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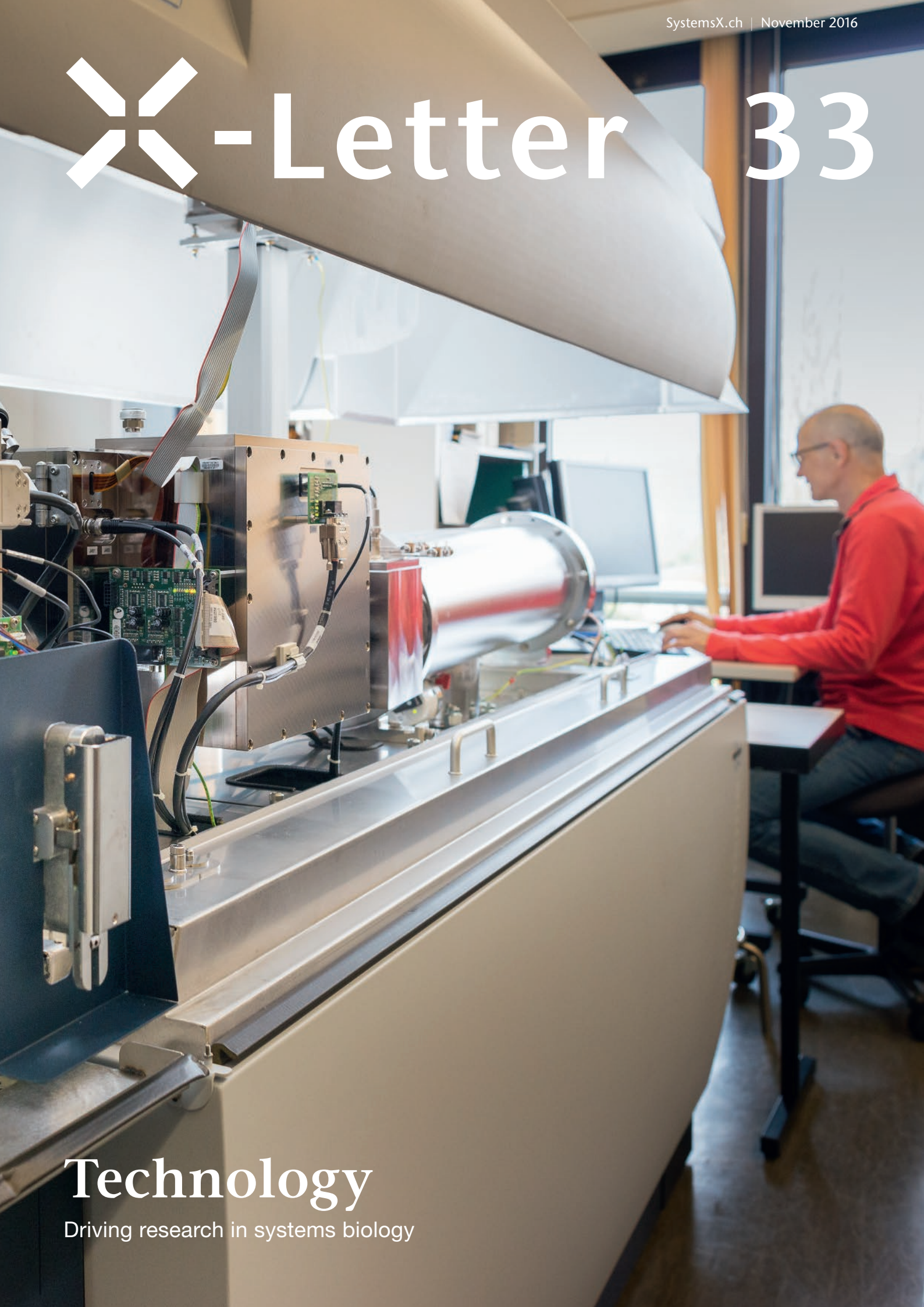

Technology

Driving research in systems biology

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- Thank you, Maja!
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- 3rd International SystemsX.ch Conference

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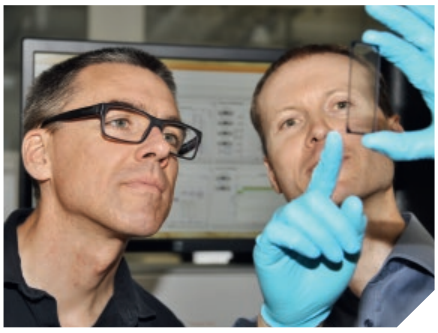

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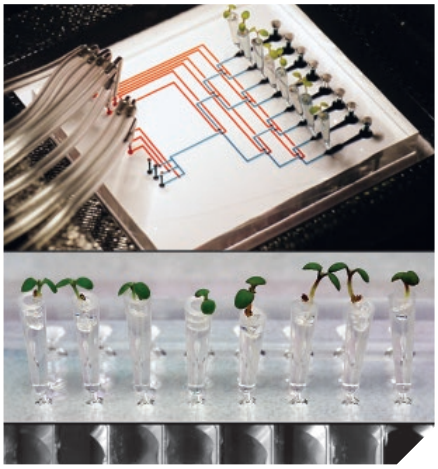

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"In a few years, many omics technologies will be routinely applicable on a single cell, and on thousands of them in parallel."

Photo: © David Schweizer

A major pillar of the progress in systems biology research is the development of new measurement technologies. SystemsX.ch has generously supported technology development over the past 9 years, and many of the discoveries made by various projects were only made possible thanks to the development of a new device, measurement technology or computational method.

In this issue of the X-Letter, we highlight the central role that technology plays in systems biology research. As outlined in an interview with Ralph Schlapbach, director of the Functional Genomics Center Zurich, one cornerstone of systems biology is made up of the omics disciplines. Not only do we see a rapidly growing number of new applications in the areas of transcriptomics, proteomics and metabolomics across the life sciences, but we also see continual improvement in their sensitivity and dynamic range. In a few years, many omics technologies will be routinely applicable on a single cell, and on thousands of them in parallel, further driving research in single-cell genomics. Exciting applications can be seen in the RTD Projects MERIC and SysGenetiX, which are described in this X-Letter.

In biology, everything depends on context, which means that translating single-cell omics technologies into methods with spatial resolution will be an important step for the future. In this regard, imaging mass cytometry is already paving the way. It allows the highly multiplexed quantification of protein abundance and their activated states within thousands of single cells *in situ,* enabling the study of the complexity of signaling networks at the single-cell level in context within tissue. This type of analysis reveals the influences that single cells have on each other, and how they collectively create microenvironments that result in heterogeneous cell behavior.

Intriguingly, many of these influences may actually derive from mechanical forces experienced by single cells. A comprehensive understanding of how heterogeneity arises in tissues and how it may go wrong in diseases such as cancer therefore requires the measurement of these mechanical forces and how they drive morphogenetic processes in animals. This is the focus of the RTD Project MorphogenetiX. A significant technological development here is the advent of light sheet microscopy, which allows the rapid imaging of large structures in three dimensions and is important for following dynamic processes in developing embryos.

I hope that you find this X-Letter, with its focus on technology in systems biology, an interesting read.

Lucas Pelkmans
Chairman of the SystemsX.ch
Scientific Executive Board (SEB)

Mechanisms of evasive resistance in cancer (MERIC)

Understanding the adaptation strategies of tumors

Targeted therapies are being increasingly employed to fight tumors. In contrast to classical chemotherapy, the drugs used target only the cancer cells, without affecting the rest of the body. Unfortunately, many tumors soon become resistant to these drugs. Researchers are now trying to understand the molecular mechanisms behind this adaptation.

Niko Beerenwinkel and Christian Beisel looking at a flow cell, which will later be filled with DNA.

Communication within a cell can be thought of as a kind of relay race. The runners are the proteins. They dock on to other proteins, where they pass their message on. When the signal arrives at the nucleus, it might turn on a gene that, for example, causes the cell to divide.

With targeted treatment, the drug attaches itself to one of the proteins in the communications relay race and blocks it. “This means that signal transmission is interrupted and, as a result, the cell dies,” says Niko Beerenwinkel of ETH Zurich, project leader of “Mechanisms of evasive resistance in cancer” (MERIC). However, cancer cells can evade a blocked signal by using an alternative signal pathway, whereby different proteins come into play that the administered drug cannot block. This is known as “evasive resistance”. The MERIC project team is now concentrating on elucidating the underlying mechanisms. Understanding these could clear the way for the development of new drugs to which cancer cells can no longer, or only much more slowly, adapt.

Analysis on three levels

To understand the mechanisms behind this molecular adaptation, the researchers are working with real cases from the University Hospital Basel. Tissue samples are taken from patients suffering from liver carcinoma (HCC). “A liver cancer is often inoperable, as the liver could be severely damaged during such a procedure," says Beerenwinkel. “Drugs are the only treatment option in many cases.”

Biopsies are carried out at regular intervals. Ideally, they are conducted before, during and after therapy. “This allows us to see exactly how the cancer cells adapt to a drug,” he explains.

The DNA, RNA, protein and protein phosphorylation levels are examined in the removed cells. “In this way, we can see how mutations in the genome affect first the RNA and then the proteins and signaling pathways,” says Beerenwinkel.

First the cancer cells’ DNA is decoded. This work is being carried out in collaboration with Christian Beisel, head of the Genomics Facility Basel. He receives prepared DNA extracted from tumor samples at the University Hospital Basel. From this, his team compiles a sort of library. “We’re not able to sequence a long DNA strand in one piece. Instead, we have to dissect it into smaller portions for our machines,” says Beisel. Each of these strands is just 200 bases long. The sections are then put into flow cells. These resemble microscope slides, but their surface is subdivided into narrow channels, in which there are docking sites for the DNA segments. They remain bound to the sites for the duration of the analysis.

The letters of life

The DNA-filled flow cell is then put into a sequencing machine. This contains tanks holding the nucleotide bases adenine (A), cytosine (C), guanine (G) and thymine (T); the building blocks of DNA. They are flushed together through the flow cell's channels. When each of the four bases meets the DNA segments, they attempt to dock on to them. If, for example, the first base of a section is a guanine, then its counterpart, cytosine, docks there. If, on the next strand, the first base is thymine, an adenine will bind to it.

"The docking bases are modified in such a way that they fluoresce, and that the docking reaction stops once a connection is created," explains Beisel. A microscope in the sequencing machine registers each successful connection as a white pixel. The machine can distinguish between the signals from A, C, G or T, and so is able to discern the first letter of each DNA segment after the first round of measurements.

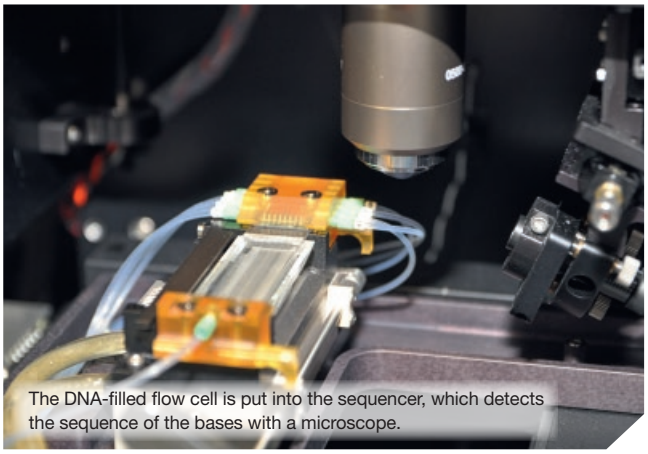
The fluorescent dyes are then cleaved and the DNA segments prepared for the next reaction step. The four bases are flushed through the flow cell once more, but now they dock on to the second location. This continues until all 200 bases of each section have been detected. In this way, the genotype of the cancer cell is sequenced within a few hours.

Evolution of resistance

The RNA is also sequenced in this way, while the proteins inside the cells are determined by means of mass spectrometry. The collected data are delivered to Beerenwinkel, who analyses them statistically. "I want to get an overview of the evolution processes," he says. Specifically, he is looking for areas in the genome that show a lot of variation and which might lead to alternative communication strategies in the cancer cell. This is where Jörg Stelling from ETH Zurich comes in, incorporating these results into a computational model of the signaling pathway. "This helps us understand how the resistance of cancer cells works at the molecular level, from gene to RNA to protein," concludes Beerenwinkel.

He and his colleagues have already analyzed cancer cell DNA and RNA from over a hundred samples. Furthermore, the cell samples are being cultivated in Petri dishes, enabling the team to create a living database.

Some of the cells are additionally implanted in mice. By means of these "xenografts", the cancer cells continue to grow in the living organism. The mice are then treated with different drugs. "This allows us to study the development of resistance in real time under controlled conditions that are as realistic as possible," says Beerenwinkel. "In the next step, we can use these findings to search for new drugs or better combinations of known treatments."



The DNA-filled flow cell is put into the sequencer, which detects the sequence of the bases with a microscope.

MERIC at a glance

Principal investigator: Prof. Niko Beerenwinkel

Research groups:

- Prof. Niko Beerenwinkel, Department of Biosystems Science and Engineering, ETH Zurich – Computational biology
- Prof. Gerhard Christofori, Department of Biomedicine, University of Basel – Tumor biology
- Prof. Michael N. Hall, Biozentrum, University of Basel – Signal transduction
- Prof. Markus Heim, Division of Gastroenterology and Hepatology, University Hospital Basel – Clinical hepatology
- Prof. Jörg Stelling, Department of Biosystems Science and Engineering, ETH Zurich – Computational systems biology

Total budget (2014–2018): CHF 5.2 million, including CHF 2.6 million from SystemsX.ch

Project type: Research, Technology and Development (RTD) Project

MERIC
Mechanisms of Evasive Resistance in Cancer

Emmanouil Dermitzakis uses next-generation sequencing to understand the function of human genome variants.

Cellular systems genetics in humans (SysGenetiX)

Turning to natural mutations to understand gene regulation

Our genes determine many aspects of who we are. But it's not just the genes themselves that play a role in establishing our biological traits. If and how they are expressed, which is crucially controlled by regulatory elements, is also a major factor. The goal of the SysGenetiX project is to closely investigate these regulatory elements, as well as their manifold interactions with genes. The findings may in future contribute to advancing our understanding of why some people are more predisposed to contracting particular diseases than others.

It makes sense that not all of our genes are expressed at all times throughout our bodies. For example, during embryo development, genes have to be turned on and off at different times according to a fixed pattern to result in the formation of distinct body parts and tissues. Even within the fully developed body, the cells of different tissues express different sets of genes so that these tissues can carry out their specialized functions. Regulatory elements make sure that the right genes are expressed at the right time in response to specific stimuli.

These regulatory elements are segments of DNA, mostly promoters or enhancers, that are coupled to particular genes. When specific proteins dock onto these elements, they trigger a whole network of reactions. These ensure that the corresponding genes are transcribed at the right moment, along with the correct amount of mRNA, which in turn prompts the production of proteins. Ideally, this all happens smoothly and according to plan.

Mistakes in gene regulation can lead to disease

However, just like the genes themselves, regulatory elements are susceptible to mutations. If they present no immediate disadvantage, mutations will be passed on, resulting in different variants of these DNA segments. These variants can either be completely neutral, have a positive effect, or entail drawbacks. That being the case, some of the mutations are being linked to predisposition to certain diseases.

"Until now, this relationship has only been shown in terms of a statistical correlation," explains Emmanouil Dermitzakis, Professor of Genetics in the Department of Genetic Medicine and Development at the University of Geneva Medical School, and SysGenetiX project leader. "What's not yet been investigated is what these variants do exactly, and how they lead to increased susceptibility to certain diseases," adds the researcher. The SysGenetiX consortium now aims to close this gap in knowledge, and is working on bringing the detailed interactions between regulatory elements and their associated genes to light.

Examining natural genetic mutations

To this end, the SysGenetiX team is following a slightly different approach than is normally used in systems biology. "Instead of interfering with a system and inspecting what happens, we're look-

Maja Schaffner

ing for and analyzing the naturally occurring mutations in the regulatory elements," states Dermitzakis.

The scientists are working with a considerable sample size, encompassing blood and skin cells from almost 300 individuals. They are quantifying how much the chromatin – that is, the DNA with all its associated proteins – is methylated and acetylated, which determines what genes are accessible to transcription. They are also looking at how much mRNA is transcribed, as well as its composition. In addition, they analyze which proteins are synthesized in what quantities. Using all this data, they are able to reconstruct the activation mechanisms and regulatory networks of the individual genes.

Dermitzakis sees every gene as a mini system comprising a network of interactions and a variable output. "Our goal is to gain an understanding of the universal rules of gene regulation, and also to find out how each individual gene is regulated," he explains. The mutations, and the way in which they perturb the system, help the scientists understand how the system normally works, and what happens when something goes wrong.

High variability

The project still has a long way to go. The first results have already been obtained, but not yet published. The ways in which the mutations affect different tissue types are still to be analyzed. However, what is already clear is that there is great variability in the ways in which different genes are regulated. "For example, we've seen the case where 20 regulatory elements regulate three genes, another case where ten of these elements regulate just one gene, and yet another where a single one regulates three genes," illustrates Dermitzakis. The team has also shown that some mutations influence the methylation and acetylation of the chromatin and hence the accessibility of the DNA, and that other mutations have an effect on the quantity of proteins that are synthesized.

The researchers now want to divide all of these very differently regulated genes into categories with similar mechanisms. They also plan to make the knowledge gained within SysGenetiX available to other scientists through an Internet database. Anyone who is interested in a particular gene and its regulation will simply be able to look it up.

A better understanding of the causes of disease

"The work we carry out on this project is basic research," emphasizes Dermitzakis. However, knowing what goes on at the level of gene regulation, and what effects mutations in the regulatory elements have, could decisively contribute to a better understanding of how diseases such as diabetes, cardiovascular disease or cancer arise. Such knowledge may indeed one day lead to early diagnosis and better treatment options. "And even," adds the researcher, "long before the first symptoms become apparent."

SysGenetiX at a glance

Principal investigator: Prof. Emmanouil Dermitzakis

Research groups:

- Prof. Emmanouil Dermitzakis, Department of Genetic Medicine and Development, University of Geneva Medical School – Population genomics and systems medicine
- Prof. Stylianos Antonarakis, Department of Genetic Medicine and Development, University of Geneva Medical School – Genomic medicine
- Prof. Philipp Bucher, Swiss Institute for Experimental Cancer Research, EPF Lausanne – Bioinformatics
- Prof. Alexandre Reymond, Center for Integrative Genomics, University of Lausanne – Disease genomics
- Prof. Sven Bergmann, Department of Computational Biology, University of Lausanne – Statistical genetics
- Manolis Kellis, MIT Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology (USA) – Computational biology

Total budget (2013–2018): CHF 6.0 million, including CHF 3.0 million from SystemsX.ch

Project type: Research, Technology and Development (RTD) Project

SysGenetiX
Cellular Systems Genetics in Humans

Part of the MorphogenetiX team (from left): David Dreher, Marisa Oliveira and project leader Damian Brunner.

Three-dimensional tissue shaping (MorphogenetiX)

The mechanics of life

Recent research findings show that tissue shaping is a particularly dynamic feedback process during which cells not only exert their own mechanical forces, but are also heavily influenced by external forces acting upon them and can even differentiate into different cell types as a result. This sounds like a case for systems biology.

When the liver of a small dog is transplanted into the body of a larger dog, the organ grows until it reaches the appropriate dimensions for the larger animal. This happens despite the fact that the liver cells were originally "programmed" for the volume of the smaller dog. But how does the donated liver "know" that it has to alter its shape, and what stops it from growing further after it has reached the ideal size? "No one is able to explain this phenomenon. The molecular interconnections involved in tissue shaping are still, to a large extent, a black box," says Damian Brunner, project leader of MorphogenetiX. The aim of the project is to study the spatial organization of cell systems, examining genetic factors, signaling networks and the physics behind these processes.

Although basic research in this field is still in its infancy, Brunner is sure of one thing: "Mechanical forces play a critical role in tissue shaping." This fact was only uncovered a few years ago, after scientists observed the fate of embryonic stem cells placed onto supports of differing stiffness, but which received the same molecular signals. Astonishingly, the cells first examined their surroundings before beginning to differentiate. "The stem cells placed on a soft substrate started developing towards a nerve cell fate, those on a semi-hard medium towards muscle cells, whereas bone cell development was initiated on the hardest surfaces," explains Brunner.

The experts therefore think that although molecular signals initiate cell differentiation, the physical properties of the stem cells' environment, along with the external forces acting on the cells, significantly influence what tissue type the cells will develop into. This is just one aspect that makes the exploration of the mechanical processes in morphogenesis so exciting, but also complex. "In order to really understand the development of tissues, we need to study them under as natural conditions as possible, and in three dimensions," states Brunner. This is why MorphogenetiX employs the most modern equipment, innovative technologies and ingenious mathematical models in search of this understanding.

High-quality images

One of these pieces of equipment is the light sheet fluorescence microscope (LSFM). It allows the scientists to examine tissue shaping either in an intact organism, or in tissue elements cultured *in vitro*. It does this with an unprecedented image quality and over a relatively long time period. A picture of the subject is taken every 30 seconds, and the images are subsequently assembled into a movie in which the tissue development can be analyzed in three dimensions. To further optimize the image quality and measurement accuracy, the MorphogenetiX team is setting up an improved prototype LSFM. With this new device, the sample can be simultaneously illuminated by two sheets of light, and observed through two objectives perpendicular to the light sheets, resulting in a considerable improvement in the image quality.

But there is a down side to this new technology, as Damian Brunner explains. "We generate around eight terabytes of data within four hours." To avoid drowning in a flood of data, the re-

searchers implement a processing pipeline before each LSFM experiment, where they define what data should be gathered, and which cells analyzed. The scientists then filter out these results from the mountain of data, deleting the rest. This step isn't easy for any scientist, but Brunner emphasizes the sheer impossibility of holding on to all of the data. Thankfully, there is a saving grace: "The movies of the tissue development can be easily reproduced at any time with the help of standardized methods."

Targeted manipulation of proteins

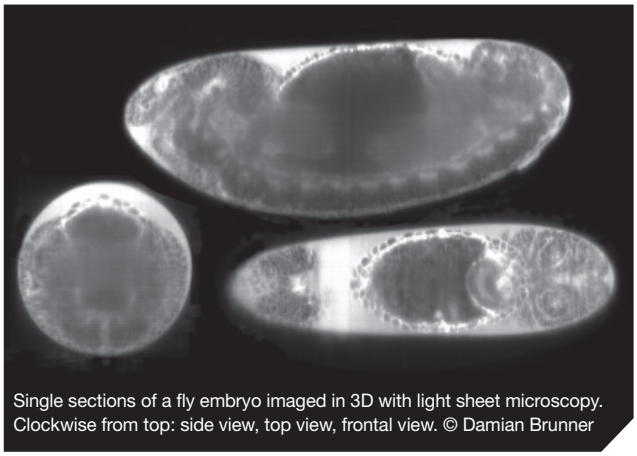
The LSFM is not the only state-of-the-art technology that Brunner and his team are making use of. "Markus Affolter has developed a method with which proteins in the cells can be turned on and off with remarkable accuracy and speed. We can even relocate proteins within the cells," reports the project leader. These methods enable the team to acutely and selectively manipulate these extremely dynamic processes and analyze the immediate consequences in the living organism, for instance during embryonic development.

Yet another innovative method involves staining each individual cell, making it easily distinguishable from its neighbors. "This gives us previously unseen insight into the behavior of cellular processes during tissue shaping," explains Brunner excitedly. The MorphogenetiX team now wants to look at the changes occurring in individual cells from each tissue type, for example in the larval wing disc, from different perspectives over a longer time period.

Finite element method

In order to use the collected data efficiently and try to understand the complex molecular processes at play, suitable mathematical models must be implemented. Here, the MorphogenetiX team is again opting for an innovative approach. "We are using models based on the finite element method, which is a routinely used approach in architecture or engineering," explains the project leader.

The finite element method allows the simulation of physical processes that would otherwise involve an enormous computational cost. A complex form is divided up into many smaller elements with simpler forms. The physical behavior of these elements can be easily calculated thanks to their simpler geometry. Both Damian Brunner's and Christof Aegerter's teams have adapted this model to meet their needs. Now the researchers are able to incorporate the third dimension in their simulations of tissue shaping and can also take into account variability amongst similar cells. "The predictive power of these models has significantly increased as a result, which in turn helps us optimize the experimental studies," says Brunner.



Single sections of a fly embryo imaged in 3D with light sheet microscopy. Clockwise from top: side view, top view, frontal view. © Damian Brunner

The clever interplay of interdisciplinary research approaches with customized models and innovative technologies makes MorphogenetiX an exemplary large-scale systems biology research project. And who knows? In the near future, it might just unravel the mystery of the mechanics of life.

MorphogenetiX at a glance

Principal investigator: Prof. Damian Brunner

Research groups:

- Prof. Damian Brunner, Institute of Molecular Life Sciences, University of Zurich – 4D tissue morphogenesis, finite elemente modeling
- Prof. Lucas Pelkmans, Institute of Molecular Life Sciences, University of Zurich – 4D tissue morphogenesis
- Prof. Christof Aegerter, Physik-Institut, University of Zurich – Biophysics, finite element modeling
- Prof. Markus Affolter, Biozentrum, University of Basel – Development of molecular tools
- Prof. Richard Smith, Max Planck Institute for Plant Breeding Research, Cologne, Germany – Finite element modeling

Total budget (2014–2018): CHF 6.2 million, including CHF 3.0 million from SystemsX.ch

Project type: Research, Technology and Development (RTD) Project

MorphogenetiX
Modeling the
3-Dimensional Shaping
of Tissue Systems

Bioinformatics is her foundation, interdisciplinary project management her element: Aitana Lebrand.

Portrait of a former Transition Postdoc Fellow

"To make things happen, there needs to be a catalyzer in the middle"

From Drosophila wing development to infectious diseases, computer models to Petri dishes: Aitana Lebrand has done it all, and finally found her place at the interface of different disciplines.

"If you see me doing something stupid, just tell me," insisted Aitana Lebrand to her colleagues as she started work in Pierre Gönczy's lab at EPFL in 2012. However, the students in the lab sometimes found it difficult to correct Aitana; after all, she was a postdoc. "A postdoc who didn't know anything, couldn't do anything and needed constant help," she clarifies. "Because she found herself conducting experiments in the lab for the very first time."

Aitana Lebrand received her PhD at the University of Lausanne in bioinformatics. Then she switched to experimental research through a SystemsX.ch Transition Postdoc Fellowship in Gönczy's lab. Previously, she had studied the role of signaling molecules in *Drosophila* wing growth using computational models and image analysis. During the postdoc, she carried out experiments to explore the molecular and cellular basis of thermal tolerance in nematodes.

The computational sciences, however, remained an important aspect of Aitana's research. In Pierre Gönczy's lab, she recorded nematodes at the single-cell stage on film under various conditions. Aitana subsequently analyzed the movies using computational methods to extract quantitative features in order to understand the mechanisms at play at the thermal limits. This is exactly the idea behind SystemsX.ch's Transition Postdoc Fellowships: Young researchers are given the opportunity to discover new research areas, while at the same time making use of their existing expertise. Interdisciplinarity is indeed a prerequisite for systems biology research, and it was exactly what Aitana was looking for. "I always wanted to work in a wet lab to see what it really meant to do the experiments and generate the data myself. And also to question what I was doing from a different perspective."

A bit of early morning soul helps

The postdoc fellowship was to be as interesting as it was labor intensive for Aitana. She did – of course – all the computing for the project herself, and was soon able to carry out simpler experiments independently. But that wasn't enough. During this time, Aitana also co-founded the EPFL Life Sciences Postdoc Asso-

ciation, which promotes networking among postdocs, organizes career events and supports future postdoc candidates. And she played the bass. “Funk, soul, groovy stuff. It gives me balance,” she says.

“First you need to accomplish your technique, then comes creativity.”

Whether in science or music, Aitana likes to do things properly. “First you need to accomplish your technique, then comes creativity. In order to make progress, you need both – in music as well as in science.” But that’s easier said than done. Full-time researcher, active member of the association and a bassist to boot. How does one manage all that? “Good time management is everything,” says Aitana. She sees these different activities not as competitive, but rather as complementary. If she played an hour of bass in the morning, she would have a clear head and be ready for the day’s work on her scientific research project.

Nowadays, the morning’s finger exercises on bass are no longer followed by pipetting in the lab. Although Aitana enjoyed her lab work – she even extended her stay after completion of the two-year postdoc program – she moved on. For her, the Transition Postdoc Fellowship was pivotal, as there she learned the importance of coordination between different disciplines. “The super-biologist, the super-modeler – sometimes they can’t even talk to each other,” she explains. “To make things happen, you often need a catalyzer in the middle who talks to the different experts, understands them, translates between them.”

A preference for applied science

Aitana sees her future at this interface, as a coordinator and project manager. And of course it wouldn’t be Aitana if she hadn’t committed herself to this notion wholeheartedly. Following the transition postdoc, she began a Diploma of Advanced Studies in Project Management at the University of Geneva, while at the same time starting work on a new research project at the Haute Ecole d’Ingénierie et de Gestion du Canton de Vaud (HEIG-VD). Working again as a bioinformatician, she helped develop a virus screening test for fish diseases in partnership with two private sector companies. This is the second important point that Aitana learned in her previous academic career: Although she enjoys basic research, she herself feels more at home doing applied research. “I like it if I can see where I need to get to,” she explains. “In basic research, you often don’t know where you’re going to end up.” For many researchers, that’s exactly what drives them, she thinks. And adds, smiling: “For me, it’s just kind of scary.”

Therefore, when the private sector partners set out their clear ideas and strict constraints on the cost and duration of each screening test, Aitana was not put off. On the contrary, it was just the right kind of challenge for her. As long as the goal is clear, she will gladly find a way there, be it convoluted or complex.

There is no master plan, but it all makes sense

Aitana’s path, on the other hand, gives a much more linear impression, even if she doesn’t have a master plan. “Each of my decisions makes sense at some point. Maybe the plan is constructed as I go,” she says. And so, her inclinations – for applied science, for interdisciplinary research, for project management – lead her after the time at HEIG-VD to the SIB Swiss Institute of Bioinformatics. Here, as of summer 2016, she co-leads a Swiss-wide working group with the goal of defining best practices for the next-generation sequencing-based analysis of microbes and antimicrobial resistance in patient care. In addition, she also coordinates the teaching activities of the SIB in clinical bioinformatics.

“When multiple individuals, who are experts in their own fields, have to work together, someone has to orchestrate them somehow.”

Aitana has found her ideal role, as an intermediary between scientists from different fields. “I see myself as a facilitator,” she says. Again, she feels it is as true for science as it is for music: When multiple individuals, who are experts in their own fields, have to work together, someone has to orchestrate them somehow. “There are different ways of doing this,” according to Aitana. “Personally, I like to play the bass. It’s not a very prominent instrument, but it sets the rhythm and ensures harmony. It’s at the interface. You only really realize how important it is when it’s not there.”

Aitana Lebrand’s SystemsX.ch project at a glance

Project title: Robustness of *C. elegans* development at thermal limits

Fellow: Dr. Aitana Lebrand, EPF Lausanne

Host research group: Prof. Pierre Gönczy, Swiss Institute for Experimental Cancer Research, EPF Lausanne

Project duration: 2012–2015

Project type: Transition Postdoc Fellowship (TPdF) – Young scientists formulate their own interdisciplinary project application and switch to a complementary discipline that is new to them.

Novel approaches for single-cell proteomics

A quantum leap in tissue imaging

With imaging mass cytometry, the team led by Bernd Bodenmiller has developed a groundbreaking approach with which a high number of cellular constituents can be simultaneously quantitatively analyzed, and even their spatial arrangement accurately determined. The scientists are now working on expanding this application for use in cancer research.

A tumor is a dynamical complex of different cells that interact strongly with each other as well as their environment. Such cancer cells try to disable immune cells, or to manipulate healthy cells in their neighborhood into helping the cancer grow. These deregulated cells go on to establish their own sort of "ecosystems" in the body of the patient. This is the focus of Bodenmiller's research at the Institute of Molecular Life Sciences at the University of Zurich.

"If we know how these ecosystems work, we can find ways of manipulating them so that the tumor dies or stops growing," says the systems biologist of his long-term research ambition. With the development of a revolutionary new approach, he is already a good deal closer to this goal.

Problem identified, problem solved

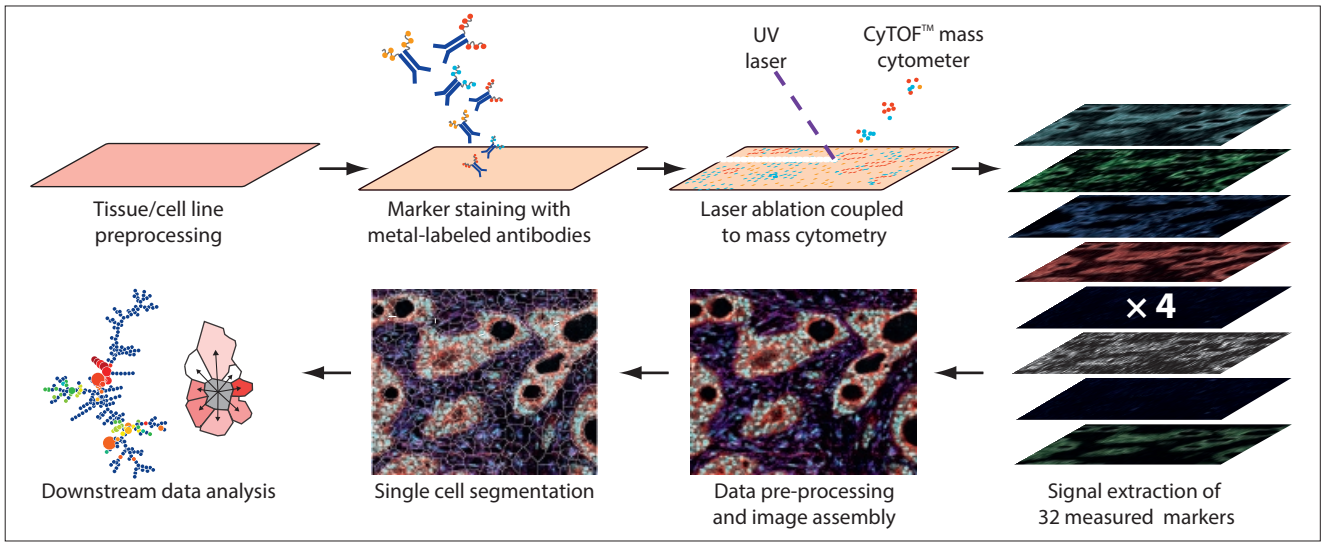
Throughout his career, the scientist has gained a great deal of experience in mass cytometry. A mass cytometer (CyTOF) is like a flow cytometer, but one where the specimen is prepared with antibodies tagged with metal isotopes instead of dyes. "At the moment, this method is able to measure up to 100 different cellular molecules and proteins at the same time," explains the researcher. It does so with an extremely high throughput rate, while the metal isotopes increase the accuracy of the cytometry measurements many times over. "This method avoids the problem with flow cytometry where the dyes can lead to color overlap, thus causing some ambiguity in the measurements."

Although mass cytometry is particularly well suited to determining cell phenotypes in a tissue sample, for instance, the technology does have a limiting drawback according to Bodenmiller. "The measurements made with CyTOF were originally performed on cells in suspension. This means that we had to dissociate solid tissues into their single-cell constituents and were therefore not able to tell where a particular cell originated in the initial tissue sample." But it is exactly this information that the scientist needs in order to investigate the interactions occurring between individual cells during cancer. Fortunately, Bodenmiller was struck by a compelling idea for the solution to this problem when he was visiting Detlef Günther's lab at ETH Zurich one day. The mass cytometer could be augmented with a laser application. And thus imaging mass cytometry was born.

Pixel by pixel

In imaging mass cytometry, the targets to be measured are also tagged with metal isotopes. "However, with this setup we no longer have to break the sample down into individual cells, meaning we can process intact tissue sections," summarizes Bodenmiller.

The isotope-labeled sample is placed in a laser ablation chamber, where an extremely fast-pulsing laser beam passes linearly

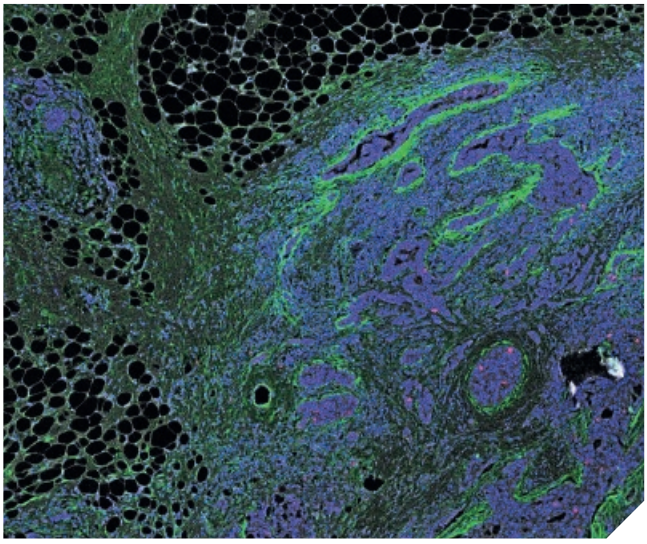


Imaging mass cytometry workflow: Tissue sections are stained with antibodies that are marked with pure metal isotopes. A laser is passed over the tissue in a raster, causing point-by-point tissue ablation. The metal isotopes bound to the tissue are then measured by the mass cytometer. Computational tools are used to generate a multiplexed image, identify the boundaries of individual cells and perform downstream analysis.
Figure: Giesen et al., Nature Methods, 2014 Apr;11(4):417-22.

over the tissue. The laser beam releases so much energy that the targeted tissue is broken down into tiny parts, which form a cloud. This aerosol is transported within a fraction of a second into the CyTOF for mass cytometry analysis. “In this way, we get a separate aerosol for each laser pulse, in which we can measure the cell constituents we marked,” describes the researcher. Since the exact coordinates of each laser pulse is known, all that remains is to combine these with the cytometry data to reveal a spatial picture of the results. “Simply put, you could say that each laser shot produces one pixel of the overall image of a tissue sample,” says Bodenmiller.

Correlations between tumor heterogeneity and clinical outcome

The scientist and his team are now applying this new technology to find out more about the tumor ecosystem and its effect on clinical outcome. For this purpose, they have also developed novel algorithms and computational tools. “We can now determine where each cell type is present in a tumor and what the microenvironment of each cell is, and we compare these results with clinical data from patients,” explains Bodenmiller. The team is looking for correlations between tumor composition, course of the disease and efficacy of the applied treatment. But imaging mass cytometry opens up still more possibilities. “We want to collect enough data to be able to model the interactions within the complex networks of the tumor itself, as well as between deregulated and healthy cells,” adds Bodenmiller.



Imaging mass cytometry of breast cancer tissue: 44 markers were measured simultaneously. Blue = histone 3, red = phosphorylated histone 3, green = fibronectin, gray = metal counter stain to reveal tissue morphology. Picture: Hartland Jackson and Raúl Catena, Bodenmiller lab

Bernd Bodenmiller contributes to various SystemsX.ch projects with imaging mass cytometry.

Patient benefit is the aim

Bodenmiller is not only lending his extensive subject knowledge to a number of SystemsX.ch projects, but makes the technical equipment available to the involved scientists, too. The common denominator in all of these projects is cancer research, for example breast cancer in the MRD MetastasiX, or prostate cancer in the RTD Project Phosphonet PPM. “In order to efficiently develop sound methods and technologies in systems biology, you have to really concentrate on a specific biological problem,” Bodenmiller is convinced.

Thanks to his collaboration on various SystemsX.ch projects, as well as his active involvement in the newly established Swiss Personalized Health Network, the chances are good that the approach he has helped develop will continue to make great progress, and even bring benefits to patients in the foreseeable future. With the success of imaging mass cytometry, and its anticipated commercial release in the near future, SystemsX.ch also leaves a lasting impact in the realm of systems biology technology.

More information is available at:
www.bodenmillerlab.org

Ralph Schlapbach is responsible for the operation of the FGCZ and the scientific coordination of research projects.

Ralph Schlapbach is head of the Functional Genomics Center Zurich (FGCZ)

Wide range of technological support for omics research

As a joint research and training facility of the University of Zurich and ETH Zurich, the Functional Genomics Center Zurich (FGCZ) offers the newest technologies and expert support for omics research. Here, we hear from the managing director Ralph Schlapbach on the center, today's technology and its potential as well as its limitations.

Why did ETH Zurich and the University of Zurich decide to found a joint technology center together?

The Functional Genomics Center offers a very wide range of technologies and services in different omics areas. Only by pooling resources were both institutions able to make such an investment. For one single institution, the investment required for this breadth of coverage would have been too high; it would have been necessary to confine the focus to one particular molecular level, such as proteomics. The broad nature of the support is also of course an advantage for the institutions themselves. Thanks to the different technologies and methods used within the FGCZ, the knowledge pool of the entire community grows.

In the FGCZ, researchers can generate and analyze their own data with support from analytical experts in the "userlab". Why did you opt for this sort of research support?

From an educational and quality standpoint, we find it very important that researchers have this access. In the userlab, they learn how the data is generated. The more they understand the data, the better they are able to interpret them. Furthermore, an active community involved in the userlab is needed to consolidate know-how and establish new methods.

What types of services does the FGCZ offer?

We distinguish between two types of services. For the traditional services, we function like a classical core facility. Here, users know more or less what they want, and they can order a sort of catalog analysis. Often, this analysis is in the area of quality assurance. In addition to these traditional services, we also provide project-based services, where the central analysis and data generation are carried out by our staff, and the up- and downstream processes such as sample preparation and data interpretation are carried out by the research groups themselves. For some projects, it makes more sense to do it this way – rather than via the userlab – for reasons of efficiency. For the standardized, robust processes, this enables us to run our equipment at optimal capacity with fewer interruptions, and in some cases achieve more consistent data quality. However, even with more complex analyses where the training time in the userlab exceeds the data generation time, it also makes sense for us to offer this service.

Christa Smith

To what extent are FGCZ staff involved in the design of a study?

We require all groups using the userlab, as well as the more complex services, to submit their project details to us with descriptions of their scientific goals. This way, we can make suggestions on the design of a study, for example by incorporating new methods or technologies that the researchers may not yet be familiar with.

Who has access to your services and technologies?

Groups from ETH Zurich and the University of Zurich are always given priority, and access to the userlab is available only to them. In addition, other academic researchers and, with limitations, industrial users have access to the services. For us it can be quite interesting to work with external research groups, since they contribute additional research questions that we might then use as pilot projects to establish new workflows. This, in turn, benefits the internal groups, too.

What does the FGCZ offer in education and training?

The main training happens through the individual project coaching of users in the userlab section. In addition, we offer condensed training courses, for example in next-generation sequencing. Over four days, the researchers are led through a complete practical workflow. The DNA and RNA sequencing courses are very popular; they even have long waiting lists. We also now offer combined genomics and proteomics courses. These are a bit longer, but are especially interesting for systems biologists, who investigate different molecular levels in order to understand a system integratively.

Which technologies are used the most, and what for?

These days, almost everything to do with nucleic acid analysis is carried out using next-generation sequencing. The FGCZ has high-throughput equipment with which many short DNA sequences can be read. This is used for example in genome resequencing or determining gene expression. For more complex sequencing questions, we employ single molecule sequencers. Although these have a much lower throughput, they are able to analyze longer fragments, and so are used for instance when sequencing a genome for the first time. For most other things, we use mass spectrometers. Here, proteins are usually broken down into fragments and measured in the form of peptides. Small molecules such as lipids and metabolites can also be measured, for example in the analysis of bodily fluids.

What are the possibilities with today's technology? And what are the limitations?

With today's technology, you often have to choose between broad or in-depth analysis. The sequencing technologies have an advantage here. With them, it's possible to measure broadly – for example the expression of every gene – and some of them are very sensitive, allowing the identification of sequence changes in only a few molecules per cell. With mass spectrometry, we're a bit more limited in both dimensions. It's not possible to quantitatively measure large numbers of proteins in a reasonable time while simultaneously achieving maximum sensitivity. The comprehensive analysis of proteins, analogous to what we already have for sequencing the nucleic acids, is unfortunately not yet possible, since proteins are biochemically much more complex. There are so many more different types, they are more difficult to separate, identify and characterize, so that a number of different methods are required.

What is missing on the technological side in order to put personalized medicine into practice?

Technologically speaking, the basis for personalized medicine is already there, but the analysis is still too expensive and too slow. Today, it still takes a matter of weeks to sequence a genome from sample to data interpretation. For diagnostics, you would need to have the results within hours, or at most days.

What is needed for the next spurt of development?

I think we've largely exhausted the possibilities with the technology available today. Of course, it can be made slightly better or faster, or we can use multiple systems in parallel, but the next growth spurt in this area will more likely be based on new technology.

Are there already some promising candidates?

In single-molecule sequencing, one such candidate is nanopores. We've started using this technology in DNA sequencing. There are still many technical limitations, but nanopores hold enormous potential. An ionic current is channeled through a nanopore, an opening with an inner diameter of the order of one nanometer. If a DNA molecule is slowly passed through the nanopore, the sequence of bases that is located in the pore at any given time affects the number of ions that make it through the pore. Reading the disrupted current signature then allows you to determine the base sequence of a strand of DNA. When this technology has been fully developed, it might be possible to unravel complex chromosomal sequences and read off hundreds of thousands of bases at once. Using multiple nanopores in parallel, this would allow genome sequencing within a matter of minutes.

In which direction will the FGCZ develop in future?

Since data generation is becoming easier in many areas, the focus of our support is shifting to the upstream and downstream workflows. With the vast amounts of data that are generated these days, we have to ensure more than ever that we're generating the right kinds of data, and that we support our research groups appropriately in the preparation of their samples. The downstream steps are also becoming more and more important. Sensible processing and presentation of the data, so that they are understandable and can be properly interpreted, is becoming increasingly difficult in the flood of data available today. In other words, bioinformatics support is more crucial than ever.

The Functional Genomics Center Zurich (FGCZ) provides technologies and services in the areas of proteomics, genomics, transcriptomics, metabolomics and bioinformatics.

More information is available at: **www.fgcz.ch**

Sunniva Förster is testing new combinations of antibiotics to combat the sexually transmitted infection gonorrhea.

The battle against resistant strains

Searching for new antibiotic combinations to fight gonorrhea

The pathogen that causes the sexually transmitted infection gonorrhea is becoming ever more resistant to various antibiotics. In her Interdisciplinary PhD Project, Sunniva Förster is investigating whether new combinations of drugs could help combat the disease. To this end, she has developed standardized high-throughput methods, custom-tailored to the bacteria that cause gonorrhea.

Until quite recently, the treatment of gonorrhea was straightforward. Upon infection after unprotected sex, the patient would simply receive a course of antibiotics. The itching, pain during urination and purulent discharges would abruptly stop.

New superbug advancing

Unfortunately, this has changed over the past few years. Many strains of the disease pathogen *Neisseria gonorrhoeae* develop increasingly comprehensive resistance against antibiotics. In 2011, one strain was even found that could not be inhibited by any of the commonly prescribed antibiotics. It's high time, then, to set out on the search for new strategies for combatting these highly adaptable and common bacteria. After all, they cause nearly 80 million new gonorrhea infections every year.

As part of her Interdisciplinary PhD Project at the Institute for Infectious Diseases at the University of Bern, the young biochemist Sunniva Förster is investigating whether new combinations of antibiotics are effective in treating gonorrhea. Her goal is to find combinations that act as synergistically as possible, together producing a greater effect than just the sum of their parts.

Lacking the right tools

In order to find suitable combinations, Förster needs standardized data for analysis. "At the start of my PhD, it became clear that the necessary tools for generating data specifically for *Neisseria gonorrhoeae* were not available," reports the young scientist. Therefore, she spent much of her time in the first year of her PhD studies adapting and developing the required automated and reproducible methods, which have long been readily available for other organisms such as *E. coli.*

To begin with, Förster developed a procedure for calculating standardized time-kill curves for *Neisseria gonorrhoeae* subject to different concentrations of single antibiotic types. These show how many bacteria survive after different periods of exposure and at different antibiotic concentrations.

With the aid of dose-response curves drawn from these data, it was also possible to estimate what kind of strategy each antibiotic uses. The shape of these curves depends on how fast the drug kills the bacteria or prohibits their replication. Förster has used this method to categorize a promising new antibiotic that is still in the development stage (see "further reading", page 17).

Although this new gonorrhea-specific method is standardized and reiterable, the bacteria still need to be pipetted by hand onto an agar plate, cultivated, and then counted by eye. This makes it much too time consuming, and inappropriate for large screenings.

New high-throughput methods

This is why the young scientist has developed a further novel method. Here, the bacteria remain the whole time in a liquid medium, where their viability is indicated by the state of the dye resazurin and simply read from a reader that detects these color changes. This optimized approach makes high-throughput screenings possible.

In getting to this stage, the scientist had to overcome a number of hurdles. First, she had to find a suitable medium in which all of the different strains of *Neisseria gonorrhoeae* thrived, with an exactly defined chemical composition (not containing any blood or yeast constituents) and which was also colorless, so as not to distort the results quantified by the reader.

In addition, just handling the bacteria, which can normally only survive within the human body, is no easy task. They must be continually kept in an incubator at 37 degrees Celsius in a controlled, 5-percent humidified CO_2 atmosphere. The researcher is therefore left with relatively little time to perform the experiments. “But the fact that the bacteria are so hard to handle also makes the whole process more interesting!” smiles Förster.

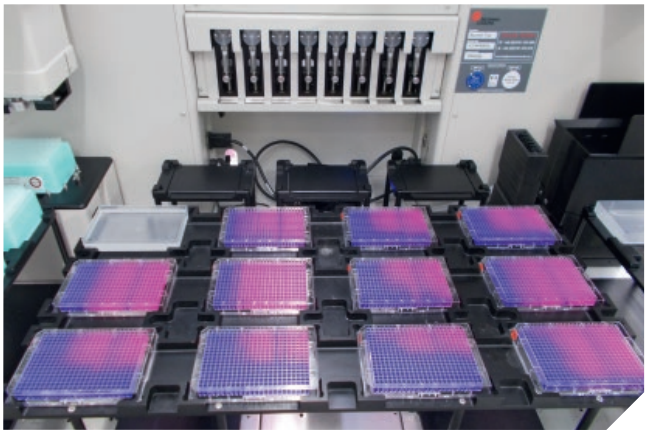
Förster discovered the sticking point in the reproducibility of the measurements thanks to the fact that she works in different laboratories. As well as at the lab in Bern, she also carries out experiments at the WHO Collaborating Centre for Gonorrhoea and Other Sexually Transmitted Infections in Sweden and makes use of a pipetting robot at the European Molecular Biology Laboratory in Heidelberg. “If it weren’t for this, it would never have occurred to me that different components of the laboratory equipment critically affect my results.”

Synergistic effects?

Förster is now using these new methods to pursue her project goal. She is looking for evidence of synergistic effects of any two antibiotics in differing concentrations applied to different strains of bacteria. Thanks to her automated methods and the pipetting robot, she has already found some potentially interesting combinations. However, these screening hits now need to be validated and characterized in detail.

“The problem is, the same combination of drugs might yield an additive, synergistic or even antagonistic effect, depending on the concentrations of the two antibiotics,” explains Förster. This means that, depending on the concentrations, some combinations of antibiotics actually work less well together than on their own. Even the results of the various strains differ quite drastically. “And that’s just the situation *in vitro.* In each individual patient, the results might look very different again,” she sighs.

However, just as the difficulties in handling the bacteria have their own charm for Förster, these complex results do nothing to deter the scientist. “Now, of course, I’m interested in why each bacterial strain reacts differently, and which genes are implicated in the process,” explains the researcher. As soon as she has all the results, she wants to take the most promising synergistic antibiotic combinations and closely examine them to find out how they work, and why they work better together. A challenging undertaking, but one which Sunniva Förster is prepared to tackle.



With the help of a pipetting robot, Förster can efficiently test the effectiveness of different combinations and concentrations of antibiotics on different gonorrhea strains. The dye resazurin shows which bacteria are killed (blue), and which survive (pink). Photo: Sunniva Förster

The project at a glance

Project title: Exploring response surfaces and synergistic interactions of antibiotic combination treatment for *Neisseria gonorrhoeae*

PhD student: Sunniva Förster, Institute for Infectious Diseases and Institute of Social and Preventive Medicine, University of Bern

Supervisors: Dr. Christian Althaus, Institute of Social and Preventive Medicine, University of Bern; Dr. Lucy Hathaway, Institute for Infectious Diseases, University of Bern; Prof. Nicola Low, Institute of Social and Preventive Medicine, University of Bern; Prof. Magnus Unemo, WHO Collaborating Centre for Gonorrhoea and Other Sexually Transmitted Infections, Örebro University, Sweden

Project duration: 2014–2017

Project type: Interdisciplinary PhD Project (IPhD) – PhD students work at the interface between two systems biology-relevant fields. During their interdisciplinary doctorate, they are supervised by a mentor from each of these two distinct subject areas.

Further reading: Foerster S. et al. (2015) Genetic Resistance Determinants, *In Vitro* Time-Kill Curve Analysis and Pharmacodynamic Functions for the Novel Topoisomerase II Inhibitor ETX0914 (AZD0914) in *Neisseria gonorrhoeae.* Front. Microbiol. 6:1377.

Short-cut to protein synthesis

Splicing helps the brain make rapid adaptations

The brain is a highly adaptable organ. Oriane Mauger, postdoc at the Biozentrum of the University of Basel, is investigating what happens in neurons when the brain adjusts itself in response to external stimuli, and how these adjustments succeed so quickly.

Oriane Mauger investigates the mechanisms behind rapid adaptations in the brain.

When a child is born, its brain is still under construction. Stimuli from other organs and the external environment influence how the different brain regions develop and connect to each other. Frequently used connections are strengthened, whereas unused ones are eliminated.

Although the building work in the brain does dwindle with increasing age, these refinements happen life-long. They allow us to learn new things, such as how to play the piano or operate a new technical device, as well as adapt to changing circumstances and demands. In light of our high life expectancy and the prospect of many changes occurring throughout our lives, this plasticity makes a lot of sense.

Part of the brain's adaptability can be attributed to events at the synapses. Here, at the junction between two neurons, signals are transferred from one cell to the next via neurotransmitters. These messenger substances dock onto specific receptors on the target cell. Depending on how many of these receptors are present, and how sensitively they react to the neurotransmitter, the incoming signal is strengthened, weakened, or not passed on at all.

Adaptable synapses

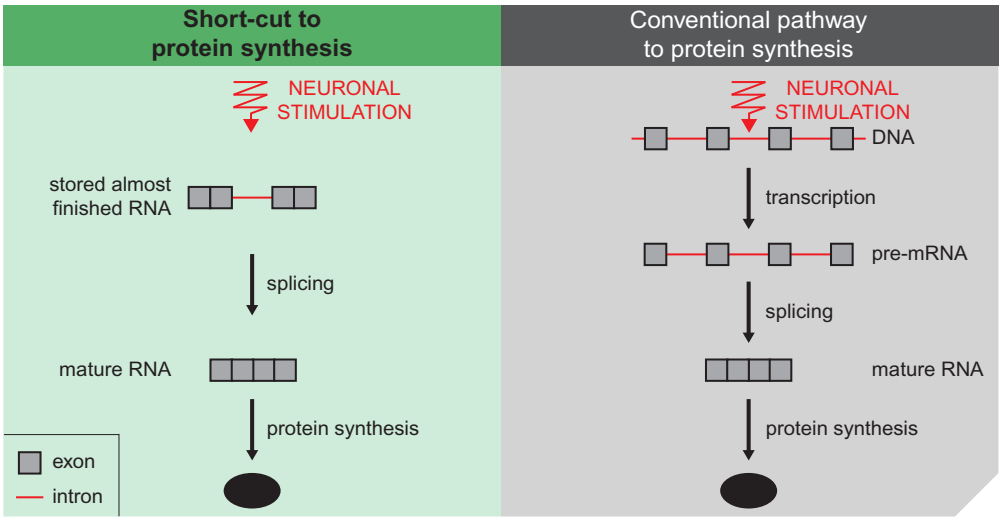
But just how sensitively a synapse reacts to a given neurotransmitter is not set in stone, and can in fact change very rapidly. Measurements show that the strength of a synapse's response can change within a few seconds or minutes of a signal being propagated. The reason for this is the presence of various feedback mechanisms regulating protein expression, which can ultimately serve to modify the properties of a synapse and thus the strength of the response that is passed on to the next neuron.

For example, increasing the number of proteins that form the receptors results in more receptors, and so to an increased sensitivity of the synapse to neurotransmitters. However, the standard pathway for producing proteins is very time consuming. "Until now it was unclear how proteins can be formed so quickly," explains postdoc Oriane Mauger. "Even just the production of the mRNA template for these proteins usually takes several hours."

Accelerated protein synthesis

"There must be a shortcut," states Mauger. Her hypothesis is that the mRNA, the blueprint for these proteins, is not produced from scratch via DNA transcription on demand, but is instead made within a much shorter time from a sort of semi-finished product that the cell has on standby. These are almost-finished mRNAs, still containing one or two introns, which are sequences that are unrelated to the protein blueprint and that have to be removed to produce the mature RNA template. According to Mauger's theory,

Maja Schaffner



Oriane Mauger has shown that in response to neuronal stimulation, there is a possible shortcut to protein synthesis: almost-mature RNAs stored in the nucleus complete their maturation within a matter of minutes, whereas the production of mature RNAs via the conventional pathway can take several hours. Figure: Oriane Mauger

these introns are only cut away in response to neuron stimulation by way of the cell's own molecular machinery, and consequently the completed mRNA is funneled into the cytoplasm, where the corresponding protein is promptly produced.

With the help of deep sequencing, a method with which all present RNA pieces can be sequenced and quantified at a given moment, and using her own specifically developed algorithm, Mauger has been investigating this hypothesis. Sure enough, she has already succeeded in verifying that many of these different, almost-finished mRNAs are in fact stored in the nucleus of the neuron, and not simply destroyed there, as would have been expected.

By inducing neuronal activity with chemicals, the young researcher has shown that some of the almost-mature mRNAs were actually spliced, meaning that the remaining introns in the blueprint were removed through the cell's own machinery. More concretely, Mauger found around 5000 such semi-finished mRNAs, of which around 250 underwent splicing. "This process happens in less than five minutes," explains the young scientist. She was also able to show that mRNAs matured in this way are in fact exported from the nucleus into the cytoplasm, where protein synthesis takes place.

"Next, I'd like to check whether the corresponding proteins are then actually produced, and whether they do indeed have an effect on the strength of a synapse's response to stimuli," describes Mauger. Needless to say, she is also interested in all the other details of this whole process: How are the almost-finished mRNAs stored in the nucleus? Why are some introns removed but others are not? And how exactly does neuronal activity lead to splicing?

Fascinated by splicing

The molecular biologist discovered her fascination for splicing during her PhD. Her eyes light up when she describes this mechanism, which, amongst other things, is responsible for producing hundreds of thousands of different proteins by using different combinations of the roughly 25,000 human genes we possess.

"After my PhD in basic research, I wanted to apply my knowledge to more concrete questions," says the young researcher. She found exactly the right environment in Peter Scheiffele's group at the Biozentrum of the University of Basel. With her Transition Postdoc Fellowship, she is able to put her knowledge to good use in neurobiology, a field that is new to her.

Connection to autism?

Mauger's research even has the potential to help elucidate the causes of autism in future. Scheiffele's group has been investigating this disorder for quite some time. Mauger speculates that in the brains of people with autism, the almost-finished mRNAs are present, but that they do not undergo splicing as usual, presumably because the molecular pathway triggering splicing is somehow faulty.

"If I am really able to show that this theory holds, I'd like to carry on researching in this direction," says Mauger. "Then it would be a case of identifying the genes that are responsible for the defect, and looking into how one could re-activate the splicing mechanism."

The project at a glance

Project title: Targeted intron retention as a novel mechanism for neuronal plasticity

Fellow: Dr. Oriane Mauger, Biozentrum, University of Basel

Host research group: Prof. Peter Scheiffele, Biozentrum, University of Basel

Project duration: 2015–2017

Project type: Transition Postdoc Fellowship (TPdF) – Young scientists formulate their own interdisciplinary project application and switch to a complementary discipline that is new to them.

Systems analysis of cell-cell communication in plant roots (RootBook)

Spatial single-cell expression map of the plant root

The team from the ERASysAPP project RootBook has not only set out to investigate cell-cell communication in the plant root tip. The scientists also want to find out what role intercellular communication plays under stress conditions. Innovative technologies and methods, as well as the international composition of the research team, constitute the ideal conditions for this endeavor.

A technical innovation stands at the center of the international RootBook project. At first glance, it looks like an inconspicuous transparent plastic block, whose interior is covered in a network of fine, geometrically arranged lines. Upon closer inspection, these lines are revealed to be channels, through which any medium can be made to flow thanks to an ingenious valve system. "This type of apparatus is known as a microfluidic device. Their use is becoming increasingly popular in biological research for the observation of miniaturized biological systems," explains project coordinator Manfred Claassen. "Based on this, our project partner Matthias Meier has developed the 'RootChip'", he adds. Its special feature is the inclusion of tiny drill holes, allowing the researchers to plant seedlings in the plastic block. Their roots then grow along the holes into the nutrient-filled channel system below. The transparent material of the block allows this process to be microscopically monitored.

Communication between root cells

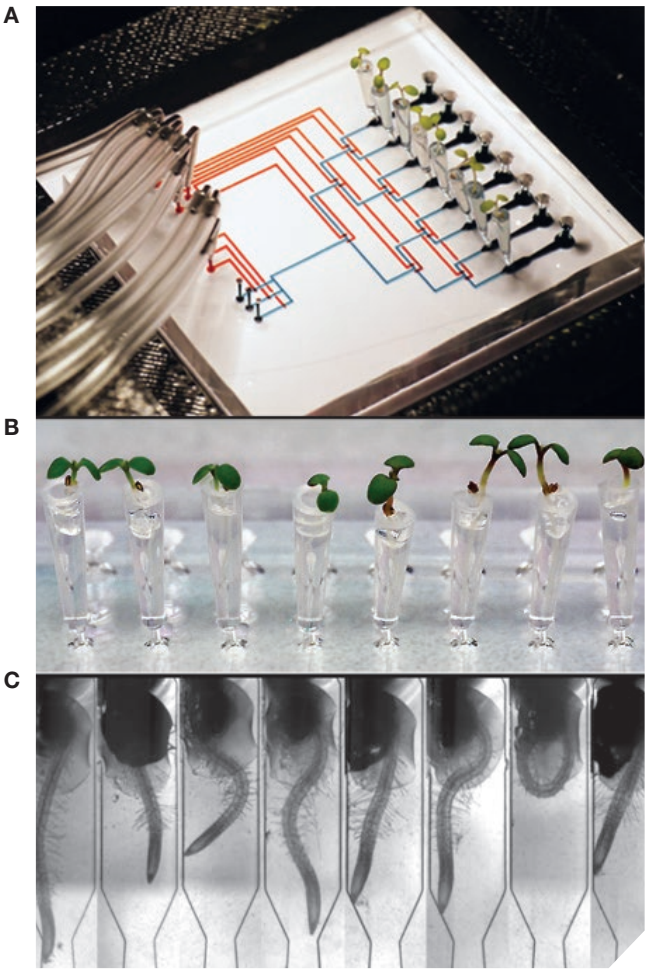
Whereas the first version of the RootChip (see picture) had the capacity to grow eight seedlings, the team of researchers is now working on a development that would allow the simultaneous cultivation of 48 plants. Claassen succinctly summarizes the main advantage of the new RootChip: "This device will allow us to measure root development in a spatial context and determine the molecular composition of individual cells." Here, the scientists are particularly interested in how the cells of the root tip communicate with each other to coordinate root growth.

Although some of the genes and mechanisms involved in this intercellular communication are already known, the RootBook team wants to be able to characterize the involved activities and correlations between individual molecular structures comprehensively and in a spatially accurate way. To this end, the scientists are making use of yet another innovative technique.

Painting an expression map with 64 colors

"In the first step, we perforate the cells of the root tip," explains Claassen. This is an intervention that requires extreme caution and precision, as the holes must be just big enough so that no molecular material leaks out of the cells. Then, colored dyes that bind specifically to previously selected protein structures are injected into the prepared cells. "In this way, we're able to mark up to 64 different mRNAs, each with a different color," says Claassen. Since an mRNA is the transcript of a particular gene and its immediate surroundings on the DNA, its concentration correlates with the activity level of the corresponding gene.

"After staining the cells, we obtain an individual picture for each cell with different numbers of colored spots in 64 different shades. These are counted using fluorescence microscopy, and from this we can ascertain which genes are expressed, and how strongly, in a particular cell at a particular point in time," explains the scientist. Although the pictures taken using the RootChip are two-dimensional, they can be extrapolated into the third dimension with



A The RootChip mounted with eight live plants.
B Top view of the eight plants in transparent tubes filled with agar and mounted onto the chip.
C Side view of the microchannels containing seedling roots 7 days after germination.
Illustration: Matthias Meier

the right computational and mathematical analysis. Using this method, the researchers can create what is known as a three-dimensional, single-cell expression map of the entire root tip, on which the measurements from each individual cell are recorded spatially accurately. “These transcription patterns will hopefully allow us to build mathematical models and understand the cell-cell communication in detail,” summarizes Manfred Claassen.

Behavior under stress conditions

If this step is successful, the RootBook team would like to examine the communication behavior under stress conditions. The growth of roots is heavily dependent on the nutrient content of the soil. “Changes in phosphate levels or salt concentration cause abiotic stress in plants, potentially resulting in the withering of the roots and subsequently the whole plant,” describes the project coordinator.

The third member of the RootBook team, plant molecular geneticist Reidunn Aalen, will take advantage of the RootChip to selectively expose the plants to differing stress conditions and systematically measure the resulting effects on intercellular communication. Claassen contemplates future applications of their findings: “This might lead us to novel solutions for maintaining plant growth even under adverse environmental conditions,” he muses.

International cooperation a great opportunity

RootBook is still very much in the conception stage, the project having only been launched at the beginning of the year. The whole team is highly motivated. “RootBook is a very exciting project. We’re investigating a fascinating, cell-transcending biological phenomenon, enabled by the innovative integration of microfluidic technologies and customized computational biology solutions,” says Manfred Claassen. The project coordinator is confident that the project will meet its ambitious goals, not least thanks to the international composition of the consortium. “A transnational collaboration has the great advantage that the involved scientists contribute to the diversity in research culture.” This results in correspondingly complementary approaches being brought together to innovatively address the research questions, which ultimately also contributes to uncovering the complex relationships of intercellular communication in the root tip.

More information on RootBook:
www.erasysapp.eu > Calls > 2nd Call > RootBook

Manfred Claassen coordinates the international RootBook project.

RootBook at a glance

Research groups:

- Prof. Manfred Claassen (project coordinator), Institute of Molecular Systems Biology, ETH Zurich – Computational biology
- Dr. Matthias Meier, Department of Microsystems Engineering, University of Freiburg, Germany – Biotechnology
- Dr. Reidunn Aalen, Department of Biosciences, University of Oslo, Norway – Plant biology

Total budget (2016–2018): EUR 1 million, including EUR 215,500 from SystemsX.ch

Project type: International Project – As a partner in the European research network ERASysAPP, SystemsX.ch has co-funded six international application-oriented projects in which Swiss consortium partners are involved.

Further reading: Grossmann G. et al. (2011) The RootChip: An Integrated Microfluidic Chip for Plant Science. The Plant Cell 23(12):4234-4240.

Sebastian Maerkl on his Special Opportunity Project

Novel high-throughput microfluidic methods

Project goal:	In this Special Opportunity Project, we aim to develop novel high-throughput microfluidic methods to enable rational protein engineering. Specifically, we are developing an integrated approach for the massively parallel on-chip synthesis, purification and characterization of hundreds to thousands of single-chain variable fragment (scFv) variants.
Origin of project idea:	Our lab had previously developed numerous microfluidic methods for high-throughput protein biochemistry. Recently, we concluded a project that combined a new gene synthesis approach with on-chip expression, purification and characterization, and applied this approach to create and comprehensively characterize hundreds of novel zinc finger transcription factors for use as building blocks in engineered transcriptional regulatory networks. In our Special Opportunity Project, we are now applying the method to immunoengineering.
Interesting facts and background:	The rapid drop in prices for synthetic gene constructs is beginning to allow researchers to order thousands of rationally designed genes. Coupled with the maturation of *in silico* protein design approaches, these two techniques promise to make rational protein engineering feasible. With our project, we aim to provide a high-throughput approach for synthesizing and characterizing the plethora of possible designs in order to obtain a functional protein, as well as to improve the design process itself.
Highlights:	In the first few months of the project, we were able to establish that functional scFv variants could be synthesized and analyzed on our devices, enabling the scaling-up of the process in the near future. We are currently establishing a number of collaborations with labs working on immunology and protein design that are interested in the platform's capabilities.
Biggest challenges:	Although the cost of gene synthesis is significantly lower than just a few years ago, the cost per gene is still around CHF 50. For the platform's analysis capacity of several thousand variants per week, genes remain too expensive. In order to reach our full capacity, we are also considering other sources such as genes obtained through library selection strategies and single-cell cDNA clone libraries.
Future applications:	The methodology has a broad range of applications, from the engineering of novel protein affinity reagents to characterization of the antibody-binding repertoire of the immune system. Furthermore, it will meet the demand for the development of novel protein binders and transcriptional regulators.
Project title:	Development of a high-throughput platform for systems immunology and protein engineering
Principal investigator:	Prof. Sebastian Maerkl, Laboratory of Biological Network Characterization, EPF Lausanne – Synthetic and systems biology, microfluidics and molecular diagnostics
Total budget:	CHF 359,000, including CHF 198,000 from SystemsX.ch (2016–2017)
Project type:	Special Opportunity Project – Highly innovative projects that promote systems biology research in the broader sense, but do not qualify for other traditional sources of funding.
Further reading:	Blackburn, M. C. et al. (2016) Integrating gene synthesis and microfluidic protein analysis for rapid protein engineering. Nucleic Acids Research 44(7):e68.
Further links:	lbnc.epfl.ch

Thank you, Maja!

The science journalist Maja Schaffner started working for SystemsX.ch in 2014. As an editor, she not only took care of the initiative's newsletter and website, but also wrote fervently about many SystemsX.ch research projects for the X-Letter. Her ability to make complex systems biology topics understandable while retaining scientific accuracy has significantly contributed to making the X-Letter an enjoyable read, not only for the involved researchers, but also for interested non-experts.

In August 2016, Maja started working as a freelance journalist again. We would like to thank her for her commitment to SystemsX.ch and wish her all the best in her personal and work life.

vdm

Welcome to the team, Marc!

Marc Mouci joined SystemsX.ch in September 2016, working 60%. The PR specialist originally studied biology at the University of Bern, later specializing in communication. At SystemsX.ch, Marc will work as an editor for the newsletter and website, as well as write scientific articles about various research projects. As of January 2017, he will also assume responsibility for SystemsX.ch communication on behalf of Christa Smith, who will be on maternity leave.

The communications professional has previously worked for a number of Swiss companies, both in media and public relations, as well as in print, online and audiovisual communication. In his free time, the art enthusiast from Aargau is a keen photographer and enjoys traveling.

We are pleased to have a new, competent colleague on board, and would like to welcome Marc Mouci to the team!

csl

3rd International SystemsX.ch Conference

The third and final international conference will bring together world-class scientists from a variety of disciplines who apply quantitative and systems-wide approaches to research in the life sciences. The conference will take place from September 4–7, 2017 at ETH Zurich, with the opening day in the main building and the following three days in Hönggerberg.

This event will represent the conclusion of SystemsX.ch. The program will reflect the current state-of-the-art in the now maturing field of systems biology research, highlighting quantitative approaches at multiple scales, from single molecules and cells to whole tissues and organisms. The main topics will be single-cell biology, systems genomics, synthetic biology, physics of living systems and medical systems biology.

After the first, omics-focused international conference in 2011, the second in 2014 centered on systems dynamics in cell- and developmental biology and genetics. This third conference will showcase an even further sophistication of the approaches to studying the dynamics of biological systems, and their applications in a wide range of contexts.

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Upcoming events

November 21–25, 2016
Joint ML Course SIB and SystemsX.ch: Machine Learning for Bioinformatics and Computational Biology
Zurich, Switzerland

September 4–7, 2017
3rd International SystemsX.ch Conference
Zurich, Switzerland

November 23–25, 2016
Physics of Biology II International Meeting
Geneva, Switzerland

February 2–3, 2017
Life Sciences Switzerland (LS^2) Annual Meeting
Zurich, Switzerland

July 5–7, 2017
International Conference on Systems Biology of Human Disease (SBHD)
Heidelberg, Germany

April 30 – May 5, 2017
Ascona Workshop 2017 Statistical Challenges in Single-Cell Biology
Ascona, Switzerland

Cover: Matrix-Assisted Laser Desorption Ionization – Time Of Flight mass spectrometer (MALDI-TOF/TOF) with cover open, not in operation. The instrument is mainly used for the determination of masses of intact proteins and peptides, the identification of proteins after enzymatic digestion (bottom-up analysis), and the determination of the N- and C-terminal sequences of proteins (top-down analysis).
Photo: © Frederike Asael, taken at the Functional Genomics Center Zurich.