB Swiss Institute of Bioinformatics (CH); 4Department of Chemical and Biological Engineering, Chalmers University of Technology (Sweden)

Toxoplasma gondii is one of the most wide-spread human pathogens worldwide. Chronic infection with this eukaryotic parasite is asymptomatic and generally not harmful for immunocompetent individuals. Yet in the case of an acquired immunodeficiency or an immunosuppressive therapy reactivation of toxoplasmosis often causes an acute and life-threatening disease. Current options for treatment of toxoplasmosis are limited, not well-tolerated, and inefficient against the encysted, chronic form. There is a clearly unmet need for new medication and metabolism is a promising source of drug targets.

Metabolic models provide an efficient framework for exploring potential vulnerabilities in metabolism of pathogenic species and thus facilitate discovery of novel drug targets. ToxoNet1 is a genome-scale metabolic model of T. gondii built in silico using state-of-the-art automated reconstruction algorithm (RAVEN toolbox). The model clarified the minimal nutritional requirements for asexual replication of T. gondii. We further defined the set of alternative precursors, salvage of which is indispensable for the parasite. Within simulated human host cell environment ToxoNet1 predicts a minimal set of 53 enzyme-coding genes to be essential for parasite replication. A number of these predictions represent readily testable hypotheses that facilitate identification of potential targets for an effective intervention against toxoplasmosis.
The Interaction Proteome of the Human Kinome

Anton Vichalkovski\textsuperscript{1}, Audrey van Drogen\textsuperscript{1}, Simon Hauri\textsuperscript{1}, Markku Varjosalo\textsuperscript{1,3}, Ruedi Aebersold\textsuperscript{1,2,4}, Matthias Gstaiger\textsuperscript{1,2}
\textsuperscript{1}Institute of Molecular Systems Biology, ETH Zurich (CH); \textsuperscript{2}Competence Center for Systems Physiology and Metabolic Diseases, ETH Zurich (CH); \textsuperscript{3}Institute of Biotechnology, University of Helsinki (Finland); \textsuperscript{4}University of Zurich (CH)

Intracellular information processing via reversible protein phosphorylation is controlled by the localization, activity, and substrate specificity of protein kinases, which to a large extent is achieved by complex formation with other proteins. Despite their critical role in cellular regulation and pathogenesis, protein interaction information is available for only a well-studied subset of the 518 human protein kinases. Here, we present the first global mass spectrometry based analysis of human kinase complexes covering 360 soluble kinases and their interactions with a total of 2816 cellular proteins. In addition to subgroup-specific functional enrichment and modularity, we uncover hundreds of novel kinase interactors, delineate their participation in specific protein complexes and establish a distinct kinase-kinase network highlighting 927 candidate regulatory phosphosites. Finally, the presented kinome interactome revealed a significant set of novel interactions with proteins genetically linked to human diseases, including cancer. Thus the presented kinase network provides a new systems level framework for the integration of genomic information from patients. This in turn may facilitate the development of promising personalized strategies for targeting kinase functions altered in human pathologies.
Sphingolipids are major and essential components of eukaryotic membranes. They serve both structural roles as well as signaling functions, possessing bioactivity potentially balancing between cellular life and death. In fact, this class of lipids has been implicated in several human pathologies, including sphingolipidoses, asthma and cancer. Despite the burgeoning appreciation of the role of sphingolipids in health and diseases, our understanding of their metabolism, structures and functions remains limited.

Advances in analytics, particularly liquid chromatography-mass spectrometry, have revolutionized lipid research and developed it into the ‘-Oomics’ field (Wenk 2010). This has allowed the determination of lipid compositions of biological systems at high resolution, creating a new knowledge-base for the ‘lipid molecular code’. Over the last years, we have developed novel lipidomics approaches and provided the most comprehensive sphingolipid profiles of the model organisms, S. cerevisiae and D. melanogaster. Combining the lipidomics tools with classical genetics as well as cell and molecular biology, we revealed new insights into the roles of the enigmatic sphingolipids in cellular physiology and organismal development (Guan et al 2006, 2009, 2013). We demonstrate the translation of this systems-based approach for therapeutic targets discovery as well as for diagnoses of protozoan infections which represent major global health and socioeconomic burdens.
Author Index

Please note that these numbers refer to the abstract number found at the top of each page, and not the page number.

A
Abbuehl, Jean-Paul 9
Abriata, Luciano Andres 10
Ackermann, Martin 58
Adametz, David 51
Aebersold, Daniel M. 45
Aebersold, Ruedi 45, 48, 54, 102, 133, 166, 181, 184
Agren, Rasmus 183
Aguilar-Rodriguez, José 121
Akhmanova, Anna 149
Albarca, Monica 122
Alon, Uri 103
Althaus, Christian 173
Ambrosini, Givanna 94
Ambühl, Mark 31
Ana-Maria, Farcas 68
Andersin, Teemu 132
Andrea, Prota 68
Andritschke, Daniel 32, 142
Anil, Kumar 68
Armenta, Ricardo 74
Arnaud, Jonathan 139
Arnal, Damien 125
Arpat, Bulak 90, 108
Arvaniti, Eirini 11
Ataman, Meric 153
Atanasoski, Suzana 56, 64
Atger, Florian 109
Auwerx, Johan 48
Aymoz, Delphine 143, 154, 162
Azarias, Guillaume 44
Aziraj, Nagjie 61

B
Baerenfaller, Katja 106
Ballmer-Hofer, Kurt 61
Banovich, Nicholas 127
Barahona, Mauricio 17
Barann, Matthias 130
Barde, Isabelle 84, 178
Barral, Yves 68, 70, 149
Baruchet, Michael 119
Bassel, George 30
Basso, Camilla 167
Baubec, Tuncay 85
Baudrimont, Antoine 86
Bayer, Mathias 149
Becskei, Attila 86, 105
Beerenwinkel, Niko 32, 171, 174
Behr, Jonas 174
Behrndt, Martin 59
Beisel, Christian 56, 64, 133, 174
Bellotto, Silvia 168
Ben-Tekaya, Houchaima 172, 176
Bensimon, Ariel 45
Berger, Imre 61
Berger, Philipp 46, 61
Bergmann, Sven 7
Berndsen, Robert 177
Berninger, Philipp 56, 64
Bersani, Alberto 13
Betizeau, Marion 47
Bevers, Roel 87
Bieler, Jonathan 144
Bigan, Erwan 16
Blackburn, Matthew 88
Blacklock, Krisin 36
Blanchoin, Laurent 139
Blattmann, Peter 48
Bocher, Philipp 114
Bodenmiller, Bernd 146
Boersema, Paul 49, 146
Boisson-Dernier, Aurélien 55
Borgers, Susanne 59
Borrell, Sonia 102, 133
Bossel Ben-Moshe, Noa 103
Bou Sleiman, Maroun 87, 169
Bucher, Philipp 82, 94
Buffing, Marieke Francisca 50
Bugarski, Milica 46
Bumann, Dirk 163
Bureau, Jean-Baptiste 145
Burger, Lukas 83, 91

C
Cabernard, Clemens 69
Califano, Andrea 125
Canella, Donatella 89
Cannavo, Rosamaria 144
Carmeli, Cristian 89
Caron, Etienne 166
Casanova, Alain 32, 176
Cassano, Marco 178
Castelo-Szekely, Violeta 90
Castro-Diaz, Nathaly 98
Cavallone, Andrea 20
Chambon, Marc 72
Chandraseelan, Jerome 156
Chen, Shiya 168
Chen, Xiuzhen 70
Chopard, Bastien 12
<table>
<thead>
<tr>
<th>Name</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choukrallah, Mohamed-Amin</td>
<td>91</td>
</tr>
<tr>
<td>Christodoulou, Dimitrios</td>
<td>14</td>
</tr>
<tr>
<td>Claassen, Manfred</td>
<td>11, 21, 26, 27, 150</td>
</tr>
<tr>
<td>Cokol, Murat</td>
<td>180</td>
</tr>
<tr>
<td>Collins, Ben</td>
<td>133</td>
</tr>
<tr>
<td>Colombi, Marco</td>
<td>51</td>
</tr>
<tr>
<td>Coluccio, Andrea</td>
<td>92</td>
</tr>
<tr>
<td>Conde-Alvarez, Raquel</td>
<td>176</td>
</tr>
<tr>
<td>Conley, Gaurasundar</td>
<td>74</td>
</tr>
<tr>
<td>Consortium, CycliX</td>
<td>132</td>
</tr>
<tr>
<td>Corsinotti, Andrea</td>
<td>84</td>
</tr>
<tr>
<td>Coscollá, Mireia</td>
<td>102</td>
</tr>
<tr>
<td>Cosentino Lagomarsino, Marco</td>
<td>20</td>
</tr>
<tr>
<td>Cosson, Pierre</td>
<td>145</td>
</tr>
<tr>
<td>Cotterell, James</td>
<td>129</td>
</tr>
<tr>
<td>Cramer, Patrick</td>
<td>77</td>
</tr>
<tr>
<td>Creus, Marc</td>
<td>170</td>
</tr>
<tr>
<td>Crotti, Pablo</td>
<td>15</td>
</tr>
<tr>
<td>Da Veiga Moreira, Jorgelindo</td>
<td>16</td>
</tr>
<tr>
<td>Daga, Neha</td>
<td>97</td>
</tr>
<tr>
<td>Dal Peraro, Matteo</td>
<td>10, 132</td>
</tr>
<tr>
<td>Dalessi, Sascha</td>
<td>7</td>
</tr>
<tr>
<td>Darbellay, Fabrice</td>
<td>65</td>
</tr>
<tr>
<td>Dattani, Justine</td>
<td>17</td>
</tr>
<tr>
<td>Dazert, Eva</td>
<td>51</td>
</tr>
<tr>
<td>De Iaco, Alberto</td>
<td>52, 92</td>
</tr>
<tr>
<td>De Simone, Alessandro</td>
<td>53</td>
</tr>
<tr>
<td>Dehio, Christoph</td>
<td>32, 97, 172, 176</td>
</tr>
<tr>
<td>Delamarche, Emmanuel</td>
<td>182</td>
</tr>
<tr>
<td>Delattre-Gubelmann, Carine</td>
<td>96, 122</td>
</tr>
<tr>
<td>Delincé, Matthieu</td>
<td>145</td>
</tr>
<tr>
<td>Delorenzi, Mauro</td>
<td>89, 93</td>
</tr>
<tr>
<td>Denervaud, Nicolas</td>
<td>160</td>
</tr>
</tbody>
</table>
Author Index

Denis K., Samuylov 68
Deplancke, Bart 82, 87, 96, 122, 132, 164, 169
Derksen, Selina 58
Dessus-Babus, Sophie 83
Desvergne, Béatrice 119
Dhar, Neeraj 158, 160
Di Palma, Serena 146
Didier, Trono 107
Diego Ulisse, Pizzagalli 147
Dilling, Sabrina 32, 142
Dimitrakopoulos, Christos 171
Ding, Xianting 177
Dormond, Olivier 177
Dreos, René 94
Drewek, Anna 95
Du, Ngoc-Hien 116
Duc, Julien 96, 117
Durandau, Eric 143, 154
Dyson, Paul 177

E
Ebhardt, H. Alexander 54
Ebrahimi, Ali 18
Ecco, Gabriela 92, 96, 98
Edgington, Matthew 19
Ehsaei, Zahra 64
Eicher, Simone 32, 172
Elitas, Meltem 160
Elowitz, Michael 2
Emmenlauer, Mario 32, 142, 172, 176
Eschbach, Katja 56, 64

F
Farcas, Ana-Maria 149
Fehling-Kaschek, Mirjam 23
<table>
<thead>
<tr>
<th>Author Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feldmann, Julia</td>
</tr>
<tr>
<td>Felekis, Dimitris</td>
</tr>
<tr>
<td>Fogelson, Ben</td>
</tr>
<tr>
<td>Fortes, Claudia</td>
</tr>
<tr>
<td>Franceschini, Andrea</td>
</tr>
<tr>
<td>Francica, Paola</td>
</tr>
<tr>
<td>Franck, Christina Maria</td>
</tr>
<tr>
<td>Fried, Patrick</td>
</tr>
<tr>
<td>Friedli, Marc</td>
</tr>
<tr>
<td>Frochaux, Michael</td>
</tr>
<tr>
<td>Fumagalli, Maria Rita</td>
</tr>
<tr>
<td>Förster, Sunniva</td>
</tr>
</tbody>
</table>

**G**

<table>
<thead>
<tr>
<th>Author Name</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gachon, Frédéric</td>
<td>101, 109, 118</td>
</tr>
<tr>
<td>Gagneux, Sébastien</td>
<td>102, 133</td>
</tr>
<tr>
<td>Ganscha, Stefan</td>
<td>21</td>
</tr>
<tr>
<td>Gatfield, David</td>
<td>90, 108, 116, 144</td>
</tr>
<tr>
<td>Geiger, Roger</td>
<td>167</td>
</tr>
<tr>
<td>Gengenbacher, Martin</td>
<td>181</td>
</tr>
<tr>
<td>Gerosa, Luca</td>
<td>14</td>
</tr>
<tr>
<td>Gilad, Yoav</td>
<td>127</td>
</tr>
<tr>
<td>Gilardi, Federica</td>
<td>119</td>
</tr>
<tr>
<td>Gili, Magüi</td>
<td>129</td>
</tr>
<tr>
<td>Gillor, Osnat</td>
<td>24</td>
</tr>
<tr>
<td>Giordano Attianese, Greta</td>
<td>119</td>
</tr>
<tr>
<td>Gitzinger, Marc</td>
<td>133</td>
</tr>
<tr>
<td>Glück, Astrid A.</td>
<td>45</td>
</tr>
<tr>
<td>Gobet, Cedric</td>
<td>144</td>
</tr>
<tr>
<td>Gonzalez-Gaitan, Marcos</td>
<td>59</td>
</tr>
<tr>
<td>Gorvel, Jean Pierre</td>
<td>176</td>
</tr>
<tr>
<td>Grandy, Aaron</td>
<td>157</td>
</tr>
<tr>
<td>Grbic, Djordje</td>
<td>135</td>
</tr>
<tr>
<td>Grellscheid, Sushma</td>
<td>136</td>
</tr>
<tr>
<td>Griffioen, Arjan</td>
<td>177</td>
</tr>
<tr>
<td>Author</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Grigoriev, Ilya</td>
<td>149</td>
</tr>
<tr>
<td>Grison, Alice</td>
<td>56, 64</td>
</tr>
<tr>
<td>Grob, Stefan</td>
<td>100</td>
</tr>
<tr>
<td>Grossmann, Jonas</td>
<td>73</td>
</tr>
<tr>
<td>Grossniklaus, Ueli</td>
<td>55, 67, 71, 111, 130</td>
</tr>
<tr>
<td>Gruber, Andreas</td>
<td>110, 131, 157</td>
</tr>
<tr>
<td>Gruenberg, Jean</td>
<td>72</td>
</tr>
<tr>
<td>Gruissem, Wilhelm</td>
<td>106</td>
</tr>
<tr>
<td>Grégory, Paul</td>
<td>68</td>
</tr>
<tr>
<td>Gstaiger, Matthias</td>
<td>184</td>
</tr>
<tr>
<td>Guan, Xueli</td>
<td>102, 133</td>
</tr>
<tr>
<td>Gubin, Matthew</td>
<td>166</td>
</tr>
<tr>
<td>Guex, Nicolas</td>
<td>72, 124</td>
</tr>
<tr>
<td>Gumienny, Rafal</td>
<td>128</td>
</tr>
<tr>
<td>Gunness, Patrina</td>
<td>73</td>
</tr>
<tr>
<td>Gustafson, Kyle</td>
<td>101, 118, 144</td>
</tr>
<tr>
<td>Guthörl, Daniela</td>
<td>71</td>
</tr>
<tr>
<td>Gygli, Sebastian</td>
<td>102, 133</td>
</tr>
<tr>
<td>Gábor, Székely</td>
<td>68</td>
</tr>
<tr>
<td>Gönczy, Pierre</td>
<td>53, 66</td>
</tr>
</tbody>
</table>

**H**

<table>
<thead>
<tr>
<th>Author</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hachet, Olivier</td>
<td>7</td>
</tr>
<tr>
<td>Hall, Michael N.</td>
<td>51</td>
</tr>
<tr>
<td>Hardt, Wolf-Dietrich</td>
<td>32, 142</td>
</tr>
<tr>
<td>Hardtke, Christian</td>
<td>42</td>
</tr>
<tr>
<td>Hart, Yuval</td>
<td>103</td>
</tr>
<tr>
<td>Hastreiter, Simon</td>
<td>159</td>
</tr>
<tr>
<td>Hathaway, Lucy</td>
<td>173</td>
</tr>
<tr>
<td>Hatzimanikatis, Vassily</td>
<td>153, 183</td>
</tr>
<tr>
<td>Hauri, Simon</td>
<td>184</td>
</tr>
<tr>
<td>Hausser, Jean</td>
<td>103</td>
</tr>
<tr>
<td>Heim, Markus</td>
<td>51</td>
</tr>
<tr>
<td>Heinis, Christian</td>
<td>168</td>
</tr>
<tr>
<td>Heisenberg, Carl-Philipp</td>
<td>40, 59</td>
</tr>
</tbody>
</table>
Author Index

Helenius, Jonne 69
Helleboid, Pierre-Yves 81, 104
Hemberg, Martin 17
Hernandez, Nouria 89, 124
Hernando-Herraez, Irene 127
Herr, Winship 124
Hersch, Micha 7
Hesketh, John 136
Heslot, Francois 20
Hilbi, Hubert 140
Hilsenbeck, Oliver 159
Ho, Chih-Ming 177
Ho, Sylvia 76
Hoffmann, Ary 169
Hofmann, Ariane 174
Holtzer, Laurent 59
Hopfgartner, Gérard 57
Hrabakova, Rita 49
Hsu, Chieh 105
Huang, Hsiao-Chun 148
Huelsken, Joerg 75
Huey Low, Shyan 32
Hughes, Tim 78
Häkkinen, Antti 156

I
Iber, Dagmar 43, 47, 64
Ihmor, Phillip 106
Ilie, Olga 22, 153
Imbeault, Michaël 81, 84, 96, 104
Isakova, Alina 82
Isalan, Mark 129

J
Jacobi, Carsten 54
<table>
<thead>
<tr>
<th>Author</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacobs, Andrea</td>
<td>146</td>
</tr>
<tr>
<td>Jahn, Sandra</td>
<td>57</td>
</tr>
<tr>
<td>Jang, Bumjin</td>
<td>67</td>
</tr>
<tr>
<td>Jang, Suk Min</td>
<td>107, 178</td>
</tr>
<tr>
<td>Janich, Peggy</td>
<td>90, 108</td>
</tr>
<tr>
<td>Jaquet, Vincent</td>
<td>105</td>
</tr>
<tr>
<td>Jenö, Paul</td>
<td>51</td>
</tr>
<tr>
<td>Jette, Lengefeld</td>
<td>68</td>
</tr>
<tr>
<td>Johnson, David</td>
<td>58, 153</td>
</tr>
<tr>
<td>Jorjani, Hadi</td>
<td>110</td>
</tr>
<tr>
<td>Jouffe, Céline</td>
<td>101, 109, 118</td>
</tr>
<tr>
<td>Jülicher, Frank</td>
<td>3</td>
</tr>
</tbody>
</table>

K

<table>
<thead>
<tr>
<th>Author</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaigala, Govind</td>
<td>182</td>
</tr>
<tr>
<td>Kalantzi, Alexandra Stylian</td>
<td>132</td>
</tr>
<tr>
<td>Kapopoulou, Adamandia</td>
<td>84, 96, 98, 117</td>
</tr>
<tr>
<td>kapopoulou, adamannda</td>
<td>178</td>
</tr>
<tr>
<td>Kardash, Elena</td>
<td>59</td>
</tr>
<tr>
<td>Karimadini, Zahra</td>
<td>43, 64</td>
</tr>
<tr>
<td>Kartal, Önder</td>
<td>111</td>
</tr>
<tr>
<td>Kaschek, Daniel</td>
<td>23</td>
</tr>
<tr>
<td>Kashyap, Aditya</td>
<td>182</td>
</tr>
<tr>
<td>Kaufmann, Stefan H. E.</td>
<td>181</td>
</tr>
<tr>
<td>Kauzlaric, Annamaria</td>
<td>92, 107</td>
</tr>
<tr>
<td>Kellogg, Ryan</td>
<td>141</td>
</tr>
<tr>
<td>Kelm, Jens</td>
<td>73</td>
</tr>
<tr>
<td>Kerkhoff, Eugen</td>
<td>142</td>
</tr>
<tr>
<td>Kessler, Aline</td>
<td>140</td>
</tr>
<tr>
<td>Khan, Tarik</td>
<td>179</td>
</tr>
<tr>
<td>Kim, Minsu</td>
<td>24</td>
</tr>
<tr>
<td>Kiparissides, Alexandros</td>
<td>153</td>
</tr>
<tr>
<td>Kleyer, Hannah</td>
<td>25</td>
</tr>
<tr>
<td>Klimovskaia, Anna</td>
<td>26</td>
</tr>
<tr>
<td>Klostermeier, Ulrich C.</td>
<td>71, 130</td>
</tr>
<tr>
<td>Author</td>
<td>Page(s)</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Kogadeeva, Maria</td>
<td>60, 167</td>
</tr>
<tr>
<td>Komljenovic, Andrea</td>
<td>112</td>
</tr>
<tr>
<td>Korem, Yael</td>
<td>103</td>
</tr>
<tr>
<td>Korf, Ulrike</td>
<td>23</td>
</tr>
<tr>
<td>Koutroumpas, Konstantinos</td>
<td>155</td>
</tr>
<tr>
<td>Krebs, Arnaud</td>
<td>83</td>
</tr>
<tr>
<td>Kreibich, Saskia</td>
<td>32</td>
</tr>
<tr>
<td>Kretzschmar, Ines</td>
<td>149</td>
</tr>
<tr>
<td>Krier, Irina</td>
<td>132</td>
</tr>
<tr>
<td>Kryuchkova, Nadja</td>
<td>113</td>
</tr>
<tr>
<td>Kuhn, Gisela</td>
<td>134</td>
</tr>
<tr>
<td>Kumar, Anil</td>
<td>149</td>
</tr>
<tr>
<td>Kumar, Sunil</td>
<td>114</td>
</tr>
<tr>
<td>Kurzawa, Laetitia</td>
<td>139</td>
</tr>
<tr>
<td>Kuznetsov, Dimitri</td>
<td>72</td>
</tr>
<tr>
<td>Kühne, Andreas</td>
<td>27</td>
</tr>
<tr>
<td>Lahav, Galit</td>
<td>137</td>
</tr>
<tr>
<td>Laman-Trip, Diederik</td>
<td>35</td>
</tr>
<tr>
<td>Lanzavecchia, Antonio</td>
<td>167</td>
</tr>
<tr>
<td>Larios, Jorge</td>
<td>72</td>
</tr>
<tr>
<td>Laurenti, Elisa</td>
<td>84</td>
</tr>
<tr>
<td>Lemaitre, Bruno</td>
<td>169</td>
</tr>
<tr>
<td>Limenitakis, Julien</td>
<td>175</td>
</tr>
<tr>
<td>Lindo, André</td>
<td>67</td>
</tr>
<tr>
<td>Link, Hannes</td>
<td>14, 50</td>
</tr>
<tr>
<td>Liphardt, Thomas</td>
<td>28</td>
</tr>
<tr>
<td>Lombardo, Angelo</td>
<td>98</td>
</tr>
<tr>
<td>Low, Nicola</td>
<td>173</td>
</tr>
<tr>
<td>Low, Shyan Huey</td>
<td>176</td>
</tr>
<tr>
<td>Ludwig, Christina</td>
<td>181</td>
</tr>
<tr>
<td>Lukas, Widmer</td>
<td>68</td>
</tr>
<tr>
<td>Lutolf, Matthias</td>
<td>84</td>
</tr>
<tr>
<td>Lygeros, John</td>
<td>155</td>
</tr>
</tbody>
</table>
Lygerou, Zoi 155
Lüönd, Fabiana 51

M
Ma, Bin-Guang 115
Macnair, Will 150
Macpherson, A. 175
Maerkl, Sebastian 75, 88, 120, 158, 160, 161
Makowska, Zuzanna 51
Manatschal, Cristina 149
Mange, François 89
Mangeat, Bastien 98
Manina, Giulia 145
Mann, Matthias 167
Manske, Christian 140
Mansouri, Maysam 46, 61
Manukyan, Liana 34, 62
Marchal, Marie 58
Marcus, Thelen 147
Marques-Bonet, Tomas 127
Martiel, Jean-Louis 139
Martin, Eva 109
Martin, Olivier 72, 124
Martin, Sophie 7
Martinez, Yannick 116
Martins, Antonio 8, 12
Marzetta, Flavia 117
Massouras, Andreas 169
Mathias, Bayer 68
Matthias, Patrick 91
Mauvoisin, Daniel 109
May, Catherine 29
Mayo, Avi 103
McCoy, K. 175
McKinney, John D. 145, 158, 160
Medo, Matúš 45
Medová, Michaela 45
Meier, Roger 97
Meireles Filho, Antonio 87
Meissner, Felix 167
Meplan, Cathy 136
Merk, Roeland 35
Mermet, Jerome 118
Mermet, Jérôme 101
Merzouki, Aziza 12
Mi Bond, Marie 86
Michel, Steinmetz 68
Milinkovitch, Michel C. 8, 12, 29, 34, 63, 62, 135
Mittal, Nitish 157
Moch, Holger 174
Moes, Suzette 51
Mogilner, Alex 139
Molina, Nacho 151
Moniatte, Marc 76
Montandon, Sophie A. 34, 62, 63
Mosca, Gabriella 30
Mukhtar, Tanzila 47, 56, 64
Munteanu, Andreea 129
Muntwiler, Simone 32, 172, 176
Murezzan, Damian 172
Myers, Gene 1
Mäcler, Philipp 44
Müller, Daniel 69
Müller, Ralph 134

N
Naef, Felix 101, 109, 118, 132, 144, 152
Naldi, Aurélien 119
Naldini, Luigi 98
Nanni, Paolo 73
<table>
<thead>
<tr>
<th>Author</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natacha, Olieric</td>
<td>68</td>
</tr>
<tr>
<td>Necsulea, Anamaria</td>
<td>65</td>
</tr>
<tr>
<td>Nelson, Bradley J.</td>
<td>55, 67</td>
</tr>
<tr>
<td>Neves, Aitana</td>
<td>66</td>
</tr>
<tr>
<td>Nicolas, Damien</td>
<td>152</td>
</tr>
<tr>
<td>Nicolet, Stefan</td>
<td>170</td>
</tr>
<tr>
<td>Nicoletti, Paola</td>
<td>125</td>
</tr>
<tr>
<td>Niederhoiltmeyer, Henriké</td>
<td>120</td>
</tr>
<tr>
<td>Nielsen, Jens</td>
<td>183</td>
</tr>
<tr>
<td>Nienhaus, Ulrike</td>
<td>67</td>
</tr>
<tr>
<td>Nowak-Sliwinska, Patrycja</td>
<td>177</td>
</tr>
<tr>
<td>O. Steinmetz, Michel</td>
<td>149</td>
</tr>
<tr>
<td>Oates, Andrew</td>
<td>41</td>
</tr>
<tr>
<td>Offner, Sandra</td>
<td>84, 96</td>
</tr>
<tr>
<td>Olieric, Natacha</td>
<td>149</td>
</tr>
<tr>
<td>Oliwia, Szklarczyk</td>
<td>68</td>
</tr>
<tr>
<td>Oppenheim, Rebecca</td>
<td>183</td>
</tr>
<tr>
<td>Or, Dani</td>
<td>18, 22, 24, 25, 33, 153</td>
</tr>
<tr>
<td>Osella, Matteo</td>
<td>20</td>
</tr>
<tr>
<td>Osman, Dani</td>
<td>169</td>
</tr>
<tr>
<td>Paek, Andrew</td>
<td>137</td>
</tr>
<tr>
<td>Palzkill, Timothy</td>
<td>10</td>
</tr>
<tr>
<td>Paul, Grégory</td>
<td>70</td>
</tr>
<tr>
<td>Paulevé, Loïc</td>
<td>16</td>
</tr>
<tr>
<td>Payne, Joshua L.</td>
<td>121</td>
</tr>
<tr>
<td>Paz Montoya, Jonathan</td>
<td>76</td>
</tr>
<tr>
<td>Pelet, Serge</td>
<td>143, 154, 162</td>
</tr>
<tr>
<td>Pelkmans, Lucas</td>
<td>72</td>
</tr>
<tr>
<td>Pe’er, Dana</td>
<td>4</td>
</tr>
<tr>
<td>Pham, Tri</td>
<td>69</td>
</tr>
<tr>
<td>Picotti, Paola</td>
<td>49, 146</td>
</tr>
</tbody>
</table>
Author Index

Planet, Evarist 98, 117
Pradhan, Rachana 122
Praz, Viviane 89, 124
Pritchard, Jonathan 127

Q
Qi, Weihong 71
Quadroni, Manfredo 109
Que, Yok-Ai 153

R
Raclot, Charlène 96
Raghav, Sunil 122, 132
Ragunath, PK. 123
Ramirez, Manuel 155
Rammensee, Hans-Georg 166
Rapsomaniki, Maria-Anna 155
Rauwel, Benjamin 107, 98, 178
Raynaud, Franck 31
Reddy, Sai 179
Reinhardt, Hagen 30
Rentero Rebollo, Inmaculada 168
Rib, Leonor 124
Ribeiro, Andre 156
Ribeiro, Samuel 156
Rieckmann, Jan 167
Rizk, Aurélien 46, 61
Robinson-Rechavi, Marc 106, 112, 113, 126
Roch, Aline 84
Roditi, Laura D.V. 150
Rodriguez Martinez, Maria 125
Rolf, Krause 147
Rolink, Antonius G. 91
Rosenberger, George 181
Rosenstiel, Philip 130, 71
<table>
<thead>
<tr>
<th>Name</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosikiewicz, Marta</td>
<td>126</td>
</tr>
<tr>
<td>Roth, Frederick P.</td>
<td>180</td>
</tr>
<tr>
<td>Roth, Volker</td>
<td>51</td>
</tr>
<tr>
<td>Rougemont, Jacques</td>
<td>132</td>
</tr>
<tr>
<td>Routier, AnneLise</td>
<td>30</td>
</tr>
<tr>
<td>Roux, Aurélien</td>
<td>39</td>
</tr>
<tr>
<td>Roux, Julien</td>
<td>127</td>
</tr>
<tr>
<td>Rowe, Helen M.</td>
<td>96, 98</td>
</tr>
<tr>
<td>Roy, Shantanu</td>
<td>128</td>
</tr>
<tr>
<td>Ruprecht, Verena</td>
<td>40</td>
</tr>
<tr>
<td>Rzepiela, Andrzej</td>
<td>157</td>
</tr>
<tr>
<td>Rämö, Pauli</td>
<td>32, 142, 172, 176</td>
</tr>
<tr>
<td>Röst, Hannes L.</td>
<td>181</td>
</tr>
<tr>
<td><strong>S</strong></td>
<td></td>
</tr>
<tr>
<td>Saez-Rodriguez, Julio</td>
<td>48</td>
</tr>
<tr>
<td>Sahin, Nil</td>
<td>180</td>
</tr>
<tr>
<td>Sailer, Christian</td>
<td>71</td>
</tr>
<tr>
<td>Salcedo, Suzana</td>
<td>176</td>
</tr>
<tr>
<td>Sallusto, Federica</td>
<td>167</td>
</tr>
<tr>
<td>Samuylov, Denis K.</td>
<td>70</td>
</tr>
<tr>
<td>Sankar, Martial</td>
<td>42</td>
</tr>
<tr>
<td>Santiago, Gonzalez Fernandez</td>
<td>147</td>
</tr>
<tr>
<td>Santoni de Sio, Francesca</td>
<td>117</td>
</tr>
<tr>
<td>Santos, Aline</td>
<td>76</td>
</tr>
<tr>
<td>Sauer, Uwe</td>
<td>14, 50, 60, 133, 181</td>
</tr>
<tr>
<td>Schaad, Olivier</td>
<td>72</td>
</tr>
<tr>
<td>Schaeli, Yolanda</td>
<td>129</td>
</tr>
<tr>
<td>Scheffold, Frank</td>
<td>74</td>
</tr>
<tr>
<td>Schelb, Mauno</td>
<td>46</td>
</tr>
<tr>
<td>Schell, Ursula</td>
<td>140</td>
</tr>
<tr>
<td>Schertler, Gebhard</td>
<td>46</td>
</tr>
<tr>
<td>Schibler, Ueli</td>
<td>132</td>
</tr>
<tr>
<td>Schlapbach, Ralph</td>
<td>73</td>
</tr>
<tr>
<td>Schmicl, Fabian</td>
<td>32</td>
</tr>
</tbody>
</table>
Schmid, Marc W. 71, 130
Schmidt, Anja 71, 130
Schmidt, Ralf 131
Schneider, Katrin 158
Schraml, Peter 174, 182
Schreiber, Robert 166
Schroeder, Timm 159
Schubert, Olga T. 102, 133, 181
Schuebeler, Dirk 83
Schuster, Heiko 166
Schwalie, Petra 122
Schwartz, Laurent 16
Schübeler, Dirk 85
Scott, Cameron 72
Selevsekk, Nathalie 73
Senger, Fabrice 139
Séréphin, Bertrand 86
Shahrezaei, Vahid 15
Shanley, Daryl 136
Sharpe, James 129
Sheftel, Hila 103
Skylaki, Stavroula 159
Smith, Richard 30
Snijder, Berend 72
Sobel, Jonathan 132
Soldati, Thierry 145
Soldati-Favre, Dominique 183
Soltermann, Alex 182
Soste, Martin 49
Sprecher, Simon 38, 74
Spühler, Isabelle 74
Stadler, Tanja 133
Stahlberg, Henning 138
Steiner Degen, Miriam 149
Steinmetz, Lars 165
Stelling, Jörg 5, 28, 37, 68, 133
Steyaert, Jean-Marc 16
Štoviček, Adam 24
Strauss, Soeren 30
Studer, Gabriel 172
Suk Min, Jang 92
Sun, Zachary 120
Suter, David 152
Syed, Afzal P. 157
Szczurek, Ewa 32
Szekely, Pablo 103
Székely, Gábor 70

T
Taraviras, Stavros 155
Tatarova, Zuzana 75
Tay, Savas 64, 141
Taylor, Verdon 47, 56, 64
Tecon, Robin 33, 153, 25
Tendler, Avichai 103
Terracciano, Luigi 51
The CycliX Consortium 89
Thomen, Philippe 20
Théry, Manuel 139
Timmer, Jens 23
Tindall, Marcus 19
Tlusty, Tsvi 6
Toffoli, Barbara 119
Trauner, Andrej 102, 133
Trono, Didier 52, 81, 84, 92, 96, 98, 104, 117, 178
Trüssel, Andreas 134
Turcatti, Gerardo 72
Turelli, Priscilla 92, 96, 98, 117
Tymoshenko, Stepan 183
Tzika, Athanasia 34, 12, 135
U
Ullate Agote, Asier 135
Unal, Erkan 43
Unemo, Magnus 173
Unzu, Carmen 98
Urdy, Severine 35

V
Vacca, Fabrizio 72
Van Beijnum, Judy 177
Van den Bergh, Hubert 177
Van der Goot, F. Gisou 76
Van der Meer, Jan Roelof 153
Van Drogen, Audrey 184
Van Nimwegen, Erik 56, 64, 79
Varesio, Emmanuel 57
Varjosalo, Markku 184
Verhovsky, Alexander B. 31
Verkhivker, Gennady 36
Verp, Sonia 84
Verpoorte, Amanda 160
Vichalkovski, Anton 184
Vignaud, Timothée 139
Villeneuve, Dominic 124
Voegeli, Sylvia 86
Vogler, Hannes 55
Volkmer, Rudolf 149
Vollmer, Jannik 47
Von Mering, Christian 32, 97
Vossio, Stefania 72

W
Wagner, Andreas 121
Waller, Manuel 71
Wang, Jingkui 109
Waridel, Patrice 109
Weber, Bruno 44
Webster, Duncan 134
Wegner, Hermann A. 168
Weiss, Andrea 177
Welz, Tobias 142
Widmer, Lukas A. 37
Wildhaber, Barbara 98
Wilson, Robin 134
Winkler, Carine 119
Wolf, Tobias 167
Wong, leong 177
Wong, Tse 177
Woodruff, Kristina 161
Wosika, Victoria 162
Wyss, Matthias 44

X
Xenarios, Ioannis 42
Xie, Ting 115
Xiuzhen, Chen 68
Xueli, Guan 185

Y
Yazdanpanah, Benjamin 84
Yli-Harja, Olli 156

Z
Zaballa, María Eugenia 76
Zamboni, Nicola 167, 27, 60
Zambrano, Samuel 151
Zampieri, Mattia 133
Zavolan, Mihaela 80, 110, 128, 131, 157
Zeller, Rolf 43
Zhang, Hong-Yu 115
<table>
<thead>
<tr>
<th>Author</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zimmer, Yitzhak</td>
<td>45</td>
</tr>
<tr>
<td>Zimmermann, Michael</td>
<td>48, 133, 181</td>
</tr>
<tr>
<td>Zoller, Benjamin</td>
<td>152</td>
</tr>
<tr>
<td>Zupanic, Anze</td>
<td>136</td>
</tr>
<tr>
<td>Zünd, Marc</td>
<td>44</td>
</tr>
<tr>
<td>Time</td>
<td>Event</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>09:00</td>
<td>Frank Jülicher</td>
</tr>
<tr>
<td>09:50</td>
<td>Antonio Martins</td>
</tr>
<tr>
<td>10:10</td>
<td>Jörg Stelling</td>
</tr>
<tr>
<td>10:35</td>
<td>Break</td>
</tr>
<tr>
<td>11:00</td>
<td>Tsvi Tlusty</td>
</tr>
<tr>
<td>11:30</td>
<td>Micha Hersch</td>
</tr>
<tr>
<td>11:50</td>
<td>Dana Pe‘er</td>
</tr>
<tr>
<td>12:35</td>
<td>Lunch and Posters</td>
</tr>
<tr>
<td>14:15</td>
<td>Andrew Oates</td>
</tr>
<tr>
<td>15:05</td>
<td>Erkan Ünal</td>
</tr>
<tr>
<td>15:25</td>
<td>Aurélien Roux</td>
</tr>
<tr>
<td>15:50</td>
<td>Break</td>
</tr>
<tr>
<td>16:30</td>
<td>Simon Sprecher</td>
</tr>
<tr>
<td>17:00</td>
<td>Christian Hartke</td>
</tr>
<tr>
<td>17:20</td>
<td>Carl-Philipp Heisenberg</td>
</tr>
<tr>
<td>18:05</td>
<td>Drinks and Posters</td>
</tr>
<tr>
<td>19:00</td>
<td>GENE MYERS</td>
</tr>
<tr>
<td>09:00</td>
<td>Patrick Cramer</td>
</tr>
<tr>
<td>09:50</td>
<td>Arnaud Krebs</td>
</tr>
<tr>
<td>10:10</td>
<td>Eric Van Nimwegen</td>
</tr>
<tr>
<td>10:35</td>
<td>Break</td>
</tr>
<tr>
<td>11:00</td>
<td>Mihaela Zavolan</td>
</tr>
<tr>
<td>11:30</td>
<td>Michael Imbeault &amp; Alina Isakova</td>
</tr>
<tr>
<td>11:50</td>
<td>Tim Hughes</td>
</tr>
<tr>
<td>12:35</td>
<td>Lunch and Posters</td>
</tr>
<tr>
<td>14:15</td>
<td>Manuel Théry</td>
</tr>
<tr>
<td>15:05</td>
<td>Hubert Hilbi</td>
</tr>
<tr>
<td>15:20</td>
<td>Break</td>
</tr>
<tr>
<td>16:00</td>
<td>Henning Stahlberg</td>
</tr>
<tr>
<td>16:30</td>
<td>Savas Tay</td>
</tr>
<tr>
<td>16:50</td>
<td>Galit Lahav</td>
</tr>
<tr>
<td>18:05</td>
<td>Lunch and Posters</td>
</tr>
<tr>
<td>19:00</td>
<td>Conference Dinner*</td>
</tr>
</tbody>
</table>

*Extra activities: prior registration and payment necessary.