University of Basel

BIOZENTRUM
The Center for Molecular Life Sciences

2015

Biozentrum Annual Report

Biozentrum Annual Report 2015

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Research at a Glance

Around 30 topics are the focus of research at the Biozentrum.

Research at the Biozentrum embraces a wide range of topics, however one main focus all the research groups share is a strong interest to understand the molecular organization of living organisms. The major areas of research are concerned with the structure and function of macromolecules, the wiring of regulatory circuits, and the general principles underlying complex biological systems and their dynamic interactions.

Currently, the research groups of the Biozentrum are grouped into five major areas of investigation: Growth & Development, Infection Biology, Neurobiology, Structural Biology & Biophysics and Computational & Systems Biology. These research areas are not strictly separated from each but rather share concepts and technologies. Furthermore, new and relevant questions often arise at the overlap between the research areas, while innovative solutions can be found arising from the expertise of each respective area.

Modern research increasingly depends on sophisticated technologies, notably in the fields of genomics, proteomics, imaging, and data analysis. To meet this challenge, the Biozentrum has established a number of so-called Technology Platforms focusing on recent developments, for example, in the fields of electron microscopy and light microscopy, proteomics, FACS (Fluorescence Activated Cell Sorting) and Research IT. The Biozentrum shares further Technology Platforms (microarray technology, next-generation sequencing, etc.) with other regional research institutes.

The Biozentrum in figures

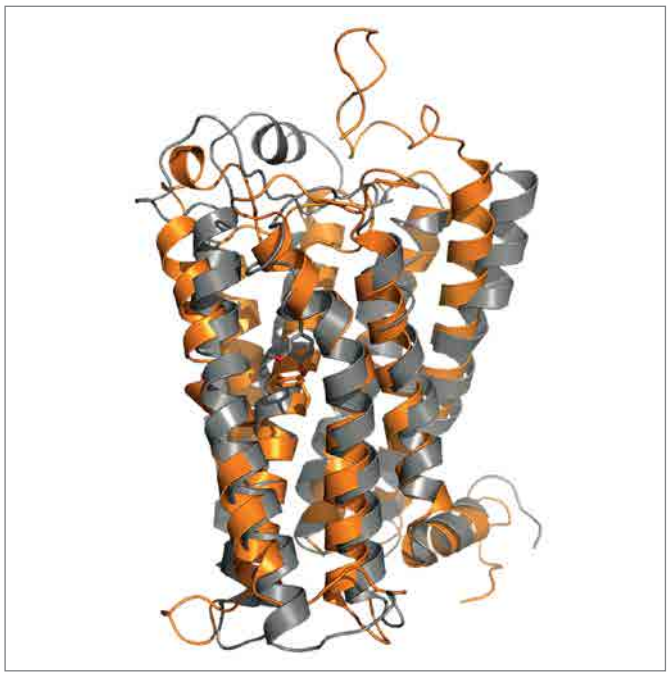
Members of staff in 2015

Total members of staff: 521
Professors: 31
Postdoctoral researchers: 122
PhD students: 123
Scientific staff, etc.: 63
Master Students: 22
Lab staff/Technicians: 122
Administration: 38

Annual financial statement 2015

Total expenditure: CHF 62.9 million
University of Basel: 64.7%
National Science Foundation: 15.1%
Swiss Institute of Bioinformatics: 2.3%
Misc. third party grants 17.9%

Computational & Systems Biology



Computational structural model of an olfactory receptor structure for the odorant eugenol.

The advent of quantitative high-throughput methods in genomics, transcriptomics, proteomics, and imaging has led to a growing need for automated analysis of large volumes of data. As a growing number of molecular cell components continue to be characterized, increasing numbers of scientists are beginning to analyze how much of the behavior of biological systems is determined by the complex dynamic interactions between these molecular components. Such developments are paralleled by an increasing demand on mathematical models and computational approaches.

The research groups involved in Computational & Systems Biology are addressing a wide range of subjects including the computer simulation of the dynamical behavior of proteins at the molecular level, methods for the inference of structure and function of proteins, to the analysis of gene regulatory networks and genome evolution. Many of these projects are undertaken in collaboration with other research groups at the Biozentrum and often also with large international consortia.

Some of the groups have access to a wet laboratory in which experiments are carried out. All group leaders from Computational & Systems Biology are also involved as research group leaders at the Swiss Institute of Bioinformatics (SIB) and support, in collaboration with the SIB, a competitive IT infrastructure that incorporates application-, database-, and web servers, large scale storage and backup facilities.

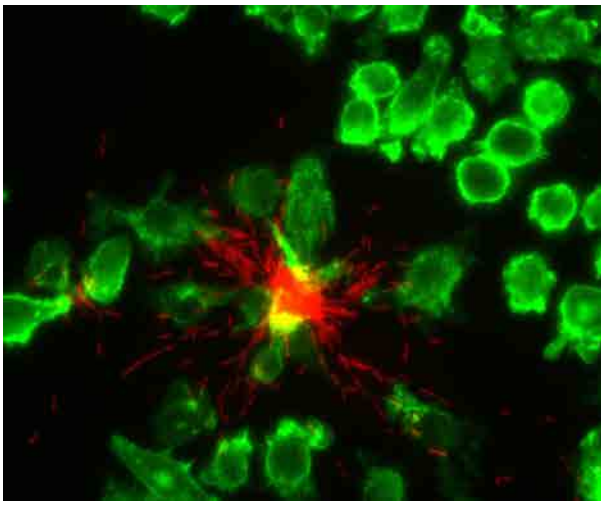
Research Groups
Prof. Attila Becskei
Prof. Torsten Schwede
Prof. Erik van Nimwegen
Prof. Mihaela Zavolan

Growth & Development

The spatial and temporal regulation of interactions between molecules is fundamental to life. Growth & Development is dedicated to understanding how these coordinated interactions lead to cell growth, cell division and the development of living organisms.

Life is more complicated than a binary interaction of two factors and its regulation; various processes need to occur in parallel for a cell to function normally. For this reason, this research area covers a broad range of aspects from signal transduction, gene regulatory networks, cell division and cell cycle control to membrane transport, protein and mRNA transport, in a variety of experimental organisms such as bacteria, yeasts, worms, flies, fish and mammals.

This broad spectrum of experimental systems and regulatory processes makes it possible to investigate the basis of organ development, cancer and muscle function. In these endeavors, collaborative arrangements with other groups at the Biozentrum, in particular those from the research areas Structural Biology & Biophysics and Computational & Systems Biology are sought. The research groups involved in Growth & Development are also involved in initiatives within the University and beyond, such as the Basel Stem Cell Network, the Basel Signaling Alliance and SystemsX.ch.



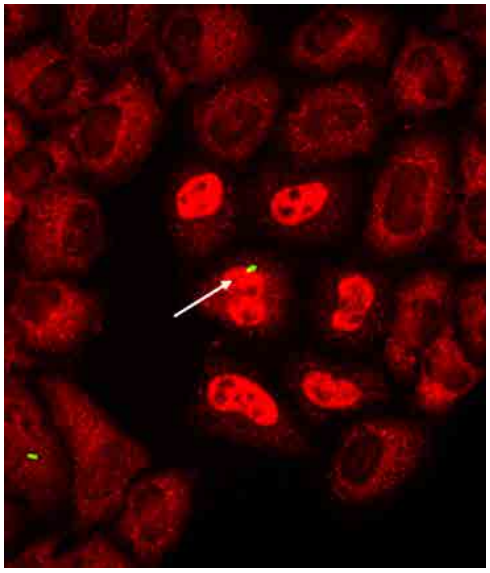
Over the course of a chronic lung infection, *Pseudomonas aeruginosa* (red) forms biofilm aggregates, which confer resistance to predation by the host immune system (cells in green).

Research Groups

Prof. Markus Affolter
Prof. Clemens Cabernard
Prof. Fiona Doetsch
Prof. Michael Hall
Prof. Christoph Handschin
Prof. Urs Jenal
Prof. Erich A. Nigg
Prof. Markus Rüegg
Prof. Peter Scheiffele
Prof. Anne Spang
Prof. Martin Spiess

Infection Biology

Research Groups
Prof. Marek Basler
Prof. Petr Broz
Prof. Dirk Bumann
Prof. Christoph Dehio
Prof. Urs Jenal
Prof. Jean Pieters



Inflammation signaling during *Shigella* infection.

The main objective of Infection Biology is to understand infectious diseases at both a cellular and molecular level in order to better control them in the future. The research groups at the Biozentrum involved in this field complement each other with their specialized skills and interests. The diversity of approaches to a common theme – the host-microbe interaction – generates a synergistic effect based on an intense exchange of scientific and technological expertise and experience.

The major research focus is on bacterial infections, in which currently nine pathogens are being studied. These represent archetypes for a range of virulence mechanisms such as intracellular replication, immune evasion, the injection of bacterial effectors into host cells, biofilm formation and persistence. The impact of this research goes beyond the field of microbial pathogenesis, addressing also basic principles in cell and molecular biology including pro-inflammatory signaling, intracellular traffic, regulation in bacteria, nanomachines, and *in vivo* microbial metabolism.

Several groups within Infection Biology have introduced systems biology as a new approach to investigate host-pathogen interactions and collaborate closely with their colleagues from other research areas, in particular from Structural Biology & Biophysics and Computational & Systems Biology.

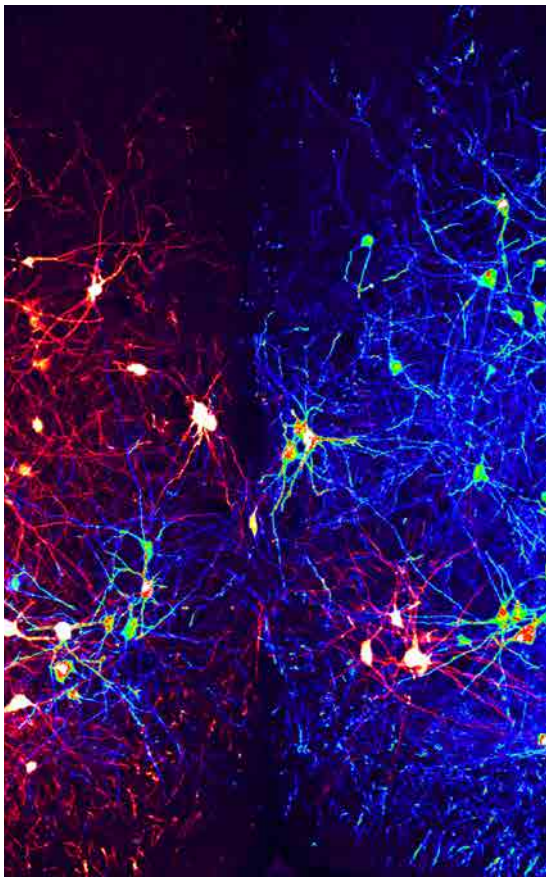
Neurobiology

One of the major challenges in biology is to understand how the nervous system forms, enabling it to appropriately respond to a broad spectrum of stimuli and to have control over complex functions, such as behavior and emotions. The nervous system must be capable of storing information, integrating it into the already existing memory and be able to retrieve it again.

The mechanisms employed are still poorly understood. The way in which neurons are generated and form meaningful functional circuits is not only of interest to developmental neurobiologists but also of great significance in regard to diseases, such as Alzheimer's disease and epilepsy, injuries to the nervous system or disturbances in body weight regulation.

The various research groups belonging to Neurobiology are concerned with neurogenesis and cell specification, the use of stem cell-based models of neural function and dysfunction, the assembly and elimination of neurons and synapses, synaptic signaling and the function of neuronal circuits.

Currently, an area of focus in this field of research is neuromuscular disorders, obesity and autism spectrum disorders – all areas involving important translational aspects, which are being further investigated in cooperation with industry. From a scientific point of view, these activities are being supported and further promoted by the Neuroscience Network Basel (NNB), a network bringing together scientists from the Biozentrum and affiliated institutions as well as partners in industry.



Visualization of spinal interneurons with direct connections to motor neurons.

Research Groups
Prof. Silvia Arber
Prof. Fiona Doetsch
Prof. Sonja Hofer
Prof. Thomas Mrsic-Flogel
Prof. Markus Rüegg
Prof. Peter Scheiffele
Prof. Kelly Tan

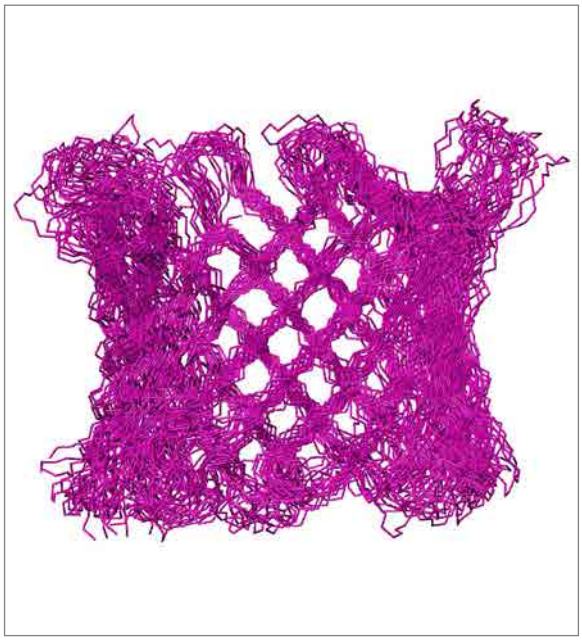
Structural Biology & Biophysics

The understanding of biological functions depends ultimately on an accurate account of biomolecular interactions in regard to structure, physical forces and their resulting dynamics. Enormous technical advances have been made in visualizing the threedimensional structures and in quantifying the dynamics of cellular components down to the atomic level. The research groups working in Structural Biology & Biophysics aim to apply and further develop these structural and biophysical techniques.

The structural techniques range from light microscopy, electron and scanning microscopy to X-ray crystallography and NMR spectroscopy. Biophysical methods encompass many different time-resolved spectroscopic techniques such as laser-flash spectroscopy and FRET, thermodynamic analytical methods such as ITC and DSC microcalorimetry, NMR imaging and *in vivo* spectroscopy. The use of rigorous combinations of these techniques enables the structure of biological matter to be determined at all magnifications; from details at the atomic level to entire cells up to small organisms.

In this way, dynamic changes can also be analyzed and quantified over a period of picoseconds or very long periods of time and the energetics and thermodynamics of biomolecular interactions can be investigated with very high precision. There is close teamwork between this branch of research and other areas of science at the Biozentrum, since many of the investigations involve projects which are carried out together with other research groups with a biochemical or biological orientation.

Research Groups
Prof. Jan Pieter Abrahams
Prof. Stephan Grzesiek
Prof. Sebastian Hiller
Prof. Roderick Lim
Prof. Timm Maier
Prof. Tilman Schirmer
Prof. Henning Stahlberg



Three-dimensional structure of the VDAC membrane protein.

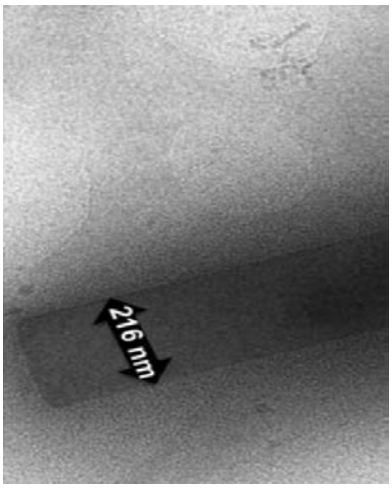
Research Group Jan Pieter Abrahams

Nano-diffraction of biological specimens

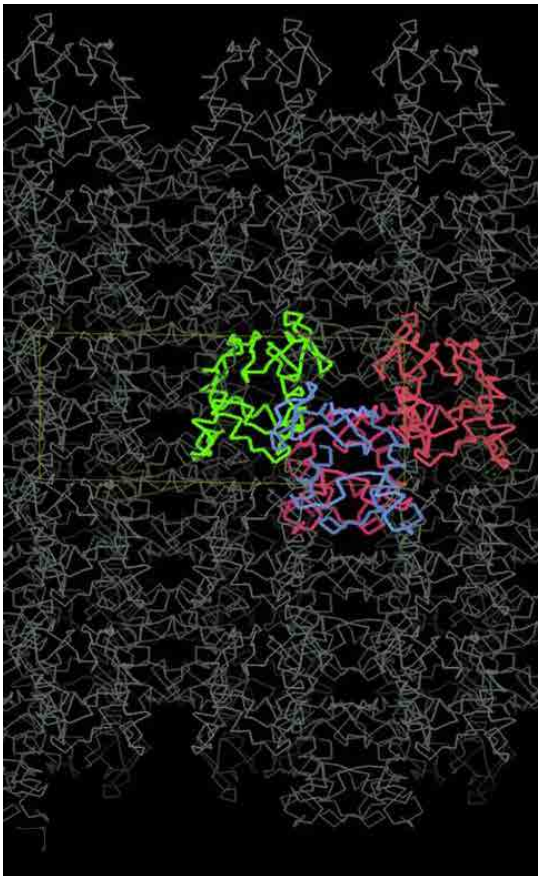
The Abrahams group develops new approaches for using diffraction to determine the atomic structures of proteins and other radiation sensitive nano-size samples. We use diffraction rather than imaging, because diffraction patterns can be measured more accurately than images.

In first instance, we concentrate our efforts on the structure determination of minute nano-crystals, too small to see even with the best light microscopes. Many proteins can only form such nano-crystals, yet these crystals are too small for standard X-ray diffraction.

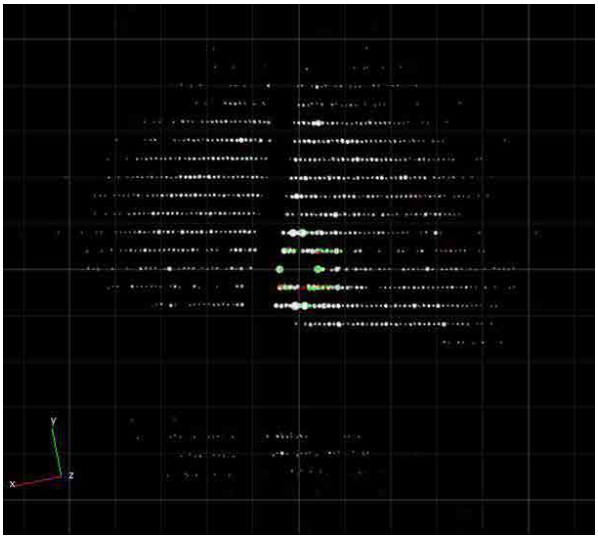
However, nano-crystals are well suited for electron diffraction, because electrons induce far less radiation damage compared to X-rays for the amount of information that their diffraction patterns provide. Electron nanocrystallography of proteins is an emerging technology and many of its aspects need to be developed and improved, while also several fundamental problems remain to be solved. To this aim, we develop novel data collection strategies, implement novel detectors for accurately and sensitively measuring electron diffraction data, we develop novel algorithms for analysing this data and (often in collaboration with others) we investigate new, unkown structures of proteins and other samples. The Abrahams group is located at C-CINA and at the Laboratory of Biomolecular Research at the Paul Scherrer Institute.



Three-dimensional electron diffraction of this protein crystal provides atomic information on its structure.



A nano-crystal of a protein that is only about 100 nm thick and 200 nm wide. Because this is far too small to see with a light microscope, it is visualised with an electron microscope.



The atomic structure of the protein crystal as deduced from the diffraction patterns.

Publications 2015

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Research Group Markus Affolter

Cell signalling and cell rearrangement during organ morphogenesis

The organization of body pattern in developing multi-cellular organisms is controlled to a large extent by cell-cell signaling. In the past two decades, the molecular components of a relatively small number of diverse developmental signaling cascades conserved throughout evolution have been identified. We have been studying two important developmental signals (Dpp/BMP and Fgf), and our efforts concentrated firstly on characterizing the signaling pathways in detail and deciphering their molecular logic, and secondly on understanding how these pathways control exquisite cellular behavior during development, both in *Drosophila* and in zebrafish. Our most intense research efforts are directed towards a profound understanding of cell behavior in branching morphogenesis, a process that leads to the ramification of epithelial structures such as seen in the lung, the kidney, many internal glands as well as the vascular system.

Cell signaling in organ formation

It has been proposed more than a century ago that the organization of body pattern might be controlled by socalled morphogen gradients. Only recently has it been possible to demonstrate that secreted proteins of the Transforming Growth Factor β (TGFβ), Wnt and Hedgehog families specify positional information by this mechanism. *Drosophila* Dpp is a member of the TGFβ superfamily and was the first secreted protein for which a morphogen function has been clearly demonstrated. Over the past ten years we have characterized the Dpp signaling pathway in detail, in collaboration with the group of Konrad Basler in Zurich.

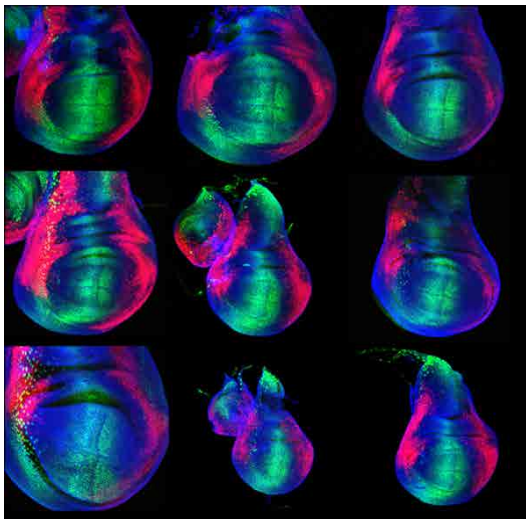

Fig. 1: Dpp gradient readout in wing imaginal discs. Wing imaginal discs of different sizes showing Brinker protein expression (red) and pMad distribution (green). Note that the pMad domain increases in size with increasing disc size, and thus scales with tissue size.

Our studies provide the molecular framework for a mechanism by which the extracellular Dpp morphogen establishes a finely tuned, graded read-out of a transcriptional repressor complex including Smad proteins and the zinc-finger protein Schnurri. Targets of this repressor complex include transcriptional regulators as well as secreted proteins involved in morphogen transport. Other morphogens, which pattern the nervous system or the limb fields in higher vertebrates, might use similar mechanisms. Our current efforts are devoted to a systems biology approach and are done in the framework of the WingX project of the Swiss initiative in Systems Biology.

The experiments we concentrate on involve genome-wide target gene identification, real-time analysis of morphogen gradient readout, and computer modelling to better understand the dynamics of the Dpp morphogen system. Just recently, we have identified a novel feedback regulator of the Dpp system which controls the spreading of the Dpp molecule and might be involved in the adaptation of the morphogen gradient to tissue size. Our studies will eventually lead to a comprehensive understanding of morphogen function in tissue growth and patterning, a key issue in modern developmental biology (**Fig. 1**).

Cell rearrangement in organ formation

To gain insight into how signaling pathways control more complex cellular decisions during the process of organ morphogenesis, we investigate the formation of the *Drosophila* tracheal system, an epithelial branched network similar to the lung, the kidney or the vasculature. Tracheal development serves as a paradigm to understand how epithelial cell sheets can be transformed by cell signaling and cell-cell or cell-matrix interactions into complex three dimensional networks, a process generally referred to as branching morphogenesis. Our approach has been to identify genes involved in the process by genetic analysis, and the characterization of relevant gene products by *in vivo* and *in vitro* analysis. In addition, we have devoted major efforts to characterize branching morphogenesis at the cellular level, using avant-garde, live imaging technology.

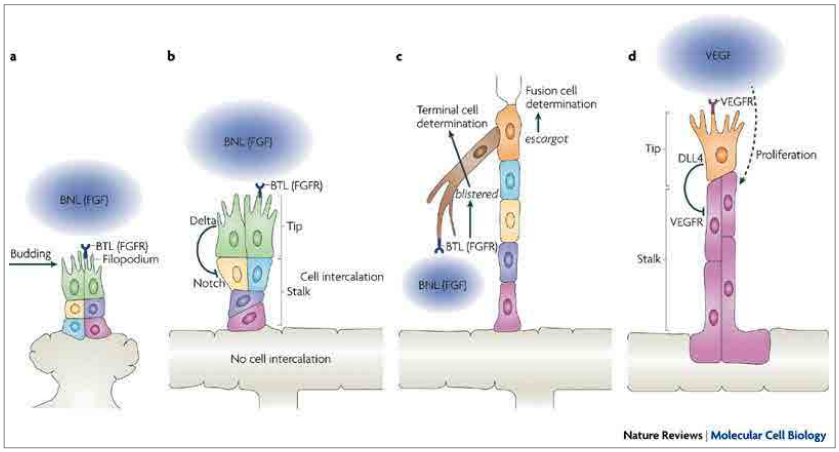

Fig. 2: *Drosophila melanogaster* trachea and vertebrate vasculature branching. Branchless (BNL), a fibroblast growth factor (FGF), acts at the top of the hierarchy of cellular events that orchestrate tracheal branching in *Drosophila melanogaster* (a to c). During vertebrate angiogenesis, vascular endothelial growth factor (VEGF) signalling determines the formation of angiogenic sprouts and controls tip cell and stalk cell identity through Delta Notch signalling. Taken from Affolter *et al.* (2009) Nat Rev Mol Cell Biol 10, 831-42.

Over the past decade, these studies have provided a framework for understanding complex processes involved in the architectural design of developing organs, including the control and integration of cell migration and cell rearrangement via cell-cell signaling and extracellular matrix components (**Fig. 2**).

Studies on the development of blood vessels in higher organisms suggest strong parallels between tracheal development in insects and tube formation in the growing vasculature (**Fig. 2**).

Interested by this possible developmental similarity, we have initiated studies aimed at a better understanding of blood vessel development in zebrafish, one of the most promising animal systems in the study of angiogenesis available at the moment. We have indeed found that our approach to studying cell rearrangement during tracheal development provides a novel insight into how cells behave during angiogenesis when applied to zebrafish. We have recently proposed a novel model for the architecture of the first vessels formed via angiogenesis, a model which is strikingly different to the one previously described. Our studies re-define the cellular routines involved in angiogenesis, and provide the basis for all future studies in the zebrafish regarding angiogenesis. We have now strengthened our efforts to study angiogenesis using live imaging combined with novel transgenic lines and strategies (**Fig. 3**). Particular emphasis is devoted to the study of blood vessel fusion, a process that has not been studied in the past *in vivo* at the cellular level.

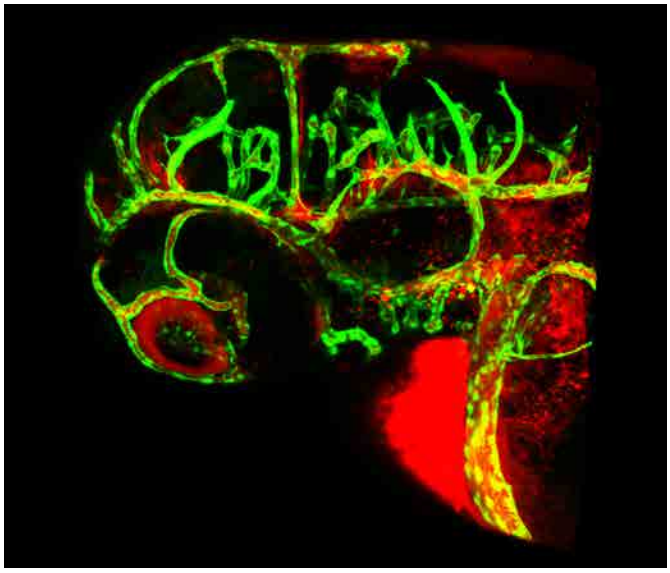

Fig. 3: Blood vessels in the zebrafish embryo. Blood vessels express Green Fluorescent Protein.

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Kumuthini Kulendra

Publications 2015

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Phng, Li-Kun; Gebala, Véronique; Bentley, Katie; Philippides, Andrew; Wacker, Andrin; Mathivet, Thomas; Sauteur, Loïc; Stanchi, Fabio; Belting, Heinz-Georg; Affolter, Markus; Gerhardt, Holger (2015). Formin-mediated actin polymerization at endothelial junctions is required for vessel lumen formation and stabilization. *Developmental Cell, 32*(1), 123-32.

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Research Group Silvia Arber

Motor circuit assembly and function

Our studies aim at identifying the principles by which neuronal circuits orchestrate accurate and timely control of motor behavior in response to stimuli such as sensory cues or voluntary initiation of movement. To decipher how motor circuits engage in the control of movement, we elucidate the organization and function of neuronal circuits by studying synaptic connectivity, genetic and molecular identities, and functional properties of motor circuits in the mouse.

The motor system is organized in a hierarchy of interleaved circuit modules, with three main overall components contributing to the control of accurate movement. These are circuits in the spinal cord responsible for rhythmicity, computation and perpetuation of motor activity, circuits communicating bi-directionally with supraspinal centers in the brain and brainstem, and sensory feedback circuits informing the nervous system about past action monitored in the periphery. We dissect these circuit elements from various angles to understand how precisely connected neuronal circuits lead ultimately to movement via activation of functionally distinct motor neurons that innervate muscles.

We use multi-facetted approaches combining many technologies. These include state-of-the-art mouse genetics, development and implementation of viral technologies for transsynaptic circuit tracing and functional manipulation, gene expression profiling of identified neuronal subpopulations, electrophysiology, and quantitative behavioral analysis.

These approaches allow us to assess connectivity and manipulate function to determine the role of defined circuit elements in animal behavior. Furthermore, we are also in a position to uncover mechanisms involved in motor circuit assembly during development, as well as circuit reorganization during learning and responses to disease or injury.

Circuit modules for motor control in the brainstem

Initiation of natural movement depends on the function of descending pathways to the spinal cord. This is strikingly obvious in patients with complete spinal cord injury who are unable to move muscles controlled by spinal segments below the lesion, despite the presence of functional circuits in the spinal cord. Descending motor control pathways are at the core of different forms of movement ranging from repetitive basic locomotor tasks such as walking to sophisticated fine motor tasks like object manipulation. Classical studies provide evidence that the brainstem is involved in the control of a variety of movements, but a deep understanding of the organization and connectivity of identified neuronal subpopulations in the brainstem, how they intersect with executive circuits at the level of the spinal cord, and how they function is currently lacking. We recently investigated the organization of the connectivity matrix between the brainstem and motor neurons in the spinal cord (Esposito *et al.*, 2014).

Upper and lower extremities in four-limbed species exhibit distinct behavioral repertoires in the execution of motor programs. In humans as in mice, forelimbs are far superior to hindlimbs in the performance of fine and skilled motor tasks. Using transsynaptic virus technology and anterograde synaptic tracking, we analyzed comparatively connection profiles between brainstem nuclei and motor neurons. More brainstem nuclei showed direct connections to forelimb-innervating

Fig. 1: Artificially rendered human hand reaching and grasping for an apple. This image illustrates the complexity of the task mediated by descending motor control circuits that can also be studied in the mouse (Esposito *et al.*, 2014).

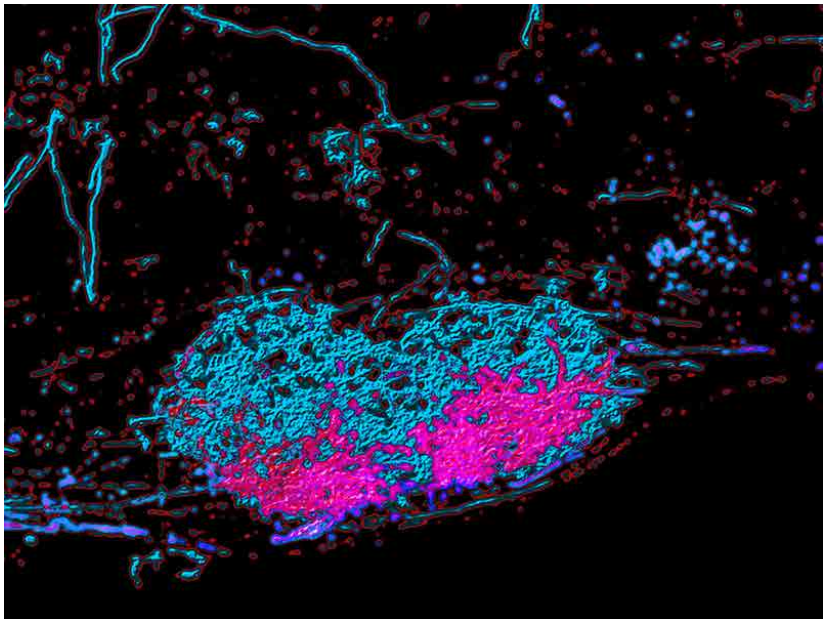

Fig. 2: Synaptic terminals of premotor neurons in the LRN (blue). Axons of a marked spinal interneuron subpopulation terminating in a specific domain of this brainstem nucleus (pink) (see Pivetta *et al.* 2014 for details).

motor neurons than hindlimb connected motor neurons and, thus, a higher degree of direct access to forelimb motor neuron populations. In a more detailed analysis of connection specificity to motor neurons innervating distinct limb muscles, a pattern of specific connections between individual brainstem nuclei and motor neuron subtypes emerged. Together, these findings reveal a highly specific and complex connectivity matrix between different brainstem nuclei and local circuits in the spinal cord, providing insight into the sophisticated interactions carrying motor control commands from the brainstem to the spinal cord (Esposito *et al.*, 2014).

We designed behavioral experiments to address the functional implications of this striking connectivity matrix, focusing on the medullary reticular formation ventral part (MdV), a brainstem nucleus with highly preferential connections to forelimb motor neurons. Within the MdV, excitatory and inhibitory neurons are intermingled and represented approximately equally. However, only excitatory (vGlut2-expressing) neurons make direct connections to forelimb-innervating motor neurons. This finding allowed us to selectively eliminate or silence glutamatergic MdV neurons by specific injection of conditional adeno associated viruses (AAV) into the MdV nucleus of vGlut2Cre mice. This led to the expression of diphtheria toxin receptor (DTR), which can be used to eliminate these neurons by application of diphtheria toxin, or a hybrid glycine-receptor binding to a designer ligand for pharmacogenetic silencing of the corresponding neurons.

Behavioral analysis of these mice revealed that glutamatergic MdV neurons are not needed for the execution of basic locomotor tasks, but are required for high-level performance in skilled motor tasks. In particular, mice with ablated or silenced MdV neurons performed poorly on a single food pellet-reaching task involving forelimbs. Analyzing the different task phases, we found that the grasping phase was affected specifically by non-functional MdV neurons, whereas the initial reaching phase towards the food pellet and the retrieval phase of the pellet to the mouth were unaffected. These findings support a model in which distinct brainstem subpopulations control aspects of motor behavior through specific targeted spinal subcircuits. More generally, they provide important insight into circuit-level mechanisms and anatomical substrates essential for task-specific motor subroutines during the execution of a complex motor program.

A copy circuit from the spinal cord to the brainstem

Movement is the behavioral output of neuronal circuits computing motor commands and performance. The muscular system acts according to instructions conveyed by supraspinal centers via descending pathways to the spinal cord. The CNS uses two circuit level strategies to monitor planned and performed motor actions. First, motor output pathways establish axon collaterals at many levels, providing internal efference copy signals of planned action to recipient neurons. Second, movement-evoked sensory feedback from the body reaches the central nervous system and reports on performed motor actions. These two information streams adjust and modify descending motor commands. Despite their undisputed role in influencing motor behavior, surprisingly little is known about the identity, composition, or synaptic organization of core circuit elements encompassing these pathways, critical information needed to understand their function.

In a recent study in mice, we revealed the connectivity matrix between neurons in the spinal cord and the brainstem (Pivetta *et al.*, 2014). In particular, we focused our attention on spinal neurons with a bifurcating connection profile to the brainstem nucleus lateral reticular nucleus (LRN). These neurons not only exhibit direct connections to forelimb-inner-

vating motor neurons but also establish ascending collaterals to the LRN. Using virus and mouse genetic tools, we demonstrated that this connection profile occurs frequently within the spinal cord for neurons with direct connections to forelimb-innervating motor neurons. Interestingly, many different functional subtypes of spinal neurons comprise such dual-connection copy-circuit modules. Moreover, we found that axon terminals of functionally distinct spinal neurons terminate in different LRN domains, demonstrating that information converging at the level of motor neuron pools diverges by function at the level of the brainstem.

Together, our findings provide evidence for precise organization of ascending spinal information to the brainstem. This encompasses many functionally distinct spinal subpopulations, which can be distinguished by site of residence in the spinal cord, developmental origin, and neurotransmitter fate. We provide the first insight into the genetic complexity of the spinal efference copy signaling system, lending support to the notion that the reporting of ongoing activity of the spinal cord to supraspinal levels is an important prerequisite for accuracy in motor control.

Motor circuit segregation by function in the spinal cord

Motor behavior can be divided often into functionally opposed phases. An important entry point to understanding differential regulation of motor output has been the comparative analysis of antagonistic motor neuron pool function at the level of the spinal cord. Extensor and flexor motor neuron pools in the spinal cord innervate distinct limb muscles, which are generally active in alternation of an "on-ground" stance and an "off-ground" swing phase during walking. Although functional antagonism is observed in individual neurons in many cases, an overall anatomical assessment of the organizational principles of neuronal circuits relaying information to functionally distinct motor neuron pools was lacking.

In recent work, we used monosynaptically-restricted transsynaptic viruses to elucidate premotor anatomical substrates for extensor-flexor regulation in mice (Stepien *et al.*, 2010; Tripodi *et al.*, 2011). We observed a medio-lateral spatial segregation between extensor and flexor premotor interneurons in the dorsal spinal cord. These premotor interneuron populations are derived from common progenitor domains but segregate by timing of neurogenesis. Proprioceptive sensory feedback from the periphery was targeted to medial extensor premotor populations and was required for extensor-specific connectivity profiles during development. This provides evidence for a discriminating anatomical basis of antagonistic circuits at the level of premotor interneurons, and points to synaptic input and developmental ontogeny as key factors in the establishment of circuits regulating motor behavioral dichotomy.

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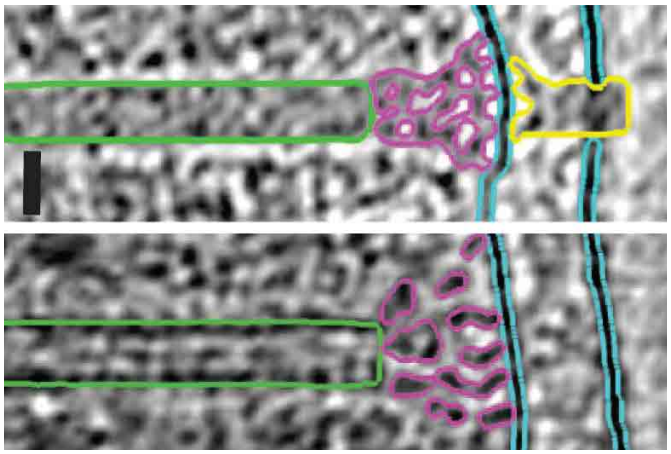
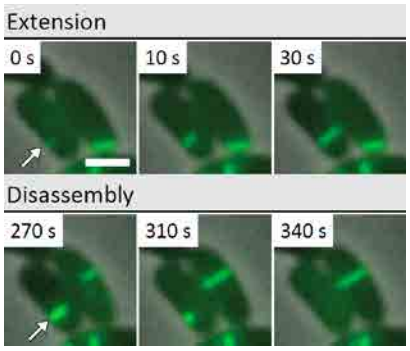
Structure, function and dynamics of type 6 secretion systems



Segmentations of densities observed in the extended (top) and contracted (bottom) structures of T6SS inside intact *V. cholerae* cells. (Basler *et al.*, Nature 2012).



Fluorescence microscopy of T6SS dynamics in *V. cholerae* cells. (Basler *et al.*, Nature 2012).

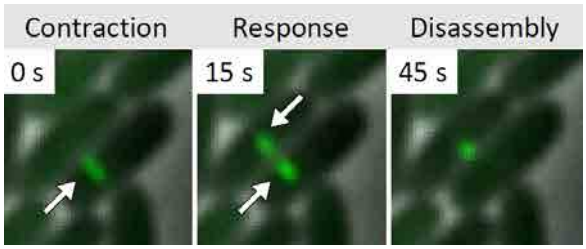
Secretion systems allow bacteria to transport macromolecules such as proteins into host cells during pathogenesis or bacterial cells during competition in various ecological settings. Type 6 Secretion Systems (T6SS) are encoded by a cluster of 15-20 genes that is present in at least one copy in approximately 25% of all sequenced Gramnegative bacteria. Although linked to virulence during host infection, species such as *Pseudomonas*, *Burkholderia*, *Acinetobacter* and *Vibrio* can use T6SS to kill competing bacterial cells by delivery of toxic proteins in a cell-cell contact-dependent process.

Several T6SS components are structural homologs of components of a contractile bacteriophage tail and assemble into a large structure that can be studied using whole cell electron cryo tomography and live cell fluorescence microscopy. A combination of these techniques allows to obtain high resolution structure of T6SS in situ and to follow T6SS assembly in time. This provides an unprecedented level of understanding of this dynamic nanomachine (Basler *et al.*, Nature 2012, Basler and Mekalanos, Science 2012). For example, live cell imaging of T6SS activity in *P. aeruginosa* revealed that these cells are able to sense an attack from neighboring heterologous bacteria and assemble its T6SS apparatus with a remarkable precision to specifically kill an attacking cell without damaging bystander cells (Basler *et al.*, Cell 2013).

The goal of our research is to understand the key molecular mechanisms underlying the assembly, substrate delivery, and regulation of T6SS function. We aim to create a detailed model of the T6SS nanomachine that would allow predicting structural changes needed to engineer T6SS with new properties. Novel approaches developed to study T6SS function will be further applied to study other molecular nanomachines as well as mechanisms of various contact dependent bacterial interactions in polymicrobial communities.

High-resolution structure of T6SS

A whole T6S apparatus was recently visualized in *V. cholerae* by whole cell cryo electron tomography. Resolution of the structure is, however, too low to identify individual components and therefore does not provide enough information to infer a mechanism of T6SS assembly. We are solving atomic resolution structures of T6SS components and analyze their mutual interactions by genetic and biochemical methods. We are developing novel strategies to improve resolution of the T6SS structure in situ in various model organisms. We aim to identify differences in T6SS assemblies in these organisms to explain the fundamental differences in their dynamics.



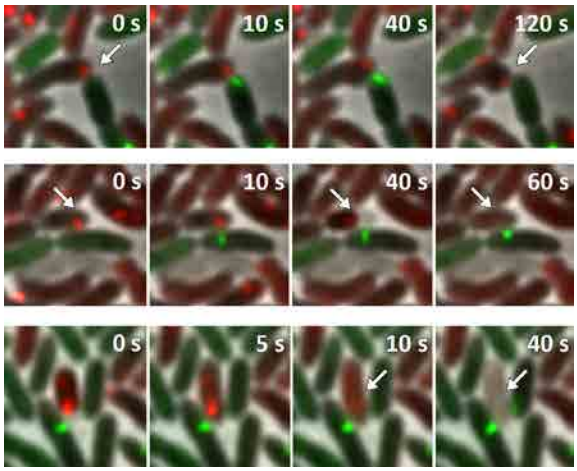
T6SS dueling between *P. aeruginosa* cells. (Basler *et al.*, Science 2012).

Visualization of T6SS activity

Our recent success in visualizing T6SS assembly and dynamics in live cells significantly improved our understanding of T6SS function in *V. cholerae* and *P. aeruginosa*. We use novel imaging approaches to describe localization of T6SS components with high spatial and temporal resolution. We are interested in understanding the process of initiation of T6SS assembly in various model organisms to further extend our knowledge about T6SS regulation. We are also using imaging to characterize the mode of action of T6SS effectors to better understand T6SS function.

Regulation of T6SS function

We are developing novel genetic methods to describe signaling processes involved in regulation of T6SS function on the transcriptional, translational, and post-translational level. We are also developing approaches to understand the role of T6SS in polymicrobial communities and to unravel the entire repertoire of secreted effectors.



Morphological changes of *V. cholerae* (red) after an attack by *P. aeruginosa* (green). (Basler *et al.*, Cell 2013).

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Research Group Attila Becskei

Epigenetic control and evolutionary optimization of cell decisions

Cell differentiation can be achieved by specifying cell fate through deterministic instructive signals or by stochastic transitions to various epigenetic states. This form of phenotypic diversity is advantageous for adaptation and survival in changing environments, as well. For example, random variations in surface antigens increase the chance of a microorganism to escape from the immune defense.

The first area of our research has focused on the understanding of epigenetic silencing. This is of paramount importance since cellular differentiation in higher eukaryotic organisms often employs silencing to package genes into the inactive heterochromatin (**Fig. 1**). The logic behind chromosomal epigenetic processes has been unclear. Our recent work unraveled spatial aspects in control of silencing in yeast cells (Kelemen *et al.* (2010) PLoS Biology). The corresponding reaction-diffusion model revealed that the same reaction mechanism that describes silencing can support both graded monostable and switch-like bistable gene expression, depending on whether recruited repressor proteins generate a single silencing gradient or two interacting gradients that flank a gene. Our experiments confirmed that chromosomal recruitment of activator and repressor proteins permits a plastic form of control; the stability of gene expression is determined by the spatial distribution of silencing nucleation sites along the chromosome. Our findings in yeast are expected to stimulate further studies to reveal the logic of chromosomal epigenetic regulation in higher eukaryotic organisms and we are starting a research project in this direction.

The second area of our research has focused on the functioning of feedback loops that arise from evolutionary gene duplication (Hsu *et al.* (2012) Nature Communications). During evolution, genetic networks are rewired through strengthening or weakening their interactions to develop new regulatory schemes. In the galactose network, the GAL1/GAL3 paralogues and the GAL2 gene enhance their own expression mediated by the Gal4p transcriptional activator. The wiring strength in these feedback loops is set by the number of Gal4p binding sites. Here we show using synthetic circuits that multiplying the binding sites increases the expression of a gene under the direct control of an activator, but this enhancement is not fed back in the circuit. The feedback loops are rather activated by genes that have frequent stochastic bursts and fast RNA decay rates (**Fig. 2**).

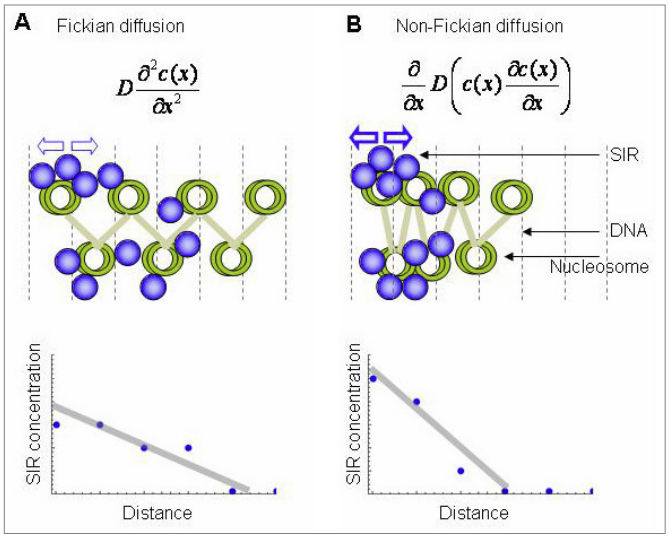

Fig. 1: Diffusion of silencing proteins (SIR) along the chromatin (Transcription, 2011).

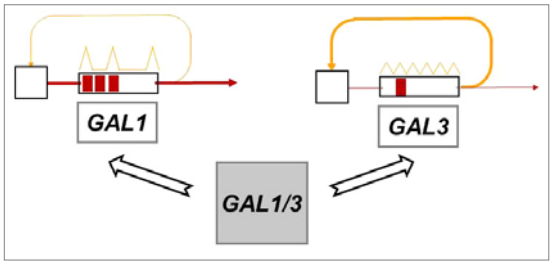

Fig. 2: The number of activator binding sites determines the strength of direct response of the gene (red arrows), while stochastic gene expression determines the activation of feedback response (orange arrows).

In this way, rapid adaptation to galactose can be triggered even by weakly expressed genes. Our results indicate that nonlinear stochastic transcriptional responses enable feedback loops to function autonomously, or contrary to what is dictated by the strength of interactions enclosing the circuit.

Since the decay rate of RNAs is fast, measurements of gene expression in the high-frequency domain is necessary. Therefore, we have started measuring stochastic gene expression at the level of single molecule RNAs (**Fig. 3**).

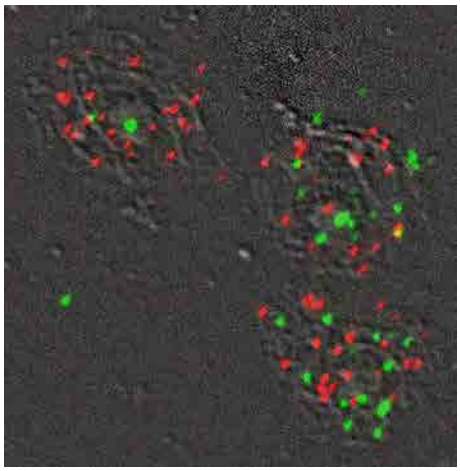

Fig. 3: Single molecule detection of mRNAs by FISH. GAL3 mRNA (red), ILS1 mRNA (green).

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Research Group Petr Broz

Host defense mechanisms against bacterial infections

The aim of our research is to understand how host cells recognize the presence of bacterial pathogens and how they eliminate this threat. We focus on the initial contact between host and pathogen, during which host defense mainly relies on the innate immune system. An important component of innate immunity are the so-called pattern recognition receptors (PRR), which detect pathogen-derived molecules known as pathogen-associated molecular patterns (PAMPs) or host-derived danger signals (DAMPs) in the extracellular or intracellular space of host cells. The best studied of the PRRs are the membrane-associated Toll-like receptors (TLRs) and the cytoplasmic RIG-I-like and NOD-like receptors (RLRs, NLRs) Upon binding of their ligands these receptors initiate a number of signaling pathways that activate anti-microbial mechanisms and initiate the recruitment of other immune cells by the secretion of inflammatory cytokines and chemokines. Altogether these responses serve to rapidly eliminate invading pathogens and to restore tissue homeostasis.

The major area of research in the lab is the study of inflammasome complexes. Inflammasomes are multi-protein complexes that are assembled in the cytoplasm by activated PRRs of the NLR and PYHIN protein families in response to inflammatory and noxious stimuli. These complexes serve as activation platforms for inflammatory caspases (caspase-1, -11 and -12 in mice) and are classified into canonical and non-canonical inflammasomes, depending on their composition and their biological effects. Besides their role in infections, unregulated inflammasome activity plays a major role in autoinflammatory diseases and during sterile inflammation (gout, atherosclerosis, type 2 diabetes).

Assembly and regulation of canonical inflammasomes

Canonical inflammasomes are the best described group of complexes and are assembled by NLRs and PYHIN receptors. In their basic form they consist of an activated receptor, an adaptor protein called ASC and pro-caspase-1, which gets activated by dimerization and autoproteolysis in the complex. Active caspase-1 directs the processing of certain cytokines (interleukins-1 and -18) and the induction of a specialized form of rapid cell death, called pyroptosis. Several different inflammasomes are known and are named after the receptor that initiates complex formation. These receptors recognize a wide variety of PAMPs and DAMPS, such flagellin and components of bacterial type 3 secretion systems (NLRC4/NAIP inflammasome), cytoplasmic DNA (AIM2 inflammasome) as well as membrane damage, changes in ion levels and ROS production (NLRP3 inflammasome) (**Fig. 1**). An essential component of all

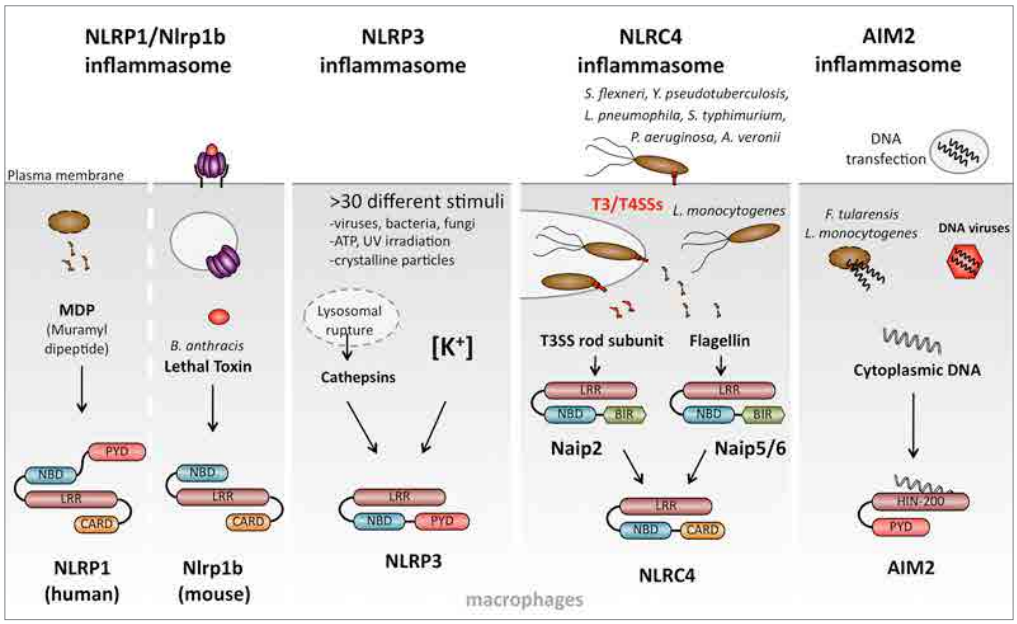

Fig. 1: Ligands and receptors of canonical inflammasomes.

canonical inflammasomes is the adaptor ASC, which has the ability to rapidly oligomerize into one single, macromolecular complex called the ASC speck. We investigate how these complexes assemble and how inflammasomes direct host immune defenses by combining cell biology, biochemistry and real-time imaging.

Non-canonical inflammasome signaling

Recently a new inflammasome pathway was discovered which resulted in the activation of caspase-11, thus referred to as the non-canonical pathway. This inflammasome is specifically activated during infections with Gram-negative bacteria but not with Gram-positive bacteria. In addition, the non-canonical caspase-11 inflammasome was linked to mortality in models of Gram-negative sepsis, highlighting the importance of this pathway. Consistently, Lipopolysaccharide (LPS) was recognized to be a ligand that results in caspase-11 activation, but other components of this pathway still remain uncharacterized. Here, we are studying host signaling pathways that determine the activation of this pathway during infections with the model pathogen *Salmonella typhimurium* and we work on characterizing and identifying components of the non-canonical inflammasome.

Links between the interferon-response, cell-autonomous immunity and inflammasomes

Since inflammasomes are important mediators of inflammation, assembly of these complexes is very tightly regulated and often requires previous induction of other signaling pathways. We have recently found that activation of caspase-11 in response to Gram-negative bacterial infections requires the production of type-I-interferons, a class of cytokines that regulates the induction of several thousands of genes involved in various aspects of host defense. In particular interferons induce a number of genes involved in cell-autonomous immunity, i.e. processes that allow cells to fight and eliminate pathogens on a single cell level. Among the most highly induced are several families of interferon-induced GTPases, which have been shown to be required to control intracellular killing bacterial and protozoan pathogens. How these GTPases attack and kill intracellular bacteria (**Fig. 2**) and how this is linked to the induction of inflammasome signaling is one of the research interests of our lab.

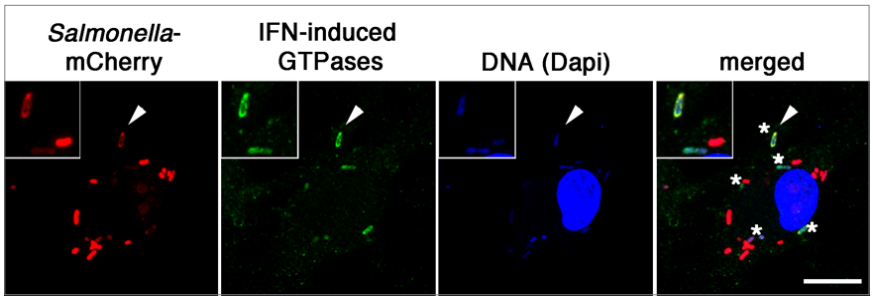

Fig. 2: Interferon-induced GTPases (green) attack intracellular *Salmonella* (mCherry, red). Inset shows *Salmonella* that have been killed and begin to loose mCherry expression. Scale bars 10 µm.

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Systems biology of *Salmonella* and *Shigella* infection

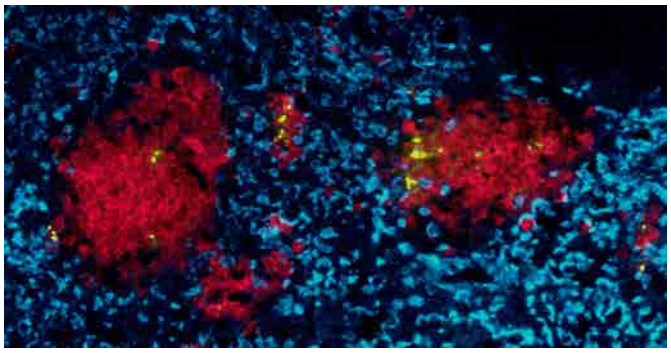

Fig. 1: *Salmonella*-infected mouse spleen (yellow, *Salmonella*; red, neutrophils; blue, red blood cells).

Infectious diseases represent a major worldwide threat to human health. Novel strategies to combat infectious disease are urgently needed because of rising resistance of pathogens to antimicrobial therapy, an increasing number of immunosuppressed patients that are highly susceptible to infection, increasing travel which enhances transmission and worldwide spread of novel and re-emerging pathogens, and potential bioterrorism threats.

The substantial progress in infection biology research in the last two decades could provide a basis for novel control strategies. However, it has remained difficult to translate this extensive knowledge into effective new control strategies. One potential reason why it is so difficult to translate basic research to effective strategies for combating infectious diseases, could be the prevailing focus on the action of individual pathogen or host components. While this reductionist approach was highly successful to identify and characterize key virulence and immune factors, it can not explain the course of complex multifactorial infectious diseases involving hundreds of interacting pathogen and host factors. Our goal is therefore to integrate the vast existing knowledge and to develop appropriate methodology to analyze interacting host/pathogen networks using FACS sorting of pathogens from infected host cells and tissues, quantitative proteomics, metabolomics, molecular genetics, animal infection models, and in silico modeling.

For our research we use *Salmonella* as well as *Shigella* as model pathogens. Both pathogens cause diarrhea and *Salmonella* can also cause typhoid fever and nontyphoidal *Salmonella* (NTS) bacteremia, which together cause over a million deaths each year. In addition to their importance as human pathogens, *Salmonella* and *Shigella* are among the best-studied model pathogens.

Metabolism

A large number of *Salmonella* proteins with detectable expression during infection have metabolic functions. Many of these enzymes could represent promising targets for antimicrobial chemotherapy. However, we have previously shown that actually only a very small minority of enzymes is sufficiently relevant for *Salmonella* virulence to qualify as a potential target. To understand the differential relevance of metabolic enzymes we systematically characterize the entire *Salmonella* metabolic network during infection by integrating large-scale data on *in vivo* nutrient availability and enzyme abundance with a genome-scale in silico model that provides a consistent largescale description of *Salmonella* metabolism during infection. The results revealed a surprisingly large diversity of host nutrients. However each of these nutrients was available in only minute amounts. This paradoxical situation ("starving in paradise") has two major consequences, i) broad nutrient supplementation buffers many *Salmonella* metabolic defects thus limiting opportunites for antimicrobials, ii) *Salmonella* growth in infected mice is rather slow and nutrient-limited. Both findings reiterate the major importance of metabolism for infectious disease outcome.

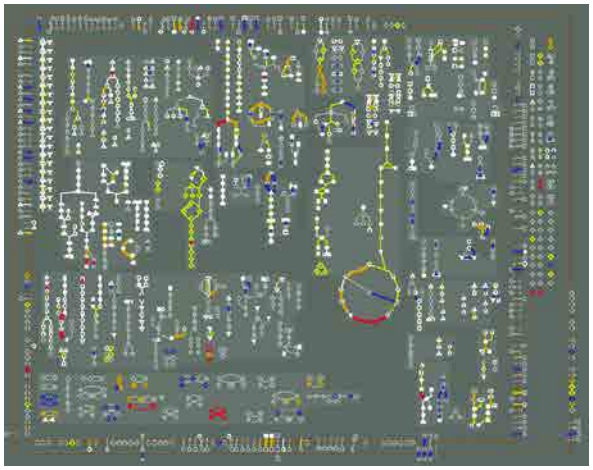

Fig. 2: Schematic overview of *Salmonella* metabolism during infection. Enzymes with detectable *in vivo* expression (yellow) and enzymes with experimentally determined relevance for virulence (red, essential; orange, contributing; blue, dispensable) are shown.

Within the framework of the SystemsX.ch RTD project *BattleX* (coordinator: Dirk Bumann) we have recently started to analyze pathogen and host metabolism in *Shigella* infections together with five collaborating groups across Switzerland. Initial results suggest that *Shigella* (like *Salmonella*) has access to diverse host nutrients. However, in this case excess nutrient quantities that support very fast pathogen growth seem to be available. These differences likely reflect differential localization of *Salmonella* in a membrane-delimited vacuole vs. *Shigella* freely residing in the host cell cytosol with unrestricted access to cytosolic metabolites. Metabolomics data suggest that *Shigella* infection causes major rearrangements of metabolic fluxes in the host cells. We currently explore such host cell activities as alternative targets for controlling infection.

Analysis of pathogen subpopulations

Salmonella reside in several distinct host microenvironments within the same infected tissue. These microenvironments differ in density of host defense cell types such as neutrophils and inflammatory macrophages and likely provide substantially different conditions for *Salmonella*. We are developing a set of complementary tools to isolate distinct *Salmonella* subpopulations from various microenvironments for systemlevel analysis. Current results suggest dramatic differences in stress exposure and growth rate in *Salmonella* subpopulations.

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Molecular and cellular mechanism of asymmetric stem cell division

Asymmetric cell division generates cellular diversity. Cell polarity, spindle orientation and cleavage furrow positioning are cellular mechanisms enabling cells to divide in a molecular and physical asymmetric manner. Stem cells in particular divide asymmetrically in order to self-renew the stem cell yet generate differentiating siblings. Many diseases such as breast cancer susceptibility, acute promyelocytic leukemia, the initiation of colon cancer but also the neurodevelopmental disorders lissencephaly or microcephaly are due to defective asymmetric stem cell division. Thus, understanding the cellular and molecular mechanisms of asymmetric cell division is important to increase our knowledge of basic stem cell biology.

Fig. 1: Asymmetric cell division generates molecularly and physically distinct siblings.

We are using *Drosophila melanogaster* neuroblasts, the precursors of the fly's central nervous system, to study the mechanism of (1) centrosome asymmetry and (2) cleavage furrow positioning during asymmetric cell division. Neuroblasts are polarized cells and divide in a stem cell-like fashion, undergoing repeated self-renewing asymmetric divisions (**Fig. 1**). The mitotic spindle invariably orients itself along the neuroblast intrinsic apical-basal polarity axis and asymmetric cleavage furrow positioning results in a physical and molecular asymmetric cell division, generating a large self-renewed apical neuroblast and a smaller differentiating basal ganglion mother cell (GMC). *Drosophila* neuroblasts provide an ideal experimental system because precise genetic manipulations are possible and superb imaging properties are available.

Mechanism and function of centrosome asymmetry during asymmetric cell division

Most metazoan cells utilize a pair of centrosomes to form a bipolar spindle. Centrosomes are microtubule organizing centers (MTOCs), consisting of a pair of centrioles, surrounded by pericentriolar matrix (PCM). Centrosomes are inherently asymmetric; centrioles duplicate in a semi-conservative manner – one centriole serves as a template for the formation of a new centriole – generating an older mother and a younger daughter centriole. Furthermore, molecular markers have been identified, specifically labeling the mother or daughter centrosome, respectively.

Fig. 2: Neuroblast centrosomes are intrinsically asymmetric.

We are using *Drosophila* neuroblasts to study the molecular mechanisms and function of centrosome asymmetry. Neuroblast centrosomes undergo an elaborate dematuration and rematuration cycle in which the older mother centriole sheds PCM right after centrosome separation and, as a consequence, loses MTOC activity and thus its position on the apical cortex. This old centriole remains PCMfree during interphase, only regaining PCM and MTOC activity from prophase onwards. The daughter centriole, however, remains stationed in the apical half of the neuroblast because it retains PCM and MTOC activity throughout interphase (**Fig. 2**). We have shown that the conserved centriolar protein Bld10 (Cep135 in vertebrates) is required to establish centrosome asymmetry in *Drosophila* neuroblasts. Failure to establish centrosome asymmetry results in centrosome mispositioning, spindle orientation defects and centrosome missegregation. We are using forward genetics and advanced imaging techniques such as in *vivo* live cell imaging and superresolution to study centrosome asymmetry during asymmetric cell division. Since mutations in Cep135 cause microcephaly, a rare neurodevelopmental disorder manifested in small but structurally normal brains, we are interested in investigating whether centrosome asymmetry is required for normal brain development.

Cleavage furrow positioning and sibling cell size asymmetry

Asymmetric cell division can result in the formation of molecularly and physically distinct siblings. We are using *Drosophila* neuroblasts to investigate how cell size differences are generated. In particular, we are focusing on the cellular and molecular mechanism of cleavage furrow positioning. Until recently, it was widely believed that cleavage furrow positioning is solely dependent on cues delivered by the mitotic spindle. However, new results suggest that two cues are used for the correct positioning of the contractile ring: (1) a microtubule-dependent cue and (2) a polarity derived signal. The novel polarity-dependent cleavage furrow positioning pathway is utilizing the two conserved polarity components Partner of Inscuteable (Pins; LGN/AGS3 in vertebrates) and Discs large (Dlg)

(Fig. 3). We are investigating how cellular polarity is translated into asymmetric Myosin localization and ultimately, asymmetric cleavage furrow positioning. To this end we started to characterize and measure Myosin dynamics using photoconversion and FRAP. We further use forward and reverse genetics, live cell imaging and biochemistry in order to identify the molecular mechanisms underlying cleavage furrow positioning in *Drosophila* neuroblasts. We are also testing the idea whether other polarized cell types utilize the "polarity-dependent" pathway to position the cleavage furrow.

The mechanics of asymmetric cell division

To better understand how sibling cell size asymmetry is generated, we started to measure physical forces during asymmetric cell division. In collaboration with the lab of Daniel Mueller (D-BSSE; ETH Zurich), we are performing stiffness measurements using Atomic Force Microscopy (AFM) (Fig. 4). We also developed methods and tools to measure pressure and cytoplasmic streaming during ACD. These methods, in combination with novel live cell imaging approaches and mathematical modeling, will enable us to gain insights into the mechanics of asymmetric cell division.

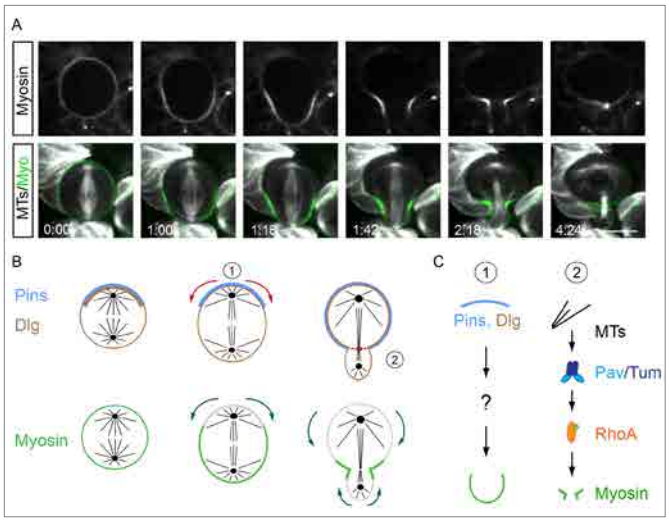

Fig. 3: Two pathways provide positional information for the placement of the cleavage furrow.

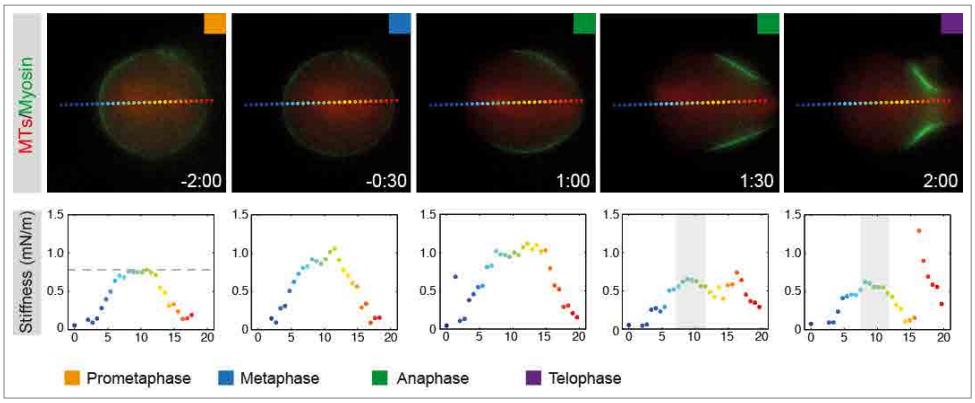

Fig. 4: Stiffness measurements during ACD.

Publications 2015

Chen, Jieyan V; Kao, Ling-Rong; Jana, Swadhin C; Sivan-Loukianova, Elena; Mendonça, Susana; Cabrera, Oscar A; Singh, Priyanka; Cabernard, Clemens; Eberl, Daniel F; Bettencourt-Dias, Monica; Megraw, Timothy L (2015). Rootletin organizes the ciliary rootlet to achieve neuron sensory function in *Drosophila*. *The Journal of Cell Biology, 211*(2), 435-53.

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Role of type IV secretion systems in the establishment of persistent bacterial infections

The aim of our studies is to gain a molecular understanding of the function of type IV secretion (T4S) systems in establishing bacterial persistence in the host. T4S systems are ancestrally related to bacterial conjugation systems that mediate interbacterial DNA transfer. Bacterial pathogens targeting eukaryotic host cells have adopted these supramolecular protein assemblies for the intracellular delivery of virulence factors from the bacterial cytoplasm directly into the host cell cytoplasm. Our longstanding research on the vascular tumor-inducing pathogens of the genus *Bartonella* revealed crucial roles of two distinct T4S systems, VirB and Trw, in the ability of these bacteria to colonize, invade and persist within vascular endothelial cells and erythrocytes, respectively (**Fig. 1**, reviewed in Dehio, 2008, Cell. Microbiol.; and Harms and Dehio, 2012, Clin. Microbiol. Rev.). More recently, we have initiated a new project to study the role of the T4S system VirB in intracellular persistence of the closely related bacterial pathogens of the genus *Brucella* that represent the etiological agents of brucellosis – the worldwide most important bacterial zoonosis. We are using a multi-disciplinary research approach including genetics, genomics, biochemistry, structural biology, cell biology, animal experimentation and bioinformatics in order to reveal the cellular, molecular and evolutionary basis of T4S in bacterial persistence of *Bartonella* and *Brucella*. Moreover, we employ a systems biology approach to reveal the host cell signaling network underlying cell entry and intracellular persistence of these pathogens in order to define novel targets for the development of innovative anti-infectiva.

T4S systems play diverse roles in *Bartonella*-host interaction: They are essential for establishing persistent infection and contribute to host adaptability

A functional and comparative genomics approach allowed us to demonstrate that both the VirB and Trw T4S systems of *Bartonella* represent essential virulence factors for establishing persistent infection in mammals. Further, these virulence devices must have played major roles during evolution in facilitating adaptation of these pathogens to their specific mammalian reservoirs (Saenz *et al.*, 2007, Nat. Genet.; Engel *et al.*, 2011, PLoS Genetics). Genetic and cell biological analysis of Trw has shown that this T4S system mediates the host-restricted adhesion to erythrocytes (Vayssier *et al.*, 2010). Important to note, during adoption of this dedicated role in host interaction this T4S system has lost its ancestral substrate transfer capability. In contrast, we have recently shown that the VirB T4S is capable of translocating DNA into endothelial target host cells in a process similar to the interbacterial DNA transfer mediated by the ancestral conjugation systems

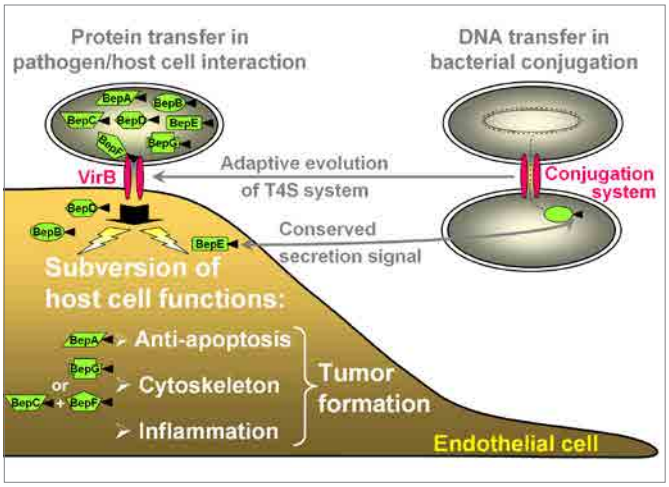

Fig. 1: Effector proteins translocated by the *Bartonella* T4S systemVirB/VirD4 subvert human endothelial cell functions.

(Schroeder *et al.*, 2011, PNAS; reviewed in Llosa *et al.*, 2012, Trends Microbiol.). However, the physiological role of the VirB T4S system is to translocate a cocktail of *Bartonella* effector proteins (Beps) into vascular endothelial cells that subvert cellular functions to the benefit of the pathogen (Schulein *et al.*, 2005, PNAS). A recent evolutionary genomics study allowed us to propose that the horizontally acquired VirB T4S system and its translocated Bep effectors facilitated adaptations to novel hosts via two parallel adaptive radiations (Engel *et al.*, 2011, PLoS Genet.). We showed that the functional versatility and adaptive potential of the VirB T4S system evolved convergently – prior to the radiations – by consecutive rounds of lineage-specific gene duplication events followed by functional diversification. This resulted in two diverse arrays of Bep effector proteins in the two radiating lineages of *Bartonella*. Together, we established *Bartonella* as a bacterial paradigm of adaptive radiation, allowing for the first time to study the molecular and evolutionary basis of this fundamental evolutionary process for the generation of organismic diversity in bacteria.

Structure/function analysis of VirB-translocated Bep effector proteins of *Bartonella*

The cocktail of Bep effectors translocated by the VirB T4S system into vascular endothelial cells mediates multiple cellular effects, including anti-apoptosis, internalization of bacterial aggregates via the F-actindependent invasome structure and proinflammatory activation (Schulein *et al.*, 2005, PNAS). Defining the cellular targets and molecular mechanisms of how these Beps interfere with eukaryotic signaling processes have become a focus of our recent studies. The C-terminal parts of the Beps carry the Bep intracellular delivery (BID) domain that serves as T4S signal, but has in several instances adopted additional effector function within host cells. A prominent example is the BID domain of BepA that binds adenylate cyclase to potentiate $G\alpha_s$-dependent cAMP production, which leads to inhibition of apoptosis in vascular endothelial cells (Pulliainen *et al.*, 2012, PNAS). The N-terminal parts of the Beps carry diverse domains or peptide motifs considered to mediate effector functions within host cells. For instance, upon translocation the effectors BepD, BepE and BepF become tyrosinephosphorylated on short N-terminal repeat motifs, thereby interfering with eukaryotic signal transduction processes (Selbach *et al.*, 2009, Cell Host & Microbe). Together with the Schirmer group (Biozentrum) we study the structure/function relationship of the Fic domains that are present in the N-terminus of multiple Beps and mediate posttranslational modifications of specific host target proteins via covalent transfer of AMP (AMPylation) (Palanivelu *et al.*, 2011, Protein Sci.). A particular focus of these studies is the identification of target proteins and the regulation of the AMPylation activity, i.e. via binding of the Fic domain to an inhibitory protein termed antitoxin (Engel *et al.*, 2012, Nature).

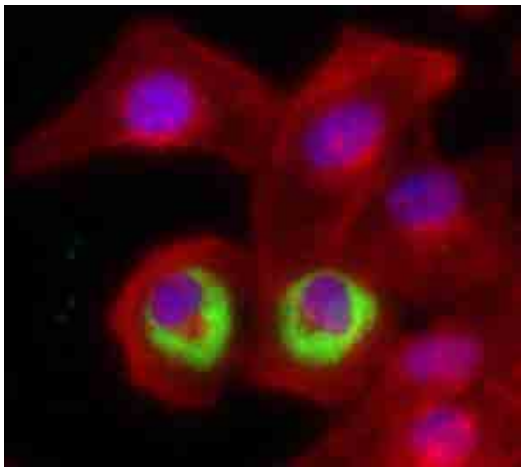

Fig. 2: Hela cells infected with GFP-expressing *Brucella abortus* (green) for 48 h and stained for F-actin (red) and DNA (blue). Intracellular bacteria replicate in an endoplasic reticulum-associated compartment localizing proximal to the nucleus.

A systems biology approach to *Bartonella* and *Brucella* entry and intracellular persistence in human cells

The goal of InfectX (www.infectx.ch) – a research and development project (RTD) of the Swiss-wide systems biology initiative SystemsX.ch – is to comprehensively identify components of the human infectome for a set of important bacterial and viral pathogens and to develop new mathematical and computational methods with predictive power to reconstruct key signaling pathways controlling pathogen entry into human cells. In the frame of InfectX we use a systems biology approach to reconstruct the host signaling processes underlying *Bartonella* and *Brucella* entry into the human model cell line HeLa that lead to the establishment of a persisting intracellular infection. For *Bartonella henselae*, the VirB T4S effector BepG or the combined activity of the effectors BepC/BepF was found to inhibit endocytic uptake of individual bacteria, thereby redirecting bacterial uptake to the invasome-mediated pathway facilitating the uptake of large bacterial aggregates (reviewed in Eicher and Dehio, 2012, Cell. Microbiol.). This unique cell entry process is mediated by massive F-actin rearrangements that depend on the small GTPases Rac1, the Rac1-effector Scar1, and the F-actin organizing complex Arp2/3 (Rhomberg *et al.*, 2009, Cell Microbiol.; Truttmann *et al.*, 2011, Cell Microbiol.) and bi-directional signaling via the integrin pathway (Truttmann *et al.*, 2011, J. Cell Sci.). The uptake process triggered by *Brucella abortus* is less well defined but considered to depend on lipid rafts and the small GTPase Cdc42. Genome-wide RNA interference screens and related modeling approaches currently performed on the basis of high-content fluorescence microscopy assays for pathogen entry and intracellular replication should facilitate the comprehensive identification of the human infectomes involved in establishing persistent intracellular infection of these pathogens as a first step towards the identification of human targets suitable for the development of a new class of anti-infectives that interfere with the function of host proteins essential for pathogen infection.

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Publications 2015

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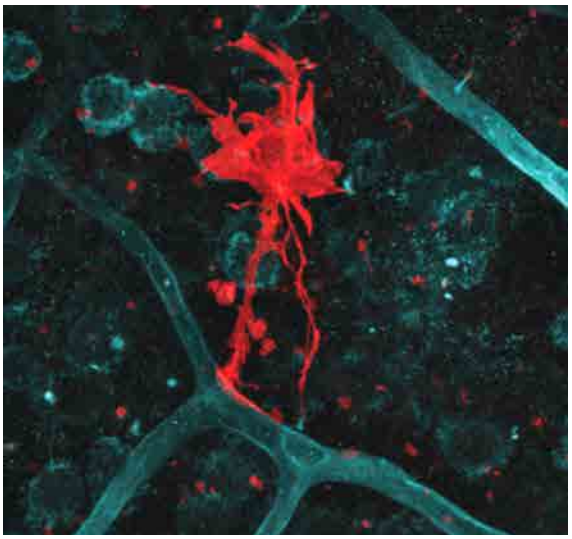
Research Group Fiona Doetsch

Stem cells and their niche in the adult mammalian brain

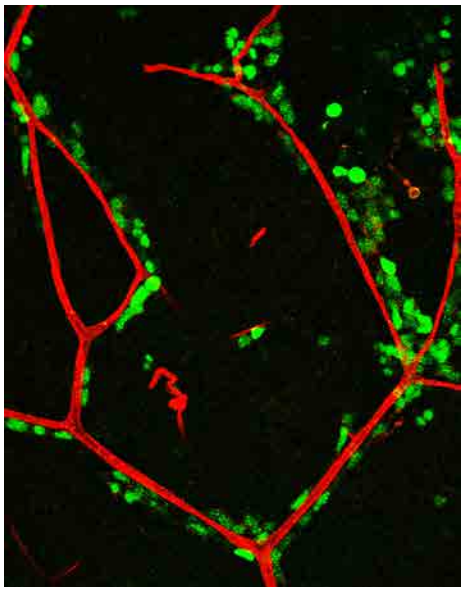
Neural stem cells continuously generate new neurons in restricted areas of the adult mammalian brain. Adult stem cells reside in specialized niches that support their lifelong capacity for self-renewal and differentiation. Intriguingly, adult neural stem cells exhibit features of glial cells, raising the possibility that glial cells elsewhere in the brain may have latent stem cell potential. The ventricular-subventricular zone (V-SVZ), adjacent to the lateral ventricles, is the largest germinal niche in the adult brain and continuously gives rise to neurons that migrate to the olfactory bulb. We are investigating the intrinsic and extrinsic (niche) signals that regulate adult V-SVZ neural stem cells under homeostasis and during regeneration.

Purification and regulation of adult neural stem cells and their lineage

A key limitation in the adult neural stem cell field has been the identification of markers that allow the *in vivo* identification and isolation of adult neural stem cells. We have recently developed a simple strategy that allows the purification of both quiescent (dormant) and activated (dividing) adult neural stem cells as well as their progeny using fluorescence activated cell sorting (FACS) (Pastrana *et al.* 2009, Codega *et al.* 2014). Quiescent and activated stem cells isolated directly from their *in vivo* niche exhibit dramatically different cell cycle, molecular, and functional properties and have unique transcriptional signatures. This FACS purification strategy provides a powerful approach to investigate the biology of adult neural stem cells. In ongoing work we are defining the heterogeneity and potential of purified populations, as well as the gene regulatory networks underlying adult neural stem cell quiescence and activation.



Quiescent neural stem cells in the adult brain have a radial morphology (red) and frequently send a long basal process that terminates on blood vessels (blue).



Dividing cells (green) are localized close to blood vessels (red) in the adult V-SVZ neural stem cell niche.

Niche regulation of adult neural stem cells

We are using a combination of novel anatomical and *in vitro* approaches to elucidate the diverse niche components that mediate adult neural stem cell behavior.

Vascular niche

The vasculature is an important component of the V-SVZ neural stem cell niche, and has unique properties (Tavazoie *et al.*, 2008). Stem cells directly contact blood vessels at specialized sites that lack astrocyte endfeet. An open question is whether vascular cells in neurogenic areas are intrinsically different from those elsewhere in the brain. We have optimized a rapid FACS purification strategy to simultaneously isolate primary endothelial cells and pericytes from brain micro-regions of non-transgenic mice. This purification strategy provides a platform to define the functional and molecular contribution of vascular cells to stem cell niches and other brain regions under different physiological and pathological states. We are comparing the effect of purified vascular cells from neurogenic and non-neurogenic (cortex) brain regions on cells isolated from each stage of the V-SVZ stem cell lineage. We are also defining endothelial and pericyte-derived factors that influence V-SVZ cells.

Cerebrospinal fluid niche

Adult neural stem cells contact the lateral ventricle via a thin process, and are continuously bathed by the cerebrospinal fluid (CSF). However little is known about the effect of the CSF compartment on adult neural stem cell function. We have found that the adult CSF compartment is a potent regulator of adult neural stem cells and their progeny and that its composition and functional effects change dynamically in different physiological states, including aging. CSF factors support the formation of multipotent selfrenewing colonies from both activated stem cells and transit amplifying cells, as well as modulate survival of neural stem cells. Notably, we have also found that the composition and functional effect of CSF changes during aging, with differential effects on distinct stages of the stem cell lineage. We are currently examining the identity of factors in the CSF that regulate adult neural stem cells and their progeny.

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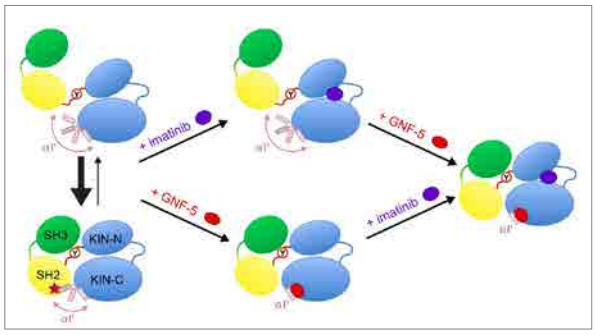
Research Group Stephan Grzesiek

Nuclear magnetic resonance spectroscopy of biomolecules

We apply and develop high-resolution Nuclear Magnetic Resonance (NMR) methods to elucidate structure, function, and dynamics of biological macromolecules.

The structural and functional projects currently encompass Abelson kinase, a prime drug target in the treatment of chronic myelogeneous leukemia; the interactions of the G-protein-coupled receptor CCR5, which is also the HIV1-coreceptor, with its chemokine ligand RANTES; the β1-adrenergic receptor, bacterial PilZ domains, which are targets for signaling via cyclic di-GMP; lipopolysaccharide, the causative agent of endotoxic shock; the TipA multidrug resistance protein of *Streptomyces lividans*; and an atomic-detail description of the unfolded states of proteins and the protein folding transitions by new NMR methods.



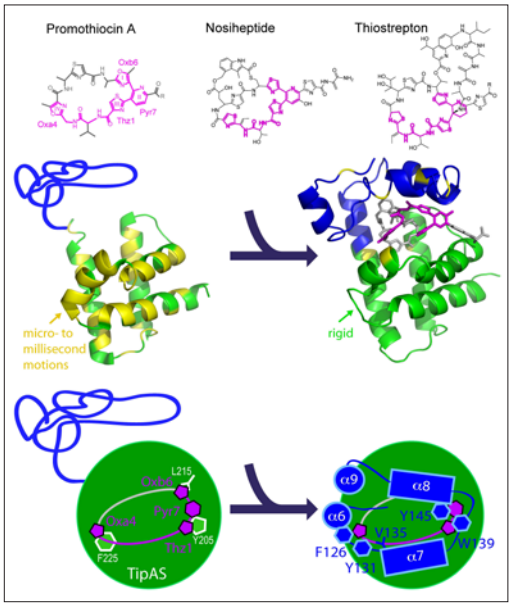
The NMR analysis of Abl, the key protein responsible for the development of chronic myelogeneous leukemia, reveals a delicate equilibrium between open and closed protein conformations in response to binding of ATP-site and allosteric inhibitors.

Abelson (Abl) kinase

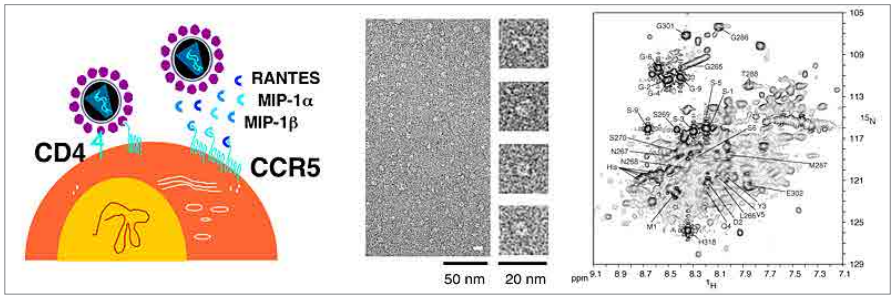
Abelson (Abl) kinase is a drug target in the treatment of chronic myelogenous leukemia (CML), against which clinically highly efficacious ATP-competitive inhibitors (imatinib and others) have been developed. However, spontaneous mutations in advanced-stage patients render these inhibitors inefficient. Recently, a new type of allosteric inhibitors was shown in preclinical studies to overcome the resistance against ATP-binding pocket inhibitors (Zhang *et al.*, Nature, 463, 501-506, (2010)). The exact mechanism of the allosteric inhibition is currently unclear. In collaboration with Novartis (Basel) we had previously determined the unknown solution conformations of the Abl kinase domain alone (Vajpai *et al.*, JBC, 283, 18292-18302 (2008)). We have now also determined the solution conformations of a much larger 52-kDa SH3/SH2/kinase domain construct under the influence of various inhibitors by solution NMR and SAXS (Skora *et al.*, Proc Natl Acad Sci USA 110, E4437-45 (2013)). The addition of imatinib induces a large structural rearrangement characterized by the detachment of the SH3-SH2 domains from the kinase domain and the formation of an "open" inactive state, which is inhibited in the ATP site. In contrast to imatinib, binding of the allosteric inhibitor GNF-5 keeps the protein in the "closed" state. Combination of imatinib with GNF-5 brings the conformation again to a "closed" state. These findings on the allosteric actions of the two classes of inhibitors reveal molecular details of their recently reported synergy to overcome drug resistance.

Multidrug recognition

Thiostrepton-induced protein A (TipA) is a minimal bacterial, multidrug resistance (MDR) protein against very diverse antibiotics of the thiostrepton class. We have determined the solution structures and dynamics of the antibiotic binding domain of TipA in complexes with several antibiotics (Habazettl *et al.*, PNAS, Dec. 8, 2014). The data give unique insights into multidrug recognition by a complete MDR system: (i) a four-ring motif present in all known TipA-inducing antibiotics is recognized specifically by conserved TipA amino acids and induces a large transition from a partially unfolded to a globin-like structure. (ii) The variable part of the antibiotic is



Mechanism of promiscuous antibiotic recognition by the antibiotic binding domain of TipA.

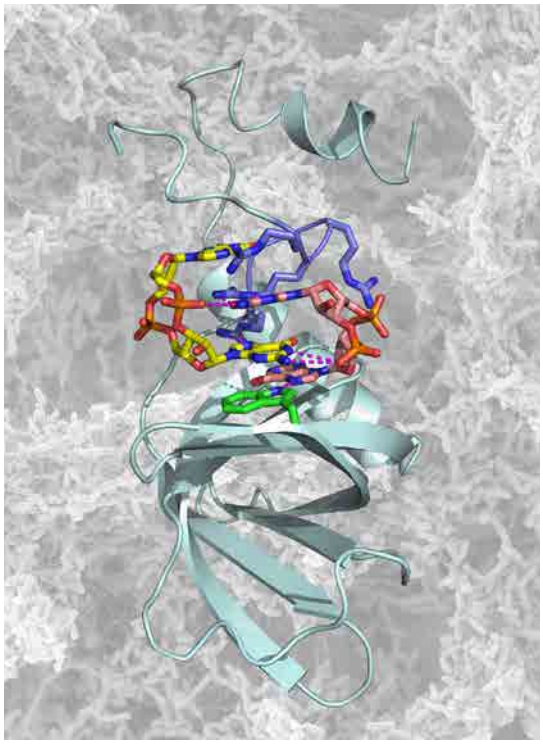


Left: docking of HIV-1 to the receptors CD4 and CCR5 on T-cell surface. Middle: electron micrograph showing detergent micelles containing CCR5. Right: 1H-15N spectrum of CCR5 in detergent micelles.

accommodated within a flexible cleft that rigidifies upon drug binding. Remarkably, the identified recognition motif is also the major interacting part of the antibiotic with the ribosome. Hence the TipA multidrug resistance mechanism is directed against the same chemical motif that inhibits protein synthesis. This makes the multidrug recognition mechanism by TipA a useful model for ribosomal thiopeptide binding and antibiotic development.

CCR5

The chemokine receptor CCR5 belongs to the class of G-protein coupled receptors (GPCRs). CCR5 is expressed on the surface of T-cells and activated after binding the endogenous chemokines MIP-1α, MIP-1β, and RANTES. CCR5 is the key molecule for HIV entrance into target cells, which proceeds via the sequential interaction of the viral protein gp120 with the host-cell factors CD4 and CCR5. Very promising HIV entry inhibitors are based on CCR5 ligands, comprising the natural ligand RANTES. In recent years, we have obtained structural information on CCR5 and its complex with RANTES by studying the interaction of the soluble protein RANTES with peptides derived from the extracellular surface of CCR5 (Duma *et al.* J Mol Biol 365, 1063-1075 (2007)). This information helped to devise more potent peptide-based HIV entry inhibitors (Chemistry & Biology 19, 1579-1588 (2012)). As part of these efforts, we have recently characterized the dynamics, oligomeric states and detergent interactions of 5P12-RANTES, an engineered RANTES variant that is currently in phase I clinical trials (Wiktor *et al.*,Biophys J, 105, 2586-97 (2013)). We have also developed methods to produce CCR5 in sufficient amounts for structural and biophysical studies (Nisius *et al.* Protein Expr Purif 61, 155-162 (2008); Van den Bergh *et al.* PLoS One. 2012;7:e35074; Wiktor *et al.* J. Biomol. NMR 55, 79–95 (2013)). The project is embedded into the EU-FP7 project CHAARM (Combined Highly Active Anti-Retroviral Microbicides), a collaborative effort to develop combinations of new and existing anti-HIV agents, which can be applied topically to reduce transmission of HIV.



NMR structure of the cyclic di-GMP receptor PA4608 from *Pseudomonas aeruginosa* in complex with its cyclic di-GMP ligand on the background of a typical bacterial biofilm.

c-di-GMP signaling

Cyclic di-guanosinemonophosphate (c-di-GMP) is a bacterial signaling molecule that triggers a switch from motile to sessile bacterial lifestyles. This mechanism is of considerable pharmaceutical interest, since it is related to bacterial virulence, biofilm formation and persistence of infection. Understanding this mechanism may offer new routes to treatment of bacterial infections. We have recently solved the structure of the PilZ homolog PA4608 in complex with cyclic di-GMP (Habazettl *et al.* J Biol Chem 286, 14304 (2011)). This complex shows large structural changes relative to the apo form. As a result of the rearrangements of N- and C-termini, a highly negative surface is created on one side of the protein complex. We propose that this movement of the termini and the resulting negative surface forms the basis for downstream signaling. We have recently also determined the exchange kinetics and equilibrium constants of various oligomeric forms of c-di-GMP (Gentner *et al.* J Am Chem Soc 134, 1019 (2012)). These projects are in collaboration with the groups of Prof. Urs Jenal and Prof. Tilman Schirmer (Biozentrum).

Lipopolysaccharide (LPS, endotoxin)

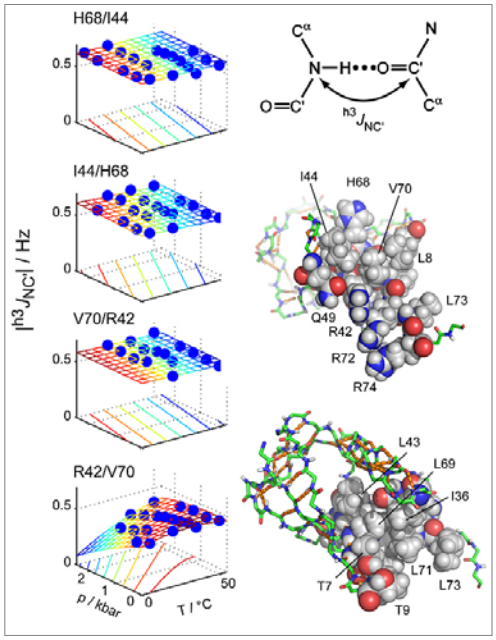
Lipopolysaccharide (LPS, endotoxin) is a major component of the outer membrane of Gram-negative bacteria, which makes it a prime target for recognition by the innate immune system. In small amounts, LPS provokes a beneficial immune response. However, in larger amounts LPS causes endotoxic shock, which is highly lethal due to the lack of effective therapeutic approaches. A detailed molecular description of the recognition events of LPS is of great medical interest and essential for the understanding of pro-inflammatory processes of the innate immune system. In collaboration with Prof. U. Zähringer (FZ Borstel, Germany) we have been able to make LPS amenable to analysis by solution NMR conditions that mimic the bacterial membrane and to determine a structure that comprises the motif responsible for the endotoxic reaction (Wang, *et al*. Angew Chem 47, 9870-9874 (2008)). Our approach presents a general methodology for the structural analysis of complex and heterogeneous LPS molecules. Current efforts are directed towards characterizing complexes of LPS with immune system receptors.

Towards an atom-scale description of order in unfolded proteins from new NMR parameters

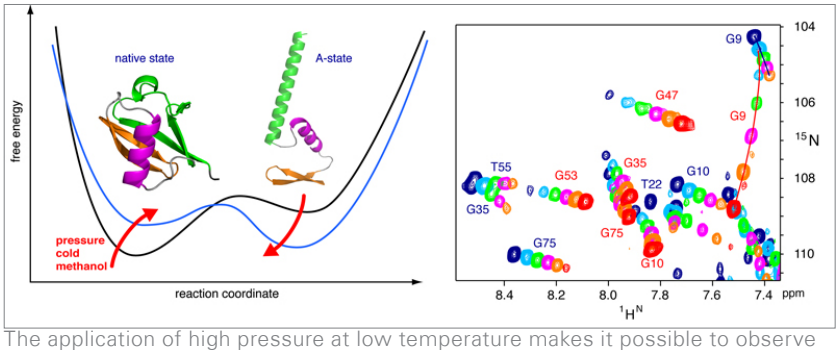
A detailed, quantitative description of the unfolded state ensemble of proteins is crucial for understanding protein folding, protein misfolding diseases such as Alzheimer's and Parkinson's, and function of intrinsically disordered proteins. The astronomical size of the conformational space of an unfolded polypeptide chain makes such a description both experimentally and theoretically very difficult. Using new NMR experimental parameters comprising residual dipolar couplings and paramagnetic relaxation enhancements, we have been able to obtain a highly detailed, quantitative description of unfolded polypeptides. The results show that unfolded states contain considerably more residual, native-like structure than previously anticipated, thereby resolving Levinthal's paradox that protein folding would need almost infinite times in an unbiased search of all accessible conformations.

Key stabilizing elements of protein structure identified through pressure and temperature perturbation of its hydrogen bond network

Hydrogen bonds are key constituents of biomolecular structures, and their response to external perturbations may reveal important insights about the most stable components of a structure. NMR spectroscopy can probe hydrogen bond deformations at very high resolution through hydrogen bond scalar couplings (HBCs). However, the small size of HBCs has so far prevented a comprehensive quantitative characterization of protein hydrogen bonds as a function of the basic thermodynamic parameters of pressure and temperature. Using a newly developed pressure cell, we have mapped pressure- and temperaturedependent changes of 31 hydrogen bonds in ubiquitin by measuring HBCs with very high precision (Nisius and Grzesiek Nat Chem 4, 711-717 (2012)). Short-range hydrogen bonds are only moderately perturbed, but many hydrogen bonds with large sequence separations (high contact order) show greater changes. In contrast, other high-contact-order hydrogen bonds remain virtually unaffected. The specific stabilization of such topologically important connections may present a general principle to achieve protein stability and to preserve structural integrity during protein function.



Highly precise measurements of h3JNC' scalar couplings across hydrogen bonds as a function of temperature and pressure show that the functionally important C-terminal part of ubiquitin is particularly stabilized against perturbations.



The application of high pressure at low temperature makes it possible to observe the cold-denatured state of ubiquitin by NMR at atomic resolution. This state contains native and non-native secondary structure elements that are every similar to the alcohol-denatured state. The transition can be studied in a continuous manner from the folded to the unfolded state.

High pressure NMR reveals close similarity between cold and alcohol protein denaturation

Proteins denature not only at high, but also at low temperature as well as high pressure. These denatured states are not easily accessible for experiment, since usually heat denaturation causes aggregation, whereas cold or pressure denaturation occur at temperatures well below the freezing point of water or pressures above 5 kbar, respectively. We have obtained atomic details of the pressureassisted, cold-denatured state of ubiquitin at 2500 bar and 258 K by high-resolution NMR techniques (Vajpai *et al*. Proc Natl Acad Sci USA 110, E368-76 (2013)). This state contains on the order of 20 % native-like and non-native secondary structure elements. These structural propensities are very similar to the previously described alcohol-denatured (A-) state. The close similarity of pressure-assisted, cold-denatured and alcohol-denatured state supports a hierarchical mechanism of folding and the notion that similar to alcohol, pressure and cold reduce the hydrophobic effect. Indeed, at non-denaturing concentrations of methanol, a complete transition from the native to the A-state can be achieved at ambient temperature by varying the pressure from 1 to 2500 bar. This method should allow highly detailed studies of protein folding transitions in a continuous and reversible manner.

Important Partners

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Research Group Michael N. Hall

TOR signaling and control of cell growth

Introduction

Cell growth is highly regulated. Cells respond to nutrients or other appropriate growth stimuli by up-regulating macromolecular synthesis and thereby increasing in size. Conversely, cells respond to nutrient limitation or other types of stress by downregulating macromolecular synthesis and enhancing turnover of excess mass. Thus, the control of cell growth involves balancing positive regulation of anabolic processes with negative regulation of catabolic processes. Growth is also controlled relative to cell division. In proliferating cells, growth is linked to the cell cycle such that most cells precisely double their mass before dividing. In other physiological contexts, such as load-induced muscle hypertrophy or growth factorinduced neuronal growth, cell growth is controlled independently of the cell cycle. Furthermore, in addition to the temporal control of cell growth described above, cell growth can be subject to spatial constraints. For example, budding yeast and neurons grow in a polarized manner as a result of new mass being laid down only at one end of the cell. Finally, in multicellular organisms, growth of individual cells is controlled relative to overall body growth such that the organs and tissues constituting the organism are properly proportioned.

The TOR signaling network

What are the mechanisms that mediate and integrate the many parameters of cell growth? In other words, what determines that a cell grows only at the right time and at the right place? Remarkably, the study of these mechanisms has been largely neglected, despite their clinical relevance and despite cell growth being, along with cell division and cell death, one

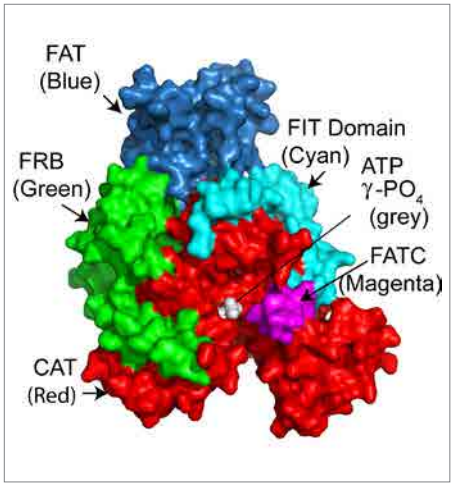

Fig. 1: Model of the catalytic region of human TOR.

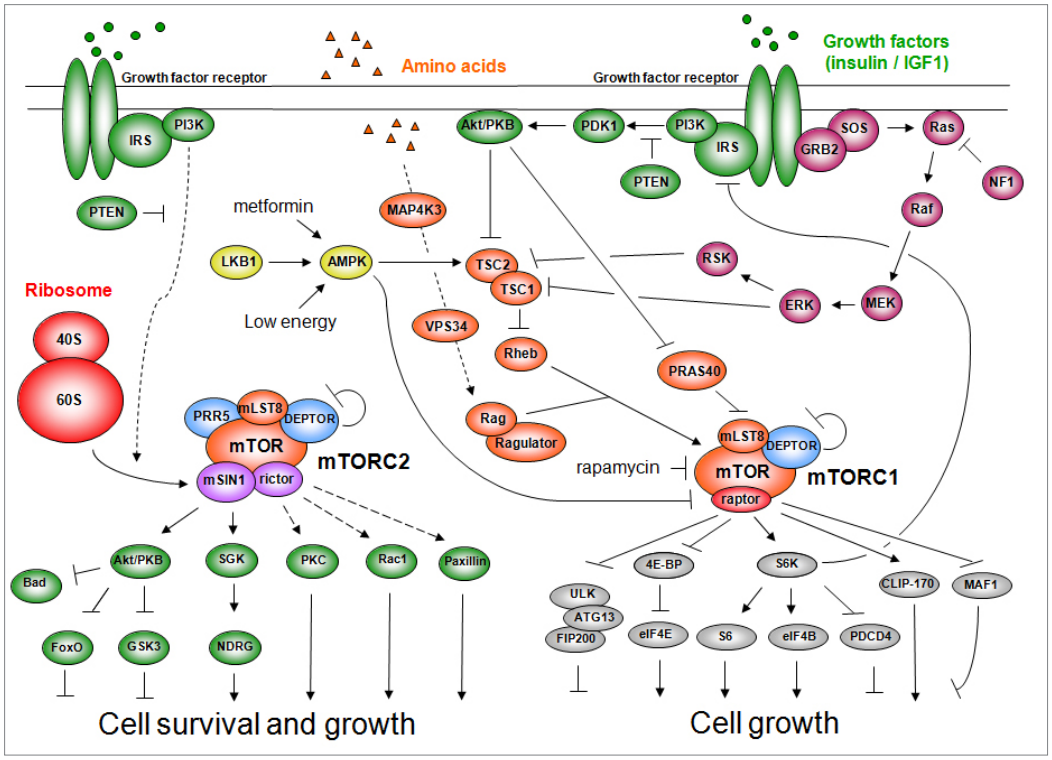

Fig. 2: The mTOR signaling network.

of the most fundamental (and obvious) features of life. Also remarkable is the finding that cell growth control, regardless of eukaryotic organism or physiological context, seems always to involve the protein kinase TOR (Target Of Rapamycin) and its signaling network. TOR has thus become known as a central controller of cell growth. Indeed, the discovery of TOR led to a fundamental change in how one thinks of cell growth. It is not a spontaneous process that just happens when building blocks (nutrients) are available, but rather a highly regulated, plastic process controlled by TOR-dependent signaling pathways. TOR, originally discovered in our laboratory, is structurally and functionally conserved from yeast to human (including worms, flies, and plants). TOR in mammals (mTOR) controls cell growth and metabolism in response to nutrients (e.g., amino acids), growth factors (e.g., insulin, IGF-1, PDGF), and cellular energy status (ATP). Nutrients are the dominant TOR input as high levels of amino acids can compensate for an absence of the other mTOR inputs but not vice versa, and only nutrients activate TOR in unicellular organisms. The growth factor signaling pathway, grafted onto the more ancestral nutrient sensitive TOR pathway, co-evolved with multicellularity. TOR activates cell growth by positively and negatively regulating several anabolic and catabolic process, respectively, that collectively determine mass accumulation and thus cell size. The anabolic processes include transcription, protein synthesis, ribosome biogenesis, nutrient transport, and mitochondrial metabolism. Conversely, TOR negatively regulates catabolic processes such as mRNA degradation, ubiquitin-dependent proteolysis, autophagy and apoptosis. TOR is an atypical serine/threonine kinase that is found in two functionally and structurally distinct multiprotein complexes, TORC1 and TORC2 (mTORC1 and mTORC2 in mammals), each of which signals via a different set of effector pathways. TORC1 is rapamycin sensitive whereas TORC2 is rapamycin insensitive. The best-characterized phosphorylation substrates of mTOR are S6K and 4E-BP1 via which mTORC1 controls translation, and Akt/PKB via which mTORC2 controls cell survival and likely other processes. Like TOR itself, the two TOR complexes and the overall architecture of the TOR signaling network appear to be conserved from yeast to human. Thus, the TOR signaling network is a primordial or ancestral signaling network conserved throughout eukaryotic evolution to regulate the fundamental process of cell growth. As a central controller of cell growth and metabolism, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, obesity, and diabetes.

We are studying the TOR signaling network in the yeast *Saccharomyces cerevisiae*, in mammalian cells, in mice, and in human tumors. A major finding in our laboratory in recent years was the fact that TOR controls cell growth via two major signaling branches. Furthermore, we discovered the two TOR complexes and demonstrated that these two complexes correspond to the two previously described TOR signaling branches. More recently, in collaboration with our in-house colleague Markus Rüegg, we introduced the mouse as an experimental system to study the role of mTOR in regulating whole body growth and metabolism. In collaboration with the clinician Markus Heim, we have initiated a translational research project aimed at defining signaling pathways that allow tumors to evade therapy. The overall goal of our studies is to elucidate how growth and metabolism are regulated in health and disease.

Fig. 3: Adipose-specific mTORC1 knockout mice are resistant to diet-induced obesity.

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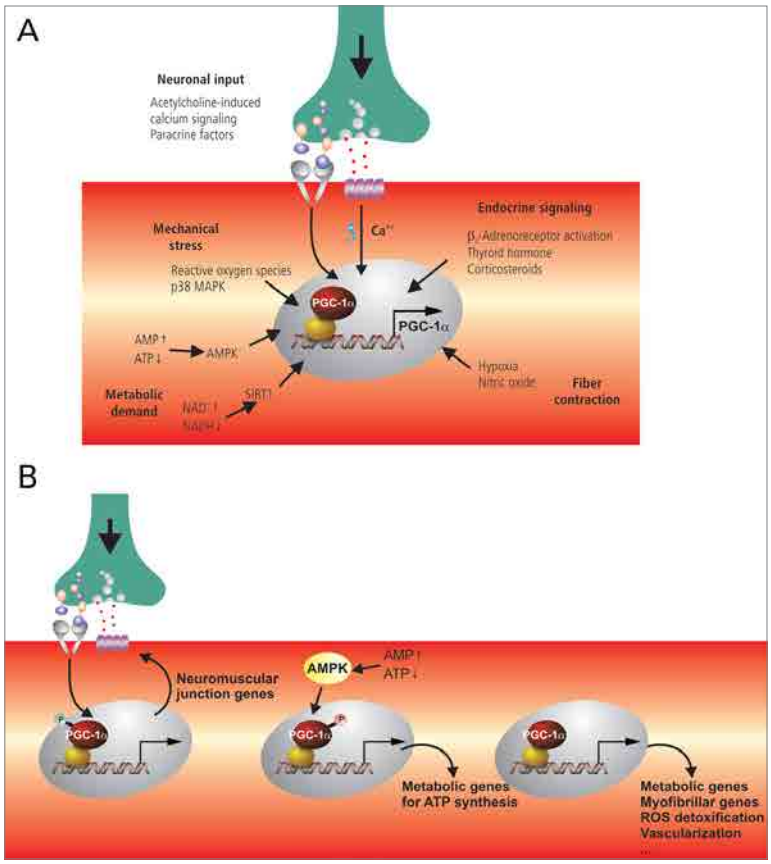
Regulation of skeletal muscle cell plasticity in health and disease



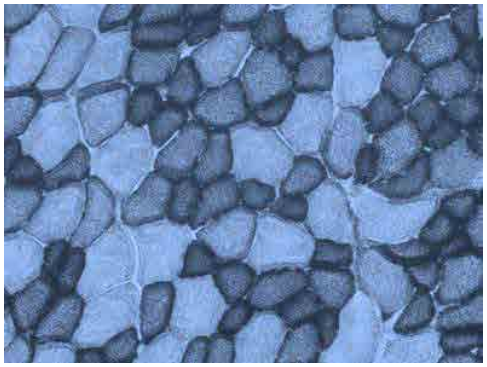
PGC-1α controls skeleta muscle plasticity in exercise. A) Every major signaling pathway in the trained muscle converges on PGC-1α by inducing PGC-1α gene expression, post-translationally modifying the PGC-1α protein, or by doing both. B) Spatiotemporal control of the specificity of the response to PGC-1α activation in muscle depending on the cellular context. Abbreviations: AMPK, AMP-dependent protein kinase; p38 MAPK, p38 mitogenactivated protein kinase; PGC-1α, peroxisome proliferatoractivated receptor γ coactivator 1α; ROS, reactive oxygen species; SIRT1, sirtuin 1.

Skeletal muscle has an enormous capacity to adapt to external stimuli including physical activity, oxygen, temperature, nutrient availability and composition. Inadequate muscle function is linked to an increased risk for many chronic diseases such as obesity, type 2 diabetes, cardiovascular disorders, osteoporosis, neurodegenerative events, mood disorders, age-related muscle wasting, and certain cancers. Inversely, regular exercise is an excellent prevention and therapeutic intervention for many of these pathologies and improves life quality and expectancy.

Skeletal muscle cell plasticity in exercise is a complex process: even a single endurance exercise bout alters the transcription of more than 900 genes in muscle. Chronic exercise leads to a metabolic and myofibrillar remodeling, increase in tissue vascularization, adaptation of the neuromuscular junction, a shift in the balance between protein degradation and biosynthesis rates, elevated heme biosynthesis, improved reactive oxygen species detoxification and a resetting of the peripheral circadian clock. Due to this complexity, it is not surprising that our knowledge about the molecular mechanisms that underlie muscle cell plasticity remains rudimentary.

The peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is one of the key factors in muscle adaptation to exercise. Muscle activity induces PGC-1α gene expression and promotes posttranslational modifications of the PGC-1α protein. In turn, PGC-1α regulates the adaptations of muscle to endurance training. Accordingly, ectopic expression of PGC-1α in muscle is sufficient to induce a trained phenotype whereas mice with a genetic ablation of the PGC-1α gene in muscle have an impaired endurance capacity.

Our group is studying the mechanisms that control muscle cell plasticity and their physiological consequences. We try to integrate molecular biology, work in muscle cells in culture and observations on mice with different activity levels to obtain a comprehensive picture of the adaptations in the active and the inactive muscle.



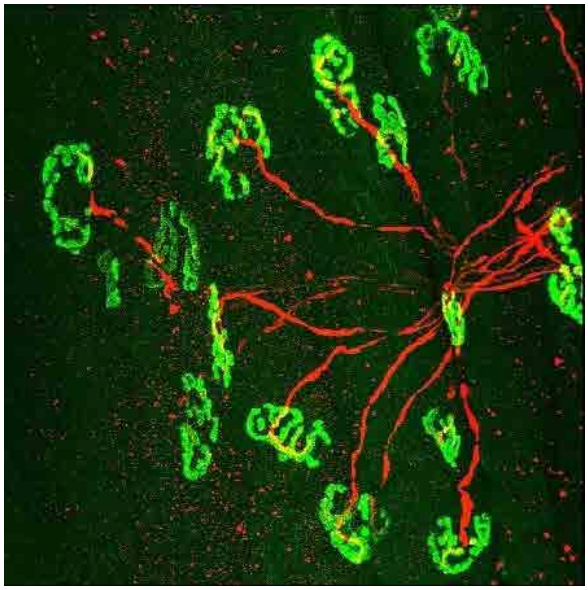
Visualization of fast (light blue) and slow (dark blue) muscle fibers in histological muscle sections with an NADH staining. Image by Joaquín Perez Schindler.

Regulation and coordination of metabolic pathways

Endurance exercise is a strong promoter of mitochondrial biogenesis and oxidative metabolism of lipids. At the same time, skeletal muscle of endurance athletes exhibits increased storage of intramyocellular lipids, similar to what is observed in muscle of type 2 diabetic patients (the "athlete's paradox"). Furthermore, the boost in mitochondrial function potentially augments the generation in harmful side-products, e.g. incomplete fatty acid oxidation products or reactive oxygen species. However, neither the lipid accumulation nor the oxidative metabolism in the exercised muscle exert detrimental effects, in stark contrast to the pathologies that develop under seemingly similar conditions in type 2 diabetes and other muscle-associated diseases. We study the coordination of anabolic and catabolic pathways in order to pinpoint the differences in substrate fluxes in the healthy and the diseased muscle.

Molecular changes in muscle atrophy and dystrophies

Muscle disuse, induced by a Western life-style or caused by diseases, leads to fiber atrophy, reduced muscle functionality and is ultimately fatal in certain inherited and sporadic muscular dystrophies. Little is known about the etiology of most of these diseases and as a result, no efficacious therapy exists for these devastating disorders. However, the induction of a trained phenotype ameliorates many of the symptoms of muscle wasting and thereby improves muscle function. For example, we have shown that using a genetic model for endurance training, PGC-1α muscle-specific transgenic mice, helps to ameliorate disuse-induced muscle fiber atrophy and Duchenne muscular dystrophy. Other groups have demonstrated that ectopically expressed PGC-1α also improves a mitochondrial myopathy, blunts muscle damage by the statin drugs and reduces sarcopenia, muscle wasting in aging in the respective animal models. We are currently studying how PGC-1α mediates this broad spectrum, health-beneficial effect on muscle and how this could be exploited therapeutically.



Histological visualization of neuromuscular junction in mouse muscles. The motor neuron is depicted in red (anti-neurofilament immunohistochemistry) and the acetylcholine receptor clusters on the muscle fiber membrane in green (using fluorescently labeled alpha-bungarotoxin). Image by Anne-Sophie Arnold.

Integration of signaling pathways and spatiotemporal control of gene expression

In exercise, PGC-1α transcription, protein levels and activity are modulated by different signaling pathways. While all of the major signaling pathways in the trained muscle converge on PGC-1α (figure part A), the consequences, the integration and the temporal coordination of these signals are not clear. Upon activation, PGC-1α controls the transcription of many different gene families in muscle to promote a trained phenotype. However, the specificity of gene regulation by PGC-1α varies according to the cellular context (figure part B). For example, the regulation of postsynaptic neuromuscular junction genes by PGC-1α is spatially restricted to subsynaptic nuclei in the muscle fiber.

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Research Group Sebastian Hiller

Structural biology of outer membrane proteins

We are interested in structural and functional details of integral outer membrane proteins and their biogenesis at the atomic level. Proteins in the outer membranes of Gram-negative bacteria and mitochondria are responsible for a wide range of essential cellular functions, including signal transduction, catalysis, respiration, and transport. Our main technique of expertise is nuclear magnetic resonance (NMR) spectroscopy, which we use together with complementary techniques to address our biological and biophysical questions. Part of our activities is the development of new and improved NMR techniques for studies of membrane proteins, such as advanced isotope labeling schemes and non-uniform data processing schemes. Such techniques are applicable to membrane protein systems but will also stimulate research on other challenging biomacromolecules.

Molecular chaperones play a key role in outer membrane protein biogenesis, forming a natural assembly line for transport of the unfolded proteins to their target membrane. We employ highresolution NMR studies of large 70-100 kDa membrane proteinchaperone complexes to provide an atomic resolution description of the underlying molecular mechanisms, such as Skp (**Fig. 1**, Burmann *et al.*, Nat. Struct. Mol. Biol. (2013)). Subsequent projects address the other chaperones. We want to know the details how the polypeptide transport is accomplished, how the substrates are recognized and how the final folding and insertion step is catalyzed.

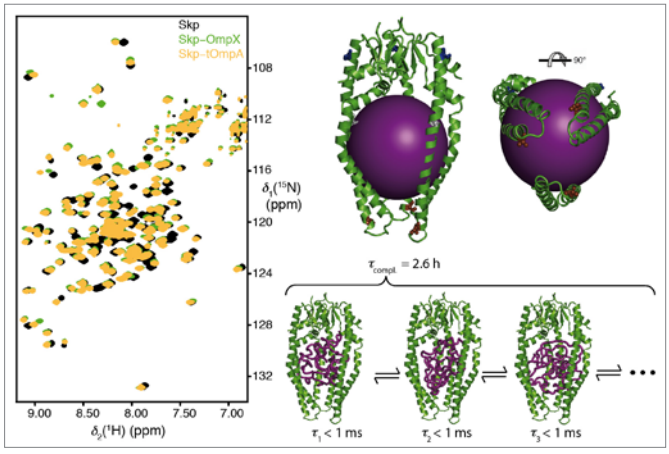

Fig. 1: NMR spectroscopy of Skp-Omp complexes.

A second line of our research addresses the folding mechanism of β-barrel outer membrane proteins. *In vivo*, as the last event in outer membrane biogenesis, Omp substrates are inserted into the membrane by members of the Omp85 family of proteins. This family comprises the proteins BamA and TamA, but also two-partner secretion systems such as FhaC in Gram-negative bacteria, and Sam50 in mitochondria. Our goals are the determination of structures and structural models of the Tam, Bam and Sam complexes and an elucidation of their functional mechanism. Our hypothesis for the functional mechanism is the formation of a hybrid barrel as the folding intermediate (**Fig. 2**, Gruss *et al.*, Nat. Struct. Mol. Biol. (2013)).

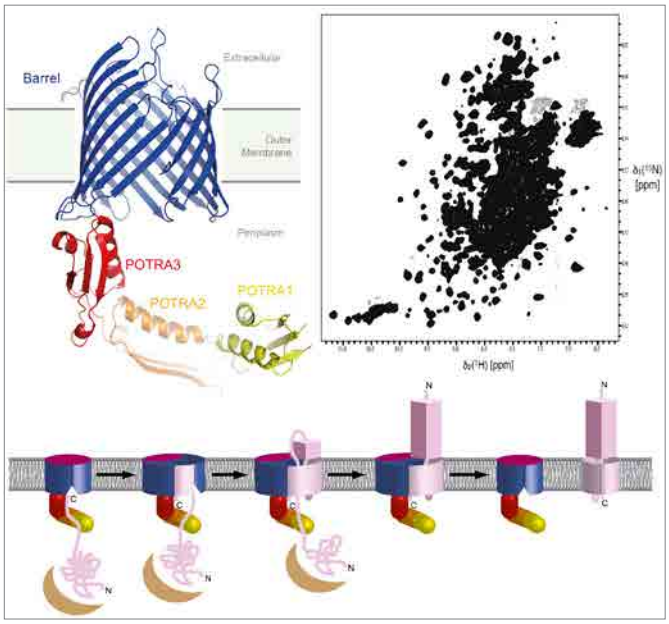

Fig. 2: Structure and a possible mechanism of TamA.

In vitro, many outer membrane proteins can fold and insert into a target membrane or membrane mimic also in the absence of an Omp85 foldase. The Omp folding process is a biophysically intriguing mechanism that is only poorly understood. We are developing new approaches to study outer membrane protein folding at atomic resolution by solution NMR spectroscopy in combination with single-molecule techniques (**Fig. 3**).

A third focus of interest is the structural biology of the mitochondrial outer membrane. From a structural biology perspective, this membrane is largely a "terra incognita". The mitochondrial outer membrane features essential biological roles in the eukaryote, such as regulation of metabolism, apoptosis and cancer. Still, so far the atomic resolution structure of just a single integral outer membrane protein is known, the voltagedependent anion channel VDAC (**Fig. 4**; Hiller *et al.* Science (2008)). We are now addressing the function of the voltage-dependent anion channel VDAC and its complexes, which play crucial roles in the regulation of the cellular metabolism. By characterizing the complexes of VDAC with its natural ligands and its protein interaction partners, we attempt to elucidate the structural bases for these functions.

The innate immune response reacts to pathogens, danger- and damage-related intracellular signals by assembling large inflammasome complexes. We have determined the structure of the mouse ASC inflammasome filament (**Fig. 5**, Sborgi *et al.*, Proc. Natl. Acad. Sci. (2015)) and characterized its polymerization process by employing a combination of NMR spectroscopy and cryo-electron microscopy. In the future, we are interested in a quantitative, atomic-resolution description of inflammasome signaling and its regulation by biomacromolecules and environmental parameters.

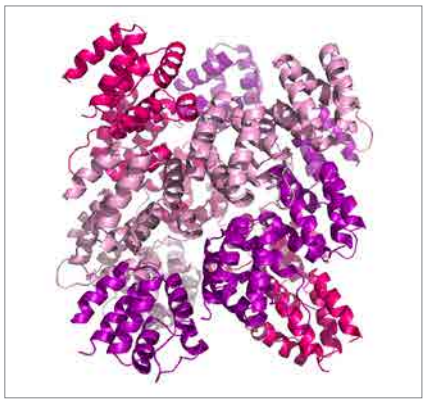

Fig. 5: Structure of the mouse ASC inflammasome.

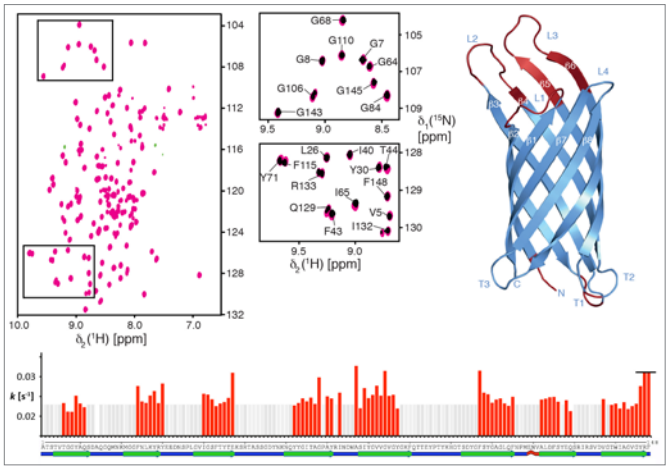

Fig. 3: Residue-specific folding kinetics of OmpX.

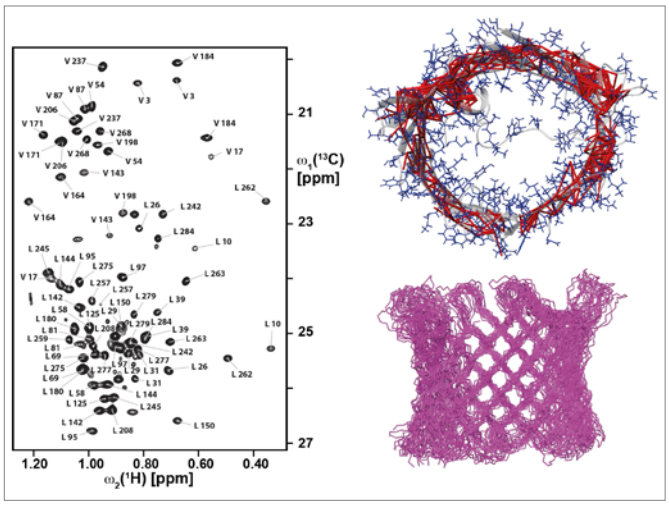

Fig. 4: 2D Methyl-TROSY NMR of VDAC in LDAO micelles.

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Research Group Sonja Hofer

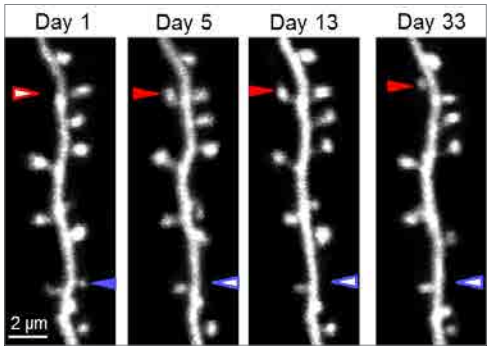
Function and plasticity of the visual system

Our research is focused on understanding how neuronal circuits process visual information coming from the eye and integrate it with other signals to enable animals to interpret the visual world and interact with their environment. Furthermore, we investigate how these circuits change during learning and new experiences, allowing the brain to store new information and to adapt to changes in the environment.

To study these questions we use a wide range of multi-disciplinary methods: *in vivo* two-photon imaging of neuronal and synaptic function and structure, extracellular and intracellular electrophysiological recordings, animal behavior and theoretical modelling, together with molecular and genetic approaches to identify different cell types, record and manipulate their function and trace specific pathways.

Changes in visual circuits during learning

Learning alters our perceptions, cognition and behavior by modifying neuronal circuits in the brain. Understanding how this happens is crucial for understanding normal brain function, and for devising therapeutic approaches for correcting disorders of information storage and retrieval such as dementia. Yet the mechanisms of learning in the intact brain are not well understood. Relatively little is known about how new information is stored in neuronal circuits and how new experiences, which are behaviourally relevant for the animal alter single cells, their connections and the flow of information through neuronal networks. One reason for our lack of knowledge is that it has long been impossible to repeatedly record activity from the same identified neurons and their connections over the course of days or weeks. The newest generation of genetically-encoded calcium indicators in combination with two-photon laser scanning microscopy now makes this possible. These indicators allow us to visualize the activity of neuronal networks with single-cell and even single-synapse resolution in the intact brain.

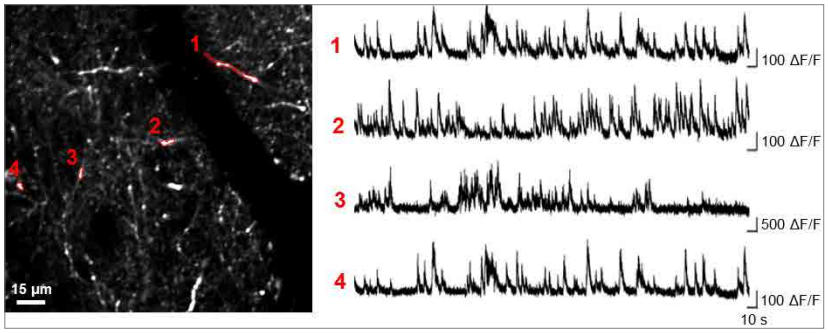


Repeatedly imaged apical dendrite from a layer 5 neuron *in vivo*. The protrusions are dendritic spines which carry the majority of excitatory synapses. Spine changes are depicted by arrows, red: spine gained, blue: spine lost.

To study learning-related changes in the brain, we are developing different behavioral paradigms for mice in which they have to learn visually-guided tasks. These tasks enable us to measure the animals' visual perception and to assess their learning progress. We are then using calcium indicators to follow directly how the function of neurons in different visual areas changes when animals make new associations during visually-guided learning. Furthermore, we are studying which circuit modifications underlie these functional changes, by following individual synapses of different pathways over the time course of learning.

The function of higher-order thalamic pathways during vision

Visual perception relies on information flow from the eye to the visual cortex, where it is relayed and transformed via a series of thalamic and cortical processing stages. In recent years it has become increasingly clear that the traditional hierarchical model of sensory processing, which is based mostly on feed-forward flow of sensory information, is incomplete. Prominent feedback projections from higher-order brain areas and information from parallel circuits involving the thalamus impinge on every cortical processing level. Such major alternative pathways may convey contextual information, such as the animal's motor output, previous experience, expectations and stimulus relevance, which can strongly modulate visual responses and influence how feed-forward sensory information is interpreted in the context of an animal's internal state and behavior. However, little is known about what information is conveyed through these different pathways and how it influences the processing of feed-forward sensory information to allow animals to actively perceive and interpret the environment based on their past experience.



Thalamic axons expressing the genetically-encoded calcium indicator GCaMP5 imaged in visual cortex and example traces of calcium transients from individual axons showing their activity in a behaving mouse.

We are studying the organization and function of one major pathway that might integrate visual and non-visual information but which is still very elusive – the input from higher-order thalamic nuclei into visual cortex. We are studying the organization of these thalamo-cortical circuits in the mouse using anatomical tracing methods and are investigating which information is conveyed to different cortical areas by higher-order thalamic signals in the behaving animal and how it influences the processing of visual information.

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Research Group Urs Jenal

Cell signaling and dynamics in bacterial growth, adaptation, and persistence

Our studies aim at understanding the molecular and cellular principles involved in the growth, differentiation and behavior of bacterial cells. We focus on the nucleotide second messenger, cyclic di-guanosine-monophosphate (c-di-GMP) and its role in bacterial cell signaling and dynamics (Schirmer & Jenal, 2009, *Nature Reviews Microbiol 7*, 724). C-di-GMP emerges as a ubiquitous signaling molecule that modulates multiple aspects of bacterial growth and behavior, including the formation of a sedentary, community-based lifestyle and its association with chronic forms of bacterial infections (**Fig. 1**) (Böhm 2010 *Cell*, 141, 107; Malone 2012 *PLoS Pathog.*, 8(6), e1002760; Steiner 2013 *EMBO J*, 32(3) :354). Our aims are to identify and characterize cdi-GMP control modules in different bacterial model organisms, to uncover and exploit the basic molecular and mechanistic principles of cdi-GMP signaling, and to probe its role in bacterial growth and persistence.

Role of c-di-GMP in cell cycle progression and cell fate determination

We use *Caulobacter crescentus* as a model to investigate the role of c-di-GMP in cell polarity and cell cycle progression. Periodic fluctuations of c-di-GMP are an integral part of the *C. crescentus* cell cycle clock and serve to control pole development in time and space and to coordinate these processes with the underlying cell cycle. Several diguanylate cyclases (DGC) and phosphodiesterases (PDE) contribute to the characteristic bimodal distribution of the second messenger during the *Caulobacter* life cycle. One of our aims is to understand their regulation in time and space (Abel 2013 *PLoS Genet* 9(9): e1003744). E.g. the DGC PleD is activated by phosphorylation prior to S-phase entry and sequesters to the differentiating *Caulobacter* cell pole where it orchestrates pole morphogenesis and replication initiation (**Fig. 2**) (Paul 2008, *Cell* 133, 452, Abel 2011, *Mol Cell* 43, 550). *Caulobacter* G1-to-S transition is mediated by a second DGC, DgcB. In the G1 swarmer cell DgcB is "neutralized" by its specific and dominant antagonist PdeA, which reduces c-di-GMP in this cell type and keeps it in the motile, replication-inert phase. Upon entry into S-phase, when cells transit into sessility, PdeA is specifically degraded leaving DgcB unopposed. PdeA dynamically localizes to the old cell pole where it is degraded by the polar protease complex ClpXP. This cell cycle dependent process is orchestrated by the single domain response regulator CpdR, which itself localizes to the old cell pole in response to its phosphorylation status, where it recruits both PdeA and ClpXP and mediates substrate delivery (Abel 2011, *Mol Cell* 43, 550). Together, PDE degradation and DGC activation result in a rapid and robust upshift of c-di-GMP, which coordinately drives pole morphogenesis and S-phase entry (Abel 2011, Mol Cell 43, 550).

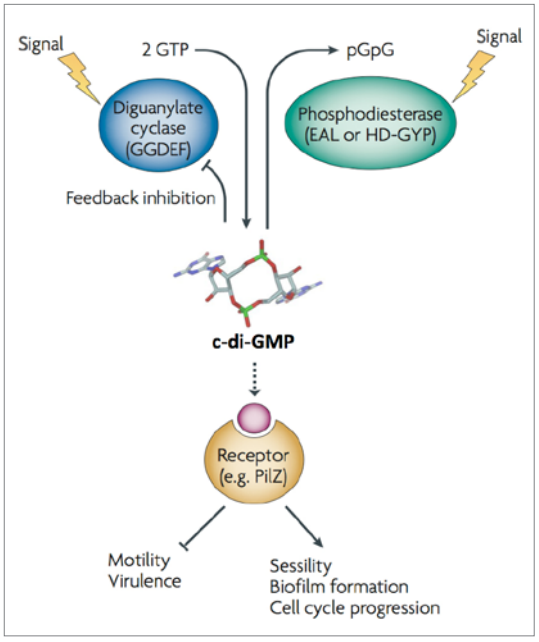

Fig. 1: Schematic of c-di-GMP mediated signaling in bacteria.

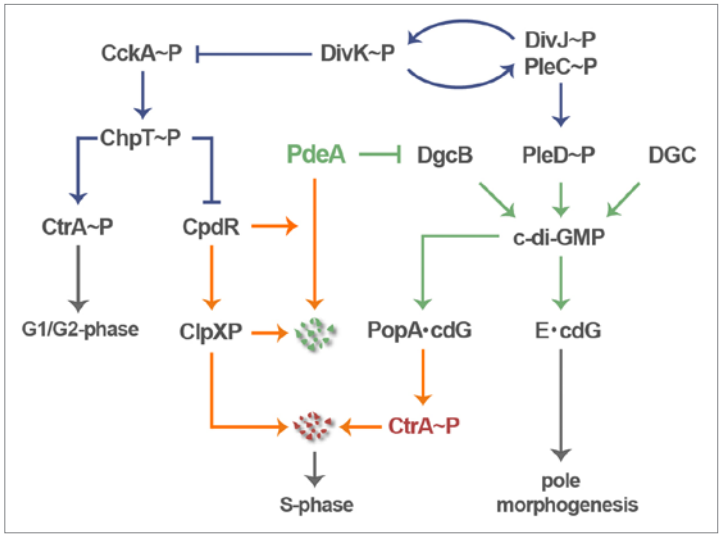

Fig. 2: Network controlling cell cycle progression and pole development in *C. crescentus*. Phosphorylation reactions (blue), c-di-GMP network (green), and protein degradation pathways (orange) are indicated. Unidentified c-di-GMP effector proteins (E) regulating pole morphogenesis are indicated.

C-di-GMP controls all aspects of *Caulobacter* polarity, including flagellar motility, pili biogenesis as well as holdfast and stalk formation (Abel 2013 *PLoS Genet* 9(9): e1003744). The mechanisms and regulatory components contributing to timing and spatial control of polar organelle formation are one main focus of our current research (Davis 2013, *Genes Dev* 27, 2049). In parallel, we are interested in how c-di-GMP modulates *Caulobacter* cell cycle progression. The c-di-GMP up-shift facilitates replication and cell division control through the controlled destruction of the replication initiation inhibitor CtrA and the cell division inhibitor KidO by the ClpXP protease complex. Cell cycle dependent degradation of these proteins entails a specific spatial arrangement where both protease and substrates transiently localize to the incipient stalked cell pole during the G1-S transition. Substrate delivery to the polar protease requires PopA, a protein that sequesters to the stalked pole upon binding of c-di-GMP (Duerig 2009, *Genes Dev* 23, 93). Our recent studies demonstrate how phosphosignaling, protein degradation, and c-di-GMP mediated regulatory processes are tightly interconnected to coordinately drive the *Caulobacter* life cycle (**Fig. 2**) (Abel 2011, *Mol Cell* 43, 550; Abel 2013 *PLoS Genet* 9(9): e1003744).

Role of c-di-GMP in biofilm formation and persistence

We have used *Escherichia coli* as a genetically versatile model organism to analyze the molecular basis of the inverse regulation of cell motility and biofilm formation by c-di- GMP. Our studies revealed that *E. coli* can fine-tune its swimming speed with the help of a molecular brake (YcgR) that, upon binding of c-di-GMP, interacts with the motor protein MotA to curb flagellar motor output (Böhm 2010 *Cell*, 141, 107). These experiments demonstrate that bacteria can modulate motor output in response to environmental cues. Our studies also led to identify c-di-GMP and ppGpp as key regulatory factors of poly-β-1,6-N-acetyl-glucosamine (poly-GlcNAc) synthesis, a polysaccharide adhesin secreted by *E. coli* as response to sub-inhibitory concentrations of antibiotics targeting the ribosome (Böhm 2009, *Mol Microbiol*. 72, 1500). The synergistic roles of ppGpp and c-di-GMP in biofilm induction, suggested that interference with bacterial second messenger signaling might represent an effective means for biofilm control during chronic infections.

Chronic *Pseudomonas aeruginosa* infections in cystic fibrosis (CF) patients can be treated with antibiotics, however full clearance is not possible due to the adaptation of infective species to a persistent lifestyle. Adaptive *P. aeruginosa* morphotypes include small colony variants (SCVs), slow growing and strongly adherent variants whose appearance correlates with poor lung function (**Fig. 3**). Our research on *P. aeruginosa* SCVs suggests that SCV-mediated persistence might be a novel target for antimicrobial chemotherapy. We characterized a tripartite signaling system called YfiBNR, mutations in which lead to the generation of SCV variants (Malone 2010, *PLOS Pathogens*, 6(3), e1000804). YfiN was shown to be a membrane-bound cyclic di-GMP synthase, whose activity is tightly controlled by YfiR and YfiB. Activation of YfiN resulted in increased levels of c-di-GMP, which in turn triggered massive

Fig. 3: Characteristic morphologies of normal "smooth" (large colony) and SCV morphotypes (small colonies) of *Pseudomonas aeruginosa*.

production of exopolysaccharides, drastically reduced growth rates, and resistance to macrophage phagocytosis. Consistent with a role for the SCV phenotype in immune system evasion, activation of YfiN significantly increased the persistence of *P. aeruginosa* in long-term mouse infections. Moreover, the Yfi system is under positive and negative selection in airways of CF patients (Malone 2012, *PLoS Pathogens*, 8(6), e1002760) driving population dynamics of persistent SCVs *in vivo*. These studies establish a firm causal link between SCV, cdi- GMP, and chronic *P. aeruginosa* infections.

Important Partners

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Selective transport control in biomimetic and cellular systems

Karyopherin-centric control of nuclear pores (SNF/SNI)

Nuclear pore complexes (NPCs) are 50 nmdiameter aqueous channels that function as the main transport hubs between the cytoplasm and nucleus in eukaryotic cells. Exclusive access is orchestrated by nuclear transport receptors (NTRs) that interact with intrinsically disordered proteins known as FG Nups that otherwise generate a permeability barrier within the NPC. Our interest is to understand how multivalent NTR-FG Nup binding impacts on FG Nup barrier function and transport kinetics at the (i) biophysical level, (ii) within individual NPCs, and (iii) in cells. Our findings thus far indicate that a classical NTR known as Kapβ1 acts as a bona fide constituent of the FG Nup barrier and plays a role in modulating selective transport control in NPCs.

Biomimetic systems for selective transport control (NCCR Molecular Systems Engineering)

Nucleocytoplasmic transport is regulated by soluble nuclear transport receptors (NTRs) that exclusively enter NPCs to accumulate selective cargoes in the nucleus against concentration gradients. Here, we want to exploit this phenomenon as a means of implementing selective transport control in biomimetic systems. To do so, we are engineering molecular adaptors that can mediate between biological and synthetic systems. This includes constructing NPCinspired biomimetic nanopores, as well as implementing two-dimensional transport control using the so-called "dirty velcro effect".

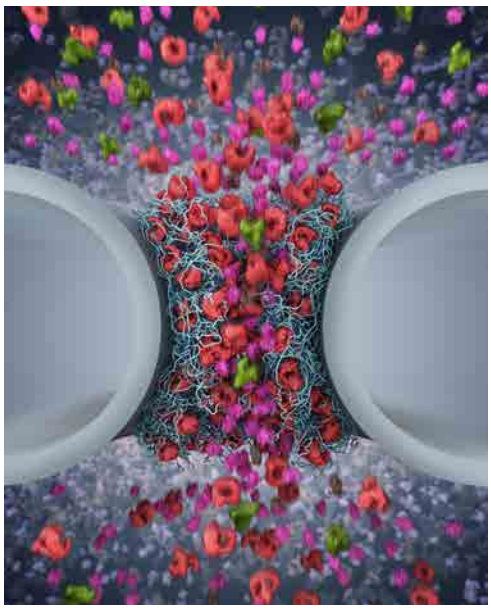


Fig. 1: Karyopherin-centric model of the nuclear pore complex. Artwork: Immanuel Wagner.

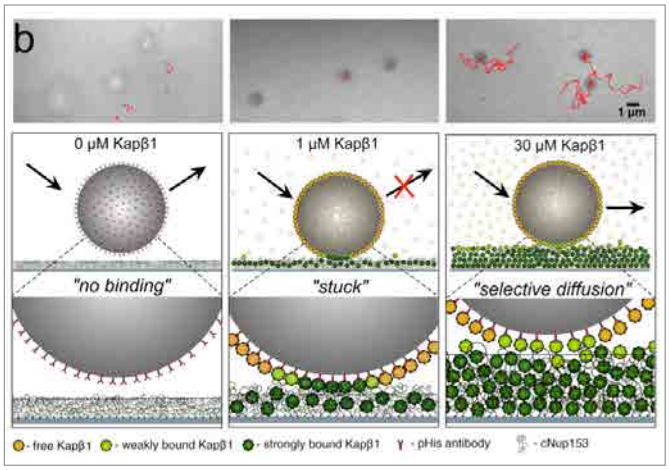


Fig. 2: Selective transport control in two-dimensions. The presence of preloaded Kapβ1 within the FG Nups results in a reduced binding of incoming Kapβ1 i.e., the "dirty velcro effect". (Schleicher *et al.*, Nature Nanotechnology 2014).

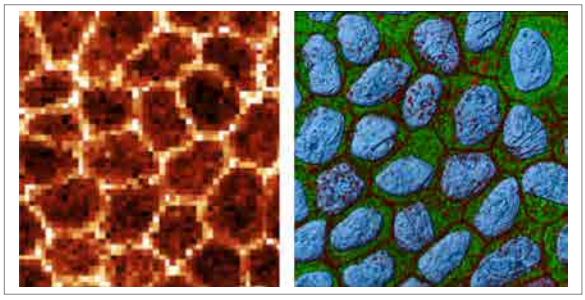
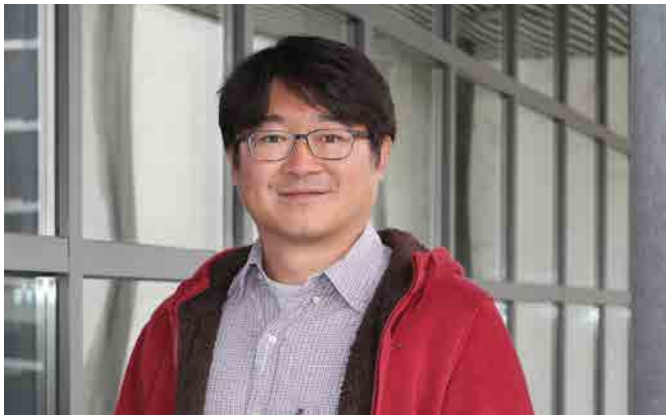


Fig. 3: ARTIDIS stiffness (left) and immunofluorescence (right) images show epithelial layer formation when cultured on native basement membrane (green) that reveals actin (red) cytoarchitecture typical for epithelial sheets *in vivo*. The cell nucleus is depicted in blue. Image: Philipp Oertle (Biozentrum, Basel) and Vasily Gurchenkov (Institute Curie, Paris).

The mechanobiological hallmarks of living tissue (NanoTera)

Cells adapt to their microenvironments via vast mechanosensory networks that have a reciprocal effect on cellular stiffness and mechanophenotype. In epithelia, these mechanosignals are transmitted from intercellular junctions and basal contacts formed on basement membranes (BMs) into nuclei. We are investigating how mechanosignaling regulates cell polarity, cytoskeletal organization, and inter-cellular communication. Interestingly, our findings reveal that epithelial cells on native BMs exhibit several key features of living tissue that are suppressed in reconstituted basement membrane (i.e., Matrigel).



ARTIDIS and nanomechanical tissue diagnostics (KTI)

We have built an atomic force microscope (AFM)-based apparatus (i.e., ARTIDIS "Automated and Reliable Tissue Diagnostics") to measure cellular stiffness with unsurpassed sub-cellular spatial resolution and high force sensitivity within living tissue. By examining unadulterated human breast cancer biopsies, we have uncovered how the "softness" of cancer cells is associated with hypoxia and is fundamentally coupled to their propensity to invade and metastasize. This has resulted in a spinoff Nuomedis® that is commercializing ARTIDIS for clinical applications of nanomechanical tissue diagnosis.

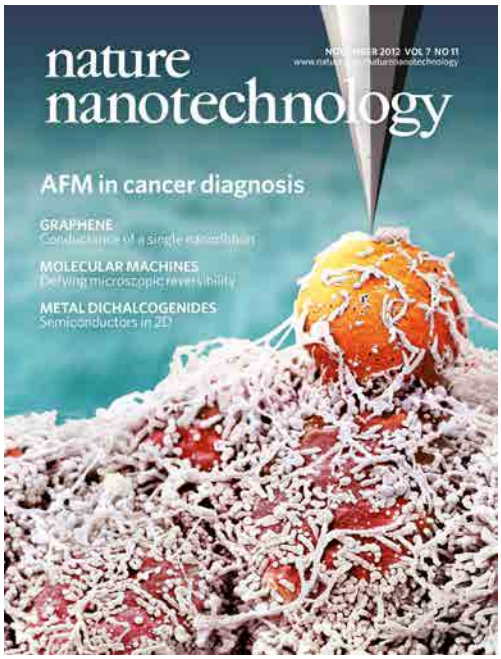


Fig. 4: Cover image depicting the principle of ARTIDIS, which uses a sharp nanometer-sized AFM tip to probe the stiffness of an invasive cancer cell that is seen protruding above a malignant matrix-embedded cluster (Plodinec *et al.*, Nature Nanotechnology 2012). Image: Eva Bieler, Marija Plodinec and Roderick Lim. Artwork: Martin Oeggerli/Micronaut 2012. Cover Design: Alex Wing.

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Research Group Timm Maier

Multienzymes and regulatory complexes in lipid metabolism

Lipid biosynthesis and degradation are essential and tightly regulated cellular processes in all organisms and are closely linked to human health. Lipids are an important source of natural chemical diversity and integrate the metabolic state with cellular processes such as inflammatory response, transmembrane signaling and trafficking. Impaired lipid and fatty acid metabolism play considerable roles in the pathogenesis of major threats to human health, including type 2 diabetes, fatty liver disease, atherosclerosis and cancer. A central aim of our work is to improve our understanding of eukaryotic lipid and fatty acid metabolism and its regulation by elucidating the structure, function and interplay of key enzymatic and regulatory protein complexes.

Recent developments in lipid metabolomics allow quantitative and realtime studies of the cellular lipidome. However, eukaryotic lipid and fatty acid metabolism remain a critical challenge for studies at the molecular and atomic scale: in contrast to simple prokaryotic systems, fatty acid metabolism in eukaryotes builds upon huge multifunctional enzymatic complexes more than all other metabolic pathways. Later steps of lipid metabolism are occurring in membrane space and rely on membraneintegral or -associated proteins, few of which are structurally characterized. We focus on multienzyme biosynthetic factories and giant regulatory complexes involved in key steps of lipid and fatty acid metabolism and homeostasis.

Multienzymes integrate several functional domains for catalysis or substrate transfer into a single protein assembly, which carries out more than one enzymatic reaction step. A landmark example are giant eukaryotic fatty acid synthases, which comprise seven types of enzymatic domains and carry out more than 40 reaction steps for the biosynthesis of fatty acids from carbohydrate-derived precursors. The interlinking, mechanical coupling and crosstalk between domains result in emergent properties of multienzymes beyond the functionality of their isolated building blocks. Multienzymes offer unique means for the consistent regulation of committed steps in fatty acid and lipid metabolism, which is critical for human health.

Information on the metabolic state of an organism and cell is shunted via complex signaling pathways to central regulatory complexes for signal integration. The mTOR kinase regulatory complex (mTORC1) senses amino acid level, cellular energy state and external growth factors and in appropriate conditions initiates the cellular program for anabolic reactions, growth and proliferation. Due to its central role in driving cell proliferation, mTORC1 is a key target for anti-cancer therapy. Combining structure determination of multienzymes and regulatory complexes in fatty acid and lipid metabolism with functional studies we elucidate regulatory mechanisms as well as fundamental principles of multienzyme architecture.

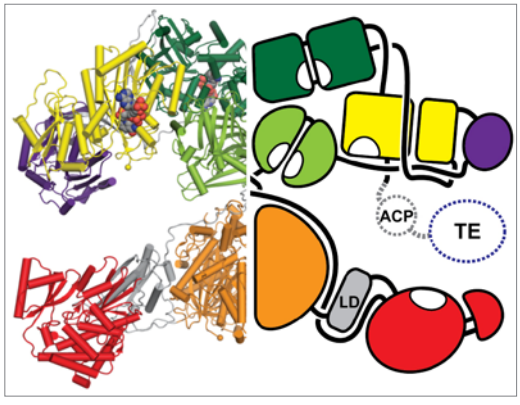

Fig. 1: The multienzyme architecture of the dimeric animal fatty acid synthase; colored by domain, left: cartoon representation of crystal structure, right: schematic representation.

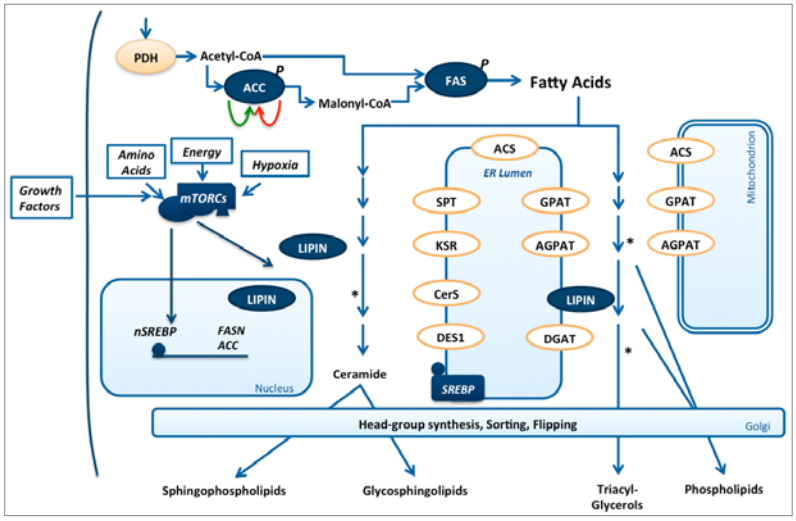

Fig. 2: Regulation of lipid and fatty acid biosynthesis pathways.

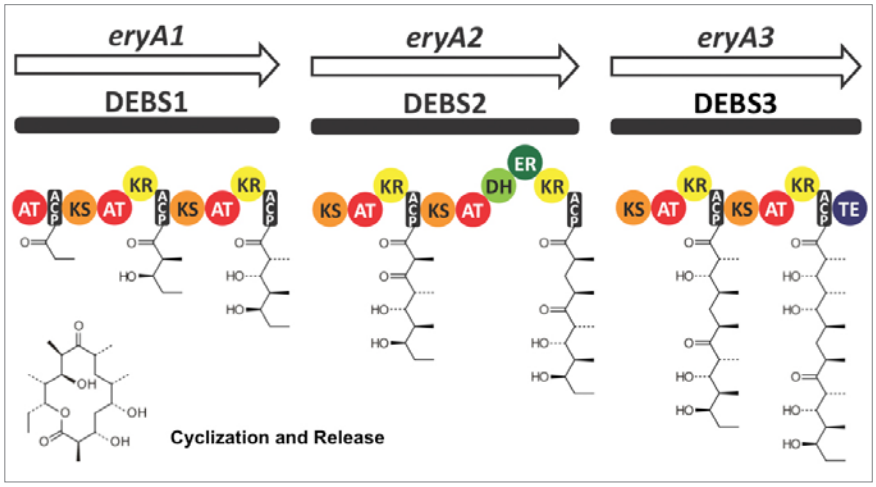

Fig. 3: Assembly line logic and collinearity from gene to chemical reactions of polyketide biosynthesis, as exemplified by the DEBS PKS involved in erythromycin biosynthesis.

Multienzymes in polyketide biosynthesis

Polyketide synthases are the most complex biosynthetic multienzymes and are built upon assembly line logic. Individual modules, each related to the mammalian fatty acid synthase, processively extend a precursor module with varying carboxylic acid building blocks. The highly diverse and complex polyketide products include numerous compounds with outstanding biological activities, e.g. antibiotics, and are a promising source of novel drug candidates. Our studies on the general architecture of polyketide synthases serve to obtain complementary information on multienzymes organization and evolution. They also provide relevant insights for combinatorial biosynthesis of novel polyketide drug candidates. Understanding the functional coupling and emergent properties in key multienzymes ultimately is a prerequisite for the rational tailoring of existing or the design of novel molecular biosynthetic factories.

Foldases, insertases and translocases in bacterial protein secretion

While the principal mechanisms of intracellular protein folding and membrane integration of α-helical membrane proteins by the Sec translocase are well understood, the assembly and insertion process of β-barrel membrane proteins by members of the Omp85 family of β-barrel proteins is still elusive. β-barrel membrane proteins are particularly prominent in the outer membrane of gram-negative bacteria and contribute to bacterial pathogenicity and persistence. In collaboration with Sebastian Hiller (Biozentrum, Universität Basel) we are studying foldase proteins of the periplasmic and extracellular bacterial space and β-barrel membrane protein assembly in a joint effort combining NMR, X-ray crystallography and biophysical characterization. We have obtained a high-resolution crystal structure of *E. coli* TamA, an outer membrane protein involved in the insertion and assembly of β-barrel autotransporters, by bicelle crystallization and seeding. TamA comprises three N-terminal POTRA domains and a C-terminal 16- stranded β-barrel. A striking feature is a kink towards the inside of the barrel formed by the C-terminal strand of the barrel. This kinked structure opens a gate to the lipidic phase for incoming substrates and creates a weak lateral contact in the barrel wall. These unique structural features suggest a mechanism for autotransporter insertion based on barrel expansion and lateral release. Sequence and structural conservation indicate that this mechanism may be of general relevance for all membrane protein insertases of the Omp85 family including the mitochondrial and bacterial general insertases, Sam50 and BamA. Further studies are required to understand how the Omp85 architecture mediates not only membrane protein insertion but also protein translocation across the membrane in specific bacterial two-partner secretion systems, exemplified by the Omp85 translocase FhaC and its transport substrate filamentous hemagglutinin. We aim to obtain further insights into the insertion mechanism and the relevance of individual protein features for insertion and translocation by combining structural and functional studies on Omp85 target proteins.

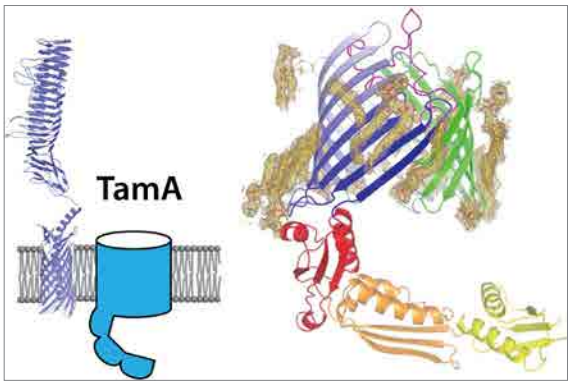

Fig. 4: Left: Schematic representation of TamA and an autotransporter substrate in the bacterial outer membrane; Right: Crystal structure of TamA with bound lipid molecules.

Catch-bond lectins in infection and inflammation

The life-time of most protein-ligand complexes is reduced under tensile force. However, some proteins exhibit catch-bond behavior: the life-time of their ligand complexes increases under tensile force up to a certain threshold. Prominent examples include carbohydrate-binding cell adhesion proteins specifically adapted to flow-induced shear forces, such as the bacterial adhesin FimH and human E-selectin. FimH is located at the tip of surface pili of *E.coli* and mediates adhesion to host-cell glycoproteins as a first step in urothelial infection. E-selectin is expressed on vascular endothelia and mediates leukocyte rolling as a prerequisite for tissue transmigration of leukocytes in the early inflammatory response. Together with Beat Ernst (Pharmazentrum, Universität Basel) and Rudi Glockshuber (ETH Zürich) we study these systems in a multidisciplinary effort combining biophysical, structural and pharmacological approaches. A key aim of our work is to provide a detailed understanding of domain interactions and their crosstalk with ligand binding as a mechanistic basis for catch-bond behavior and to define and confirm relevant states for ligand design and pharmacological intervention.

A hybrid approach to structural biology

Our approach to structural biology builds on combining X-ray crystallography as a key method to obtain insights at atomic resolution with electron microscopy, NMR spectroscopy and Small-angle X-ray scattering for studies of non-crystalline proteins. Chemical biology approaches to the stabilization and trapping of transient complexes as well as massspectrometric distance-restraint generation by chemical crosslinking (XL-MS) and molecular dynamics simulations provide further information on dynamic complexes and states. Biophysical characterization of macromolecular interactions and biochemical analysis of biological function provides critical complementary results. The lab is well set for all aspects of modern protein production and structural analysis. Facilities are available for large-scale protein expression in bacterial, yeast, insect and mammalian systems. Crystallization is aided by microfluidic screen preparation and nanoliter robotics for crystallization setup and automated seeding. We are using combined SONICC, UV-two photon excited fluorescence and trace-label fluorescence in an automated manner for reliable crystal and nanocrystal detection in standard and lipidic phase crystallization. Crystallographic data collection is carried out at the nearby Swiss Light Source. NMR and electron microscopic work is carried out in collaboration with the groups of S. Hiller and H. Stahlberg at the Biozentrum. Excellent support in proteomics and biophysical characterization of molecular interactions is provided by central service facilities. The focal area Structural Biology & Biophysics provides a highly collaborative environment between groups with expertise in all major techniques in structural biology.

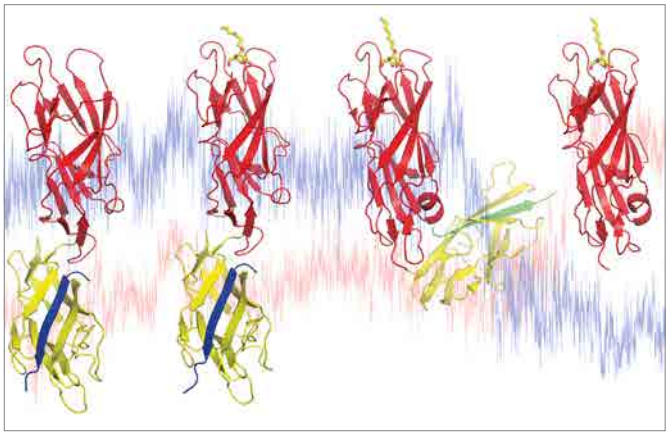

Fig. 5: Structural analysis of catch-bond properties of the bacterial FimH adhesin. Individual functional states are resolved by highresolution structural analysis, the transition between states are analyzed using molecular dynamics simulations.

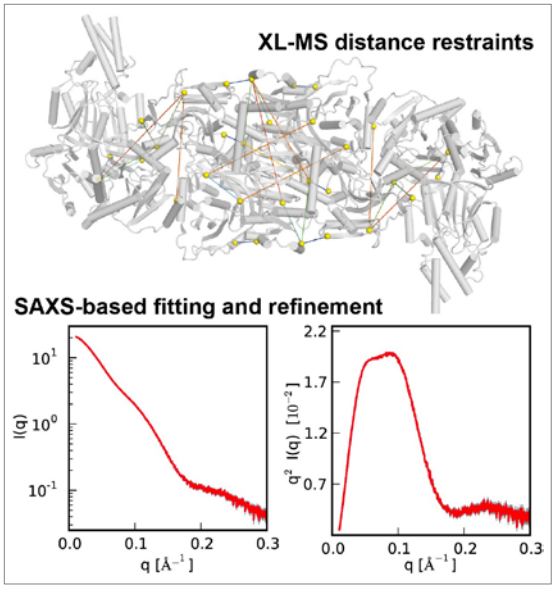

Fig. 6: Integration of structural data from chemical crosslinking mass spectrometry (XL-MS) and small angle X-ray scattering with high-resolution structural information from electron microscopy, NMR spectroscopy and X-ray crystallography provides a comprehensive representation of macromolecular complexes.

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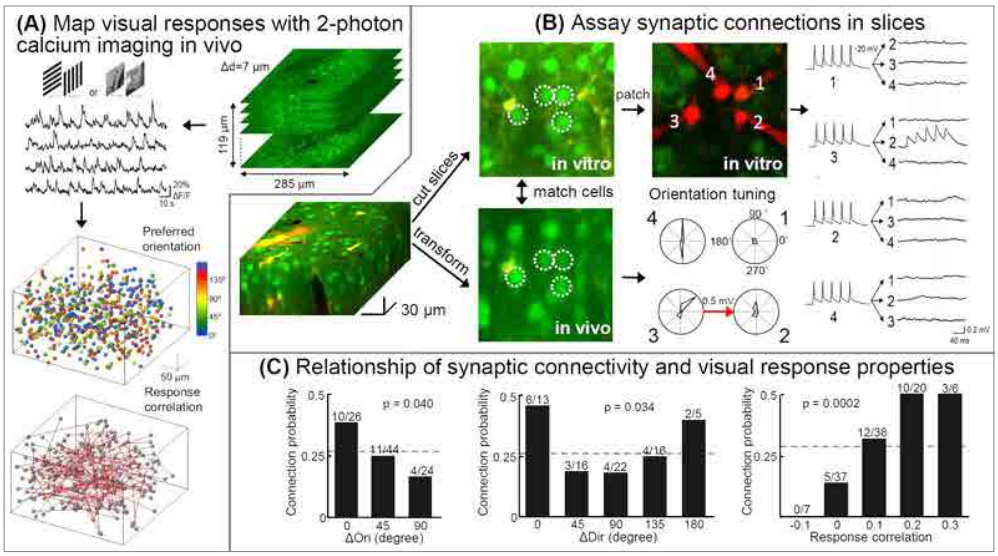
Research Group Thomas Mrsic-Flogel

Organisation and function of neuronal networks in visual cortex



The research in my lab aims to understand how sensory function emerges from highly complex synaptic interactions in large neuronal populations. I believe the best way to understand this complexity is to (i) record activity in single neurons and large neuronal ensembles in the intact brain during sensation, (ii) map connections between identified components of this circuit, and (iii) use computational models to understand which circuit components are important for single neuron and network function.

Methods

We are addressing how different components of the visual cortex circuit give rise to its function using a combination of methods, including two-photon calcium imaging in anesthetised and behaving mice, *in vitro* whole-cell recordings, *in vivo* whole-cell and extracellular recordings, optogenetics, visual behavioural tasks, and computational modelling.

Functional organisation of microcircuit connectivity in visual cortex

Determining how the organisation of neural circuitry gives rise to its function is a major challenge for understanding the neural basis of perception and behaviour. In order to determine how different regions of the neocortex process sensory information, it is necessary to understand how the pattern and properties of synaptic connections in a specific sensory circuit determine the computations it performs. We have begun forging the relationship between synaptic connectivity and neuronal function in primary visual cortex (V1) with the aim of revealing circuit-level mechanisms of sensory processing. To this end, my laboratory has developed a new method, by which visual response properties of neurons are first characterised with two-photon calcium imaging *in vivo*, and then synaptic connections between a subset of these neurons are assayed with multiple whole-cell recordings in slices of the same tissue (Ko, Hofer *et al.*, 2011, Nature). Using this approach, we found that connection probability between nearby excitatory neurons is intimately related to the similarity of their responses to oriented stimuli and natural movies. This functionally organised pattern of local excitatory connections reveals the fine-scale specificity by which neurons may influence each other through local excitatory connections, and points to the existence of subnetworks dedicated to processing of related sensory information.

Interactions between excitatory and inhibitory neuronal populations

The capacity of the neocortex to process sensory information depends on neuronal interactions between excitatory and inhibitory cell types. However, the relationship between connectivity, receptive field properties and network dynamics of different cell types are not well understood. We have shown that the differences in connectivity between excitatory neurons and fast-spiking inhibitory (parvalbumin-expressing) interneurons in V1 influence the specificity by which these inhibitory subpopulations respond to sensory stimuli, and the extent to which their activity is influenced by visual input (Hofer, Ko *et al.*, 2011, Nature Neuroscience).

Development of functional microcircuits

Using imaging *in vivo*, connectivity mapping *in vitro* and network modelling, we found that the functionally specific arrangement of connections in visual cortex is instructed by patterns of feedforward input after eye opening (Ko, Cossell *et al*., 2013, Nature, in collaboration with Sonja Hofer).

Current projects

- Functional circuit mapping: forging the relationship between the organization of excitatory and inhibitory synaptic connections and the detailed sensory response properties of neurons in visual cortex.
- Description of neuronal population dynamics during visual information processing in relation to excitatory and inhibitory cell types.
- Determining the influence of stimulus context, reward or expectation on neural representations in visual cortex, using electrophysiological recordings in single neurons and population imaging in behaving animals.
- Optogenetic manipulation of the circuit to investigate the impact of different neuronal classes and cortical areas in visual processing in anaesthetised and behaving mice.
- Building a biophysically realistic network model of the visual cortex microcircuit, relying on connectivity data and biophysical properties of cortical cell types (in collaboration with Angus Silver, University College London).

Publications 2015

Poort, Jasper; Khan, Adil G; Pachitariu, Marius; Nemri, Abdellatif; Orsolic, Ivana; Krupic, Julija; Bauza, Marius; Sahani, Maneesh; Keller, Georg B; Mrsic-Flogel, Thomas D; Hofer, Sonja B (2015). Learning Enhances Sensory and Multiple Non-sensory Representations in Primary Visual Cortex. *Neuron, 86*(6), 1478-90.

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Control of chromosome segregation and centrosome duplication in human cells

Cell proliferation depends on passage of cells through a series of biochemical reactions that are collectively termed "cell cycle". This fundamental process is indispensable for the development of an entire organism from a single cell (fertilized egg), as well as the constant renewal of most cells throughout adult life. Key events during cell cycle progression include the duplication of the chromosomes (the genome) and their subsequent segregation to two nascent daughter cells. Chromosome segregation occurs during a cell cycle phase known as "mitosis", a highly dynamic and spectacular stage of the cell cycle (**Fig. 1**). The main goal of our research is to elucidate the mechanisms that regulate mitosis in time and space and thereby ensure the error-free segregation of chromosomes. A better understanding of mitosis will hopefully illuminate the origins of the chromosome aberrations (aneuploidies) that give rise to birth defects and constitute hallmarks of aggressive human tumors.

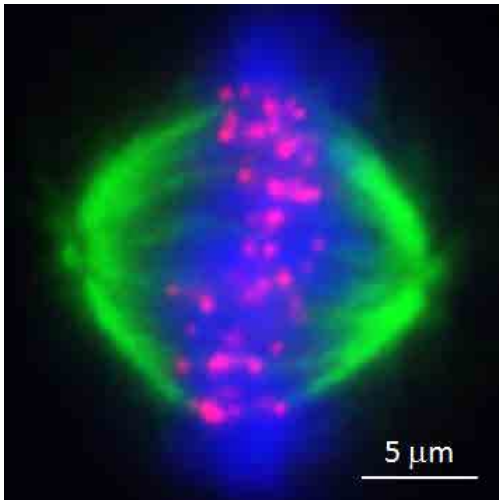


Fig. 1: A dividing human cell was stained with antibodies against tubulin (green) and a kinetochore marker (red); condensed chromosomes are visualized by staining with DAPI (4',6-diamidino-2-phenylindole; blue). Size bar: 5 µm.

Central to mitosis is the spindle apparatus, a complex and highly dynamic microtubule-based structure that captures chromosomes through specialized protein structures termed kinetochores (**Fig. 2**). Hence, we study the composition, regulation and dynamics of the mitotic spindle and kinetochores. In addition, we aim at elucidating the function of a surveillance mechanism – the spindle assembly checkpoint – that monitors the complete attachment of all mitotic chromosomes to the spindle.

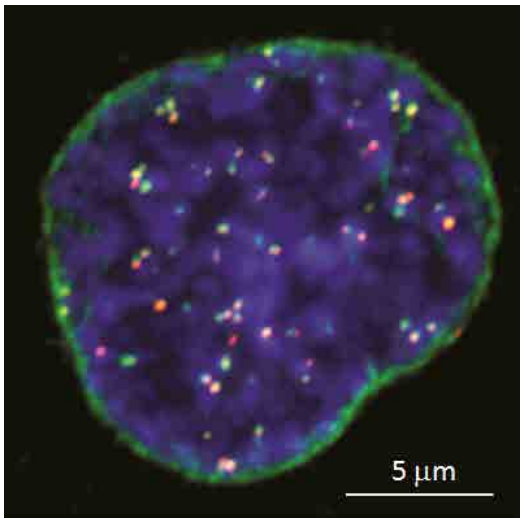


Fig. 2: A cultured human cell was stained with antibodies against two kinetochore components, Mad1 (green) and Mad2 (red); DNA was stained by the dye DAPI (4',6-diamidino-2-phenylindole; blue). Also visible is the nuclear envelope (stained by anti-Mad1 antibodies; green). Size bar: 5 µm.

At the two poles of the spindle apparatus are tiny organelles known as "centrosomes" (**Fig. 3**). The biogenesis, duplication and function of centrosomes (and their constituent centrioles) constitute a second major research focus of our laboratory. Centrosomes function to organize microtubule arrays in most animal cells and are present as only one or two copies per cell, depending on cell cycle stage. At the core of each centrosome are two microtubule-based cylindrical structures called "centrioles", embedded in a matrix of pericentriolar proteins. Deregulation of the centrosome/centriole duplication cycle is believed to constitute a major cause of chromosome mis-segregation during the development of human cancers. Furthermore, certain brain diseases (notably microcephaly) and some forms of dwarfism have been causally linked to mutations in specific centrosomal proteins. Importantly, centrioles function also as basal bodies for the formation of cilia and flagella in quiescent cells, and mutations in genes coding for centriole/basal body proteins contribute to a multitude of diseases and syndromes (ciliopathies) that reflect the absence or malfunction of the basal-body/ciliary apparatus.

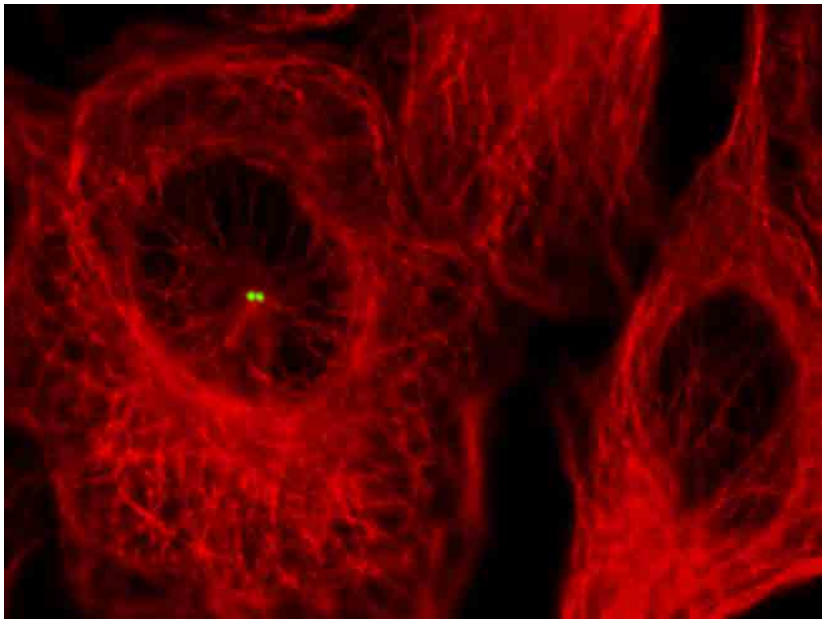


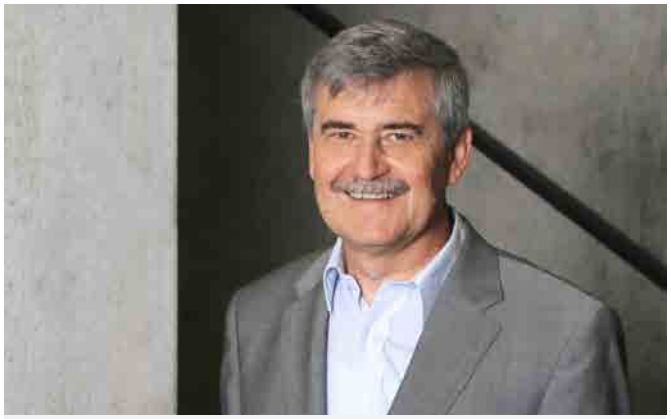
Fig. 3: Centrosomes organize microtubule arrays. A cultured human cell was co-stained with antibodies against the protein kinase Plk4, a key regulator of centriole duplication (green), and antibodies against the cytoskeletal component tubulin (red). Size bar: 5 µm.

Our laboratory combines reverse genetics (e.g. RNA interference), immunocytochemistry (including structured illumination super-resolution microscopy) and multiple biochemical techniques (notably mass spectrometry) to unravel the molecular mechanisms that ensure correct centrosome duplication and chromosome segregation in human cells. Many of our studies focus on phosphorylation (a reversible protein modification controlled by kinases and phosphatases). Studying mostly human cells in culture, we have used mass spectrometry to establish inventories of proteins and phosphorylation sites in the spindle apparatus, the kinetochore and the centrosome. More recently, we focus on the wiring of key regulatory circuits, as defined by kinases, phosphatases, and selected substrates. We anticipate that our work will lead to a better understanding of the regulation of chromosome segregation and centrosome duplication in normal cells, as well as provide insights into the deregulation of these processes in disease.

In the recent past, we have discovered and characterized several novel spindle components and proteins implicated in centriole duplication. Of particular interest is our discovery of Plk4 as a key regulator of centriole biogenesis and the demonstration that a ternary complex of Ska proteins (Ska1, 2 and 3) plays a major role in stabilizing the attachment of spindle microtubules to kinetochores. Ongoing work concerns the function and regulation of several cell cycle-regulatory kinases, including Polo-like kinases (notably Plk1 and Plk4), Aurora kinases and spindle checkpoint kinases (Mps1 and Bub1).

One major challenge in contemporary biological and biomedical research concerns the development of technologies that will permit the acquisition of quantitative information about the abundance, localization and dynamics of proteins and protein modifications under physiological conditions. We anticipate that such technologies will become increasingly important not only in systems biology but in life science research altogether. Hence, we have optimized massspectrometry based procedures (selected reaction monitoring) that allow us to monitor, in quantitative terms, the abundance of key components involved in both centrosome duplication and chromosome segregation. In parallel, we have begun to use somatic gene targeting approaches that should allow us to visualize and quantify a subset of these very same components in time and space.

The cell cycle field holds considerable promise for the development of novel therapeutic approaches. In particular, it appears legitimate to hope that new information on the mechanisms that govern chromosome segregation and cell division will contribute to the design of novel strategies to thwart cancer growth. This has been widely recognized not only in Academia, but also in the Pharmaceutical and Biotechnology industry, providing ample opportunities for collaboration and translational research.



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Research Group Jean Pieters

Role of signal transduction in pathogen persistence and lymphocyte survival

Our laboratory is investigating signal transduction processes that are involved in the body's responses towards environmental stimuli. One important part of our research aims to understand the mechanisms of immune cell activation. Also, we are interested in elucidating how pathogens cause disease despite the presence of a functioning immune system. Immune cell activation, as is the case for all eukaryotic cells, strongly depends on the triggering of cell surface receptors in order to transmit signals to diverse signaling pathways that allow the cell to respond appropriately. A major aim in the laboratory is to unravel the biochemical and molecular mechanisms involved in such signal transduction.

Coronin 1 signaling in leukocytes

A major focus in the laboratory concerns the analysis of coronin 1-dependent signaling. We originally defined coronin 1 as a host protein utilized by *M. tuberculosis* to survive within macrophages (Cell (1999) 97:435; Science (2000) 288:5471). To understand the mechanisms of action of coronin 1 as well as its normal function in leukocytes, we generated coronin 1 deficient mice. This approach allowed us to molecularly dissect how coronin 1 modulates the survival of *M. tuberculosis* inside macrophages: upon infection by *M. tuberculosis*, coronin 1 is responsible for the activation of the Ca^{2+}-dependent phosphatase calcineurin, thereby preventing mycobacterial killing within lysosomes (Cell (2007) 130:37). Recent work from the laboratory suggests that upon inflammatory stimuli, coronin 1 activates phosphoinositol (PI)-3-kinase in order to induce the rapid elimination of pathogens (Bosedasgupta and Pieters, PloS Pathogens, 2014).

Importantly, *in vivo*, coronin 1 is required for the maintenance of T lymphocytes in peripheral lymphoid organs (Mueller *et al.*, Nature Immunol., (2008), 9:424). We uncovered that coronin 1 is essential for the activation of the Ca^{2+}/calcineurin pathway following T cell activation thereby regulating T cell homeostasis (see figure). As a consequence, in both mouse and humans, coronin 1 depletion results in a profound depletion of naïve T cells (Jayachandran *et al.*, Nature Review Immunology, 2014). Interestingly, mice lacking coronin 1 are resistant towards a variety of autoimmune stimuli (Siegmund *et al.*, J. Immunol. (2011) 186:3452). Ongoing studies aim to understand the role for coronin 1 in maintaining naïve T cells.

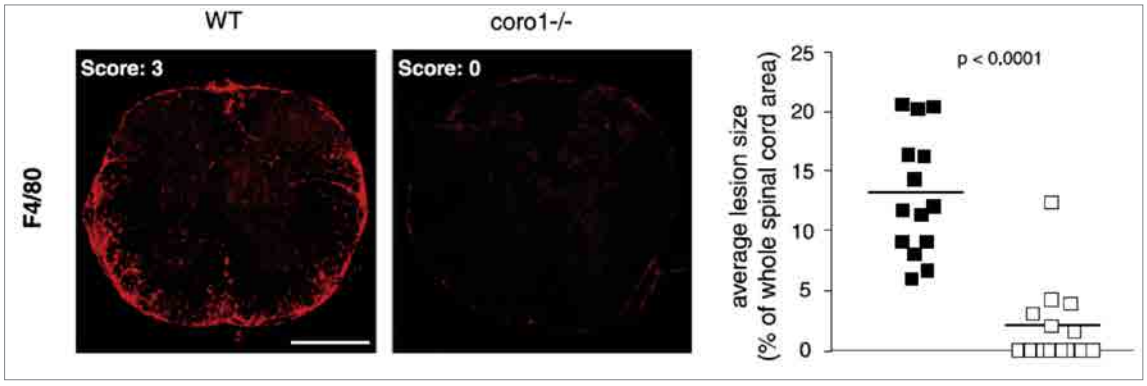

Fig. 1: The role of coronin 1 in the induction of autoimmune pathology. Coronin 1-deficient (Cor 1-/-) and wild-type (WT) mice were immunized with MOG35-55 peptide in CFA to induce active EAE. Left panels: Immunohistological analyses (F4/80) of spinal cord sections (lumbar). Right panel: Quantification of lesion sizes. From Siegmund *et al.* (2011) J. Immunol 186:3452.

Coronin 1-dependent activation of neurons

Recent work followed up on the realization that besides immune cells, also a certain class of neurons, the excitatory neurons, express coronin 1. Interestingly, coronin 1 is encoded in a genomic region associated with neurobehavioral dysfunction. In recent work we found that coronin 1 plays an important role in cognition and behavior by regulating the cyclic AMP (cAMP) signaling pathway: when cell surface receptors are activated, coronin 1 stimulates cAMP production and activation of the protein kinase A. Coronin 1 deficiency resulted in severe functional defects at excitatory synapses. Furthermore, in both mice and humans, deletion or mutation of coronin 1 causes severe neurobehavioral defects, including social deficits, increased aggression, and learning disabilities. Strikingly, treatment with a membrane-permeable analogue of cAMP restored synaptic plasticity and behavioral defects in mice lacking coronin 1. Together this work not only shows a critical role for coronin 1 in neurobehavior but also defines a new role for the coronin family in regulating the transmission of signals within cells (Jayachandran *et al*., PloS Biology, 2014).

A conserved pathway sensing cell surface stimulation?

Coronin 1 is one of seven coronin molecules expressed in mammalian cells, and whether or not there is redundancy among the different coronins remains unknown. We have recently initiated a project in which we analyze the role of the coronin 1 homologue in the lower eukaryote *Dictyostelium discoideum*, that only expresses one single short coronin isoform. It turns out that in *Dictyostelium*, coronin is required for the initiation of the developmental processes associated with starvation, that also involves activation of the cAMP/protein kinase A pathway (see Vinet *et al*., MoBC, 2014). Current work aims to unravel the molecular details of the coronin-mediated activation of the cAMP/PKA pathway leading to *Dictyostelium* multicellular development.

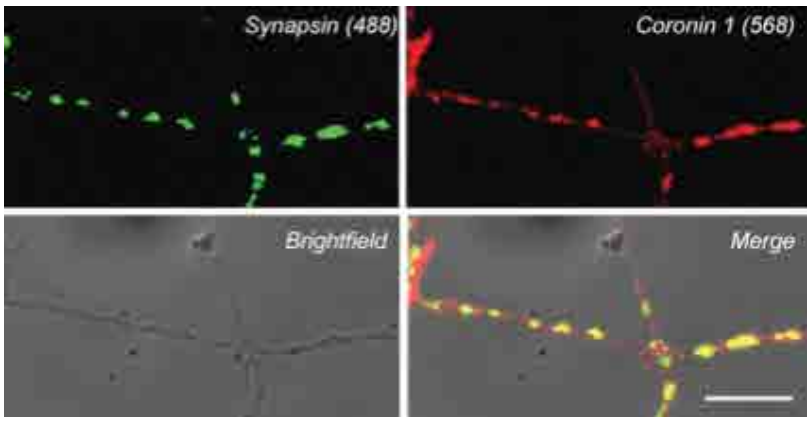

Fig. 2: Localization of coronin 1 at synapses: Coronin 1 co-localizes with synaptic markers in excitatory neurons. In such neurons of the amygdala, coronin 1 modulates activation of the cAMP/Protein kinase A pathway. As a consequence, coronin 1 depletion results in severe neurobehavioral defects. See Jayachandran *et al*., PloS Biology 2014.

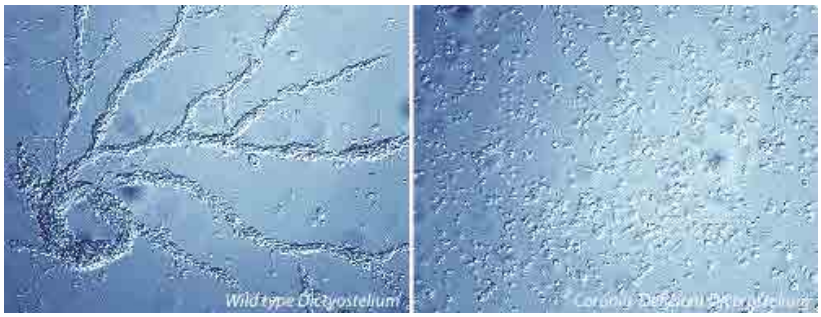

Fig. 3: In the absence of coronin, *Dictyostelium discoideum* fails to initiate multicellular aggregation upon starvation (Vinet *et al*., MoBC 2014).

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Molecular mechanisms involved in synapse formation and neuromuscular disease

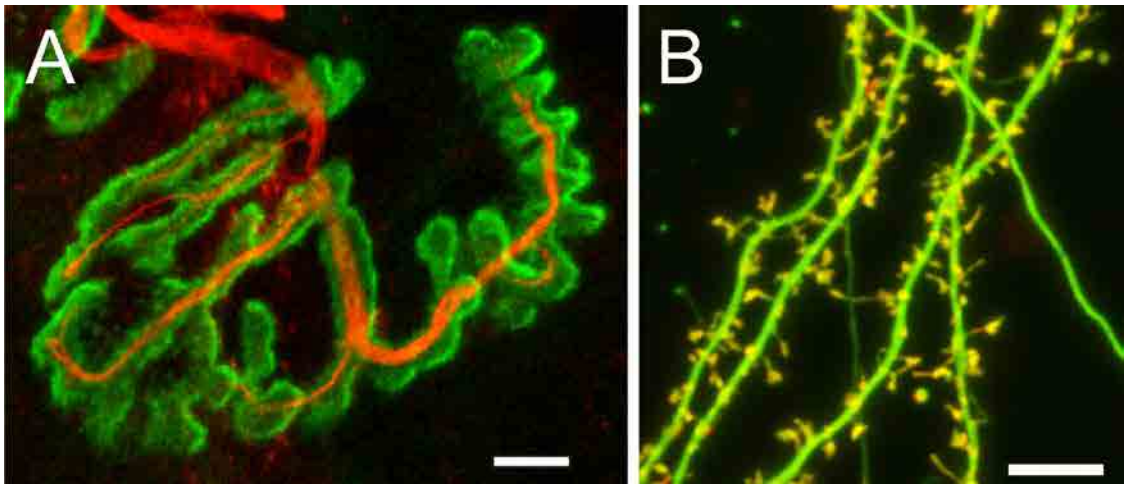

Fig. 1: Comparison of neuromuscular junctions and synapses in the brain. (A) At the NMJ, the presynaptic motor nerve, visualized by the staining with antibodies to neurofilament and synaptophysin (red), perfectly matches the postsynaptic structure, stained with an agent that binds to acetylcholine receptors. (B) At neuron-to-neuron synapses, actin (yellow) is highly enriched at postsynaptic spines on the dendrites of cultured hippocampal neurons (green). Thus, the structure of synapses is similar at the NMJ and at neuron-to-neuron synapses but there is a big difference in size. Scale bar = 10 µm.

The overall research topic concerns the molecular mechanisms important for the function and dysfunction of synapses at the neuromuscular junction (NMJ) and between neurons in the brain (**Fig. 1**). Pathological changes at the NMJ, as for example seen in myasthenia gravis (**Fig. 2**) or muscle dystrophies (**Fig. 3**), impair muscle function and can be life-threatening. Our group investigates different signaling pathways important for the establishment and function of synapses. Using this knowledge, we also try to find new ways to treat pathological alterations at the NMJ and in skeletal muscle. All these projects synergize with each other as we are using the same systems to answer related questions.

The role of mTORC1 and mTORC2 in brain and skeletal muscle

Both, neurons and skeletal muscle fibers are postmitotic and thus their growth largely depends on changes in cell size and not cell number. The mammalian target of rapamycin (mTOR), which was discovered in yeast by Michael Hall and collaborators, assembles into two multiprotein complexes called mTOR complex 1 (mTORC1) and mTORC2. The two complexes are characterized by the presence of particular proteins that are necessary for their function, such as raptor (mTORC1) and rictor (mTORC2). While mTORC1 is inhibited by the immunosuppressant rapamycin, mTORC2 is not inhibited by this drug. We are investigating the role of mTORC1 and mTORC2 in brain and skeletal muscle in mice using the Cre/loxP technology. In both tissues, deletion of mTORC1 causes the organ to be smaller. In the brain, mTORC1 is essential for brain development while mTORC2 mutants survive. In the brain, mTORC2 deficiency causes a strong decrease in its size and the mice display behavioral abnormalities. Neurons show deficits in dendritic arborization and synaptic circuitry. We are currently investigating the detailed molecular mechanisms underlying these phenotypes as well as the function of mTORC1 and mTORC2 at adult synapses.

Like in the brain, mTORC1 deletion in skeletal muscle results in a more severe phenotype than mTORC2 deletion. Here, mTORC2 deletion does not cause any overt phenotype whereas mTORC1 mutation affects the metabolism and function of skeletal muscle. Importantly, mTORC1 deficiency causes a severe myopathy and leads to the death of the mice at the age of 4 to 6 months. Moreover, the mice display a general wasting syndrome that is not restricted to skeletal muscle. Current projects study the mechanisms that trigger this wasting and investigate the contribution of mitochondrial biogenesis to the overall phenotype. In addition, we examine the effect of activating mTORC1 in skeletal muscle by the deletion of its inhibitor TSC1.

Copine 6, a novel calcium sensor involved in synapse structure

Changes in synaptic activity alter synaptic transmission and ultimately change neuronal network dynamics. Structural changes induced by electrical activity are often mediated by calcium-dependent processes. Initiated by gene expression studies at the NMJ, we focus on the function of Copine 6, a member of a small family of calcium-binding proteins. We have shown that Copine 6 affects spine formation and maintenance in hippocampal neurons. Copine 6 shuttles from the cytosol to postsynaptic sites upon NMDA receptor-dependent calcium influx. It binds to the Rho GTPase Rac1 and mediates its translocation to membranes upon calcium influx. These results strongly suggest that Copine 6 serves as a calcium sensor that links neuronal activity to the subsequent changes in synaptic structure. In the current projects, we investigate the function of Copine 6 and additional family members *in vivo* and we try to identify additional binding partners.

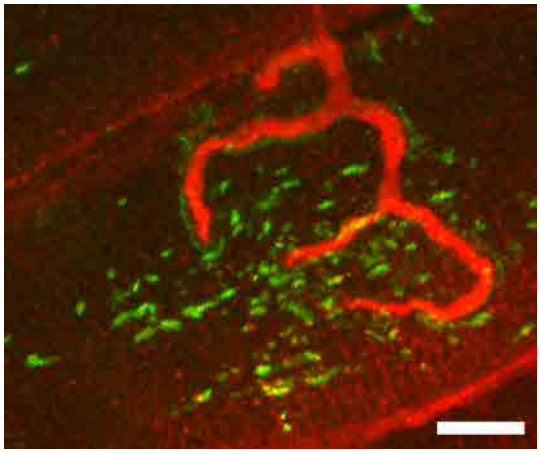

Fig. 2: Experimentally induced myasthenia gravis causes a severe fragmentation of postsynaptic structures (green) in sternomastoid muscle so that neuromuscular transmission initiated by the presynaptic nerve terminal (red) is largely abrogated. Scale bar = 10 µm.

NMJ and disease

There are sporadic and genetic neuromuscular diseases (NMDs). Although they are often severe, they affect only a small proportion of the human population and most of the diseases are still not treatable. One of the acquired NMDs is myasthenia gravis that is caused by auto-immune antibodies directed to components of the NMJ. While most of the antibodies are directed against the acetylcholine receptor, in about 10% of the cases antibodies are directed against the receptor tyrosine kinase MuSK. Interestingly, the clinical symptoms are clearly distinct between the two subgroups. We have recently shown that the phenotypic difference between the two subtypes of myasthenia gravis can also be reiterated in a murine model of experimental autoimmune myasthenia gravis (EAMG). The symptoms in MuSK-EAMG mice are a severe kyphosis, weight loss and signs of neuromuscular hyperactivity, which are all distinct from the symptoms in EAMG induced by acetylcholine receptor antibodies. At the NMJs, MuSK-EAMG causes fragmentation and often loss of innervation (**Fig. 2**). Interestingly, like in human patients, the pathological changes observed at NMJs differ between muscles. Current studies aim at elucidating the molecular mechanisms that are responsible for this difference between muscles.

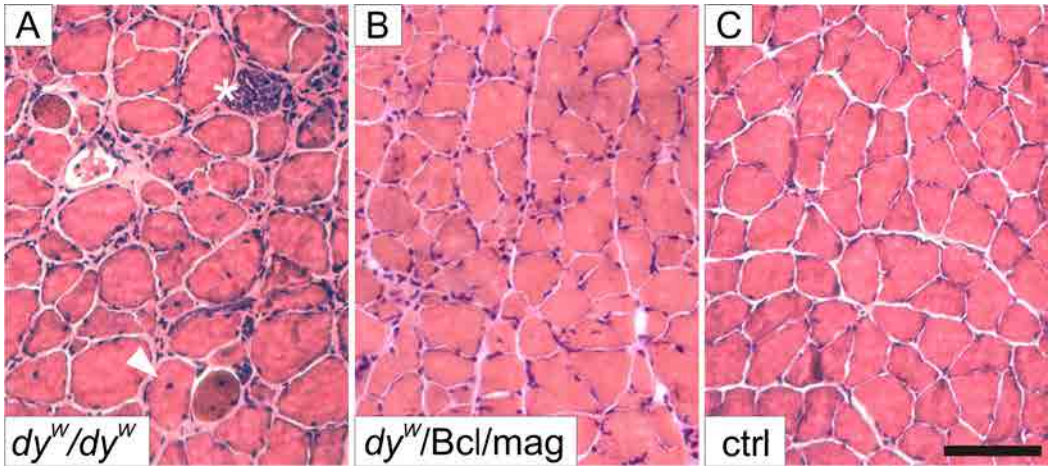

Fig. 3: Cross-sections of skeletal muscle from mice stained with hematoxilin & eosin to visualize their structure. (A) Muscle from mice suffering from a severe muscular dystrophy due to loss of laminin-211 (dyW/dyW) with signs of degeneration (white arrowhead) and fibrosis (asterisk). (B) The muscle from mice with the same muscular dystrophy that also express Bcl2 and mini-agrin (dyW/Bcl/mag) is much improved and looks like muscle from healthy, wildtype mice (ctrl; C). Scale bar = 100 µm.

Another set of NMDs are muscular dystrophies, where the skeletal muscle fibers and not the NMJs are affected. Although the mutations causing muscular dystrophies are distinct, the severe loss of muscle mass due to the degeneration of muscle fibers is common to all diseases. As a consequence, muscle becomes replaced by fibrotic tissue (**Fig. 3A**). In one project we develop new methods to treat some of the muscular dystrophies in experimental mouse models. For example, we have recently shown that transgenic expression of a miniaturized form of the extracellular matrix molecule agrin (mini-agrin) can substantially compensate for the loss of laminin-211. This compensation is even more complete when cell death of muscle fibers is prevented by additionally expressing the anti-apoptotic protein Bcl2 (**Fig. 3B**) or treating mice with the anti-apoptotic agent omigapil. Such combination therapy restores the structure of the muscle to control levels (**Fig. 3C**). In current projects we test additional pharmacological agents for their potential to ameliorate the disease.

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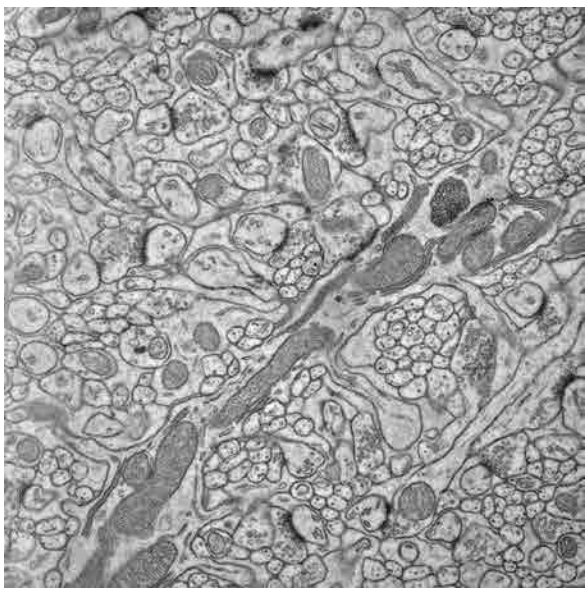
Research Group Peter Scheiffele

Neuronal circuit assembly and synapse formation

The goal of research in the Scheiffele Lab is to understand molecular mechanisms underlying the formation of neuronal circuits in health and disease. Synapses are the key processing units in neuronal circuits. Therefore, we are examining mechanisms of synapse formation and synaptic re-arrangements in the central nervous system. We are exploring the trans-synaptic signals that coordinate the choice of synaptic partners, assembly of synaptic junctions and stabilization of appropriate contacts.

Coupling of postsynaptic neurotransmitter complexes to synaptic adhesion molecules

Synaptic adhesion molecules have important roles in organizing synaptic structures. In the past years we have focused on one pair of synaptic adhesion molecules called the neuroligin-neurexin complex which spans the synapse and contributes to the organization of pre- and postsynaptic membrane compartments. In cell biological studies we identified a novel mode of lateral coupling between neuroligins and neurotransmitter receptors in the postsynaptic membrane. We demonstrated that neuroligin-1 recruits NMDA-type glutamate receptors through interactions via the extracellular domains of the protein. These interactions are critical for physical retention of a pool of NMDA-receptors at glutamatergic synapses *in vivo* and regulate NMDA-receptor-dependent synaptic plasticity in the mouse hippocampus (Budreck *et al.*, *PNAS*, 2013). These findings highlight the possibility that neurotransmitter receptors and adhesion molecules assemble into complexes that have structural roles at central synapses.



Synapses in the mouse cerebellum.

Molecular diversification of recognition molecules by alternative splicing

Neuronal networks in the mammalian brain represent one of the most complex examples of a highly organized biological system. The finite number of protein-coding genes in the human genome severely limits the genetic resources that can be employed for generating molecular diversity. Therefore, highly polymorphic cell surface receptor families arising from extensive alternative splicing provide attractive candidates for neuronal recognition. Neurexins are highly polymorphic synaptic cell surface receptors that are extensively modified by alternative splicing. Alternative splice variants of neurexins differ in biochemical interactions with neuroligins and other binding partners and may underlie an adhesive code at central synapses. We discovered that neurexin alternative splicing is regulated by neuronal activity. The KH-domain RNA-binding protein SAM68 binds directly to the neurexin-1 pre-mRNA and is essential for activity-dependent splicing regulation (Iijima *et al.*, Cell, 2011). SAM68-like proteins (SLM1 and SLM2) exhibit highly selective expression patterns in interneuron populations in the mouse brain. These findings provide an entry point to unraveling the cell type-specific neurexin repertoires and their contribution to neuronal connectivity.

Synaptic defects in autism-spectrum disorders

Autism-spectrum disorders are amongst the most heritable neurodevelopmental disorders known to date. Human genetic studies conducted over the past 10 years have led to the identification of several candidate genes that may confer susceptibility to autism but also environmental risk factors might exist. The study of neuronal circuit alterations in autism has been most advanced for monogenic forms of syndromic autism, such as Fragile X and Rett's Syndrome, where specific alterations in synaptic transmission have been identified. We focused our studies on a mouse model of a non-syndromic form of autism, carrying a mutation in the synaptic adhesion molecule neuroligin-3. Using a combination of electrophysiological, anatomical, and behavioral studies we identified a remarkable convergence in the synaptic pathophysiology in neuroligin-3 knock-out mice and a rodent model of Fragile X, characterized by a defect in metabotropic glutamate recep-

tor-dependent synaptic plasticity. Importantly, the synaptic defects could be reversed by re-expression of neuroligin-3 in adult animals highlighting a substantial reversibility of the neuronal phenotypes in this model (Baudouin *et al.*, Science, 2012). In ongoing studies we are now testing pharmacological interventions in transgenic mouse and rat models of autism to identify treatment strategies for the disorder.

Emergence of synaptic specificity in the pontocerebellar projection system

A key question in neural development is how axons choose their appropriate synaptic partners. We performed a detailed anatomical analysis to unravel how target specificity of ponto-cerebellar mossy fiber projections emerges during development. We observed that mossy fibers form transient synapses with Purkinje cells (an "inappropriate target") before precise connectivity with granule cells is established. We discovered that Purkinje cell-derived bone morphogenetic protein 4 (BMP4) acts as a retrograde signal that drives the destabilization of mossy fiber contacts (Kalinovsky *et al.*, PLoS Biology, 2011). Interestingly, the bone morphogenetic protein signaling pathway continues to be active in the adult cerebellum. Therefore, we are now examining functions of this signaling system in learning-dependent plasticity in mature cerebellar circuits.

Publications 2015

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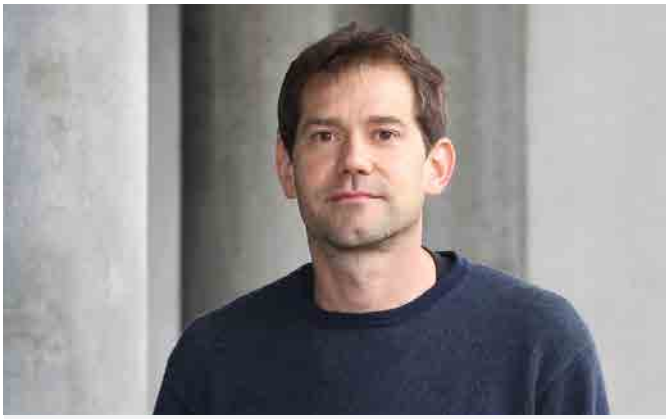

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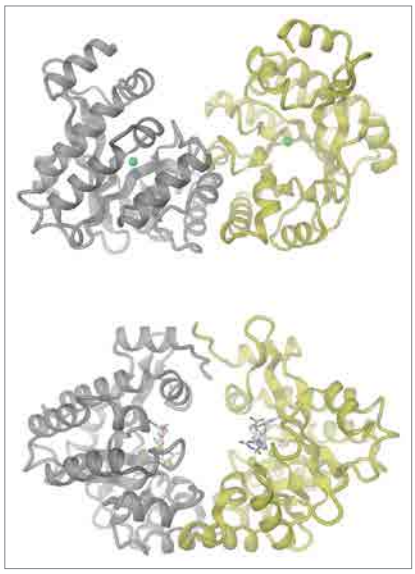
Molecular mechanisms of c-di-GMP signal transduction and AMP transferases

We are employing crystallographic and biochemical/ biophysical techniques to reveal the structural basis for the catalysis and regulation of c-di-GMP related proteins. Our second focus is on bacterial type IV secretion system (T4SS) effector proteins with AMP transferase activity.



Close of the EAL dimer of YahA (*E. coli*) induced by binding of c-di-GMP. Adopted from Sundriyal *et al.*, 2014.

Make, break and recognition of c-di-GMP

Recent discoveries show that a novel second messenger, c-di-GMP, is extensively used by bacteria to control multicellular behavior, such as biofilm formation. Condensation of two GTP to the dinucleotide is catalyzed by GGDEF domains that usually occur in combination with sensory and/or regulatory modules. The opposing phosphodiesterase activity is provided by EAL domains that are similarly regulated.

In collaboration with the Jenal group (Biozentrum) and based on crystallographic and functional studies we have studied the catalytic and regulatory mechanisms of diguanylate cyclases, the enzymes that synthesize the second messenger. It appears that the general mechanism of activation relies on signal induced dimerisation of its regulatory domains that ensures productive of the two GTP loaded catalytic GGDEF domains. Currently, we are studying the molecular basis of phosphodiesterase regulation that are the antagonistic enzymes that degrade c-di-GMP. Although the active site is completely contained with their EAL domain, the domain is active only as a homodimer. This generic property of the catalytic domain is probably utilised in many of the full-length proteins to control their activity, very similar to the situation in diguanylate cyclades.

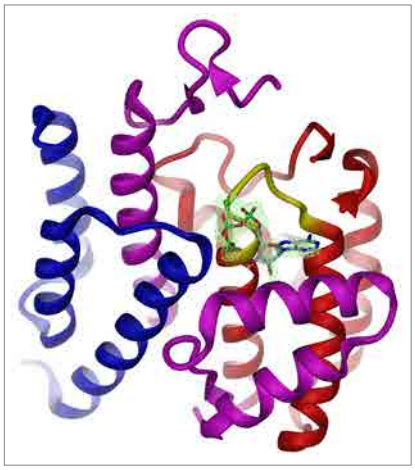
Thus our results provide clues about how this class of enzymes can be regulated in a modular and universal fashion by their sensory domains.

Recently we have started to elucidate the structures and binding modes of newly discovered c-di-GMP receptors. Furthermore, we are studying c-di-GMP regulated histidine kinases that thus provide a link between two-component and c-di-GMP signaling. The investigations will contribute to our knowledge of the complete c-di-GMP signal cascade.

Regulation of AMP transferases with FIC fold

Type IV secretion systems (T4SS) are utilized by many bacterial pathogens for the delivery of virulence proteins or protein-DNA complexes into their eukaryotic target cells. Together with the Dehio group (Biozentrum) we are working on a class of effector proteins that are composed of a FIC and a BID domain responsible for pathogenic action in the host cell (AMPylation of specific target proteins) and translocation, respectively.

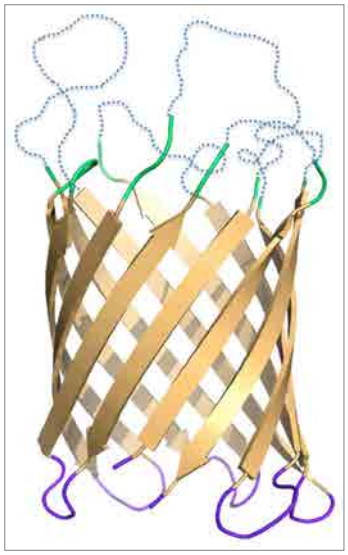
Based on crystallographic analyses, we have found that FIC proteins are expressed in an inhibited form and are, thus, catalytically silent under normal circumstances. Inhibition is caused by partial obstruction of the ATP binding site by a helix that, depending on the Fic class, is provided by a cognate anti-toxin or is part of the enzyme itself. For the latter class, we are currently investigating the mechanism of auto-inhibition relief. Furthermore, we are interested in the structural basis of target recognition and, particular, target specificity. This knowledge may be utilized for drug development to target FIC proteins of bacterial pathogens.



Crystal structure of Fic protein VbhT in complex with anti-toxin VbhA (blue). Also shown is the ATP substrate (with electron density). Adopted from Engel *et al.*,2012.

Porins

Porins are integral membrane proteins from the outer membrane of Gram-negative bacteria. They allow the uptake of nutrients by passive diffusion through an intrinsic pore that extends along the axis of the transmembrane β-barrel structure. After extensive work on the general trimeric porins OmpF and OmpC from *E. coli*, we have recently determined the high-resolution 12-stranded β-barrel structures of NanC from *E. coli* and KdgM from *Dickeya dadantii*, representatives of a porin family that is specific for the translocation of negatively charged poly-saccharides.



KdgM porin folded to a small 12-stranded hollow β-barrel. Adopted from Hutter *et al.*, 2014.

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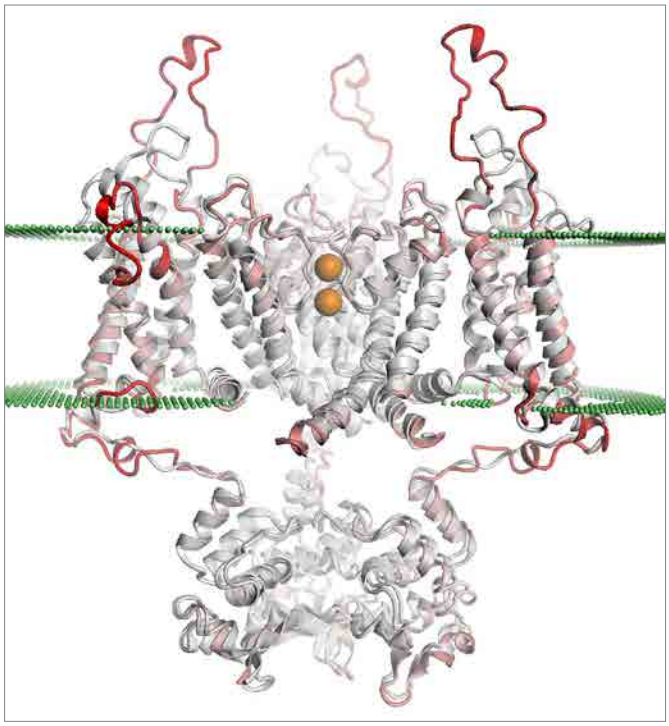
Research Group Torsten Schwede

Computational structure biology

Protein structure modeling

The main interest of my group is the development of methods and algorithms for molecular modeling and simulations of three-dimensional protein structures and their interactions. One of the major limitations for using structure-based methods in biomedical research is the limited availability of experimentally determined protein structures. Prediction of the 3D structure of a protein from its amino acid sequence remains a fundamental scientific problem, and it is considered as one of the grand challenges in computational biology. Comparative or homology modeling, which uses experimentally elucidated structures of related protein family members as templates, is currently the most accurate and reliable approach to model the structure of the protein of interest. Template-based protein modeling techniques exploit the evolutionary relationship between a target protein and templates with known experimental structures, based on the observation that evolutionarily related sequences generally have similar 3D structures.

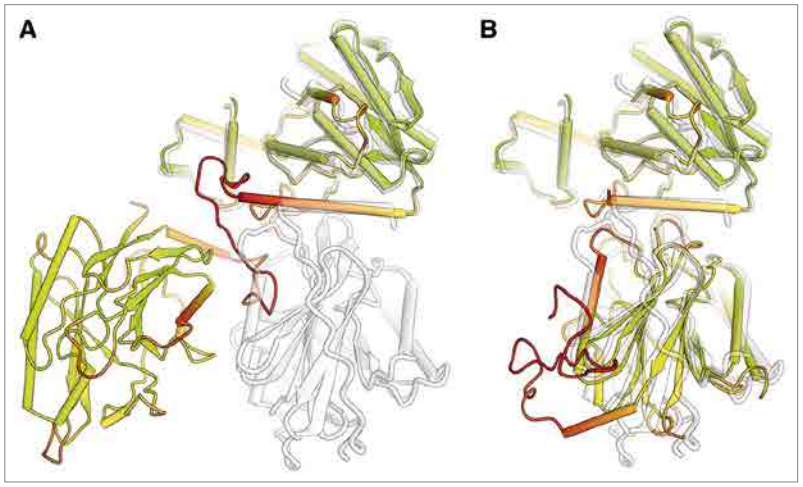


Comparative model of a Potassium channel in comparison with the corresponding crystal structure. The model is colored by a membrane-specific mean force potential. (Image generated with OpenStructure).

The SWISS-MODEL expert system developed by our group is a fully automated web-based workbench, which greatly facilitates the process of computing of protein structure homology models. SWISS-MODEL currently counts more than 300 000 registered users and calculates about 1 model per minute. Up to today SWISS-MODEL relevant publications have been cited more than 15 000 times by biomedical researchers world-wide.

Mean force potentials for model quality estimation

Ultimately, the quality of a model determines its usefulness for different biomedical applications such as planning mutagenesis experiments for functional analyses or studying protein-ligand interactions, e.g. in structure based drug design. The estimation of the expected quality of a predicted structural model is therefore crucial in structure prediction. Especially when the sequence identity between target and template is low, individual models may contain considerable errors. To identify such inaccuracies, scoring functions have been developed which analyze different structural features of the protein models in order to derive a quality estimate. To this end, we have introduced the composite scoring function QMEAN, which consists of four statistical potential terms and two components describing the agreement between predicted and observed secondary structure and solvent accessibility. We have shown that QMEAN can not only be used to assess the quality of theoretical protein models, but also to identify experimental structures of poor quality. Specific potentials for transmembrane regions are required for the correct assessment of TM proteins such as receptors and channels. As membrane proteins play crucial roles in many biological processes and are important drug targets, QMEANBrane further extends our approach to membrane protein structures.



Comparison of a predicted two-domain protein structure model (colored according lDDT score) with its reference structure (shown in gray). The model is shown in full length (A), with the first domain superposed to the target. For graphical illustration, (B) shows the two domains in the prediction separated and superposed individually to the target structure. (Bioinformatics, 2013, 29:2722-2728).

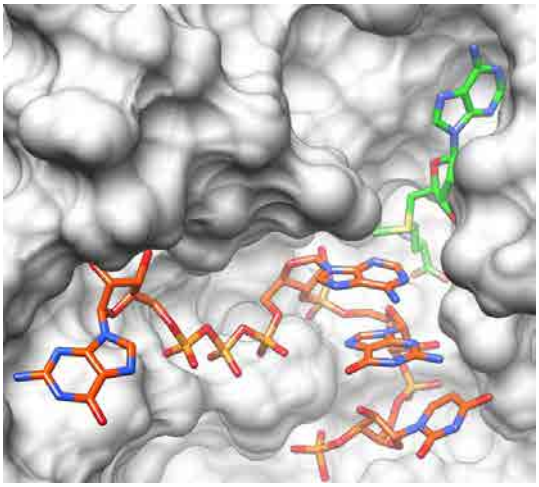
CASP and CAMEO: Critical assessment of structure prediction methods

Methods for structure modeling and prediction have made substantial progress of the last decades, but still fall short in accuracy compared to high-resolution experimental structures. Retrospectively assessing the quality of a blind prediction in comparison to experimental reference structures allows benchmarking the state-of-the-art in structure prediction and identifying areas which need further development. The Critical Assessment of Structure Prediction (CASP) experiment has for the last 20 years assessed the progress in the field of protein structure modeling based on predictions for ca. 100 blind prediction targets which are carefully evaluated by human experts. The "Continuous Model EvaluatiOn" (CAMEO) project aims to provide a fully automated blind assessment for prediction servers based on weekly pre-released sequences of the Protein Data Bank PDB. CAMEO requires the development of novel scoring methods such as lDDT, which are robust against domain movements to allow for automated continuous operation without human intervention. CAMEO is currently assessing predictions of 3-dimensional structures, ligand binding sites, and model quality estimation.

Molecular modeling of Dengue virus RNA methyltransferase

Dengue fever is a viral disease that is transmitted between human hosts by *Aedes mosquitoes*, particularly *Aedes aegyptii*. According to the CDC, dengue virus is a leading cause of illness and death in the tropics and subtropics, with more than onethird of the world's population are living in areas at risk for infection, and as many as 400 million people are infected yearly. There are not yet any vaccines to prevent infection with dengue virus and the most effective protective measures are those that avoid mosquito bites. One of the viral proteins encoded in the Dengue genome, RNA methyltransferase (MTase), appears as interesting target for the development of novel inhibitors of Dengue virus as it is necessary for virus replication. In a public-private partnership with Schrodinger (New York) and the Novartis Institute for Tropical Diseases in Singapore, we have used a structure based virtual screening approach to identify novel inhibitors of Dengue virus.

In order to better understand the catalytic mechanism of the MTase, we applied a diverse set of computational methodologies as well as experimental isothermal titration calorimetry (ITC) based assays. Based on a structural model of the enzyme bound to the RNA substrate and the SAM cofactor, we establish an in-silico protocol to identify the effect of single point mutations. The protocol employs MD simulations to analyze effects on the geometric arrangement between cofactor, substrate and active site residues, an MM-GBSA approach to analyze cofactor binding free energies and mixed QM/MM simulations to estimate activation barriers. With this knowledge, we hope to facilitate the rational development of novel inhibitors against dengue fever and related diseases caused by flavivirus and we believe that our protocol gives valuable contributions for future drug resistance predictions.



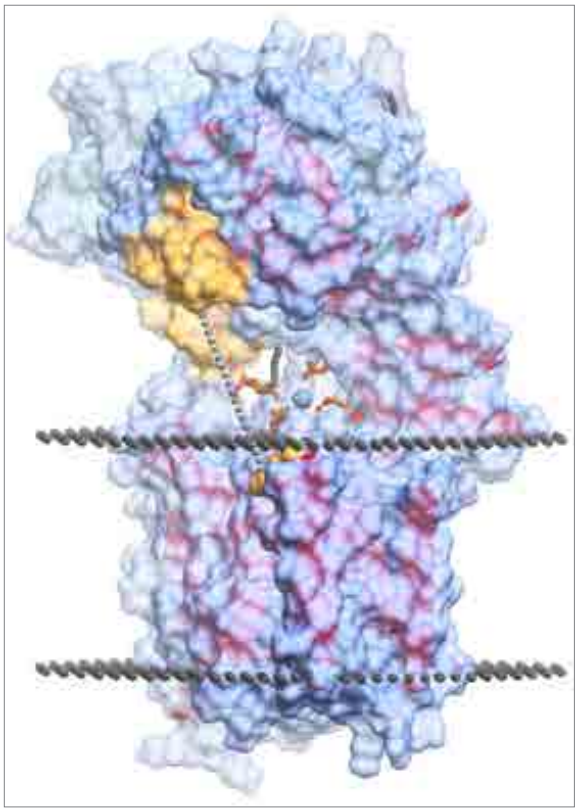
Model of the viral RNA bound to the Dengue virus RNA methyltransferase structure.

Structure-guided protein engineering and *in vitro* evolution of enzymes

Three-dimensional models of proteins are valuable tools for the design of protein engineering and *in vitro* evolution experiments. In the following, some exemplar projects involving molecular modeling of protein-ligand interactions at different levels of model resolution are briefly presented.

Conjugate vaccines in which polysaccharide antigens are covalently linked to carrier proteins belong to the most effective and safest vaccines against bacterial pathogens. The current production process of conjugate vaccines is a laborious, chemical multi-step process. The discovery of N-glycosylation in bacteria allows for protein glycosylation in recombinant bacteria by expressing the N-oligosaccharyltransferase PglB of *Campylobacter jejuni* in *Escherichia coli*. We are collaborating with GlycoVaxyn AG (Schlieren) and EMPA (St. Gallen) on a project funded by the KTI on structure-guided protein engineering of PglB in order to improve the efficiency of in *vivo* synthesis of novel and well characterized immunogenic polysaccharide/protein complexes for use in vaccines.

Other projects involve studying Zinc-selective inhibition of the promiscuous bacterial amide-hydrolase DapE and the implications of metal heterogeneity for evolution and anti-biotic drug design (Marc Creus), or the design of protein kinases with altered substrate specificity.



The N-oligosaccharyltransferase PglB is used for linking polysaccharide antigens to carrier proteins in the recombinant production of conjugate vaccines.

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Principles of intracellular organization

Asymmetry is an inherent property of most cells. Proteins and mRNA have to be distributed at specific cellular locales to perform their assigned function or to be translated in a spatially and temporally regulated manner. Although the localization of mRNAs is restricted to the cytoplasmic face of intracellular organelles or the plasma membrane, proteins and lipids have to be localized to these organelles to provide a platform on which mRNAs and/or proteins can be recruited and form a domain or a small compartment. In general this compartmentalization is achieved by intracellular transport through exocytic (secretory pathway) and endocytic avenues. Communication between different organelles is maintained in large parts by transport vesicles and direct contact sites. While the transport vesicles transport proteins and lipids, the contact sites are places for ion and lipid exchange. These different kinds of communications not only are essential for cell asymmetry establishment and maintenance they further allow to mount a rapid cellular response in case of stress.

Our research interests center around questions like: how intracellular traffic regulates cellular asymmetry and stress response. How do RNA localization, translation and metabolism contribute to asymmetry and defense against stressors. To answer these questions we take advantage of different model systems such as yeast, *C. elegans* and tissue culture to enable us to study these problems from the single cell to organ and animal level.

The regulation transport along the secretory pathway

In order to understand how microdomains or compartments are formed on different organelles and the plasma membrane, we have to understand how proteins reach their specific compartments. Therefore, we study on one hand the formation of transport containers at different levels of the secretors pathway. Since the formation of transport containers is mostly dependent on the small GTPase Arf1, we study its regulation through ArfGEFs and ArfGAPs (Ackema *et al.*, 2013, 2014, Estrada *et al.*, 2014, Spang *et al.* 2010, Spang 2009, 2013). Activated Arf1 will interact with adaptor complexes and coat components that will on one hand recruit the cargo that needs to be transported and on the other hand deform the donor membrane to allow the generation of a transport vesicle. We have identified a novel adaptor complex, exomer, which only transport a subset of proteins from the trans-Golgi network (TGN) to the plasma membrane (Trautwein *et al.*, 2006, Zanolari *et al.*, 2010, Rockenbauch *et al.*, 2012, Spang 2015, Huranova *et al.*, in press). Yet the cargoes are all exquisitely regulated in terms polarized localization through the cell-cycle and upon stress response (Zanolari *et al.*, 2010, Ritz *et al.*, 2014). One of the cargoes, Pin2, contains a prionlike domain, which serves as a TGN retention signal, in particular under stress conditions (Ritz *et al.*, 2014). The prion-like aggregates may also serve to sequester other cargo proteins, hence providing a novel mechanism for compartmentalization and domain formation.

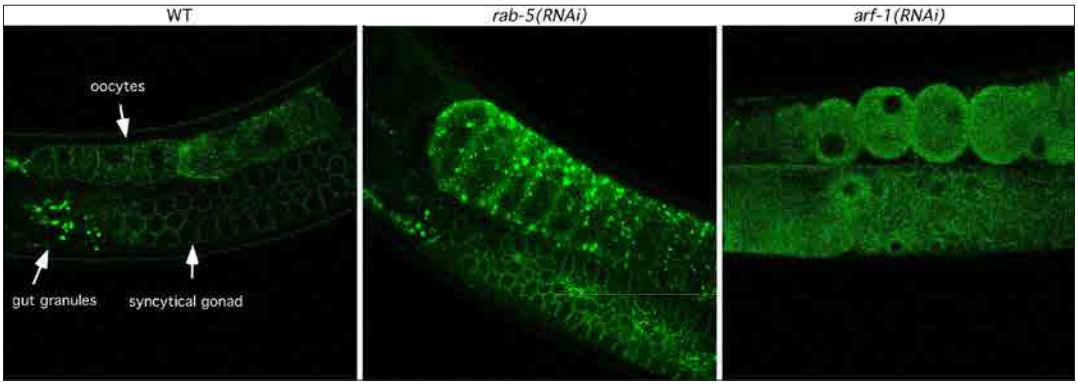

Fig. 1: The architecture of the Golgi is disturbed upon knockdown of the small GTPases RAB-5 and ARF-1. Worms expressing the Golgi marker UGTP-1::GFP (green) under the pie-1 promoter, which drives expression in the gonad and in early embryos were subjected to RNAi by feeding. The distribution of UGTP-1::GFP was analyzed by confocal microscopy. The Golgi morphology was greatly altered upon RNAi against RAB-5 and ARF-1. In particular, arf-1(RNAi) let to a dispersal of Golgi structures.

The regulation of early-to-late endosomal transport

However not only export to the plasma membrane is essential for maintaining cell polarity and asymmetry, the endocytic route plays an equally important role. We have identified a factor, SAND-1/Mon1, which acts as a switch in early to late endosome maturation by on one hand displacing the GEF for the early endosomal GTPase RAB-5, RABX-5, from early endosome and by actively recruiting the late endosomal GTPase RAB-7 onto the maturing endosome (Poteryaev and Spang 2005, Poteryaev *et al*., 2007, 2010). With these results, we demonstrated the existence of endosome maturation. But then the next question is how is maturation regulated? Rab conversion from RAB-5 to RAB-7 positive endosomes is an important but not the only step along the maturation process. We are studying currently how recycling and sorting, pH and lipid changes, interactions with the cytoskeleton, incoming cargo from the TGN and signaling pathways influence endosome maturation and how these different processes are coordinated. Recently, we demonstrated that the SM subunits of the HOPS and CORVET tethering complexes (Solinger and Spang, 2013) are important to control the flow through the endocytic pathway (Solinger and Spang, 2014). This project led us also to the discovery of a novel set of tethering complexes in the endocytic pathway.

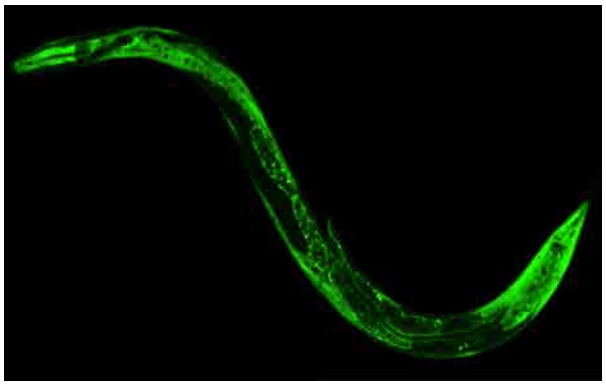

Fig. 2: Adult worm expressing SAND-1::GFP.

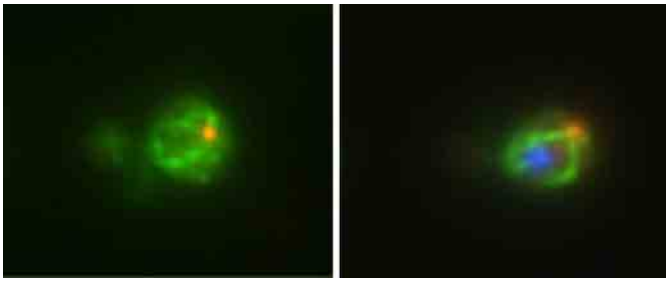

Fig. 3: Asymmetrically localized mRNA to the distal pole of yeast cells. FISH/IF picture of 2 yeast cells. mRNA is in red, actin in green and DNA in blue.

The regulation of mRNA metabolism and transport

This research direction was inspired by our finding that the poly A binding protein, Pab1, associates with Arf1 and COPI vesicles in an mRNA-dependent manner and that Arf1 is required for ASH1 mRNA localization to the bud tip of yeast cells (Trautwein *et al*., 2004). The subsequent analysis allowed us to identify the first distal pole-localized mRNA in yeast (Kilchert and Spang, 2011). We performed screens to identify mRNAs that are restricted to certain sites in the cell, and we are currently investigating the mechanism of the localization of a subset of mRNAs. Moreover, we realized that arf1 mutant caused the formation of processing bodies (Pbodies), which are the main mRNA degradation platform in yeast (Kilchert *et al*., 2010). Interestingly, the P-bodies formed in arf-1 and other secretory pathway mutants were morphologically distinct from the ones formed under glucose starvation and required signaling through Ca^{2+}/calmodulin. This led to the hypothesis that P-bodies may sequester a distinct set of mRNAs depending on the stressor. To test this hypothesis, we established a protocol that allowed us to purify Pbodies and to determine the RNA content through RNAseq. Likewise, we have established ribosome profiling to be able to get a systems view on how the cell regulated its proteome through mRNA degradation and translation under various conditions. This latter part was based on previous findings that polysome-associated proteins negatively control P-body formation under normal growth conditions (Weidner *et al*., 2014).

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Publications 2015

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Research Group Martin Spiess

Topogenesis and intracellular sorting of membrane proteins

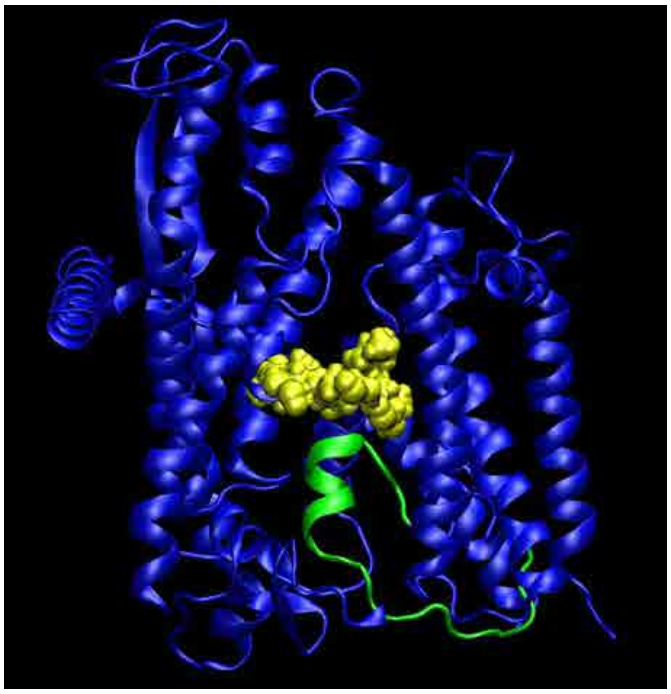

Fig. 1: "One ring to rule them all": View of the interior of the closed Sec61 translocon with the plug domain in green and the constriction ring in gold (Sefer Baday).

Proteins synthesized on cytosolic ribosomes must be sorted to the specific compartment(s) in which they perform their function. Proteins of the endoplasmic reticulum (ER), the Golgi apparatus, the plasma membrane, the endosomal/lysosomal system and the cell exterior are all first targeted to the ER, translocated across the membrane or inserted into the lipid bilayer, and then distributed via the secretory pathway. Our research focuses on (1) how membrane proteins are inserted into the ER membrane and acquire a defined topology, and (2) how transport vesicles are formed at the trans-Golgi or endosomes, or *in vitro* from purified components and liposomes. In close collaboration with Jonas Rutishauser, we furthermore study the mechanism by which trafficking mutants of provasopressin cause dominant Diabetes insipidus.

Topogenesis of membrane proteins

Hydrophobic signal or signal-anchor sequences target newly synthesized proteins to the translocon in the ER membrane. By systematic mutation of substrate proteins, we analyze the determinants that define their orientation in the membrane and the mechanism by which topogenesis occurs. Signal hydrophobicity, flanking charges, and the position of the signal within the protein determine the mode of insertion as well as the final orientation in the membrane.

The translocon is a compact helix bundle that forms a pore for protein translocation and a lateral gate for the integration of transmembrane segments. In its empty state, the pore is closed by a lumenal plug domain and a hydrophobic constriction ring (**Fig. 1**). By random or targeted mutagenesis, we explore the contributions of the translocon to signal acceptance and orientation, as well as to transmembrane domain integration. The plug domain stabilizes the closed state of the translocon and defines the stringency of signal recognition, whereas the constriction ring defines the hydrophobicity threshold for membrane integration. In collaboration with Dominic Höpfner (Novartis), we identified novel fungal translocation inhibitors by chemogenomic profiling and selected for resistant translocon mutants to study the inhibitors' mode of action.

Post-Golgi protein sorting

Endosome identity, morphology, and transport are regulated by rab GTPases and their effectors. We are studying the role of rabaptin-5, an effector of rab4 and rab5, that associates with rabex5, the exchange factor of rab5. Based on mutational analysis, rabaptin-5 is found to control endosome morphology without affecting transferrin transport (determined by automated microscopy) in a manner that is incompatible with the prevailing model of rab5 feed-forward loop.

Little is known about how proteins exit the *trans*-Golgi. We use sulfation, a *trans*-Golgi-specific modification, to characterize the exit pathway and kinetics to the cell surface. If necessary, proteins of interest are tagged to introduce tyrosine-sulfation sites or short sequences for the attachment of (heavily sulfated) glycosaminoglycans (GAG). In this manner, we found GAG-attachment to accelerate exit kinetics and to change the exit pathway of model proteins. Similarly, the proteoglycan form of the amyloid precursor protein exits in a manner distinct from that of GAG-free splice variants.

Sulfation is a *trans*-Golgi-specific modification useful to study post-Golgi traffic. To introduce sulfation sites, we have tagged proteins with short sequences for the attachment of (heavily sulfated) glycosaminoglycans (GAG). Interestingly, GAG attachment was found to affect protein traffic by inhibiting endocytosis and by accelerating *trans*-Golgi-to-cell surface transport both for secretory and membrane proteins. We are analyzing the mechanistic and physiological implications for proteoglycan sorting. In endocrine cells, prohormones and granins are sorted at the *trans*-Golgi network into dense-core secretory granules by an entirely different mechanism. We found expression of granule cargo to be sufficient to generate granule-like structures in nonendocrine cells. Deletion analysis of chromogranin A showed that the same segments that are required for granule sorting in endocrine cells produce granule-like structures in fibroblasts. The results support the notion that self-aggregation is at the core of granule formation and sorting into the regulated pathway.

Diabetes insipidus: a degenerative trafficking disease

Autosomal dominant neurohypophyseal Diabetes insipidus results from mutations in the precursor protein of the hormone vasopressin. Mutant precursors are retained in the ER of vasopressinergic neurons and cause cell degeneration. We discovered that pro-vasopressin mutants form disulfide-linked oligomers and develop large, fibrillar aggregations in fibroblast and neuronal cell lines (**Fig. 2**). Purified mutant pro-vasopressin spontaneously formed fibrils *in vitro*. Dominant Diabetes insipidus thus belongs to the group of neurodegenerative diseases associated with fibrillar protein aggregates. We identified the vasopressin nonapeptide in the precursor sequence to be primarily responsible for aggregation in the ER, i.e. the same sequence that had been proposed to be responsible for amyloid aggregation into secretory granules at the *trans*-Golgi. The sequence physiologically important for cargo aggregation into the regulated secretory pathway thus is responsible for pathological aggregation of mutant precursors in the ER.

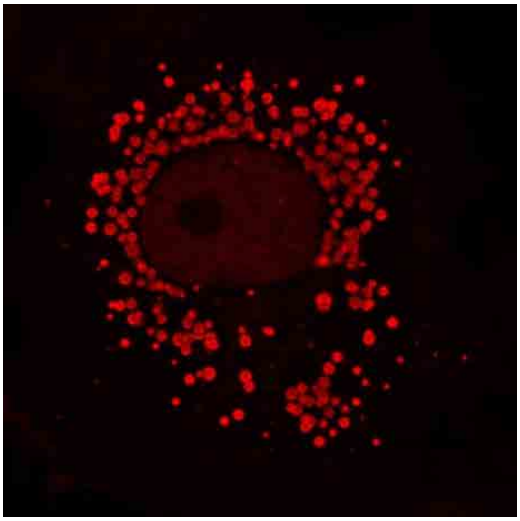

Fig. 2: Pro-vasopressin aggregates in COS-1 cells.

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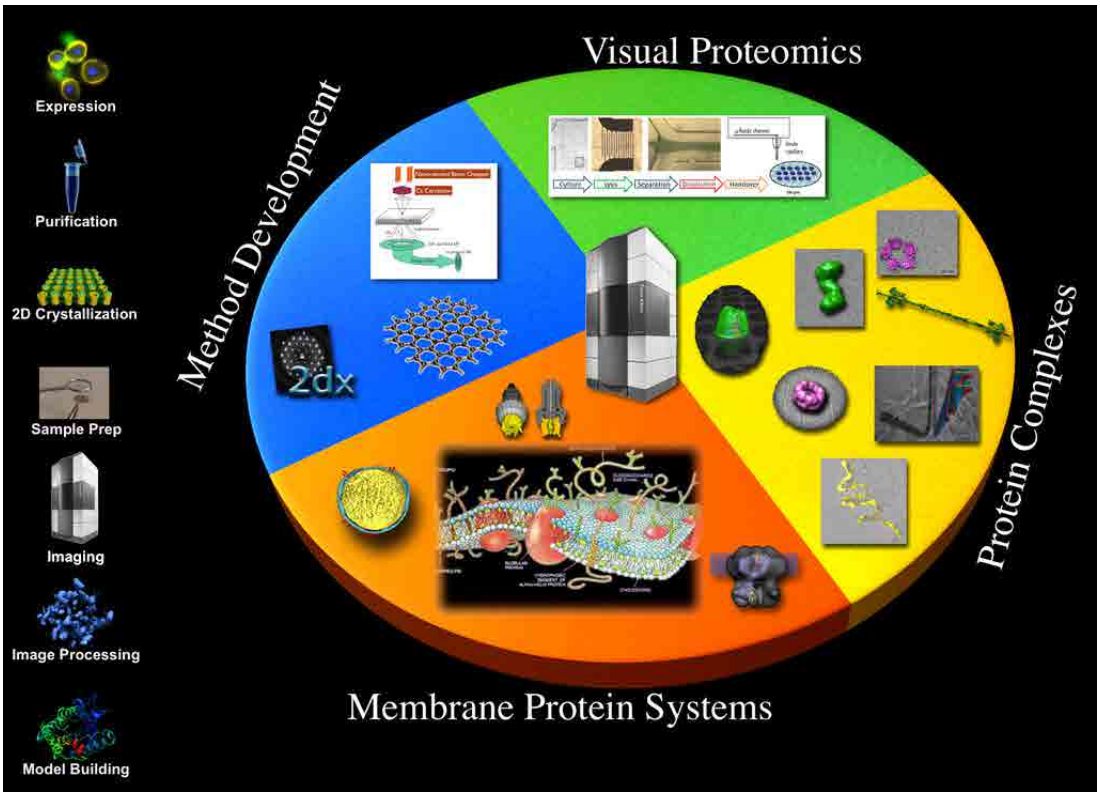
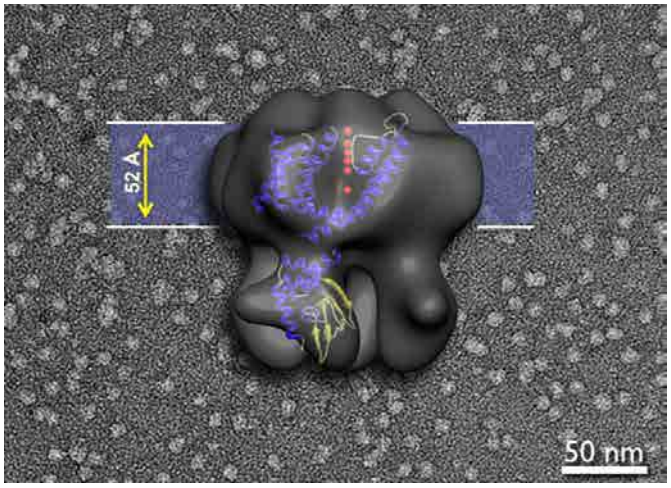


C-CINA studies Membrane Protein Systems and also other protein complexes. We develop methods for sample preparation, microscopy hardware, and image analysis software. In a systems-biology project for Visual Proteomics we are developing a tool for the cellular total content analysis by microscopy and ther methods.

The Center for Cellular Imaging and Nano Analytics (C-CINA) is studying membrane protein systems at different size and length scales with a combination of various light and electron microscopy methods. C-CINA is located in the D-BSSE building in the northern part of Basel, and is supported by the Swiss systems biology initiative SystemsX.ch.

The Stahlberg group studies biological membranes and the contained membrane proteins at several length scales and resolution levels. We use fluorescence light microscopy, and combine the obtained localization information about fluorescentically labeled protein complexes or viruses with Serial Blockface Scanning Electron Microscopy (SBF-SEM), in collaboration with the Friedrich Miescher Institute. SBF-SEM can characterize the 3D structure at 20nm resolution of large specimen areas of thousands of human cells at a time, thereby extending light microscopy to higher resolution. We also employ electron tomography (ET) in a transmission electron microscope, to study small specimens like individual bacteria at even higher resolution.

To this end, C-CINA operates an FEI Titan Krios transmission electron microscope (TEM), which is one of the worlds most advanced high-resolution electron microscopes for the study of biological specimens. Further instruments in C-CINA include atomic force microscopes and a scanning transmission electron microscope (STEM). The latter is used to determine the mass-distributions of biological particles, which are adsorbed to ultra-thin carbon films and freeze-dried. We apply these different methods to the same specimens, enabling correlative light and electron microscopy (CLEM). The study of biological specimens at different levels of resolution and scale allows understanding the biological system at the cellular, molecular and submolecular level.



The cyclic nucleotide gated potassium channel MloK1 is studied in C-CINA by single particle electron microscopy and by cryo-EM of two-dimensional membrane crystals.

Visual proteomics

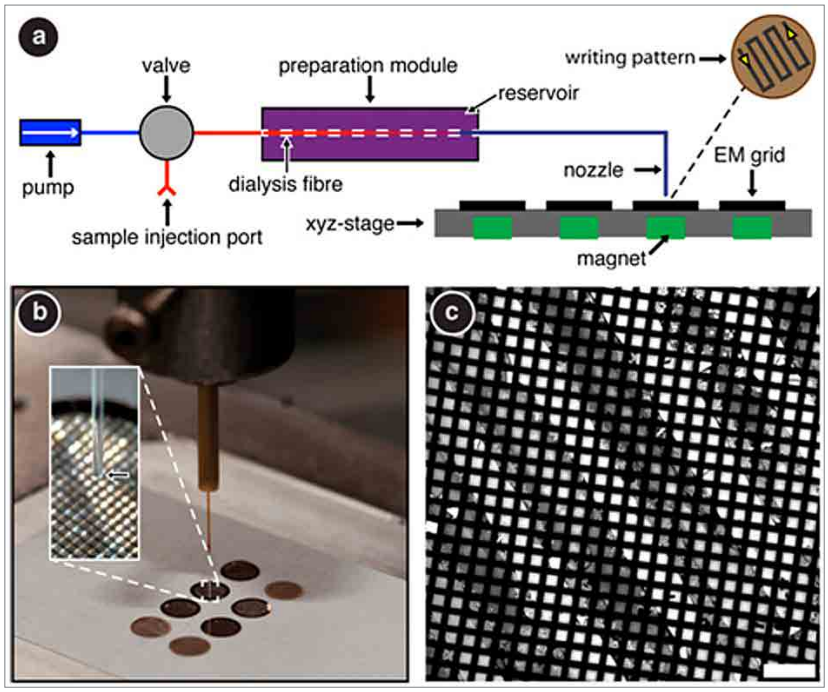
As part of the SystemsX.ch funded project, we develop a visual proteomics platform to study the 3D structure, and size and mass distribution of the proteome of a biological cell. In collaboration with the Hierlemann group at the D-BSSE, we develop a microfluidics setup to pick individual cells, lyse and fractionate them, and cross-link the cytosolic content of a single cell, which is then stained and deposited on a TEM grid for automated 3D imaging to obtain structural information about the entirety of the proteome. Samples can also be freeze-dried and their mass analyzed by the scanning transmission electron microscope (STEM). This platform will also be combined with mass spectrometry in collaboration with the Zenobi laboratory at the ETHZ, and with the multi-array optical tweezers system developed in the Vogel laboratory at the EPFL.

Membrane proteins

Membrane proteins are of central importance for health and disease. We study the high-resolution structure of membrane proteins by electron crystallography, and also characterize the arrangement of larger membrane protein complexes or the dynamic conformation of certain membrane protein systems in the biological membrane by multiresolution microscopy, including electron tomography. In collaboration with Crina Nimigean, Cornell University, NY, USA, Joe Mindell, NIH Bethesda, USA, and Horst Vogel, EPFL, Lausanne, Switzerland, we study the structure and function of gated ion channels, transporters, and receptors by single particle EM and electron crystallography.

Software development

We are also developing software for the computer evaluation of the recorded data. We distribute a software package called 2dx for the computer image processing of 2D crystal images of membrane proteins (available at http://2dx.org). This MRC-based software is now used by over 400 external users, and features a user-friendly graphical user interface, and optionally fully automatic image processing, merging, and 3D structure reconstruction. In collaboration with Niko Grigorieff, Brandeis University, MA, we have developed a maximum-likelihood module, so that high-resolution structures of membrane proteins can also be determined in the absence of large well-ordered 2D crystals. We have developed a software algorithm for projective constraint optimization, to improve the resolution of the reconstruction, also in the direction perpendicular to the viewing direction of the microscope (effectively filling the so-called missing cone). We are also developing software solutions for the structure analysis of *in vivo* membrane protein systems by electron tomography, by enabling user-friendly tomographic high-contrast reconstructions and tomographic molecular structure averaging.



The visual proteomics imaging platform has as its central part an automated sample staining and desalting module for mico-patterning of electron microscopy grids. (a) Schematic representation of the main components and the meander-type writing pattern. (b) Nozzle positioned above an EM grid (enlarged inset, arrow indicates the nozzle tip) on the xyz-stage. (c) TEM image of a micropatterned grid showing a section of the six 200-300-µm-wide lines of a stained sample (dark grey) diagonally to the EM grid (black) with empty carbon film in between (bright grey). Scale bar, 200 µm.

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Remodeling of basal ganglia circuitries in Parkinson's disease

Voluntary movements are orchestrated by a complex network of central nervous system nuclei, the basal ganglia. Among these nuclei, the substantia nigra pars reticulata (SNr) is the output nucleus; receiving, integrating and transferring information to diverse specific output regions. As such the SNr represents a crucial node in the processing of motor control. This ability depends on the SNr anatomical organization, its cellular, morphological, and functional connectivity with both the input basal ganglia nuclei and other diverse output targets, as well as its synaptic plasticity ability, which allows flexibility when integrating motor signals. This implies that such a complex circuit organization relies on specific subcircuits and each of them may be responsible for a precise aspect of motor tasks.

SNr circuitry

The heterogeneity of GABAergic neurons has been well described in the cortex or in the hippocampus. However the SNr has been neglected so far. Identifying and characterizing each cell population to understand its role in the dynamic of the overall basal ganglia is crucial to assess its impact at the level of motor behavior.

Our strategy relies on the use of cell type-specific transgenic mouse lines combined with viral-mediated gene delivery (optogenetic tools). This allows the identification and manipulation of one SNr neuron subtype at a time. *In vitro* and *in vivo* electrophysiology is performed to assess the local SNr functional connectivity as well as the input and output partners. Imaging techniques (confocal and electron microscopy) are valuable tools to further confirm neuronal identity and connectivity. We are developing motor-based behavioral tests that when combined with optogenetic stimulation of SNr neuronal subpopulations provide us with powerful means to assess their specific role in a precise motor task.

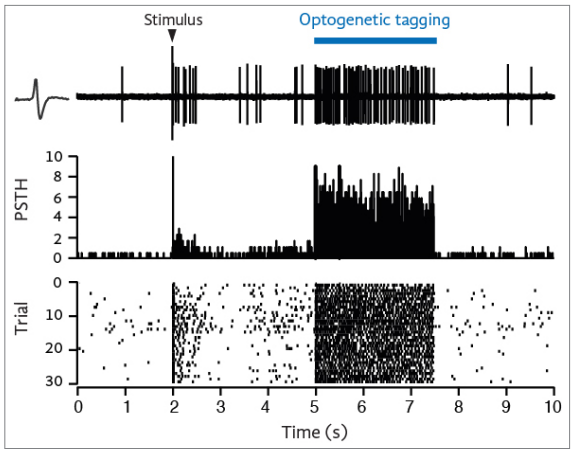

Fig. 1: *In vivo* recording of an optogenetically-identified neurone.

Dopamine neuromodulation

Dopamine is a key modulator of synaptic function in the basal ganglia. It is produced by dopamine neurons of the substantia nigra pars compacta and released in different nuclei of the basal ganglia, including the substantia nigra pars reticulata. Such neuromodulation plays a major role in amplifying or decreasing the activity of specific subcircuits with the famous direct and indirect pathways, leading to coordinated locomotion. Here we aim at refining our knowledge and understanding of these two pathways considering the heterogeneity of the SNr.

Remodelling of the SNr circuitry in pathological conditions

In Parkinson's disease, the degeneration of dopamine cells leads to a loss of dopamine neuromodulation modifying neuronal morphology and their connectivity, which impacts synaptic transmission. Such reorganization drives the typical clinical motor symptoms of the disease, specifically a difficulty in initiating movements, resting tremor, stiffness, slowing of movement and postural instability. We are using a chemically induced mouse model of Parkinson's disease. A complete investigation of the alterations induced after dopamine depletion will provide a ground to develop *in vivo* optogenetic manipulations in order to reverse or compensate the behavioral motor symptoms.

This study of the SNr circuitry will offer insights into the still poorly understood physiological mechanisms linking cell-type specificity and synaptic function to basal ganglia network activity and behavior. In addition, the study of alterations/remodeling within this system in a model of Parkinson's disease will provide detailed knowledge of the cellular basis of motor disorders, which may lead to novel therapeutic strategies.

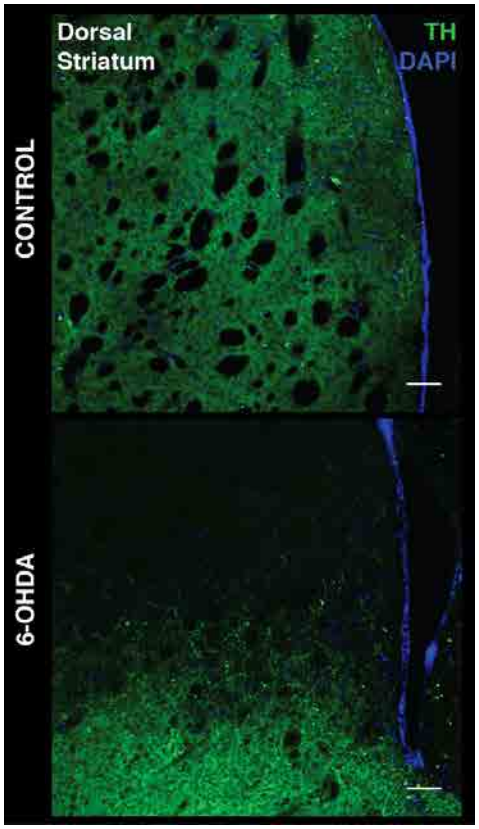

Fig. 2: Chemically-induced dopamine neurone degeneration.

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Structure, function, and evolution of genome-wide regulatory networks

Most projects that we pursue concern the functioning and evolution of genome-wide regulatory systems in organisms ranging from bacteria to humans. The type of large-scale questions that we aim to address include understanding how regulatory systems are integrated on a genome-wide scale, how regulatory networks are structured, how these systems control and potentially exploit the inherent noise in gene regulatory processes, how stable cell types are defined and maintained, how gene regulation evolves, and understanding under what conditions regulatory complexity can be expected to increase in evolution. Another major topic of interest in our group is the discovery and analysis of quantitative laws of genome evolution. We are particularly interested in using high-throughput genomic data to put theories of genome evolution on a strictly empirical footing. Our group pursues both theoretical/computational and experimental approaches and our projects can be roughly divided into "dry lab" projects in which we develop regulatory genomics tools for studying gene regulatory networks in higher eukaryotes, and "wet lab"projects in which we study gene regulation at the single-cell level in *E. coli*.

Regulatory genomics tools: from binding site constellations to genome-wide regulatory programs

In spite of the large volumes of data gathered by high-throughput measurements technologies in recent years, our knowledge and understanding of the genome-wide regulatory networks that control gene expression remains extremely fragmentary. We are still very far from being able to do meaningful quantitative modeling of these gene regulatory networks. Consequently, and understandably, the focus in most of the regulatory genomics community has remained firmly on data gathering efforts. In our opinion, this continuing primacy of experimental data gathering has led to a fairly careless attitude toward data analysis methods in many studies. We feel that the lack of rigorous and robust methodologies for interpreting high-throughput data is currently a major stumbling block in regulatory genomics research, and our group aims to help remedy this situation by developing analysis tools that use methods of the highest rigour, that can be automatically applied to raw data-sets, and that provide robust predictions with concrete biological interpretations. We typically strive to make our tools available through automated interactive web-services, thereby empowering experimental researchers to perform cutting-edge analysis methods on their own data.

For over a decade we have been developing Bayesian probabilistic methods that combine information from high-throughput experiments (e.g. RNA-seq, ChIP-seq) with comparative genomic sequence analysis. Very roughly speaking, our projects concern identifying regulatory sites genome-wide in DNA and RNA, understanding how constellations of regulatory sites determine binding patterns of transcription factors and, ultimately, gene expression and chromatin state patterns. Finally, we aim to develop quantitative and predictive models that describe how dynamic interactions between transcriptional and post-transcriptional regulators implement gene regulatory programs that define cellular states and the transitions between them.

With regards to the predictions of regulatory sites in DNA and RNA, we have been working on extending the well-known position-specific weight matrix models of transcription factor (TF) binding specificity into dinucleotide weight tensor models that take arbitrary dependencies between pairs of positions into account. We have also been developing a completely automated procedure, called CRUNCH for analysis of ChIPseq data, including comprehensive downstream motif analysis of binding regions. Using CRUNCH we have analyzed large-collections of ChIP-seq datasets in humans, mouse, and Fly, and have been using these to curate comprehensive sets of regulatory motifs in these model organisms. All our genome-wide regulatory site predictions are available in various formats through our SwissRegulon database and genome browser.

To take a first step toward modeling how constellations of regulatory sites determine genome-wide expression patterns we developed an approach, called Motif Activity Response Analysis (MARA), that models the expression of each gene as a linear function of the binding sites that occur in its promoter, and unknown "motif activities" that represent the condition-dependent activities of the regulators binding to these sites. Since the original presentation of this approach, in the FANTOM4 collaboration with the RIKEN Institute in Yokohama, Japan, we have been working both on completely automating the MARA approach and on extending it in a number of ways, including using MARA to model chromatin dynamics in terms of local constellations of regulatory sites. MARA is now available through a fully automated webserver, called ISMARA (integrated system for motif activity response analysis), available at ismara.unibas.ch , where users can perform automated motif activity analysis of their micro-array, RNA-seq, or ChIP-seq data, simply by uploading raw data (**Fig. 1**). The system has already been successfully used to predict key regulatory interactions in substantial number of studies, and we are working on various further improvements and extensions of the system. This includes extension to additional model organisms such as *Drosophila* and *E. coli*, signifi-

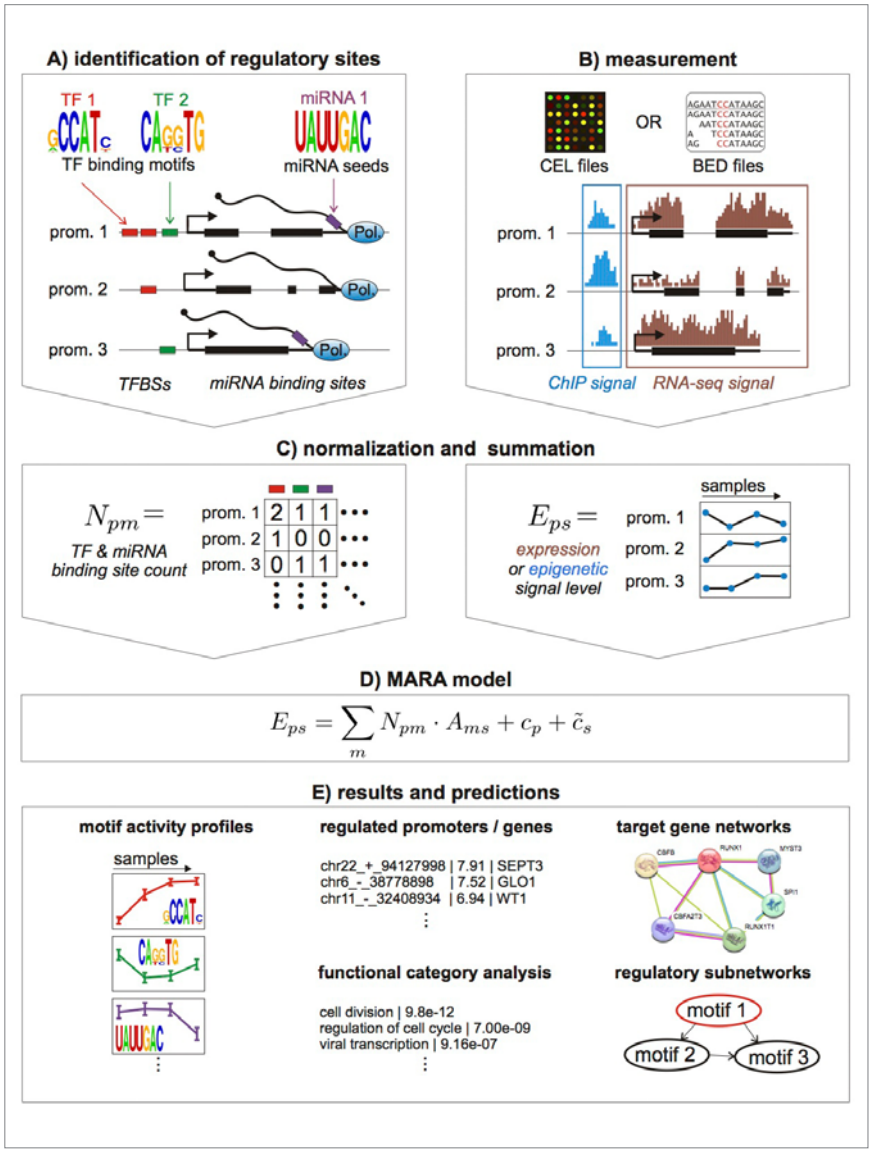

Fig. 1: Outline of the Integrated System for Motif Activity Response Analysis. A: ISMARA starts from a curated genome-wide collection of promoters and their associated transcripts. Transcription factor binding sites (TFBSs) are predicted in proximal promoters and miRNA target sites are annotated in the 3' UTRs of transcripts associated with each promoter. B: Users provide measurements of gene expression (micro-array, RNA-seq) or chromatin state (ChIP-seq). The raw data are processed automatically and a signal is calculated for each promoter and each sample. C: The site predictions and measured signals are summarized in two large matrices. The matrix N contains the total number of sites for each motif m in each promoter p and the matrix E contains the signal associated with each promoter p in each sample s. D: The linear MARA model is used to explain the signal levels in terms of regulatory sites and unknown motif activities, which are inferred by the model. E: As output, ISMARA provides the inferred motif activity profiles of all motifs across the samples sorted by the significance of the motifs. A sorted list of all predicted target promoters is provided for each motif, together with the network of known interactions between these targets and a list of Gene Ontology categories that are enriched among the predicted targets. Finally, for each motif, a local network of predicted direct regulatory interactions with other motifs is provided.

cantly extending the set of regulatory motifs that it uses, and incorporation of distal cis-regulatory modules. In addition, several of ISMARA's recent applications involve systems that are highly medically relevant and we plan to adapt ISMARA in ways that aim to increase its medical relevance. In particular, we want to extend ISMARA to allow it to infer the effects of single nucleotide polymorphisms on gene expression and regulatory programs genome-wide. Finally, we are currently working to adapting ISMARA to be able to analyze data from single-cell RNA-seq experiments.

Gene regulatory dynamics at the single-cell level in bacteria

Since 2010 our group also includes a wet lab component where we study gene regulation at the single-cell level in *E. coli*. We are particularly interested in stochastic aspects of gene regulation at the single-cell level, how fluctuations in the physiological state of the cell couple to gene expression fluctuations, how stochastic fluctuations propagate through the regulatory network, and the role of stochasticity in the evolution of gene regulation.

In the first major wet lab project of our lab, we set out to study how natural selection has shaped the noise characteristics of *E. coli* promoters. In particular, using FACS selection in combination with error-prone PCR, and starting from a large collection of random sequences, we evolved a collection of synthetic promoter sequences in the lab. Surprisingly, we found that these synthetic promoters generally exhibit noise levels that are lower than those of most native *E. coli* promoters. In particular, native promoters that are known to be regulated by one or more TFs tend to exhibit elevated noise levels. Since our synthetic promoters were selected solely on their mean expression and not on their noise properties, this allowed us to conclude that native promoters of regulated genes must have experienced selection pressures that caused their noise levels to increase.

To explain these observations we developed a new theory for the evolution of gene regulation that calculates the "fitness"of a promoter as a function of its coupling to transcriptional regulators and the noise levels of these regulators (Fig. 2). This analysis shows that noise propagation from regulators to their target genes can often be functional, acting as a rudimentary form of regulation. In particular, whenever regulation has limited accuracy in implementing a promoter's desired expression levels, selection favors noisy gene regulation. The theory provides a novel framework for understanding when and how gene regulation will evolve, and shows that expression noise generally facilitates the evolution of gene regulatory interactions.

The results of this project suggest that gene expression noise to a significant extent results from propagation of noise from regulators to their target genes. This in turn implies that noise levels should be condition-dependent and we are currently investigating how noise levels change as growth conditions change.

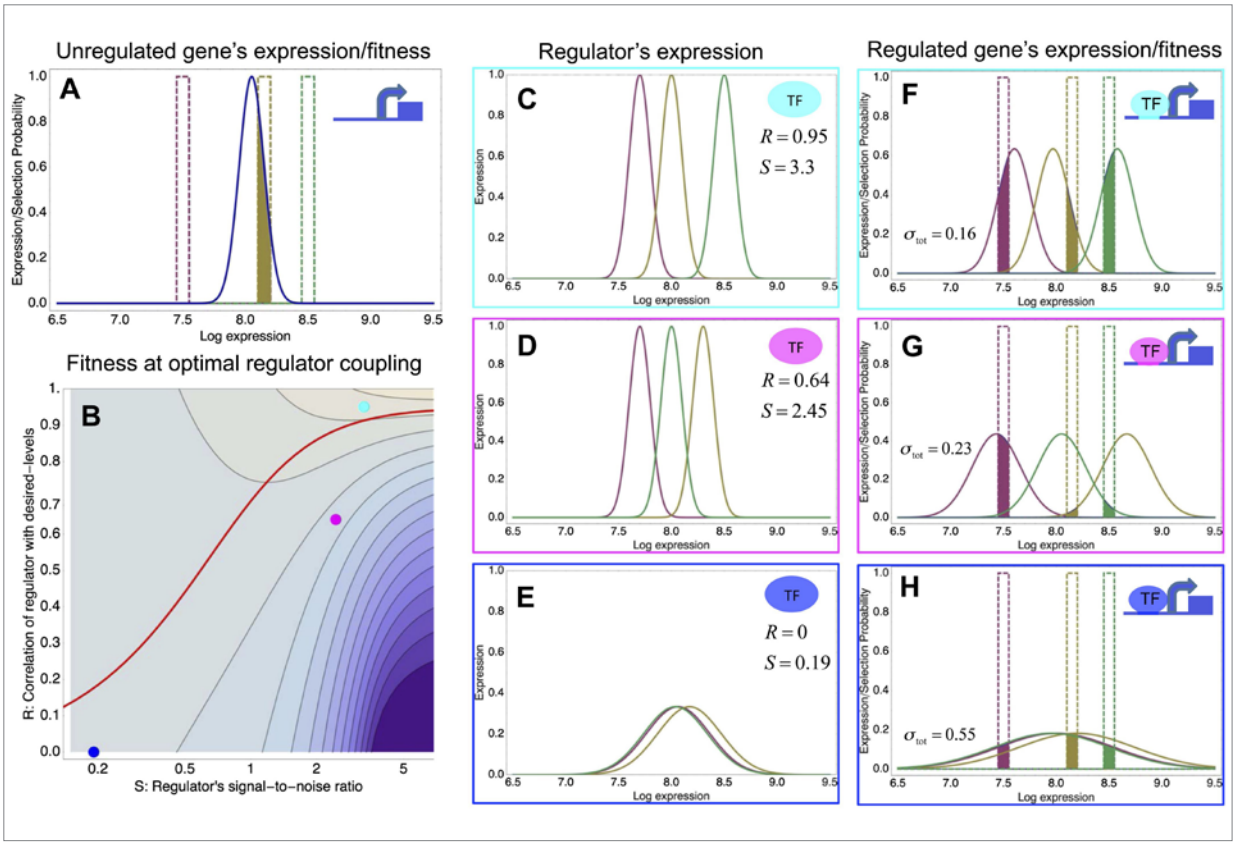

Fig. 2: A model of the evolution of gene expression regulation in a variable environment. (A) Expression distribution of an unregulated promoter (blue curve) and selected expression ranges in three different environments, that is, the red, gold, and green dashed curves show fitness as a function of expression level in these environments. The fitness of the promoter in the gold environment is proportional to the shaded area. (B) Contour plot of the log-fitness change resulting from optimally coupling the promoter to a transcription factor (TF) with signal-to-noise ratio S and correlation R. Contours run from 7.5 at the top right to 0.5 at the bottom right. The three colored dots correspond to the TFs illustrated in panels C–H. The red curve shows optimal S as a function of R. (C–E) Each panel shows the expression distributions of an example TF across the three environments (red, gold, and green curves). The corresponding values of correlation R and signal-to-noise S are indicated in each panel. (F–H) Each panel shows the expression distributions across the three environments for a promoter that is optimally coupled to the TF indicated in the inset. The shaded areas correspond to the fitness in each environment. The total noise levels of the regulated promoters are also indicated in each panel. The unregulated promoter has total noise σ tot = 0.1.

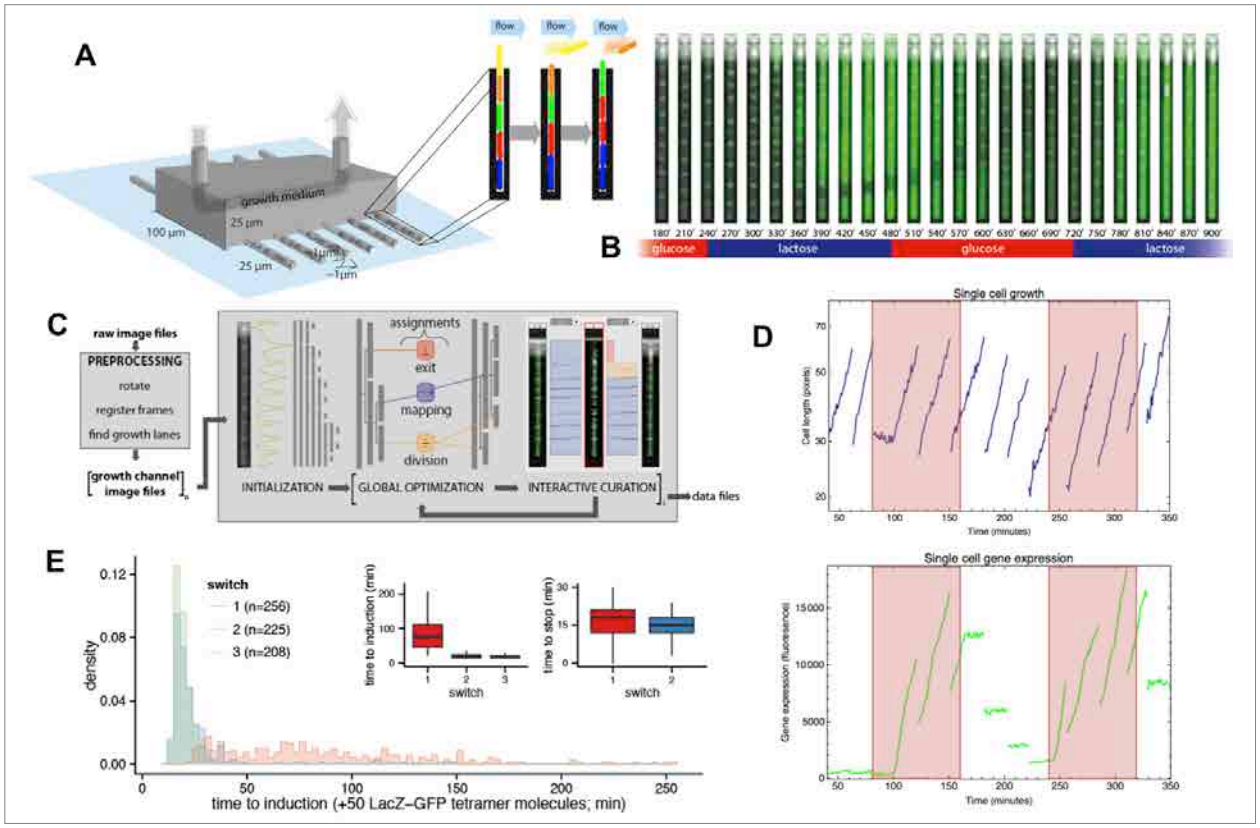

Fig. 3: A microfluidic setup for tracking growth and gene expression in lineages of single *E. coli* cells across dynamically changing growth conditions. A: The mother machine microfluidic device containing a main flow channel and dead-end growth channels in which *E. coli* cells grow. Our "dial-a-wave" modified design of this mother machine device, allows arbitrary switching between and mixing of two input media. B: Snap shot images of *E. coli* cells, carrying a GFP reporter construct reporting expression from the lac operon, in one growth channel of our microfluidic device. Snap shots are 30 minutes apart with time increasing from left to right, and the growth medium switching between glucose and lactose every 4 hours. C: Outline of the Mother Machine Analyzer software which segments the images and tracks the cells. D: Examples of the cell size (top, shown on a logarithmic scale) and gene expression (bottom) of a lineage of single cells across the time course. Note the temporary halt in growth after the first switch from glucose to lactose. Note also how, upon switching to lactose, lac operon expression is induced after an initial stochastic waiting period, and how protein production ceases during growth in glucose, with expression levels approximately halving at each cell division. E: Distribution of the lag times for lac induction for the first (red), second (blue), and third (green) change to lactose. The insets show the summary of lag times for lac operon induction (left) and for lac expression to cease upon switching to glucose (right).

A microfluidic framework for studying single-cell gene expression and growth dynamics

Beyond studying single-cell gene expression using flow cytometry, we aim to directly track both the growth and gene expression dynamics of single-cells as they are experiencing changing growth conditions. To this end we have worked on establishing a micro-fluidic setup in our lab that allows us to track growth of single cells and expression of fluorescent reporters in these cells using time-lapse microscopy. Our novel design is an extension of the so-called Mother Machine design (**Fig. 3**), which allows us to dynamically mix different growth media, thereby allowing us to arbitrarily vary the growth conditions that the cells experience. In addition, through a successful collaboration with the group of Gene Myers (MPI, Dresden), we have developed image analysis procedures that automatically segment and track the cells, allowing us to obtain accurate tracking of size and gene expression in a large number of single cell lineages. Using this microfluidic setup we are currently studying the stochastic response of single *E. coli* cells to switches in growth conditions.

Integrated Genotype/Phenotype evolution in *E. coli*

The availability of large numbers of complete genome sequences has led, over the last 15 years, to a revolution in our understanding of genome evolution and the identification of a number of surprising "quantitative laws" of genome evolution. However, whereas the insights gained from analysis of genomic data have been impressive, they have taught us surprisingly little about what selective pressures in the wild are driving genotype dynamics. In this project we aim to learn about selection pressures that are acting in the wild by combining information on genotype evolution in closely related bacterial strains with extensive quantitative characterization of their phenotypes. In particular, using next-generation

sequencing we have determined complete genomes of 91 wild *E. coli* isolates that were all obtained from a common location at the shore of lake Superior (Minnesota, USA). In parallel we have been characterizing the phenotypes of these strains by assessing their growth in a wide variety of conditions using automated image-analysis of cultures on agar plates. We have started developing theoretical models to describe the joint evolution of genotypes and phenotypes of the strains. We are aiming in particular to develop rigorous quantitative measures of the extent to which different phenotypic traits have been under natural selection in the history of these strains, and to infer how this has impacted their genomes. As part of this project we have also recently developed a new method, called REALPHY, for automatically inferring phylogenies from raw next-generation sequencing data.

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Publications 2015

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Research Group Mihaela Zavolan

Regulation of gene expression by small RNAs

For many years transcription factors held the center stage in the regulation of gene expression. This paradigm has changed with the discovery of Piwi-protein-associated small RNAs that regulate gene expression at either transcriptional or post-transcriptional level. Among these, the microRNAs (miRNAs) have initially been discovered in the worm *Caenorhabditis elegans*, but in recent years they have been found in the genomes of organisms as varied as viruses, plants and humans. miRNAs play essential roles in development, metabolism, immune responses, and they can either suppress or enhance specific pathogenic processes such as infections and cancer.

Combining high-throughput experimental approaches with data analysis and computational modeling, the group of Mihaela Zavolan studies post-transcriptional regulatory circuits that control cellular differentiation. MiRNAs are an important component of these circuits, being predicted to target the majority of human genes. The Zavolan group contributed to the development of Argonaute crosslinking and immunoprecipitation (CLIP)-based approaches to the isolation of miRNA targets. Computational prediction of miRNA targets remains however essential for guiding experimental approaches to the study of miRNA function. Initially, the Zavolan group used comparative genomics data to develop ElMMo, which is one of the most accurate miRNA target prediction programs currently available. More recently, in collaboration with Erik van Nimwegen, the group used their previously obtained CLIP data to infer a biophysical model of miRNA-target interaction (MIRZA). MIRZA enables accurate identification of both canonical miRNA targets, which are identified by other methods as well, and non-canonical targets, which have not been previously predicted with good accuracy. The group combines predictive modeling of miRNA binding sites with analyses of their functions. In particular, they showed that evolutionarily conserved miRNA target sites and target sites that are associated with the degradation of

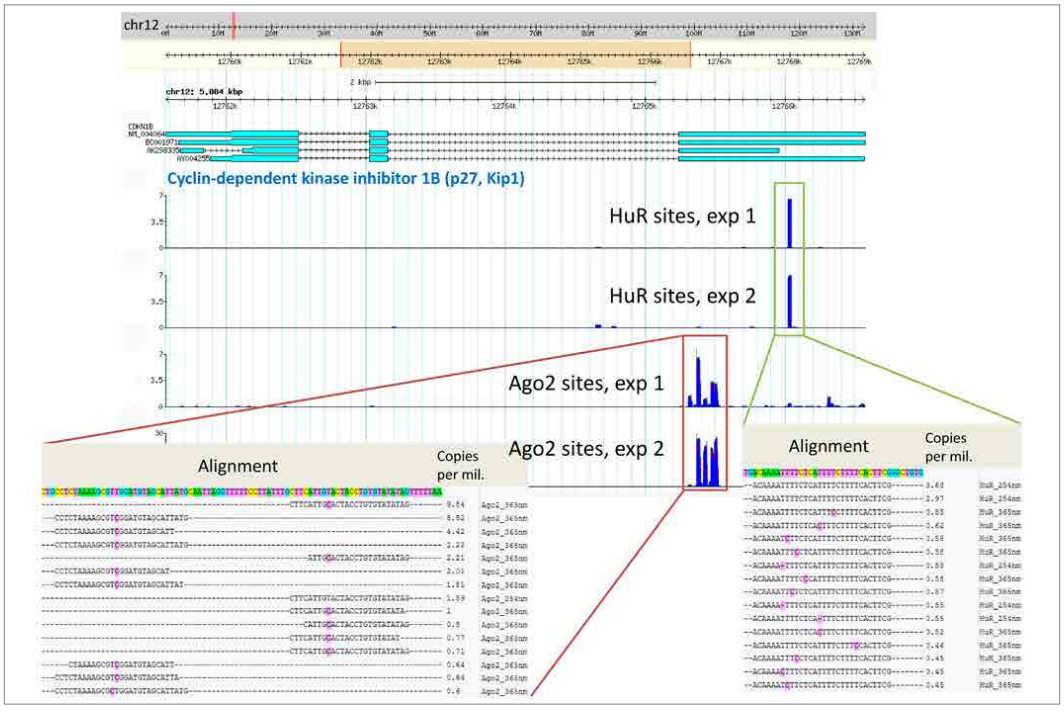

Fig. 1: Location of binding sites of Argonaute 2 and HuR proteins in transcripts of the p27 cell cycle regulator. The binding sites were determined by crosslinking the Argonaute 2 and HuR proteins to mRNAs with UV light, isolation and deep sequencing of RNA fragments that were bound by these proteins. The alignment shows the transcript sequence at the top, with each following track representing a unique sequenced fragment. The number of times each fragment was observed in the sample is indicated on the right of the corresponding track in the alignment. Differences between the sequenced reads and the genomic sequence are indicated by the color boxes. Crosslinking induces diagnostic T-to-C mutations.

target mRNAs have similar properties, indicating that mRNA degradation is a common, important outcome of miRNA-target interaction. Furthermore, by modeling the combined effects of transcription factors and miRNAs on the transcriptome of various cells, the group aims to uncover regulatory cascades that are triggered by miRNAs in the context of various differentiation processes.

One of the most intriguing features of miRNA-dependent regulation is that most mRNAs that carry highly conserved miRNA target sites respond only mildly to changes in miRNA concentrations. It is therefore believed that miRNAs mostly "fine-tune" gene expression. Understanding the mechanisms behind this fine-tuning function is one of the current projects of the group. Availability of high-throughput data sets provides ample opportunities for testing computational models of gene expression regulation. A surprising recent finding of the group is that loading of miRNAs into the Argonaute proteins may constitute an important bottleneck that limits the speed of miRNA-dependent gene regulation. This finding has important implications, suggesting for example the necessity of active miRNA turnover in the Argonaute protein for rapid regulation of target expression.

Generation of a mature mRNA involves many steps (transcription initiation, splicing, 3' end processing) that can be independently regulated to give rise to multiple transcripts with different properties. In different phases of their cycle, cells can for e.g. express transcript forms that translate into the same protein, but have different susceptibilities to post-transcriptional regulation. In collaboration with Walter Keller, professor emeritus at the Biozentrum, the Zavolan group has mapped binding sites of 3' end processing factors transcriptome-wide and related these to 3' end processing sites that were identified in the same cell type as well as in cells in which various 3' end processing factors underwent siRNA-mediated knockdown. With this approach, two components of the mammalian cleavage factor I (CFIm) have been found to be important for the regulation of 3'UTR lengths. Various other factors have been recently reported to be able to globally alter the polyadenylation site usage when overexpressed or inhibited. The group is currently studying the mechanism underlying systematic changes in 3' end processing sites that are observed in relation to specific cellular states such as proliferation.

Much of the work in the Zavolan group is collaborative, involving application of the computational tools developed in the group to various experimental data sets. Examples are the work in the context a Sinergia project entitled "Post-transcriptional regulation of germ cell apoptosis in *C. elegans*", and the collaborative SystemsX.ch grants entitled "Controlling and exploiting stochasticity in gene regulatory networks" and "TargetInfectX: multi-pronged approach to pathogen infection in human cells".

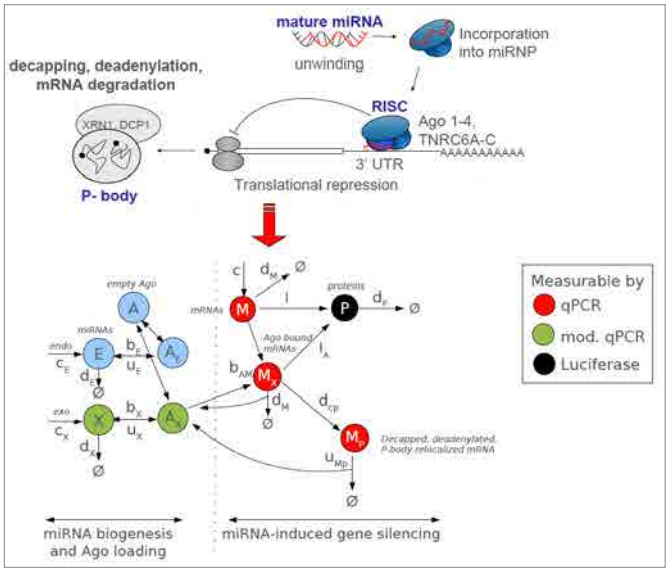

Fig. 2: A detailed model of miRNA-dependent regulation. It serves as a working model in our attempt to quantify the magnitude of the effects that miRNAs exert at different steps of mRNA processing and translation.

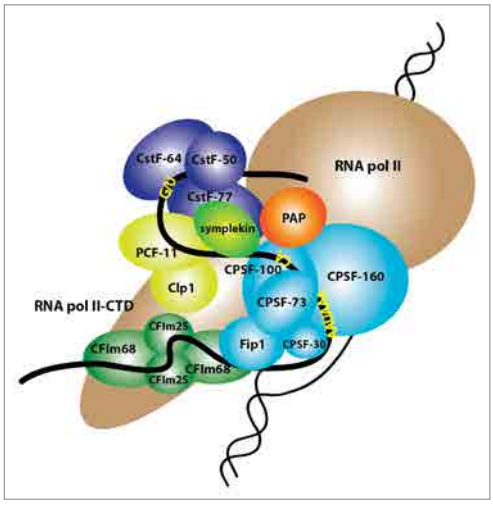

Fig. 3: Sketch of the components of the core 3' end processing complex. By crosslinking and immunoprecipitation of these components together with the RNA fragments that are bound to them, we are attempting to unravel the grammar of 3' end recognition and processing in mammalian cells.

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Biophysics Facility (BF)

Biophysical answers to biological questions

The Biophysics Facility supports researchers in the use of sophisticated instrumentation to measure the interactions, reactions, stability and size of biological molecules with accuracy and precision.

A growing range of techniques has been developed to characterise the physical properties of biological macromolecules such as proteins and nucleic acids. These biophysical techniques enable us to investigate how those molecules gain and retain structure, perform reactions, and interact when they assemble into larger structures, in order to play their essential roles in living cells.

Choosing the best methods to answer your question

We identify the best combination of methods to answer each question. The facility has instruments for micro-calorimetry, circular-dichroism and fluorescence spectroscopy, surface plasmon resonance, analytical ultracentrifugation and light-scattering measurements. A few examples of typical applications are:

- Measuring the energies of macromolecular interactions
- Determining the size and shape of complexes
- Assessing the impact of mutations on protein structure and stability
- Screening small molecules libraries to identify chemical probes that bind to a specific macromolecular target

Cells made of quartz glass are used to hold biological samples for absorbance, circular dichroism and fluorescence measurements.

Dr. Timothy Sharpe
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Xiaochun Li Blatter

We support researchers who wish to use these techniques, from the first stages of experimental design to the final stages of data interpretation and presentation. Depending on individual requirements, we can perform experiments for users or train them to operate instruments and to collect high-quality data. We are also responsible for maintaining and improving the instruments to meet the future needs of the Biozentrum.

Publications 2015

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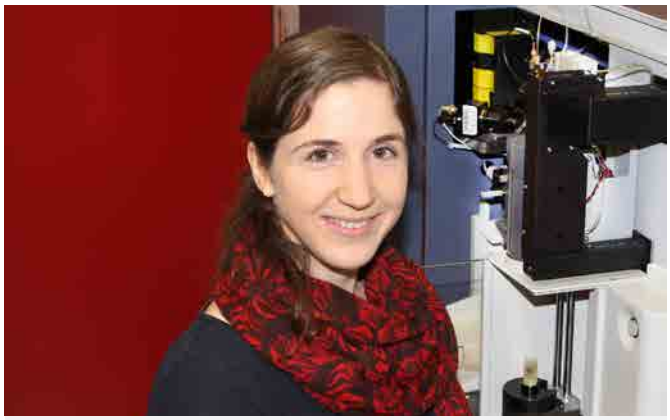
FACS Core Facility (FCF)

Staining, analysis and sorting of cells

Fluorescence Activated Cell Sorting (FACS) enables a qualitative and quantitative analysis of mixtures of cells, as well as the sorting of individual particles using a flow cytometer. The application of this methodology is available to all research groups at the FACS Facility at the Biozentrum.

Fluorescence Activated Cell Sorting (FACS) is a technology with which mixtures of cells can be analyzed, counted and separated with a special instrument, the flow cytometer. This procedure is already being routinely applied in medical diagnostics in hematology and immunology, in order to identify specific cell types in samples. Furthermore, in various research fields such as cell biology, neurobiology and infection biology FACS is being used more and more frequently and offers completely new possibilities for the analysis and purification of cells and cell organelles.

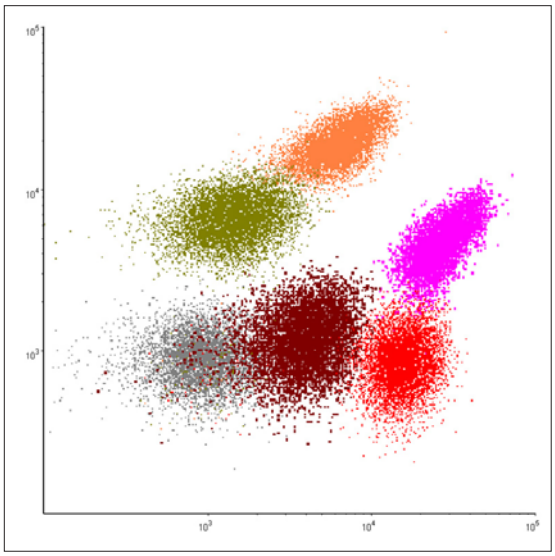


Janine Zankl

» further information

The procedure

The analysis can be carried out on suspended cells with a size range between 0.2 und 100 micrometers, which are first stained with different fluorescent markers such as fluorescent proteins like GFP and RFP, fluorescently labeled antibodies and/or many other stains. In the flow cytometer, these particles/cells pass a laser at up to 130 km/h. The scattered light reveals the size and internal structure of the cell, while the fluorescence indicates which stain the cell contains. Cells displaying the desired characteristics are identified and counted. Finally, the fluid stream is divided into many miniscule droplets. Droplets which contain a desired cell are electrically charged and diverted by an electric field into different collecting tubes. The various separated cells can subsequently be investigated using microscopicy, biochemistry and functional experiments.



Detection of 6 subpopulations in a mixture of various *Salmonella* strains.

The service

All research groups at the Biozentrum are invited to use the services of the facility and receive advice for upcoming experiments. They will be supported and guided in the planning and carrying out of experiments as well as the subsequent analysis. Besides support and teaching, the service facility FACS is concerned with further development and optimization of various aspects of the FACS methodology.

Imaging Core Facility (IMCF)

Imaging Core Facility at the Biozentrum

During the past decade the importance of light microscopy increased tremendously in all types of biological research. In addition to the image acquisition at various types of microscopes, data handling and image analysis is becoming more and more important.

Microscopes and support for image analysis is available for all research groups of the Biozentrum

The Imaging Core Facility (IMCF) provides infrastructure for all microscopy techniques currently used in life sciences. The microscopes in the facility span the entire range from standard wide-field microscopy to confocal microscopy and super-resolution microscopy. The newly established facility started to operate in autumn 2011. There are 6 point scanning confocal microscopes, 2 spinning disk confocal microscopes, 3 wide-field microscopes, a serial 2-Photon tomography microscope, a lightsheet microscope, a slide scanner and a 3D-SIM super-resolution microscope available. The aim of the IMCF is not only to provide the facility users with well-maintained modern microscopy systems as well as with theoretical and technical support but also to develop and adapt new microscopy techniques and image analysis routines.

Areas of interest

Besides the microscopy service for the Biozentrum, research at the IMCF focuses on the development of (i) optimal system performance in terms of image acquisition speed and experimental flexibility thereby enabling users to do all types of experiments under optimal conditions, (ii) image analysis and automation solutions, and (iii) a data storage and database solution (in collaboration with Research IT).

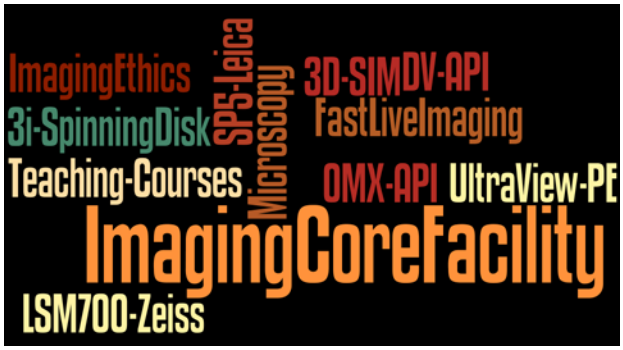


Fig. 1: Overview of the systems and activities of the Imaging Core Facility (Word cloud created with wordle.net).

Areas of activity

The IMCF supports projects requiring specific light and fluorescence microscopy setups. Preferably, the IMCF should be involved at every stage: in the planning of a new imaging/microscopy project (selection of fluorescent dyes, choice of microscopy system), during the initial phase (helping with the actual experimental setup including the soft- and hardware settings), and in image analysis and quantification (advice on usage of image analysis software such as Icy, Arivis, Imaris and Fiji/ImageJ). Only this allows the IMCF to provide the best support at all stages of the project and to adapt the experimental settings to the specific needs.

Fig. 2: Zeiss LSM700 inverted scanning confocal with incubation chamber for live imaging, large samples, and FRAP.

We also provide a weekly open support (every Wednesday morning), where users can come and discuss their issues with members of the IMCF, without having to take an appointment.

Specific services and resources

In detail, we will provide the following state of the art microscopy systems for research groups:

Point Scanning Confocal Microscopes

- Inverted Leica SP5 I system with resonance scanner for fast scanning (live cell imaging), multiphoton laser, and incubation chamber.
- Inverted Leica SP5 II system with resonance scanner for fast scanning (live cell imaging), high sensitivity HyD-detectors, Matrix screening software, and incubation chamber.

- Inverted Leica SP8 system with resonance scanner for fast scanning (live cell imaging), and incubation chamber. !BSL2 system!
- Zeiss LSM 700 upright
- Zeiss LSM 700 inverted
- Zeiss LSM 800 inverted with incubation chamber for live cell imaging and Airyscan

Spinning Disk Confocal Microscopes

- PerkinElmer Ultraview with dual camera setup for high speed imaging
- 3i Spinning Disk with highly sensitive Photometrics Evolve EM-CCD camera

Wide-field live cell imaging systems

- Applied Precision DeltaVision system
- FEI MORE system with TIRF

Standard wide-field system:

- Leica DM 6000, upright microscope with color camera for histology images

Serial 2-photon Tomography system:

- TissueVision TissueCyte, to image whole organ imaging at high resolution without clearing procedures

Lightsheet system:

- Zeiss Z1 lightsheet, for live & fixed large samples

Slide scanner system:

- Zeiss Axio Scan.Z1, fully automated for high throughput

Super-resolution 3D-SIM system:

- GE Heathcare OMX v4 Blaze, for fixed and live samples, with structured illumination (3D-SIM), Ring TIRF (works with PALM or TIRF techniques)

Data visualization and image analysis software

For 3D-rendering, tracking, and animations of microscopy data:

- Arivis
- Icy
- Imaris
- Volocity
- FiJi/ImageJ

For reconstruction of 3D multi-position stacks (registration, stitching):

- XuvTools

For image analysis:

- Python/SciPy/NumPy
- Matlab
- CellProfiler
- In-house plugin or macro development for specific image analysis solutions

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Steering committee

To coordinate the Imaging Core Facility activities at the Biozentrum a steering committee has been established in 2011. The steering committee is composed of five group leaders from different focal areas of the Biozentrum that are heavily involved in microscopy, and representatives from Research IT and from the Biozentrum coordination office.

Outlook

Light microscopy is developing at a tremendous pace and several new microscopy techniques have been developed to bypass Abbé's diffraction limit of light. These new super resolution technologies are either based on structured illumination, nonlinear fluorophore responses, or on the precise localization of single molecules. Depending on the type of method used, it is now possible to improve the maximal resolution from 200 nm to values of 50 to 100 nm.

After careful evaluation of the different techniques, the IMCF of the Biozentrum is planning to provide super-resolution techniques at the facility. Super-resolution microscopy will open up a new dimension for research at the cellular and subcellular level. Hitherto non-resolvable small subcellular structures such as centrioles, lipid rafts, neuronal dendrite spines, nuclear pore complexes, bacterial polar complexes, and many other macromolecular structures with less than 200 nm in diameter – so far exclusively accessible by electron microscopy – will be visualized by light microscopy, and eventually live cell imaging.

Proteomics Core Facility (PCF)

Proteomics at the Biozentrum

Proteomics rapidly evolves from a discovery-oriented technique to a robust and sensitive quantitative tool in biological research to study changes in protein expression and protein modifications in a high-throughput manner.

Proteomics service is available for all research groups of the Biozentrum

The Proteomics Core Facility (PCF) provides infrastructure for the identification and quantification of proteins and their modifications. This includes profound expertise in phosphopeptide enrichment strategies, various platforms for protein and peptide separations, state-of-the-art mass spectrometry (MS) for discovery based MS and LC-MS/MS experiments as well as directed and targeted MS workflows for sensitive and consistent quantitative monitoring of pre-selected sets of proteins. The PCF continuously develops and adapts new sample preparation techniques, MS approaches and software tools to provide optimal analytical services for the individual research groups and their projects.

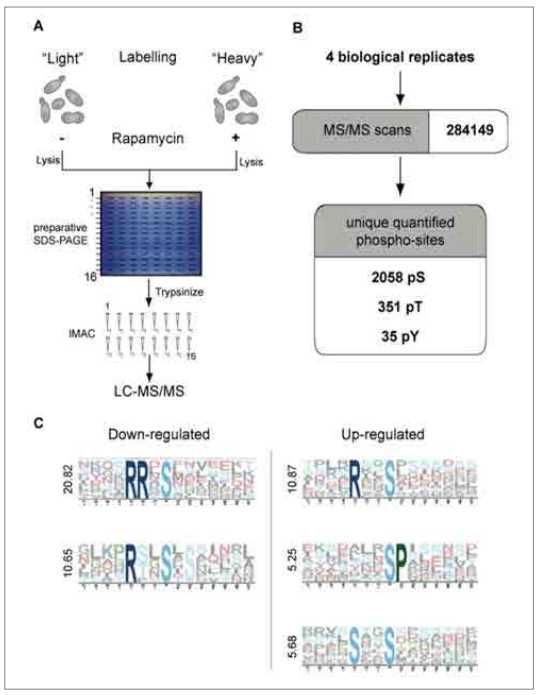


Fig. 1: Quantitative analysis of the rapamycin-sensitive phosphoproteome by SILAC. A) Two yeast cultures are metabolically labeled with normal or isotopically labeled Lysine and Arginine (heavy culture). The heavy culture is treated for 15 minutes with rapamycin. Cell lysates mixed in a ratio of 1:1 are separated by preparative SDS-PAGE, sliced into horizontal bands and proteins are digested. Phosphopeptides are enriched via IMAC and measured in an LTQOrbitrap. B) Four independent experiments yielded 972 phosphoproteins, corresponding to 2.383 unique phosphopeptides. C) Motif analysis with Motif-X of all down-regulated and upregulated phosphopeptide sequences. Motifs are ranked from top to bottom according to their score.

Areas of interest

Besides the analytical service for the Biozentrum, research at the PCF focuses on the development and application of (i) quantitative phosphoproteomics for tracking complex cellular phosphorylation events (Jenoe lab) and (ii) data-independent MS workflows for proteome-wide quantitative studies of microbes and the specific monitoring of proteins and their modifications in complex systems such as human cell lines (Schmidt lab).

Specifically, snapshots of signaling pathways in hepatocellular carcinomas (HCC) are acquired by quantitatively comparing proteomes and phosphoproteomes of biopsies taken from HCC patients. The aim is to capture the proteome and the phosphorylation status of signaling pathways by analyzing serial biopsies of patients before and during treatment with the protein kinase inhibitor Sorafenib. Other projects focus on proteome-wide studies of various human pathogens, identification of protein-protein interactions and determining precise concentrations of outer membrane proteins using targeted proteomics assays. Furthermore, extensive quantitative datasets of human cancer cells showing chromosome instability (CIN) were acquired to identify key factors and cellular processes involved in the formation and tolerance of CIN.

Areas of activity

The PCF supports projects requiring the identification and quantification of proteins and protein modifications. Preferably, the PCF should be involved at every stage: in the planning of new research projects, during the initial phase and while the project progresses. Only this allows the PCF to provide the best analytical tools at all stages of the project and to adapt the analytical strategies to the specific needs. Furthermore, this facilitates the interpretation of the data and its communication in a user-friendly and plain manner.

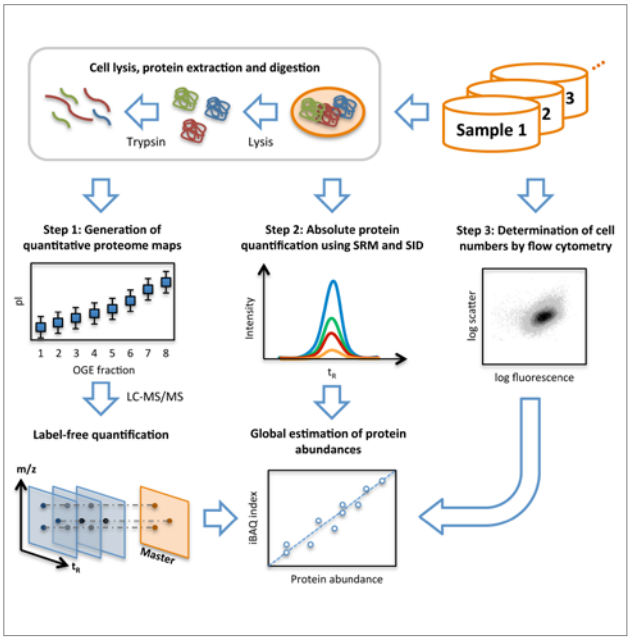


Fig. 2: Workflow of system-wide protein abundance determination. The workflow comprised three steps. First, cells of the various samples were lysed, proteins extracted and proteolyzed using trypsin. The peptide mixtures were either further fractionated using OFFGEL electrophoresis (OGE) or directly analyzed in biological triplicates by shotgun LC-MS/MS and quantified by label-free quantification. Second, the cellular concentrations of 41 proteins covering all components of the glycolysis pathway were determined across all samples by selected-reaction monitoring (SRM) and stable isotope dilution (SID). Therefore, for each protein, heavy labeled reference peptides (selected from the shotgun LC-MS/MS experiment) were synthetized. After spiking known amounts of these references into each sample, absolute quantities were determined for the corresponding proteins by SRM. Third, the numbers of cells taken for LC-MS/MS analyses were determined for each sample by flow cytometry. With this information, the protein concentrations determined by SRM/SID could be transformed to protein copies/cell and a quantitative model was built to translate MS-intensities of all quantified proteins to cellular abundance estimates using the Intensity Based Absolute Quantification (iBAQ) approach.

Specific services and resources

In detail, we provide the following state of the art MS instrumentation and methods for the research groups:

LC-MS/MS platforms:

- Two High-resolution hybrid LTQ Orbitrap-Elite coupled online to an Easy-nLC-system (both from Thermo-Fisher Scientific) for discovery-driven workflows
- TSQ Vantage Triple Stage Quadrupole Mass Spectrometer coupled online to an Easy-nLC-system (both from Thermo-Fisher Scientific) for hypothesis-driven workflows using selected-reaction monitoring for protein quantification
- Q-Exactive HF coupled online to an Easy-nLC-system (both from Thermo-Fisher Scientific) for discovery-driven and data-independent MS workflows

Sample preparation and fractionation instruments:

- 3100 OFFGEL Fractionator for peptide separation using isoelectric focusing (Agilent)
- Capillary liquid chromatograph for peptide separation and fractionation (Agilent)

Software:

- Database search tools: Mascot, Sequest and XTandem for tandem mass spectra interpretation, also in combination with the trans proteomic pipeline
- Scaffold (Proteome software) for communicating proteomics results in a user-friendly format
- Progenesis QI label-free quantification software (Nonlinear Dynamics)
- MaxQuant for quantification of isotopically labeled samples
- SpectroDive/spectronaut/Skyline for analysis of data-independent protein quantification experiments
- In-house software tool SafeQuant for absolute protein quantification and statistical analysis of large quantitative datasets

Methods:

- Protein identification, including posttranslational modifications
- Absolute and differential protein quantification (label-free, isobaric isotope labeling-based)
- Enrichment and quantification of phosphopeptides
- Data-independent MS for consistent and accurate protein quantification in large-scale proteomics projects

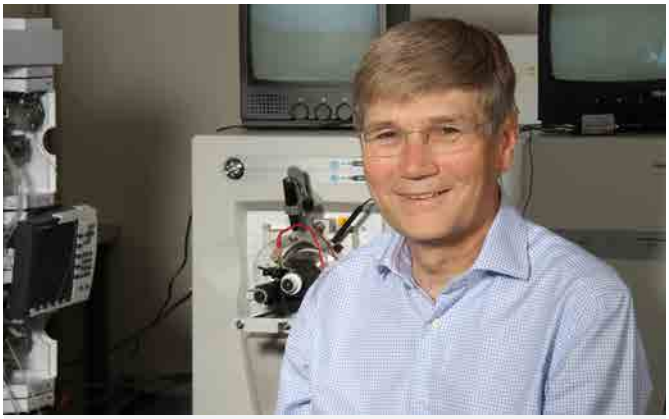
Steering board

To coordinate Proteomics activities at the Biozentrum a PCF steering board was established in 2010. The steering board committee is composed of four group leaders who are strongly committed to proteomics, the Head of Administration of the Biozentrum and the two co-directors of the PCF.

Outlook

With the continuing advances in MS instrumentation (and the purchase of the new QE-HF), analytical methodology and software tools, proteomics is well suited to meet the requirements for modern biological projects on a system-wide level. In particular, data independent (DIA) proteomics workflows have great potential in reproducibly and extensively quantifying entire proteomes over large sample numbers. The new LC-MS platform is ideally suited for this type of analysis, which is currently integrated and tested for several in-house projects. We will go one step further and develop optimized protocols for targeted quantification of post-translational modifications (PTMs), like phosphorylation and acetylation, as well as protein isoforms directly from cell lysates without the need for enrichment steps and considerably reducing required sample amounts. Besides, we are currently establishing a new solid phase extraction protocol that only requires minimal sample load and is particular useful for precious samples that are in short supply.

Dr. Alexander Schmidt
» further information



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Publications 2015

Gasser, Véronique; Baco, Etienne; Cunrath, Olivier; August, Pamela Saint; Perraud, Quentin; Zill, Nicolas; Schleberger, Christian; Schmidt, Alexander; Paulen, Aurélie; Bumann, Dirk; Mislin, Gaëtan L A; Schalk, Isabelle J (2015). Catechol siderophores repress the pyochelin pathway and activate the enterobactin pathway in *Pseudomonas aeruginosa*: an opportunity for siderophore-antibiotic conjugates development. *Environmental Microbiology,* Epub ahead of print.

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Research IT

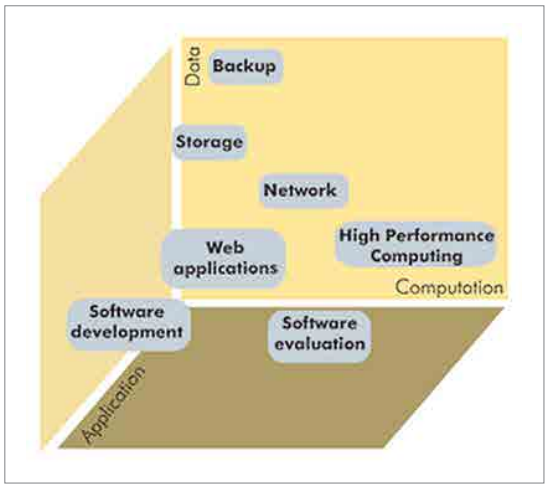
Research IT support for the Biozentrum

The Research IT technology platform supports research and administration projects with increased information technology demands. Our goal is to accompany projects from their inception to a stable productive solution.

We provide and integrate information technology (IT) systems that help our institute's members to organize and execute their work easily and efficiently. We support the Biozentrum's groups and platforms in complex IT questions, for instance when dealing with large data sets.

Three main aspects characterize our service:

- Coordination and support of large-scale IT projects that are of strategic benefit to the Biozentrum, arising from scientific and business needs.
- Operation and maintenance of production systems and services (resulting from above projects or otherwise), alone or in collaboration with internal partners, e.g. the Basel University central IT services, or the BC2 computational infrastructure for bioinformatics.
- Support of requests and questions by Biozentrum members on a daily basis ("open-door consulting").



Research IT Solution Space.

Areas of interest

In the past years, we have led projects that cover the whole spectrum from scientific research projects (with direct involvement in the science and the analysis of data) to core infrastructure projects (where we develop a tool or technology for widespread use in the institute). We are working with the Biozentrum's research groups, technology platforms and administration units to address these challenges. Common to our projects is the focus on the management of information, such as:

Platforms for external and internal information exchange

Biozentrum Intranet:
With more than 500 members, the Biozentrum needs a central place to efficiently exchange information, for administrative and organizational purposes as well as for committees or research groups. Our solution is based on an enterprise-level wiki platform, which we have customized to our requirements, including specific feature and corporate look and feel.

Biozentrum web site:
In time for our institute's 40-year anniversary, our web site was due for an overhaul. Research IT led the project team, further composed of Scientific Affairs, Communications, and external contractors. The goal was to evaluate target audiences and their needs to provide an optimal platform for information access to diverse visitor groups. Our technical goal was to integrate and leverage existing data sources into a central, common content management system. Both goals were met with the launch of the first version kicking off the Biozentrum's 40 year festivities.

Research data management and analysis

Biological data sets are growing exponentially. These data need to be efficiently stored, annotated and retrieved. We are aligning scientists' needs and technical implementation of storage systems, in partnership with the University central IT services. In this way, we can provide for the storage of large scientific datasets with appropriate disaster recovery strategies, as well as develop processes for archiving to slower, cheaper storage media.

Experimental data must be annotated with meta-information necessary to preserve the context in which it was generated. For specific experimental approaches, we use Imagic, OpenBIS and Omero as scientific data management systems to capture and organize annotated data, as well as our own SampleQueuer application, which is used to manage mass spectrometry samples and derived data. For general laboratory data, we are introducing wiki-based "paper-on-glass" electronic lab notebooks to the Biozentrum's research groups.

We work closely with the computational experts embedded in the other Biozentrum technology platform, as a large part of the research data to be managed and analyzed is generated here.

Automated data processing

Increased automation in the production of large-scale data (e.g. siRNA screens) require the development of automated systems to capture, validate, and store the data, as well as trigger automated analysis on high-performance computing infrastructure. This allows biologists to perform analyses of data sets requiring significant computational power without the significant learning curve associated with setting up intensive calculations on large datasets.

The open-source iBRAIN2 software developed in our group within the SystemsX.ch SyBIT project has become the processing backbone of the InfectX project, a multi-group, multi-pathogen project lead by Prof. Christoph Dehio to elucidate key host-pathogen interactions in infection. Terabytes of images acquired from genome-wide siRNA screens are now routinely analyzed using an image processing pipeline within iBRAIN2.

Oversight

A steering board oversees the activities and strategic orientation of Research IT. It is currently composed as follows: Dr. O. Biehlmaier (Head, IMCF), T. Jelk (Head, BioPhIT), Prof. Dr. Ch. Dehio, Prof. Dr. Ch. Handschin, Prof. Dr. E. Nigg (ex officio), Dr. M. Podvinec (Head, Research IT), Marcel Scheiwiller (Head of Administration), Prof. Dr. T. Schwede (Chair), Dr. A. Schmidt (Co-director PCF)

Dr. Michael Podvinec
» further information

Research Associates
Dr. Adam Mazur
Dr. Rainer Pöhlmann

Publications 2015

SIB Swiss Institute of Bioinformatics Members (2015). The SIB Swiss Institute of Bioinformatics' resources: focus on curated databases. *Nucleic Acids Research,* (database issue).

C-CINA

Center for Cell Imaging and Nano Analytics (C-CINA)

Prof. Dr. Henning Stahlberg
» further information

The Center for Cell Imaging and Nano Analytics (C-CINA) combines a wide range of microscopy equipment, methods and software tools to investigate biological specimens in three dimensions.

C-CINA examines biological specimens at various magnifications. Different types of light and electron microscope are used to produce three-dimensional images of proteins at different magnifications and from various aspects.

Combined use of different microscopes

Researchers in the C-CINA use the serial block face scanning electron microscope to determine the rough three-dimensional structure of biological tissues. They then examine individual cells from interesting areas of the specimen using high resolution electron tomography. And, finally, the atomic structure of individual proteins in the cells can be reconstructed.

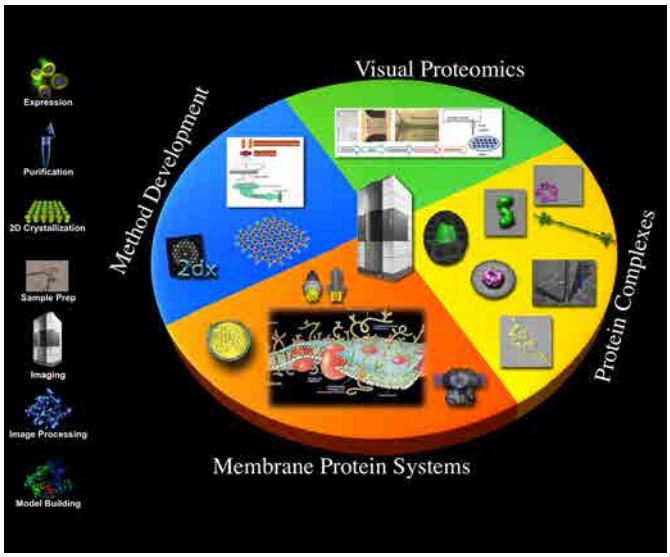
The microscopes function at different magnifications, ranging from hundreds of micrometers to less than a fraction of a nanometer. The key element of C-CINA's equipment is the very high resolution microscope called "Titan". This machine is 4.5 meters high and operated exclusively by computerized remote control.

Computer image processing gives us insight into 3D

Two-dimensional images from the various microscopes are collated into three-dimensional data by computer image processing. Just like Google Maps, the computer allows the combination of different magnifications. C-CINA also uses many different methods and types of equipment to prepare biological specimens for examination under the electron microscope.

Method development at C-CINA

C-CINA is also active in developing methods for specimen preparation, the microscopy itself, and computer-based evaluation of the images.



C-CINA is active in research into the structure of membrane protein systems and protein complexes, the development of methods for microscopy, and visual proteomics.

sciCore Center for Scientific Computing

sciCore – the Center for Scientific Computing

Infrastructure for scientific high-performance computing.

Ranging from storage and organization of large data volumes, to statistical data analysis and the simulation of complex systems, computer supported methods play a central role in today's research. sciCORE provides the central infrastructure and services in the field of scientific computing for researchers at the University of Basel.

The application of modern research technologies generates increasing volumes of data that not only requires efficient storage but also meaningful analysis and interpretation. In addition to the traditional pillars of science, theory and experimentation, computer supported simulation has established itself as a third pillar. Computer simulations assist researchers in modeling complex systems to better understand their behavior. In order to successfully master these tasks, alongside an efficient and powerful IT infrastructure (high-performance computing, HPC), highly sophisticated software tools and expertise in computational sciences are required.

Scientific high-performance computing, data management, training and support

The services provided by the sciCORE Facility are available to all researchers at the University of Basel. sciCORE offers efficient technical solutions in high-performance computing and advises scientists on matters of data management and storage as well as the use of high-performance computing. More specifically, the tasks of sciCORE are:

- Provide and maintain state-of-the-art infrastructure for high-performance computing (HPC) and large-scale storage systems for big data volumes
- Provide advice to scientists on most efficient usage of HPC resources (consulting)
- Maintain an up-to-date software stack
- Host and operate HPC equipment on behalf of university departments
- Provide courses and training in the use of HPC resources
- Participate in computational projects of national scope (e.g. SystemsX.ch; PASC, CRUS P2)
- Support users in computational aspects of grant applications
- In collaboration with the SIB Swiss Institute of Bioinformatics, host and maintain several worldwide utilized bioinformatics resources and provide active scientific support for research projects in the field of life sciences.

In addition, sciCORE organizes monthly informal user meetings to inform about latest developments, updates on ongoing projects and plans for the future. sciCORE currently supports a broad spectrum of scientific projects in the fields of physics, computational chemistry, life sciences, medicine and economics.

Prof. Dr. Torsten Schwede
» further informations

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Life Sciences Training Facility

The LSTF allows researchers to understand gene function in health and disease

The Life Sciences Training Facility (LSTF) is an academic facility that provides access to deep-sequencing and microarray technologies and contributes to the identification of novel molecular pathways.

Until recently, researchers were able to study only single or just few genes related to a particular biological question. Novel genome-wide methods now allow for studying all genes of an organism. These technologies pave the way towards new discoveries related to the regulation and function of genes. The LSTF provides researchers a fully functional platform to use such technologies for their projects.

RNA: a key molecule mirroring gene function

The LSTF provides guidance to the research community for various projects related to DNA and RNA applications. Specifically, the study of RNA, a key molecule mirroring gene function, represents one of the current research foci within the LSTF. Indeed, the regulation of RNA molecules is key to understand the way cells function or dysfunction. The strength of recent technologies is to provide a simultaneous assessment for all genes: the entire populations of mRNA, non-coding RNA or microRNA are quantified. Thus, we cover all aspects of gene expression profiling from the experimental design to basic data analysis.

Microarrays for the analysis of the human genome processed at the LSTF.

Prof. Dr. Andreas Papassotiropoulos
» further information

More than a classical Core Facility

For close to 15 years, the LSTF has been helping researchers to perform genome-wide studies. We serve the University of Basel, in particular the Department of Biomedicine and the Biozentrum. Experiments are designed and executed in an interactive way, i.e. both the specific research team and the LSTF staff are involved. The facility charges solely reagent costs. We work in close collaboration with the D-BSSE in Basel, which has ample expertise related to deep sequencing.

Microscopy Center

Electron microscopy for research and teaching purposes

The Center for Microscopy (ZMB) of the University of Basel provides electron microscope equipment of every description for research group projects and also plays a key role in education.

The ZMB accepts microscopy commissions from all disciplines in Life Sciences and Medicine, supporting the research groups in their projects. The ZMB also carries out its own research projects to develop and refine methods of preparation, imaging techniques, and image processing software. The most important thing is to keep up with state-of-the-art microscopy and make the necessary new methods available to research.

ZMB for teaching purposes

Another of the ZMB's tasks is training laboratory staff and students on the electron microscopes. We also offer courses for students, so that they can acquire basic knowledge in microscopy. These courses are organized as part of the curriculum in Biology and Nanosciences. The head of the ZMB, Dr. Markus Dürrenberger, has a contract with the Faculty of Philosophy and Natural Sciences, University of Basel, to run the courses.

History of the Center

The Center for Microscopy of the University of Basel has been in existence since September 2001. It resulted from the merger of the Biozentrum's Interdepartmental Electron Microscopy (IEM) unit and the Scanning Electron Microscope (SEM) Laboratory of the University of Basel, at the time when Prof. Richard Guggenheim was appointed emeritus professor. The goal of the merger was to create a central platform for services related to electron microscopy, to be provided to researchers as well as for teaching purposes.

Administration of the ZMB is integrated into the University of Basel's Biozentrum. The president of the ZMB Users' Board, the professor of Microscopy at the Biozentrum, is at the same time the scientific director of the ZMB. Prof. Ueli Aebi of the Biozentrum's M.E. Müller Institute was the scientific director until 2010. Prof. Henning Stahlberg, the successor to Prof. Andreas Engel, was appointed head of the C-CINA and awarded the chair in Microscopy at the Biozentrum in 2010. He is currently the Scientific Director of the ZMB and President of the Users' Board.

Dr. Markus Dürrenberger
» further information

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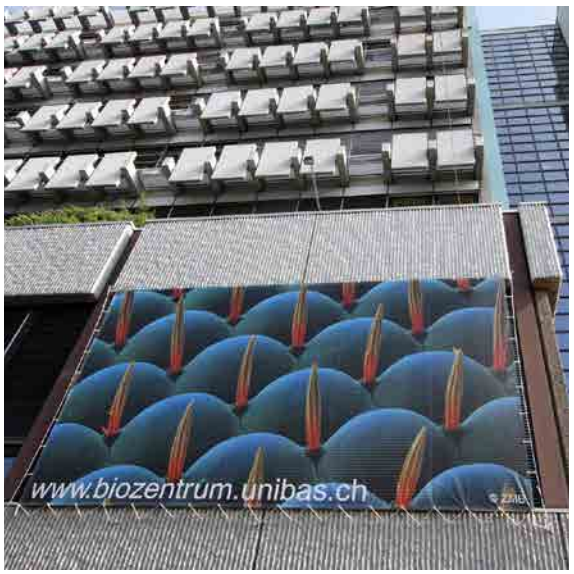


The greatest achievements of the ZMB were put on display at the façade of the Biozentrum on the occasion of the 2nd University-night; a compound eye of a fruit fly sized 6 by 9 meters (1 million final magnification).

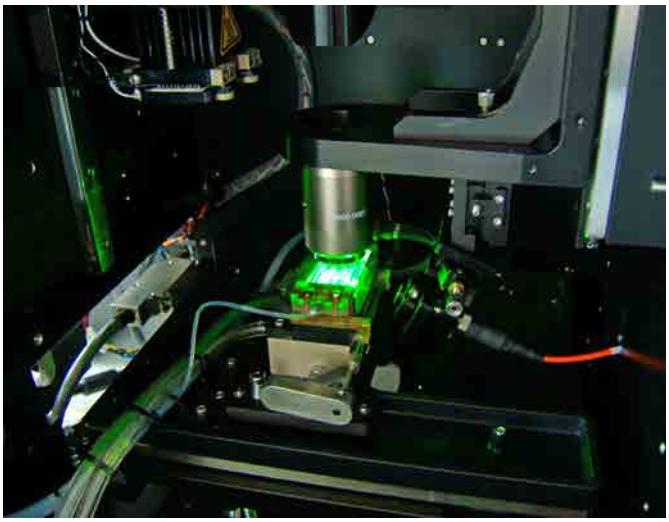
Quantitative Genomics Facility

Next Generation Sequencing

The Quantitative Genomics Facility (QGF) is a central research and service facility located in the Department of Biosystems Science and Engineering (D-BSSE) of the ETH Zurich in Basel, supported and run jointly with the University of Basel and the Friedrich Miescher Institute (FMI) for Biomedical Research. The QGF team provides technical support for next generation sequencing applications in genomics and epigenomics, including high-throughput data management and analysis.

In parallel with the human genome sequencing effort, several new technologies have emerged that allow sequencing at unprecedented throughput and low cost. These technologies are generally referred to as "Next Generation Sequencing (NGS)". They have enabled a large diversity of applications from genome resequencing to identify variations within populations to quantification of mRNA and small RNA expression and the abundance of various epigenetic marks.

In order to take advantage of these powerful technologies, scientists from the Department of Biosystems Science and Engineering (D-BSSE), University of Basel (DBM, Biozentrum) and FMI established a NGS unit, which is housed by the D-BSSE. It currently comprises an Illumina GAIIx and a HiSeq2000 sequencing machine as well as storage and a data analysis pipeline.



The interior of a genome analyzer, located in the Quantitative Genomics Facility at D-BSSE.

Chromatin-IP combined with NGS (ChiP-Seq) to identify binding sites of proteins on DNA or specific histone modifications is one type of application frequently making use of the QGF facility. Another comes from the new field of metagenomics that emerged due to the ability to sequence DNA from diverse biological communities in ecosystems or in infectious diseases. Sequencing of hundreds of cancer genomes is yielding an unprecedented wealth of information about how this deadly disease restructures the genome. It has become evident that NGS technologies will revolutionize many areas of biology and medicine.

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Silvia Arber (Second Deputy)
Manuela Holzer (Secretary to the Director)

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