### Who's Who?

**BNI — Brain Mind Institute**
- 
  - Lab Study of Neurodegenerative Diseases
  - Computational Neuroscience
  - Molecular and Cellular Biology of Alzheimer's Disease
  - Computational Nanobiology
  - Neurovascular Imaging

**IBI — Institute for Research in Biomedicine (Bellinzona)**
- 
  - Marcel Tanner: Director, Swiss Tropical Institute (Basel)
  - Maurizio Molinari: Professor at the Institute for Research in Biomedicine (Bellinzona)

**EPFL School of Basic Sciences**
- 
  - William Pralong: Director, Life Sciences and Technology Bachelor/Master Programs

**EPFL School of Engineering**
- 
  - Marcel Tanner: Director, Swiss Tropical Institute (Basel)
  - Maurizio Molinari: Professor at the Institute for Research in Biomedicine (Bellinzona)

**School of Life Sciences**
- 
  - Marcel Tanner: Director, Swiss Tropical Institute (Basel)
  - Maurizio Molinari: Professor at the Institute for Research in Biomedicine (Bellinzona)

**SIC2008 — Swiss Institute for Cancer Research**
- 
  - Adjunct Professors
  - UDL and CNR Associate Professors
  - Director, Center for Translational Biomedical Informatics (EPFL-CNRI)
  - Director, Lung Center at University Hospital (CHUV)
  - Director, Genomics Center at Swiss Tropical Institute (ITC)
  - Director, Life Sciences and Technology Bachelor/Master Program
  - Director, Institute for Research in Biomedicine

**Center for Phenogenomics**
- 
  - Core facilities & Technology platforms
  - Bioimaging & Optics
  - Flow Cytometry
  - Microscopy & Electron Microscopy
  - Bioinformatics & Bioinformatics
  - Center for Transgenomics

**Public Health**
- 
  - Epidemiology, Vaccines, and Toxins
  - Cell Biology of Bacterial Infection

**Epidemiology, Vaccines, and Toxins**
- 
  - Van der Goot: Cell Biology of Bacterial Infection
  - Trono Lab: Virology and Genetics

**Global Health Institute**
- 
  - Lemaître Lab: Mechanisms of Microbial Infection
  - Cole Lab: Microbial Pathogenesis

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  - Lemaître Lab: Mechanisms of Microbial Infection
  - Cole Lab: Microbial Pathogenesis

**GHI — Global Health Institute**
- 
  - Lemaître Lab: Mechanisms of Microbial Infection
  - Cole Lab: Microbial Pathogenesis

**Swiss Institute for Experimental Cancer Research**
- 
  - Bucher Group: Computational Cancer Genomics
  - Trumpp Lab: Cancer Genes and Stem Cells
  - Radtke Lab: Molecular Mechanisms of Cancer

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**School of Life Sciences**
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  - Marcel Tanner: Director, Swiss Tropical Institute (Basel)
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Preamble

Biomedical research will increasingly rely on quantitative approaches and high-end technologies, and the future of life sciences lies at the crossroads of biology, basic sciences, informatics and engineering. Accordingly, the EPFL School of Life Sciences trains a new breed of researchers whose combined skills in these various fields are set to address fundamental biological questions and to attack the major medical problems of our times with the true spirit of systems biologists. The School of Life Sciences hosts some forty-five research groups that apply this philosophy to broad questions including cancer, infectious diseases and mental or neurological disorders, pushing for integrated approaches that span a range of disciplines from functional genomics to high-tech bio-engineering, and from computational neurosciences to structural modeling. A bachelor’s degree (Life Sciences and Technology), two master’s degrees (Life Sciences and Technology, Bioengineering and Biotechnology), and three Ph.D. programs (Biotechnology and Bioengineering, Neurosciences, Molecular Biology of Cancer and Infection), constitute the educational arms of our school, hosting some six hundred students from all geographic and scientific horizons.

2008 was a landmark year for our school. We celebrated the graduation of our first class of master’s students (Congratulations, class of 2008!), our brand new life sciences building opened its glossy doors, and the integration of ISREC within our institution was formalized. All of this happened while new research groups continued to join us and while our ties with other EPFL schools and other institutions in Switzerland and elsewhere, kept growing through joint research and educational programs in a truly multi-disciplinary spirit. Only six years old, the EPFL School of Life Sciences lives resolutely exciting times!

Professor Didier Trono
Professor & Dean of the School of Life Sciences
Ecole Polytechnique Fédérale de Lausanne (Switzerland)
http://sv.epfl.ch
Main Scientific Events


July 7 to September 5: The second international Summer Research Program for undergraduate students offered intensive research training opportunities to 24 talented students who are interested in research careers in the life sciences.  http://sv.epfl.ch/page60602-en.html

August 21 - 23: During the third EPFL Life Sciences Symposium (LSS08) on 'Cancer and the Cell Cycle,' the prestigious 'Debiopharm Life Sciences Award' was given to Spanish scientist Dr. Manel Esteller for his outstanding research in both basic and translational epigenetic oncology research.  http://lss08.epfl.ch

September 17: The EPFL campus hosted the third annual 'Bio Alps Networking Day'.  http://www.bioalps.ch

Public-Oriented Events

March 4: Inaugural lecturers on how the brain works & mammalian intelligence were given by Prof. Pierre Magistretti & Prof. Henry Markram in video & podcast versions on the School of Life Sciences Channel.

April: In close collaboration with our UNIL, CHUV & the 'Jules Gonin' Hospital colleagues in Lausanne, we actively participated in Gene Days which hosted Swiss high school students.  http://sv.epfl.ch/page66519.html

October 4: The first generation of Life Sciences and Technology Engineers received their Master's degree at the EPFL Dies Academicus.

Novembre 19: As part of the School of Life Sciences community outreach events, we hosted five famous international scientists from the 'Colloque Wright' (Geneva) to talk about epidemics. The public was free to ask 'crazy' and 'not so crazy' questions to learn more about the mysterious world of microorganisms.  http://sv.epfl.ch/page73562.html

Honors-Awards-Announcements

January: Prof. Carmen Sandi was named Project Coordinator of one of the first collaborative projects financed by the Seventh Framework Program of the European Union (3 year duration) studying 'Synaptic mechanisms of memory loss : «Novel cell adhesion molecules as therapeutic targets».

February: A Debiopharm oncology chair which is funded through a CHF 2.5 million endowment, was established at the ISREC-EPFL. The Chair will support the research work of Assistant Professor Dr. Joerg Huelsken, whose focus is on the role of the Wnt signalling pathway in tumorigenesis and stem cell maintenance.

February: Melody Swartz, head of the Lab of Mechanobiology and Morphogenesis (IBI-EPFL) was awarded one of the European Research Commission (ERC) Starting Grants.

September: The «CTI Medtech Award 2008» went to Professor Yann Barrandon, Head of the Stem Cell Dynamics Lab (SV - IBI) and Dr Frédéric Neftel, General Manager and President of Debiotech SA, for research work on a novel approach involving the use of microneedles, which enable doctors to administer medicines at the exact dosage and penetration depth needed. This method of drug delivery offers the added benefit of being virtually painless for patients.

October: Ilaria Malanchi, biologist at ISREC-EPFL, was awarded one of the four Latsis Prizes for young scientific researchers. This prize is awarded each year for the successful research undertaken by a young scientist of less than 40 years old.

November: Five Life Sciences professors (Denis Duboule, Pierre Gönczy, Bruno Lemaître, Joachim Lingner & Jeffrey Hubbell) received the prestigious European Research Commission (ERC) Advanced Grants.

November: EPFL announced the creation of a large world-class Center of Neuroprostheses. This pioneering Lausanne-based facility will include five Chairs and is situated at the crossroads between fundamental research, clinical applications and market opportunities. The Center was inaugurated on January 1, 2009, and is formally part of EPFL's School of Engineering, in collaboration with the School of Life Sciences and the School of Computer and Communication Sciences.
The Undergraduate Studies

The Life Sciences curriculum aims to educate a new generation of engineers who can master the technical and scientific skills needed for studying life processes and developing the biomedical technologies of tomorrow. This educational program, established under the direction of Prof. William F. Pralong, M. D., is unique in Switzerland and Europe.

Bachelor’s program (3 years)

The first two years provide basic courses followed throughout the EPFL, such as analysis, linear algebra, physics, chemistry (general and organic), and numerical methods. Specific courses in Life Sciences begin with biochemistry, cellular, molecular and developmental biology. In the first two years, life sciences courses make up less than 20% of the total academic load.

In the third year, engineering courses (signals and systems, electronic and electrical systems) and typical life sciences courses such as genetics, immunology, and functional genomics applied to biological development, bio-computing, neuroscience, molecular biology, systems biology via the study of human physiology are integrated. Physiology training also gives the opportunity to apply the engineering and biological knowledge acquired up to this point. During this year, the students also fine tune their training by choosing some of their credits from one of the specializations offered in the masters’ program. This includes a bachelor project either in bioengineering and biotechnology or in neurosciences and molecular medicine.

Master’s programs (2 years)

Master’s in Life Science and Technology includes several specializations. Among these are neurosciences, molecular medicine, and bio-computing. Each specialization is made up of 15 credits of required courses plus 15 credits of optional courses.

Master’s in Biotechnology and Bioengineering, includes two specializations in Biotechnology and Bioengineering. Each one requires taking 15 specific and obligatory credits together with 15 optional credits. An additional specialization is offered as a minor in Biomedical engineering consisting of 22 optional credits with a mandatory project worth 8 credits. This specialization is organized with the School of Engineering.

Both degree programs share a common basic curriculum that aims to provide students with the knowledge of the modern technologies used in the life sciences such as imaging, bio-computing and optical systems applied to biology. In addition, courses in management, economics, applied laws and ethics for the life sciences are offered. A large portion of the master’s program (60 credits) is dedicated to laboratory work and projects.

The Graduate Studies

All three graduate programs comprise a combination of coursework, laboratory-based research, in-house seminars, and national or international conferences.

The Doctoral Program in Biotechnology and Bioengineering aims at providing doctoral students with the education necessary to be leaders in the fast-growing industrial and academic biotechnology and bioengineering sectors, i.e. a depth of knowledge and competence in their specific research area as well as a breadth of knowledge in biology, bioengineering and biotechnology. These program themes include: genomics and proteomics, biomolecular engineering and biomaterials, stem cell biotechnology, cell and process engineering, biochemical engineering, orthopaedic engineering, biomechanics, mechanobiology, cell biophysics, computational biology, biomedical imaging as well as molecular, cell and tissue engineering. [http://phd.epfl.ch/edbb]

The Doctoral Program in Neuroscience provides its students with training from the genetic to the behaviour level including molecular, cellular, cognitive, and computational neuroscience. Students matriculate in the highly dynamic and interdisciplinary environment of the BMI-EPFL of the SV. The program is further strengthened by research and training opportunities in collaboration with the Universities of Lausanne and Geneva. [http://phd.epfl.ch/edne]

The Doctoral Program in Molecular Biology of Cancer and Infection is a joint program between the Swiss Institute for Experimental Cancer Research (ISREC-EPFL) and the Global Health Institute (GHI-EPFL). The program provides training and research opportunities to highly motivated doctoral students in key areas of modern biology in Lausanne, Switzerland. Highly qualified applicants worldwide are chosen twice a year through a competitive selection procedure. [http://www.international-phd.ch/index2.html]
Our Core Facilities & Technology Platforms

To enhance the training and research capabilities of its students and scientists, EPFL and the School of Life Sciences have made a significant investment over the past few years to establish state-of-the-art technology platforms and core facilities. These facilities are directed and managed by dedicated teams of highly trained and experienced staff and are run on a fee-for-service basis. They offer training, access to technology, assistance with experimental design and high level data analysis, and collaborations.

In addition, scientists from our School of Life Sciences closely collaborate with other services in the Lemanic region, including the 'Center for Biomedical Imaging' ([http://www.cibm.ch](http://www.cibm.ch)) and the 'Lake Geneva Area Genomics Core Facility' ([http://unil.ch/dafl](http://unil.ch/dafl)).

Currently, the following Life Sciences related core facilities and technology platforms are available at the EPFL School of Life Sciences:

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<th>Facility Type</th>
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<td>BIOIMAGING &amp; OPTICS</td>
<td><a href="http://biop.epfl.ch">http://biop.epfl.ch</a></td>
<td>Dr Arne Seitz</td>
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<td>BIOMOLECULAR SCREENING</td>
<td><a href="http://bsf.epfl.ch">http://bsf.epfl.ch</a></td>
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<td>FLOW CYTOMETRY</td>
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<td>Dr David Hacker</td>
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<td>HISTOLOGY</td>
<td><a href="http://hcf.epfl.ch">http://hcf.epfl.ch</a></td>
<td>Dr Jessica Dessimoz</td>
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<tr>
<td>BIOINFORMATICS &amp; BIOSTATISTICS</td>
<td><a href="http://bbcf.epfl.ch">http://bbcf.epfl.ch</a></td>
<td>Dr Jacques Rougemont</td>
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<tr>
<td>BIO-ELECTRON MICROSCOPY</td>
<td><a href="http://cime.epfl.ch">http://cime.epfl.ch</a></td>
<td>Head of Bio-EM at CIME: Dr Graham Knott</td>
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<tr>
<td>CENTER FOR PHENOGENOMICS</td>
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<td>TRANSGENIC CORE FACILITY</td>
<td><a href="http://tcf.epfl.ch">http://tcf.epfl.ch</a></td>
<td>Dr Friedrich Beermann, Dr Isabelle Barde</td>
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BMI - The Brain Mind Institute
The Brain Mind Institute is dedicated to exploring the emergence of higher brain function across multiple levels ranging from gene expression to cognition and covering multiple brain regions. Constructed on a foundation of high-end technology, in a manner that can be recurrently interconnected with labs around the world, it has the vision to go beyond established concepts. The spectrum of BMI’s faculty projects spans across several domains of the neurosciences, including: Molecular Neuroscience, Cellular Neuroscience, Systems Neuroscience, Behavioral Neuroscience, Cognitive Neuroscience, Computational Neuroscience, and, in collaboration with clinical partners, the neurobiological bases of Neurodegenerative and Psychiatric diseases.

'http://bmi.epfl.ch'
Introduction
During the last decade, our laboratory has primarily focused on the use of viral vectors to mimic in a reasonable time frame both neuropathological and behavioral features of diverse human conditions such as Parkinson’s disease, amyotrophic lateral sclerosis and Alzheimer’s disease. The biological characteristics of viral vectors have also been investigated to assess various therapeutic concepts, playing with transgene and capsid characteristics, as well as the route of administration. Altogether, the recent developments of viral vectors for both modeling and treating CNS disorders have widely helped understanding critical interactions between genetic defects, cellular dysfunctions and environmental factors leading to the demise of specific neuronal populations.

Keywords
Disease modelling, gene therapy, animal models, Parkinson’s disease, Amyotrophic lateral sclerosis, Alzheimer’s disease, viral vectors, lentivirus, adeno-associated virus, cell encapsulation

Results obtained in 2008
The involvement of alpha-synuclein in familial forms of Parkinson’s disease suggests a potential causative role in the pathogenesis. We have explored the possibility of generating animal models of Parkinson’s disease by overexpressing alpha-synuclein in the nigrostriatal pathway using viral vectors. We have also evaluated the effect of phosphorylation on the formation of alpha-synuclein toxic species. Both lentiviral and adeno-associated vectors (AAV) efficiently transduce dopaminergic neurons in the substantia nigra, and transgenic expression of alpha-synuclein leads to the progressive loss of neurons positive for dopaminergic markers, with the formation of intraneuronal alpha-synuclein aggregates. Due to their high tropism for nigral dopaminergic neurons, AAVs are valuable tools to monitor dopaminergic function using spontaneous and drug-induced behaviour. Virus-based rodent alpha-synuclein models thus provide a valuable approach for the preclinical testing of therapeutics.

Beyond the disease modeling approach, viral vectors are promising tools for gene therapy. With the aim of maximizing the delivery of therapeutic proteins in the context of neuromuscular diseases, AAV serotype 6 (AAV6) expressing small hairpin RNAs targeting mutant superoxide dismutase 1 (SOD1) were injected intravenously in the G93A SOD1 mouse model of amyotrophic lateral sclerosis (ALS). This approach resulted in a systemic transduction profile, corresponding to transduction of the entire skeletal muscle as well as heart and liver. In addition, motor neurons at all levels of the spinal cord and brain stem were transduced, amounting to 3-5% of the lower motor neuron pool. SOD1 protein levels were reduced by >50% in all the muscles that were examined. Crucially, this silencing profile did not alter the course of the disease in this ALS model, thereby providing compelling evidence that SOD1-mediated damage within skeletal muscles does not contribute to death of motor neurons in ALS. Furthermore, this study demonstrates that motor neurons can be transduced across the length of the spinal cord through a single noninvasive delivery of recombinant AAV. Other routes of administration, i.e. intraspinal, intramuscular, are under investigation and should allow to better understanding the role of specific cell populations in ALS pathogenesis.

At present, the animal models of Alzheimer’s disease (AD) show synaptic malfunction and a behavioural deterioration similar to AD. However, the pathology is still partially replicated, with few if any neu-
ronal loss observed in APP-based transgenic models. Therefore, they are not suitable for the development and assessment of plasticity-promoting treatments for instance. We have thus started to use AAV vectors carrying various combinations of tau and Aβ mutations to further assess the effect of pathogenic protein expression in selected brain regions, and ideally correlate pathologic features with AD-like behavioural impairments. Preliminary results show that AAV6 vectors can efficiently target hippocampus and cortical neurons. In vivo evaluation of tau/Aβ constructs are underway.

Finally, we have evaluated the use of a novel passive immunotherapy approach as an AD treatment based on the intracerebral implantation of encapsulated myoblasts genetically engineered to chronically release single-chain Fv antibody fragments targeted to the N-terminus of Aβ. These immuno-isolated brain implants have been shown to prevent the production and aggregation of Aβ in the APP23 AD mice. Functional effects six-month post-implantation in mice showed significant behavioural recovery of specific anxiety, exploration and memory traits. The chronic release of antibodies from encapsulated myoblasts constitutes a promising approach for neurobiological diseases including AD.

Selected publications


C. Towne, C. Raoul, B.L. Schneider, P. Aebischer, Systemic AAV6 delivery mediating RNA interference against SOD1: Neuromuscular transduction does not alter disease progression in fALS mice, Molecular Therapy, 16: 1018-1025, 2008

B.L. Schneider, R. Zufferey, P. Aebischer, Viral vectors, animal models and new therapies for Parkinson’s disease, Parkinsonism & Related Disorders, 14 Suppl. 2: S169-S171, 2008


Introduction
We focus our investigations on the bodily perception and self-consciousness, which is considered one of the greatest mysteries in science. How does the brain generate the experience of being someone, of being the subject of conscious experience and the first-person perspective? Our projects rely on the investigation of healthy subjects as well as neurological patients by combining cognitive paradigms with neuroscience/neuro-imaging techniques, virtual reality and neuroscience robotics.

Keywords
Multisensory perception, bodily awareness, self-consciousness, intracranial human electrophysiology, neuro-imaging, EEG, neuropsychology, cognitive neurology, experimental epilepsy, optical body tracking, virtual reality, neuroscience robotics

Results obtained in 2008
With several major publications our research has elucidated how self-consciousness relates to multisensory bodily processing. Using virtual reality technology, we have shown that the “I” of conscious experience and the subject’s first-person perspective depends on the integration of synchronous visual and somatosensory signals. Employing our interdisciplinary expertise - bridging cognitive neurology, experimental epilepsy, intracranial electrophysiology, experimental psychology and neuro-imaging - we have further developed engineering-based approaches for the cognitive neuroscience of bodily perception and self-consciousness. The installation of a virtual reality neuro-imaging platform (with a portable 256 channel EEG system; VR-EEG) was a major achievement and has allowed us to carry out cognitive experiments in highly realistic, ecologically valid environments that close the perception-action loop while the participants’ brain activity is continuously monitored. (Soon brain damaged neurological patients will participate.) This technique and studies using fMRI compatible neuroscience robotics have allowed us to describe the electrophysiological activity in medial prefrontal cortex, temporo-parietal cortex, and medial posterior cortex that reflects experimentally altered states of self-consciousness.

Selected publications


**Merging virtual reality technologies and robotics with neuroimaging in the cognitive neurosciences**

**Left.**
In a novel research platform that is installed at LNCO, participants are asked to wear a suit with 20 active infra-red markers strategically positioned on their joints (not shown). The three-dimensional positions of these markers can be recorded by detectors (black bars), further processed by a motion capture computer and then used for real-time animation of a virtual body, which was shown from the back in a virtual room (see inset) on a back-projection screen in front of the participant. A target (here a transparent cylinder) or any other visual or auditory stimulus can be represented within the virtual room. Participants in one experiment on motor performance (gait) and conscious action awareness were asked to move the virtual body to the target by means of actually walking in the 4 x 4 m sized motion capture area. In one of our experiments, targets were randomly selected from several locations, and the visual feedback of the virtual body’s location seen by the participant was either faithful or systematically deviated towards the left or right as a function of the virtual body’s distance from the onset of perturbation. The setup is fully equipped with a high density EEG system allowing to measure electrophysiological brain activity in fully moving human agents.

**Right.**
Data from a recent neuroimaging experiment using functional magnetic resonance imaging (fMRI) in combination with robotic fMRI compatible interfaces (in collaboration with Prof. Dr. R. Gassert) for the study of conscious action and own body awareness. The fMRI data reveal an activation of a dedicated network of own body awareness in the following brain areas: right extrastriate body area (rMidTemp), right temporo-parietal junction (rTPJ), and right precentral and postcentral gyrus (rPrec) (only the right hemisphere activations are shown).
**Introduction**

The Laboratory of Molecular and Cellular Biology of Alzheimer’s Disease is focusing on better understanding the molecular, cellular and biochemical mechanisms of Alzheimer’s disease. Our laboratory is also implicated in the design and development of new therapeutic strategies to slow down the pathogenesis of Alzheimer’s disease.

**Keywords**

Molecular and cellular biology of Alzheimer’s disease, γ-Secretase, Amyloid-beta peptides (Aβ), intramembrane-cleaving proteases, therapeutic targets

**Results obtained in 2008**

Alzheimer’s disease (AD) is the most common form of neurodegenerative diseases in humans, characterized by the progressive accumulation and aggregation of amyloid-β peptides (Aβ) in brain regions subserving memory and cognition. The 39–43 amino acids long Aβ peptides are generated by the sequential proteolytic cleavages of the amyloid-β precursor protein (APP) by β- and γ-secretases, with the latter being the founding member of a new class of intramembrane-cleaving proteases (I-CliPs) characterized by their intramembranous catalytic residues hydrolyzing the peptide bonds within the transmembrane regions of their respective substrates. Although the two primary forms of Aβ are the 40- and 42- amino acid variants (Aβ40 and Aβ42), the longer and more hydrophobic Aβ42 has been particularly implicated in amyloid plaque formation and in the pathogenesis of AD.

Three-dimensional structure of γ-secretase.

γ-Secretase is an unconventional aspartyl protease that processes many type 1 membrane proteins within the lipid bilayer. Because its cleavage of APP generates the amyloid-β protein (Aβ) of Alzheimer’s disease, partially inhibiting γ-secretase is an attractive therapeutic strategy. Despite this evidence, the structure of the protease remains poorly understood. In 2006, we used electron microscopy and single particle image analysis on the purified enzyme to generate the first 3D reconstruction of γ-secretase, but at low resolution (15Å, Lazarov/Fraering et al., 2006). The limited amount of purified γ-secretase that can be produced using currently available cell lines and procedures has prevented the achievement of a high-resolution crystal structure by X-ray crystallography or 2D crystallization. Because of this observation, we generated and characterized a new mammalian cell line (S-20) that overexpresses strikingly high levels of all four γ-secretase components (presenilin, nicastrin, Aph-1 and Pen-2). We then used these cells to develop a rapid protocol for the high-grade purification of proteolytically active γ-secretase (Cacquevel et al., 2008). Next, we used these cells and purification methods to solve the 3D structure of the γ-secretase complex at 12Å resolution as obtained by cryoelectron microscopy and single-particle image reconstruction (Osenkowski et al., 2008). The structure revealed several domains on the extracellular side, three solvent-accessible low-density cavities, and a potential substrate-binding surface groove in the transmembrane region of the complex.

Substrate-targeting γ-secretase modulators.

Collectively, it is accepted now that strategies that decrease the accumulation of Aβ, in particular Aβ42, might be therapeutically beneficial if they do not interfere with the other γ-secretase functions (e.g. interference with Notch processing leads to severe gut toxicity because of interference with intestinal stem cell differentiation). For example, selective lowering of Aβ42 with Notch-sparing small molecule γ-secretase modulators (GSMs) is a promising therapeutic approach for AD. Because the precise molecular target of GSMs has not been established, novel biotinylated photo-activatable GSMs have been syn-
thesized to identify the target responsible for the selective lowering of Aβ42 (Kukar et al., 2008). First, we found that these GSM photoprobes altered Aβ42 production, but did not label any of the four primary protein components of the γ-secretase complex. Instead, GSM photoprobes labeled APP, APP carboxyl-terminal fragments and Aβ. Next, labeling of an APP γ-secretase substrate was more efficient than labeling of a Notch substrate. Finally, GSMs altered the production of cell derived Aβ oligomers. Taken together, these findings demonstrate that substrate targeting by GSMs results in two therapeutic actions: (1) alteration in Aβ42 production and (2) inhibition of Aβ aggregation, that may synergistically reduce Aβ deposition in AD. They also provide the first proof of this concept with respect to the feasibility of substrate targeting by small molecule compounds.

Selected publications


γ-Secretase-dependent intramembrane processing of APP and Aβ production. The membrane-bound 99-residue C-terminal fragment of the amyloid precursor protein (APP-C99) serves as a substrate for γ-secretase, which catalyzes an unusual proteolysis within the transmembrane region to generate the toxic amyloid-beta peptides (Aβ) and release the APP intracellular domain (AICD). Following association with the adaptor protein Fe65 and nuclear translocation, AICD is able to suppress the expression of the major apoe/lipoprotein receptor LRP1 by binding directly to its promoter. Thus, APP processing is also involved in the regulation of brain apoE (a major genetic determinant of AD) and cholesterol metabolism.
Introduction
The Laboratory of Computational Neuroscience uses theoretical methods from mathematics, computer science, and physics to understand brain function. Questions addressed are: what is the code used by neurons in the brain?; how can changes of synapses lead to learning?

Keywords
Modeling, Hebbian learning, spike-timing dependent plasticity, simulation, spiking neuron models

Results obtained in 2008

We have been active in three different, but connected areas:

Single-Neuron Modeling: We have shown that the electrical behaviour of neurons under somatic current or conductance injection can be well described by simplified neuron models with only one or two equations. The parameters of these neuron models can be directly extracted from experimental data. Our work has answered, in this context, two questions: first, what is the best simplified neuron model – the answer is exponential integrate-and fire model combined with adaptation and/or refractoriness (Badel et al 2008). Second, is there a way to quantify the heterogeneity of neurons – the answer is yes, since model parameters can be estimated reliably and on a neuron-by-neuron data from a few seconds of electrophysiological data (Badel et al. 2008c). The mathematical properties of such neuron models have been analyzed (Naud et al 2008). To compare our approach with other approaches, we have organized an international competition – and indeed the simplified neuron models from our and one other lab wer the winners (Jolivet et al. 2008, 2008b). This work involves collaborations with the labs of Henry Markram and Carl Petersen.

Modeling synaptic plasticity. We have developed a model that combines induction of synaptic plasticity with consolidation of synapses. The model of induction accounts for induction of Long-Term Potentiation under protocols of voltage-dependent and Spike-Timing Dependent Plasticity and leads to the tagging of the synapse. The model of consolidation combines a bistable dynamics with a triggering process for protein synthesis (Clopath et al. 2008). The model accounts for a large variety of tagging protocols.

Network Simulation. We have analyzed the dynamics of visual illusions in the framework of a neural field model of cortical activity (Hermens et al. 2008). This project which is finished, now involves collaboration with the laboratory of Michael Herzog. The collaboration with the Herzog lab is continued by a PhD student who connects models of decision processes with psychophysical data on rapid visual processing.

Selected publications
Laurent Badel, Sandrine Lefort, Thomas K. Berger, Carl C. H. Petersen, Wulfram Gerstner and Magnus J. E. Richard-


### Introduction

The theme of our research is the neuro-anatomical bases of emotional, social and cognitive difficulties in autism. To address these questions, we use behavioral data acquisition as well as anatomical and functional brain imaging in young adults with high functioning autism or Asperger syndrome. We are particularly interested in trying to find biomarkers of autism and in exploring whether cognitive behavioral training may induce brain plasticity together with improvement in symptomatology.

### Keywords

Functional and anatomical brain imaging, cognition, emotion, autism, plasticity

### Results obtained in 2008

Our lab has been working at not only performing research on autism spectrum disorders, but also on informing the public about this condition, and organizing meetings with parents’ associations to provide, in collaboration with other labs at EPFL, an overview of what is done in autism research.

We have been publishing a number of papers examining the mechanisms of social cognition in normal controls, as well as underlining the potential involvement of the different brain mechanisms in autism.

Autism spectrum disorders (ASD) are characterized by inflexible and repetitive behavior. Response monitoring involves evaluating the consequences of behavior and making adjustments to optimize outcomes. Deficiencies in this function, and abnormalities in the anterior cingulate cortex (ACC) on which it relies, have been reported as contributing factors to autistic disorders. In collaboration with the group of Dara Manoach at Harvard, we investigated whether ACC structure and function during response monitoring were associated with repetitive behavior in ASD. Our findings suggest that in ASD, structural and functional abnormalities of the ACC compromise response monitoring and thereby contribute to behavior that is rigid and repetitive rather than flexible and responsive to contingencies.

ASD is also characterized by language and communication impairments. In collaboration with the group of Helen Tager-Flusberg in Boston University, we examined semantic functions in adolescents with ASD compared to typically developing adolescents. Our results suggest differences in semantic organization, approaches to the semantic task, or efficiency in semantic processing in ASD adolescents relative to typically developing adolescents.

Facial expression and direction of gaze are two important sources of social information, and what message each conveys may ultimately depend on how the respective information interacts in the eye of the perceiver. Direct gaze signals an interaction with the observer, but averted gaze amounts to «pointing with the eyes», and in combination with a fearful facial expression may signal the presence of environmental danger. We used fMRI to examine how gaze direction influences brain processing of facial expression of fear. Our results indicate that the direction of gaze prompts a process whereby the brain combines the meaning of the facial expression with the information provided by gaze direction, and in the process computes the behavioral implications for the observer.

In parallel, we have been pursuing, in collaboration with our group at the Harvard Medical School in Boston, our research on migraine, its physiopathology and its long-term effect on the brain. We have described that repetitive migraine attacks can induce a thickening of the cortical mantle in areas that are specialized in the sensation of the face and the head, and that they also change connections between areas of the brain that are processing pain.
Selected publications


Meeren HK, Hadjikhani N, Ahlfors S, Hämäläinen MS, de Gelder B. Early category-specific cortical activation revealed by visual stimulus inversion. *PLoS ONE* 2008;3(10)e3503


*MEG evidence in group data that face-like objects activate face-specific cortex as early as 165ms. (Hadjikhani & al., NeuroReport 2009, 20:403–407)*
**Introduction**

In humans, vision is the most important sensory modality. Surprisingly, the mechanisms of even the simplest forms of visual processing, such as spotting a pen on a cluttered desk, are largely unknown and for this reason robots are still "object blind". Our research aims to understand how and why humans can cope with visual tasks so remarkably well.

The main goal of our research is to characterize the interplay of spatial integration and temporal binding processes with the help of psychophysics, TMS, EEG, mathematical modelling, and clinical investigations. Main topics of research are: feature integration, contextual modulation, time course of information processing, and perceptual learning. In clinical studies, deficits of visual information processing are investigated in schizophrenic patients.

**Keywords**
Vision research, spatio-temporal vision, schizophrenia research, psychophysics, TMS, EEG, modelling

**Results obtained in 2008**
Spatial and temporal visual processing are usually considered as largely independent. Consequently, there is very little cross talk between the two research areas. We have shown that this assumption is not justified. The spatial layout of a stimulus determines largely the temporal processing (Duangudom et al., 2008). This finding is at odds with all models of visual masking. For this reason, we developed a new mathematical model which takes spatial aspects into account. Surprisingly, many spatial aspects occur in this model not by explicit coding of spatial features, but by the intrinsic dynamics of spatial processing (Hermens et al., 2008) - showing again the complex relationship between spatial and temporal processing. These results were strongly supported by further experiments from highly different research fields such as contrast processing (Saarela & Herzog, 2008), masking (Breitmeyer et al., 2008), feature fusion (Scharnowski et al., 2008a,b), and even schizophrenia research (Schutze et al., 2008).

Many neural network models attribute the emergence of consciousness usually to certain brain functions modelled by complex networks. We showed that this approach falls short by a simple gedankenexperiment (thought experiment) highlighting that all up-to-date theories imply implicitly that consciousness would also emerge in small networks of less than 10 neurons - leading to a kind of unacceptable panpsychism (Herzog et al., 2008).

**Selected publications**


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Figure 1. Dynamics of non-retinotopic feature integration. Leading up to the behavioral response, the red areas showed decreased activity when two features integrated in a sequential metacontrast paradigm. The timeline in the center of the figure indicates time relative to the button press. The lower leftmost plot shows the integration effect in the Middle Temporal Gyrus (MT) at 530 ms before the response. Activity in all areas decreased when the two features integrated. The interaction closely resembled behavior: with integration, discrimination accuracy was around chance. This resemblance is corroborated by linear regression of estimated current densities on accuracy, depicted in the second plot from the left, green line. The only area where activity increased with feature integration was the Middle Frontal Gyrus (MFG), at around -300 ms. In this area the interaction changed sign, as can be seen in the two lower right plots. Here too, a strong correlation with accuracy was observed. Error bars denote 95% confidence intervals.
Introduction
Research in the Lashuel laboratory is focused on applying chemical, biophysical, and structural biology approaches to understanding molecular and structural basis of protein misfolding and self-assembly and the mechanisms by which these processes contribute to the physiological and pathogenic properties of specific proteins implicated in neuro-degenerative diseases. In addition, we are interested in applying the same approaches to understanding the role of quaternary structure in modulating protein function in health and disease. Current research efforts cover the following topics: (1) Elucidating the molecular and cellular determinants of underlying α-synuclein aggregation and toxicity in Parkinson’s disease and related disorders. (2) Elucidating the structural basis of amyloid-associated toxicity in neuro-degenerative diseases, including Alzheimer’s, Parkinson’s, and Huntington’s disease; (3) developing innovative chemical approaches and novel tools to monitor and control protein folding/misfolding and self-assembly in vitro and in vivo; (4) developing cellular models of neuro-degeneration in Parkinson’s disease; (5) developing novel therapeutic strategies to treat neuro-degenerative diseases based on modulating protein aggregation and clearance.

Results obtained in 2008
α-Synuclein phosphorylation (Refs 1, 4, 6): Increasing evidence suggest that phosphorylation may be an important regulator of alpha-synuclein (α-syn) aggregation, Lewy body (LB) formation and neurotoxicity in Parkinson’s disease (PD) and related synucleinopathies. We demonstrated for the first time that phosphorylation at serine 129 inhibits rather than promotes. In addition, we showed that the phosphorylation mimics (S129E/D) do not reproduce the effect of phosphorylation on the structural and aggregation properties of α-syn in vitro. Our findings have had significant implications for current strategies to elucidate the role of phosphorylation in modulating protein structure and function in health and disease and provide novel insight into the underlying mechanisms that govern α-syn aggregation and toxicity in PD.

AB aggregation and toxicity (Ref 5): Increasing evidence from genetics, clinical, and cell culture studies suggests that the ratio of Aβ40 to Aβ42, rather than the total amount of Aβ, is an important determinant of Aβ aggregation, fibrillogenesis, and toxicity in AD. We have uncovered new aspects of Aβ aggregation equilibria by investigating the ratio-dependent effects of monomeric Aβ40 on the fibrillogenesis of monomeric and protofibrillar forms of Aβ42 and the dynamic reassembly of short Aβ42 fibrils. By studying well defined aggregation states of Aβ, our findings provide critical new mechanistic insights about how Aβ40 and Aβ42 interact at different stages along the amyloid formation pathway. These findings have allowed us to develop a new working model of Aβ42 and Aβ40 interactions, which could serve as mechanistic explanation for the aggregation, clearance, and distribution of Aβ in vivo.

Hsp104 (Ref 6, 8): The molecular mechanisms by which Hsp104 modulates protein aggregation and fibrillogenesis remains unclear. Using Aβ as a model system, we demonstrated that the Hsp104 strong anti-aggregation activity is mediated by its ability to act at different stages and target multiple intermediates on the pathway to amyloid formation. Hsp104 interacts with both Aβ monomers and protofibrils, (ii) inhibits conversion of protofibrils into amyloid fibrils, (iii) arrests fibril elongation and reassembly, and (iv) abolishes the capacity of protofibrils and sonicated fibrils to seed the fibrillation of monomeric Aβ.

Novel chemical tools and approaches to study protein folding and self-assembly (Refs 2, 7, 9, 10): In collaboration with Prof. Manfred Mutter, we extended the switch peptide concept, based on the controlled induction and/or disruption of peptide secondary structure, to develop novel model peptide systems and high-throughput screening approaches to study the molecular mechanism of amyloid formation and disassembly. The experimental data offer new insights into the stability and dynamic structural properties of amyloid fibrils. Furthermore, these studies demonstrate for the first time that controlled induction of amyloid dissociation is possible and that...
Structural changes in polypeptide regions, not involved in amyloid formation, could have a significant influence on the stability and structural dynamics of amyloid fibrils.

**Macrophage Migration Inhibitory factor (MIF):**
MIF is a multifunctional protein and a major mediator of innate immunity. Despite the extensive knowledge about the structure of the MIF trimer, very little is known about the molecular and structural determinants that govern the oligomerization of MIF and the role of quaternary structure in modulating its functions in health and disease. Using biochemical and computational approaches, we investigated the role of the C-terminal region in modulating the tertiary structure, thermodynamic stability, receptor binding and enzymatic activity of MIF. Our results suggest that the C-terminal region is required for stabilizing the tertiary structure of MIF and maintaining it in an enzymatically active conformation, but does not play a significant role in modulating the oligomerization of MIF.

**Selected publications**


El-turk F., Michele C., Oueratani-Sakouhi H., Zweckstetter, M., Leng L, Bucala R., Röthlisberger U., Lashuel HA*. "The role of the carboxy terminal residues 104-114 in modulating the conformational flexibility and catalytic activity of Macrophage migration inhibitory factor (MIF)". *Biochemistry* 2008, 47(40):10740-10756


Introduction
The Laboratory of Functional Neurogenomics (LNGF) uses high-throughput gene expression profiling and other molecular approaches to elucidate new aspects of brain function and neurodegenerative disease. In this manner, we are able to study the diverse effects of pharmacologic agents, environment, and disease-causing proteins on the expression of the entire mammalian genome. This information is then used to understand which molecules are responsible for a particular brain-related process. Currently, our efforts focus on the molecular study of hereditary polyglutamine neurodegenerative diseases, primarily Huntington’s disease, and the normal function of the brain regions involved in Huntington’s disease.

Keywords
Neurodegenerative disease, Huntington’s disease, striatum, cerebral cortex, motor cortex

Results obtained in 2008
1. Intrinsic effects of mutant huntingtin in striatal neurons. Our molecular assessments of Huntington's disease in primary neuron-based models of HD have shown that the effects of mutant huntingtin on the expression of other genes are not dependent on brain circuitry, but represent disease-related changes within striatal neurons (Runne et al., 2008).

2. Heterogeneity of molecular subtypes of cells derived from the embryonic ganglionic eminence. We have shown that cells derived from the caudal ganglionic eminence phenotype represent a subset distinct from its medial or lateral subdivisions. Moreover, additional subregional heterogeneity suggest that a large number of independent GABAergic neuronal populations may arise from this structure (Willi-Monnerat et al., 2008).

3. Negative regulation of AP-2- and clathrin-dependent endocytosis by calpain. We have shown that alpha and beta-2-adaptins are in vivo substrates for calpains in and that the presence of calpain-generated beta-2-adaptin fragments can specifically decrease endocytosis of specific receptors and sensitize neurons to excitotoxic cell death (Rudinskiy et al., J. Biol. Chem., in press).

Selected publications


Model for calpain-driven sensitization of neuronal cells to excitotoxicity via decreased endocytosis. The left panel represents normal (non-degenerative) conditions, where calcium homeostasis is normal and calpain activity is low. The right panel represents a potential disease state where primary abnormalities of protein aggregation, energy metabolism, endoplasmic reticulum stress, or dysregulated neurotransmission could potentially sensitize cells to excitatory neurotransmitters such as glutamate. In this case, calpain cleavage of endocytic proteins is envisaged to decrease excitatory receptor endocytosis, which would result in their increased presence on the neuronal surface and thereby mediate excitotoxic damage or death. Not shown is the possibility that an abnormal cell surface turnover of receptors could also result in their being abnormally trafficked to extrasynaptic sites, which could further sensitize them to negative effects of excitatory signals.
Introduction

Our laboratory has two main lines of research. The principal one is to try to understand the cellular and molecular mechanisms of the interactions between neurons and glial cells (astrocytes), termed neuro-metabolic coupling and to investigate this coupling in other aspects of brain function and dysfunction, such as the sleep-wake cycle, learning and memory as well as neurodegeneration.

The second line of research, which is a joint effort with the laboratory of Christian Depeursinge, is represented by the neurophotonics project to develop a new type of microscope for the study of dynamic cellular processes by non-invasive, on-line, three-dimensional visualization of cells with a spatial resolution in the order of the nanometer and a millisecond temporal resolution.

Keywords

Neuro-energetics, neuro-glia interactions, brain metabolism, neuronal plasticity, glial plasticity, high-resolution optical imaging, digital holographic microscopy, cell dynamics, neurodegeneration, sleep, functional brain imaging, dialogue between neurosciences and psychoanalysis

Results obtained in 2008

Neuro-energetics and neurodegeneration (Igor Allaman, Mireille Belanger) Alterations of brain energy metabolism and oxidative-stress status. Aβ (25-35 peptide) appeared to trigger changes of astrocytic phenotype similar to those observed with inflammatory mediators such as increased glucose uptake and its various metabolic fates (glycolysis, tricarboxylic acid cycle, pentose phosphate pathway and incorporation into glycogen). Further studies showed that the aggregation and binding of Aβ peptides to class A scavenger receptors (SR-A) induced a deleterious cascade leading to the modification of astrocytic phenotype and ultimately to neurotoxicity. These results indicate that perturbations of neuro-energetics are linked to neuro-degeneration in AD.

Glucose metabolism in the central nervous system of rats defective for genes implicated in the anaplerotic cycle using NMR spectroscopy (Sylvain Lengacher, Maude Marti) It is well accepted that neurons are more oxidative and astrocytes more glycolytic and that enzymes involved in energy production are different in the two cell types. For example glutamine synthetase and pyruvate carboxylase are found almost exclusively in astrocytes. We took advantage of this specific gene expression to study the interaction of astrocytes and neurons related to energy metabolism. To evaluate the role of some key enzymes, we have established a technique to inject RNA interference (RNAi) into the brain via a cannula connected to an osmotic pump. This results in a reduction of enzyme expression in the living rat and thereby will allow the subsequent measurement of cerebral glucose and glycogen metabolism using 1H nuclear magnetic resonance (NMR) spectroscopy.

Metabolic plasticity during learning (Julia Parafita, Sylvain Lengacher, Elsy Dunand) Given the tight coupling that exists between synaptic activity and energy metabolism, it is likely that the processes
that underlie synaptic plasticity may also be reflected at the energy metabolism level, resulting in correlated metabolic adaptations which could be defined as “metabolic plasticity”. To explore the mechanisms of metabolic plasticity, we have mapped glucose utilization at different phases in a well-established paradigm of spatial learning - the eight-arm radial maze. In collaboration with Professor Jean-Philippe Thiran (STI, EPFL), we have developed a new software for the analysis of 2-deoxyglucose (2DG) autoradiograms in a faster, more efficient and unbiased manner, compared to classical systems, such as MCID, in which the regional cerebral 2DG uptake is assessed by optical densitometry of selected brain regions on the autoradiograms. This new software (named JULIDE) reconstructs a virtual brain by registering the successive 2D autoradiography images (10 x 10 microns resolution, 200 microns between slices). Using both types of analysis (MCID and JULIDE), we found increased metabolic activity in the hippocampus and the latero-dorsal nucleus of the thalamus the first day of training, which decreases in time during learning in favour of the cingulate and the retrosplenial cortex. At day 9, the last day of training, the striatum was the region which presents a higher activation consistent with some form of habit formation over the nine days of training.

**Neurophotonic project** (P. Marquet, P. Jourdain, B. Rappaz, D. Boss, A. Hoffmann, C. Moratal) In a collaborative effort with the Prof. Depeursinge’s group (STI, EPFL), we have developed an optical digital holographic microscopy technique (DHM), that allows to observe non-invasively and in real time the 3D cellular dynamics with an axial resolution of a few tens of nanometers Within the field of cell biology, DHM phase signal provides information about the observed specimen, including cell structure and dynamics as well as intracellular refractive index related to intracellular content. Consequently, different technical developments aiming to adequately interpret the DHM phase signal in terms of the underlying biological processes have been developed. We are exploring the possibility to optically and non-invasively measure transmembrane electrical currents, allowing to interpret DHM quantitative phase image at each pixel as a non-invasive optical ‘electrode’. Consequently, DHM can be used to resolve local neuronal network activity by recording electrophysiological events in addition to the cell morphology information provided by the quantitative phase images.

**Selected publications**


Gavillet M, Allaman I, Magistretti PJ. Modulation of astrocytic metabolic phenotype by proinflammatory cytokines. *Glia,* 2008 Mar 27; [Epub ahead of print]

Introduction
The Laboratory of Neural Microcircuitry focuses on understanding the circuitry formed between neurons and how these circuits give rise to emergent intelligence and cognition. LNMC is also focused on what goes wrong in the circuits of the brain in different diseases such as autism. Using an animal model of autism we found that circuits are enhanced in their sensitivity to stimulation as well as their ability to learn and we have therefore put forward a radical new theory for this disorder called "the intense world theory of autism".

Results obtained in 2008
The Laboratory of Neural Microcircuitry is dedicated to understanding the structure, function and plasticity of the microcircuitry of the neocortex in particular, as well as other brain regions.

The neocortex constitutes nearly 80% of the human brain and is made of a repeating stereotypical microcircuit of neurons. This neural microcircuit lies at the heart of the information processing capability of the neocortex, the capability of mammals to adapt to a rapidly changing environment, memory, and higher cognitive functions.

Our goal is to derive the blue print for this microcircuit. The neocortical microcircuit exhibits omnipotent computational capabilities, meaning that the same microcircuit of neurons can simultaneously partake in an unrestricted number of tasks. This capability allows the neocortex to be parcellated into multiple overlapping functional vertical columns (0.3-0.5 m in diameter) that form the foundation of functional compartmentalization of the neocortex. In order to derive the blueprint of this microcircuit, we study the components (the neurons) of the microcircuit, how the neurons are interconnected (anatomical properties of connections), and the functional structure of the microcircuitry (physiological & plasticity properties of connections). A neocortical column contains several thousand neurons interconnected in a precise and intricate manner.

To study the different types of single neurons we employ whole-cell patch clamp studies in neocortical slices to obtain the electrophysiological profile of neurons, to aspirate cytoplasm for single cell multiplex RT-PCR studies and to load the neurons with dyes to allow subsequent 3D anatomical computer reconstruction of each neuron. This approach enables us to derive the electrophysiological behavior,
The anatomical structure, as well as the genetic basis of the anatomy and physiology of each type of cell. The microcircuit contains at least 9 major anatomical classes of cells, 15 major electrophysiological classes and 20 major molecular classes. Precise anatomical and physiological rules also operate to connect the different types of neurons. In order to derive these rules, we obtain multiple patch-clamp recordings from pre-selected neurons. This allows repeated analysis of the major connections and derivation of the signatures of connectivity as well as the physiological and plasticity principles for these connections.

With the growing set of precise multidimensional parameters that characterize the microcircuit, it has now become possible to assess the integrity of the microcircuit to support functions and what may go wrong in different diseases. This is allowing a new generation of experiments that could reveal microcircuit changes caused by interacting with the environment and by disease. A current project is aimed at isolating the microcircuit deficits that may underlie autism. In addition to obtaining the genetic, structural, functional and plasticity principles that make up the blueprint of the neocortical microcircuit, we are systematically reconstructing this microcircuit in large scale computer models. These theoretical studies are focused on simulating the entire microcircuit, constructing genetic algorithms that could grow microcircuits based on genetic information, constructing algorithms to allow a model microcircuit to learn and adapt to a rapidly changing environment, exploring principles of information processing at different levels of the microcircuit and practical implementations in robotics. In summary, we believe that the neocortical microcircuit is the essence of neocortical computation and that deriving this blueprint is essential for a comprehensive understanding of high cognitive functions.

Virtually all neurological and psychiatric disorders involve the neocortex at some stage and at some level. The blue print to the neocortical microcircuit could therefore provide the foundation for developing interventions that could «surgically» correct microcircuit deviations. Furthermore, this neocortical microcircuit exhibits computational power that is impossible to match with any known technology. Deriving the blueprint and its operational principles could therefore spur a new generation of neuromorphic devices with immense computational power.

**Selected publications**


Rinaldi T, Perrodin C, Markram H., Hyper-connectivity and hyper-plasticity in the medial prefrontal cortex in the valproic Acid animal model of autism, *Front Neural Circuits*. 2008;2:4


Rinaldi T, Kulangara K, Antonielo K, Markham H., Elevated NMDA receptor levels & enhanced postsynaptic long-term potentiation induced by prenatal exposure to valproic acid, *Proc. Natll. Acad.Sci. USA.* 2007 Aug 14;104(33):13501-6


**Schematic overview of autism as an intense and aversive world syndrome: from the triggering events and genetic predisposition to the observable behaviors mediated through a hyper-reactive and hyper-plastic neocortex**
Introduction

The Blue Brain Project accomplished the proof-of-concept that it is possible to introduce simulation-based research into neuroscience. In order to devise a detailed biophysical tissue model of the brain, extensive reverse engineering of the biological specimen is used and the data is combined with highly automated workflows for building, simulating and visualizing models on a BlueGene/L supercomputer.

Keywords

Neocortex, simulation-based research, reverse engineering, high performance computing

Results obtained in 2008

Three years after the project start, the Blue Brain Project had succeeded to develop a novel modeling facility that allows the automatic creation of models of neural circuitry in a completely data-driven fashion. Applying this process to years of Prof. Markram’s electrophysiology, morphology and genetic data of the somatosensory cortex of young rats, 1 mm³ of rat neocortex is now being modeled at the cellular level. This is the first proof-of-concept that sufficiently quantitative data can be combined with automated modelling procedures to allow “in silico” research in neurobiology that can verify against published detailed anatomical and electrophysiological experiments and even predict novel aspects to be studied.

In the year 2008, the primary focus was to validate the biological accuracy and in a close iteration cycle with experiment, the modeling process (and consequently the models) from ion channels, to single cells, from individual synapse behavior to connections has been refined. Using other labs’ published data on anatomical findings (e.g. EM data) and electrophysiology (e.g. network experiments in slice with voltage or calcium dye imaging), the model generalization has been carefully tested.

Those results were presented to the scientific community during the Forum of European Neuroscience Symposium (FENS 2008) in a satellite workshop, a special session and numerous poster presentations. Not only new methods to large-scale modeling have been contributed (Hines 2008a, 2009b), but also the first modeling insights have been published (Druckmann 2008, 2009) that prepare the grounds for the neuroscientific insights currently in preparation for journal publication. Furthermore, first tools have
been rolled out to the community (King 2009).

Meanwhile, the Blue Brain project represents a facility not only to synthesize existing biological data in a comprehensive and consistent framework, but also a novel tool for neuroscientists to recreate experiments done typically over months in the wetlab in that matter of hours to days in silico. Such a capability will prove useful in the future to do joint experimental/simulation studies and to eventually go to full in silico research.

Selected publications


![Visualization of an entire neocortical column with network activity. The membrane voltage is shown in false colors. This provides a view into the neocortical column at a level inaccessible to experimental techniques to date.](image-url)
**Introduction**
Sensory perception is an active process in which neurons in the brain construct an internal representation of the world. Our goal is to obtain causal and mechanistic explanations for simple forms of sensory perception in mice at the level of individual neurons and their synaptic connections.

**Keywords**
Neocortex, synaptic transmission, sensory perception, learning, whisker behaviour, barrel cortex, cortical circuits, whole-cell recording, voltage-sensitive dye imaging, calcium-sensitive dye imaging, two photon microscopy, Lentivirus

**Results obtained in 2008**
Brain states strongly affect the processing of sensory information. We have examined the mouse primary somatosensory barrel cortex, which is known to process tactile information relating to the movements of the mystacial vibrissae. During quiet wakefulness sensory responses to a single brief whisker deflection are large and propagate across a large area of sensorimotor cortex. On the other hand, during active behaviours, for example when the mouse is moving its whiskers, the same whisker deflection evokes only small sensory responses, which are localised to a small part of the barrel cortex and do not propagate (Ferezou et al., 2007). Because of these prominent differences in processing of sensory information comparing these two behavioural states, we have begun to analyse the underlying differences in brain states between quiet and active behaviours.

Our analyses are based on single and dual whole-cell recordings of membrane potential dynamics of excitatory neurons in awake head-restrained mice during quantified whisker behaviour (Poulet & Petersen, 2008). During quiet wakefulness slow, large-amplitude membrane potential changes are prominent in the mouse barrel cortex. These slow 1-5 Hz oscillations are highly synchronised in nearby neurons and they define a resting state of the mouse brain, which may be similar to the EEG alpha waves observed in human visual cortex during rest. When the mouse is active, the slow oscillation is dramatically reduced in amplitude. Higher frequency membrane potential fluctuations play a more prominent role during this active state and they are less correlated in nearby neurons. During active behavioural states, membrane potentials in nearby cortical neurons therefore become more independent from each other, which may help increase the total processing power of the neocortex.

The correlated cortical activity occurs at the level of subthreshold membrane potential dynamics and we did not observe synchronous action potential firing in nearby neurons. Indeed action potential activity in the excitatory neurons of the supragranular layers of awake mouse barrel cortex appears to occur at low frequencies, suggesting a sparse coding of information. In order to examine the events driving these action potentials, we specifically analysed the membrane potential dynamics leading up to action potential initiation. Single action potentials were driven by large, brief and specific excitatory input, which was not present in the membrane potential of another nearby neuron. Action potentials, although rare, are therefore not random noisy events, but appear to be driven by highly specific mechanisms.

These data provide the first insights into the membrane potential dynamics and correlations of cortical neurons in an awake mammal. However, we know little about how the different patterns of activity arise. Interestingly, the brain state changes appear to be generated internally within the central nervous system and do not arise from differences in sensory input generated by the active behaviour. It will therefore be of great interest in future studies to define the underlying central mechanisms generating the brain state changes in somatosensory cortex during whisking and to determine whether they relate to direct effects of neuromodulators in the neocortex or to changes in corticocortical or thalamocortical input.
**Selected publications**


Aronoff R, Petersen CCH (2007) Layer- and column-specific knockout of NMDA receptors in pyramidal neurons of the mouse barrel cortex. *Front Integr Neurosci.* 1: 1


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The membrane potentials of two cortical neurons were recorded during filming of whisker-related behaviour. Superimposed images over a 100 ms period during quiet wakefulness and during active whisking (above). The membrane potential (Vm) of cell 1 (red trace) and cell 2 (black trace) are highly correlated during quiet wakefulness when the whisker is not moving (whisker angle is plotted in green trace). During active whisking the slow large-amplitude membrane potential oscillations are suppressed and the membrane potentials decorrelate (Poulet & Petersen, 2008).
Introduction
The Laboratory of Behavioural Genetics investigates the impact and mechanisms whereby stress affects brain function and cognition, with a focus on learning and memory processes and on psychiatric disorders - such as anxiety, depression, and violence.

Keywords
Stress, learning, memory, violence, neuroplasticity, cell adhesion molecules, glutamate receptors

Results obtained in 2008
Stress at learning facilitates memory formation by regulating AMPA receptor trafficking through a glucocorticoid action
Stress and glucocorticoids can facilitate memory formation. However, the molecular mechanisms mediating their effects are largely unknown. AMPA receptor (AMPAR) trafficking has been implicated in the changes in synaptic strength at central glutamatergic synapses associated with memory formation. Using the water maze spatial task involving different stress levels, mice trained under more stressful conditions (water at 22°C) showed better learning and memory, and higher post-training corticosterone levels, than mice trained under lower stress (water at 30°C). Strikingly, this facilitated learning by stress was accompanied by enhanced synaptic expression of GluR2 AMPARs that was not observed in mice trained under less stressful conditions. Interfering with glucocorticoid actions by injecting the glucocorticoid synthesis inhibitor, metyrapone, blocked both the memory facilitation and the enhanced GluR2 trafficking induced by stressful learning. Intracerebroventricular infusion of the peptide, pep2m, that blocks GluR2 synaptic trafficking by interfering with the interaction between N-Ethylmaleimide-Sensitive Factor (NSF) and GluR2, impaired immediate performance at learning as well as long-term memory retrieval, supporting a causal role for GluA2 trafficking in stress-induced facilitation of spatial learning and memory. We also found evidence for the involvement of the neural cell adhesion molecule N-cadherin in interaction with GluA2. These findings underscore a new mechanism whereby stress can improve memory function (Conboy and Sandi, in press).

Neural and molecular correlates of abnormal aggression
Early life stress enhances the risk for psychopathologies, including excessive aggression and violence. Infancy and adolescence are important maturation phases during which critical neuro-developmental events occur in brain regions associated with motivation, emotion and cognition. We have developed an animal model of abnormal aggression in rats induced by exposure to stress during the peri-puberty period. Using 14C-2-deoxyglucose autoradiography and immunohistochemistry of the immediate early response proteins (IREs), we have found that stress alters the expression of IREs in the hippocampus and prefrontal cortex, regions involved in memory and emotion regulation. These findings suggest that stress can disrupt the balance between excitatory and inhibitory neurotransmission, leading to abnormal aggression.
gene c-fos, we found increased basal metabolism in different amygdala nuclei and in the bed nucleus of stria terminalis, both areas related to anxiety. After an aggressive encounter, peri-puberty stressed rats showed increased c-fos expression in the medial and central amygdala nuclei and a lower expression in the medial orbitofrontal cortex. We also found increased expression of the serotonin transporter and mono-aminooxidase A (MAO-A) genes in the prefrontal cortex in peri-puberty stressed rats, as well as evidence for the involvement of epigenetic mechanisms on the observed changes in MAO-A expression. These findings underscore new mechanisms whereby early stress can have an enduring impact in individuals’ aggression (Marquez et al., in preparation).

**Modelling stress effects on behaviour**

In collaboration with the group of Wulfram Gerstner at the BMI, we used a reinforcement learning approach for model-based analysis of rodent learning in two tasks: the Morris water maze and the hole-box conditioning. Using two inbred strains of mice (C57BL/6 and DBA/2), we investigated how different stressors – extrinsic (e.g. prior exposure to an elevated platform) and intrinsic (e.g. food deprivation in the hole-box or water temperature in the water maze) – affect animal learning and behavioural performance. In the hole-box study, we also pharmacologically manipulated the noradrenergic system. Then, we interpreted the effects of experimental manipulations using best-fitting parameters of reinforcement learning models, such as the learning rate, the exploration-exploitation balance, and the future reward discounting. We observed that stress and noradrenaline led to higher exploitation of current knowledge and smaller consideration of future rewards, although its effects were dependent on genetic strain, stress type, and other factors (Luksys et al., in press).

**Selected publications**


Introduction
In the brain, nerve cells are arranged in intricate neuronal networks, and communicate with each other at synapses, in a process called «synaptic transmission». Synaptic transmission is the only means of fast information transfer between neurons. Therefore, a detailed understanding of the signalling mechanisms in synaptic transmission is an important pre-requisite to understand how information is processed in neuronal circuits.

Keywords
Synaptic transmission, nerve terminal, neurotransmitter, synaptic vesicle, exocytosis, short-term plasticity, Synaptogenesis

Results obtained in 2008
Ca2+ dependent vesicle fusion and its molecular regulation. In these projects, we study the presynaptic signalling mechanisms during synaptic transmission, with emphasis on the opening of voltage-gated Ca2+ channels and the triggering of vesicle fusion by Ca2+. For these and other studies of our lab, we use a large glutamatergic model synapse in the lower auditory brainstem circuit, the calyx of Held. At this synapse, direct presynaptic whole-cell patch-clamp recordings can be performed. In 2008, we have shown that in developing calyx of Held synapses, the spatial coupling between Ca2+ channels and membrane-docked (readily-releasable) transmitter vesicles becomes tighter, so that a significantly smaller Ca2+ current in the nerve terminal is sufficient to trigger a given amount of transmitter release (see Figure). On the other hand, the intrinsic Ca2+ sensitivity, as measured in presynaptic Ca2+ uncaging experiments, is unchanged in the same developmental period (Kochubey, Han & Schneggenburger 2009, J. Physiology).

In an ongoing study, we investigate the role of distinct residues and domains of the synaptic vesicle protein Synaptotagmin-2, in the Ca2+ triggering of vesicle fusion at the calyx of Held. For this purpose, we have developed an adenovirus-mediated overexpression approach at the calyx of Held (O. Kochubey, ongoing). Furthermore, we have adapted the Cre-lox system in mice to allow tissue-specific gene deletion in the lower auditory system of mice (Y. Han, ongoing). With this approach, we have begun to study the role of the presynaptic active zone protein Rim-1/2 (rab-interacting molecule) in presynaptic Ca2+ signalling and transmitter release (Y. Han et al., ongoing). In addition, we have completed a molecular characterization of the expression of all Synaptotagmin isoforms in calyx of Held-generating neurons, using a novel method of single-cell qPCR (Xiao, Han, Runne, Murray, Kochubey, Lüthi-Carter, Schneggenburger, submitted).

Mechanisms of short-term plasticity and modulation. Using the calyx of Held, we have shown that facilitation of Ca2+ current in the nerve terminal, although observed reproducibly, plays only a minor role for the classical «paired pulse facilitation» (Müller et al., 2008). In a further study, we found that when transmitter release is pre-depressed, the synapse shows a larger susceptibility to the effects of short-term facilitation as compared to the «naïve» synapse. This can be explained by a heterogeneity of intrinsic Ca2+ sensitivities as probed by the Ca2+ uncaging technique (Müller, Goutman, Kochubey and Schneggenburger, in preparation). These findings are important for a mechanistic understanding of short-term plasticity and its effect on neuronal network communication.

Potentiation- and second messenger modulation of synaptic transmission. We have finished a study that shows that both munc-13 (a presynaptic active zone protein with a role in vesicle priming) as well as protein kinase C (PKC) contribute to the diacylglycerol - (DAG) and phorbol ester-mediated increase in transmitter release at the calyx of Held (Lou et al., 2008; (see Figure). We are now investigating the physiological pathways that lead to DAG production in the nerve terminal, and to the activation of the munc13/PKC signalling pathway (O. Genc, ongoing).
**Molecular mechanisms of calyx of Held development.** Calyces of Held are abruptly formed shortly after birth, but the molecular mechanisms of their development are unknown. In 2008, we have developed approaches of genome-wide gene expression analyses in distinct lower auditory brainstem nuclei (Le Xiao, ongoing; with the aid of the DNA Array facility Lausanne; DAFL). This might allow us in the near future to identify nuclei-specific/enriched genes and to test their role in signalling cascades that lead to the generation of the large calyceal synapses.

**Selected publications**


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**Munc13-1 is critically involved in the phorbol ester-induced potentiation of spontaneous release at the calyx of Held.** Experiments with a Munc13-1 point mutation in which phorbol ester binding is abolished.

*Taken, with permission, from Lou et al. 2008, J. Neuroscience 28: 8257.*
IBI - Institute of Bioengineering & Co-Affiliates

The Institute of Bioengineering sits at the interface of the life sciences and engineering, being situated in both the Faculty of Life Sciences and the Faculty of Engineering and reporting to both deans. This dual affiliation allows great diversity in hiring faculty from different backgrounds and with different research perspectives, all focused on basic biological sciences using quantitative and systems analyses, as well as translating the biological and biochemical sciences into therapeutics and diagnostics. The dual affiliation also provides a rich educational environment, both at the BS/MS and PhD levels; at present, a joint MS in Bioengineering is being developed between the two Faculties.

In pursuit of basic biological mechanisms, IBI faculty investigate questions such as: How the cellular microenvironment controls cellular differentiation and morphogenetic processes; How stem cell processes, such as self-renewal and differentiation, are determined; How cell migration and trafficking in complex environments is modulated; How complex biological networks such as metabolism, gene expression and protein trafficking are regulated; and How biophysical and biomolecular signals interact in controlling cellular behavior. Our goal is to transform knowledge gained from our studies into clinical applications. To that end, the IBI faculty develop novel technologies in areas including: interventional and diagnostic biomedical microdevices, synthetic and biosynthetic biomaterials for delivery of small molecule drugs, proteins and DNA, materials in bionanotechnology, immunotherapy based on active biomolecules and nanomaterials, novel molecules for photodynamic therapy, and tissue engineering for therapeutics as well as physiological modeling based on biomolecular and stem cell approaches. 'http://ibi.epfl.ch'
Introduction
The research of the Laboratory of Integrative and Systems Physiology (LISP) aims to understand how regulatory proteins, including nuclear receptors, membrane receptors and transcriptional cofactors, act as sensors for molecules of nutritional, metabolic or pharmacological origin, and translate this into altered gene expression and protein patterns affecting metabolic function.

Keywords
Diabetes, genetics, metabolism, metabolic disease, phenogenomics, transcription

Results obtained in 2008
The Auwerx/Schoonjans laboratory was amongst the pioneers to unravel the wide-ranging implications of the three PPARs, PPARα, PPARβ/δ, and PPARγ, in metabolic control. Perhaps most striking in this context was our discovery of an association between the PPARγ Pro12Ala gene variant with type 2 diabetes and obesity, identified long before the era of genome-wide association studies, and as such the first gene tied with these common complex diseases. We established how the enterohepatic nuclear receptors, LRH-1 and SHP, govern hepatic lipid and bile acid metabolism, regulate mucosal immune homeostasis, and control fertility via their commanding role on steroid production. We furthermore identified bile acids as endocrine regulators of energy expenditure, through the activation of a novel membrane receptor, TGR5. Finally, we established that transcriptional cofactors, such as the acetyltransferases (SRC2/TIF2 and SRC-3) and the deacetylases (such as SIRT1), fine-tune energy homeostasis by changing the acetylation status of PGC-1α, the master regulator of mitochondria. Since altered signaling by nuclear receptors and cofactors, contributes to the pathogenesis of type 2 diabetes, obesity and atherosclerosis, our research paved the way for novel preventative and therapeutic strategies for these common diseases. The importance of these discoveries is testified by the fact that several compounds targeting these receptors and/or cofactors have made it into the clinic. Examples of drugs for which our research contributed to clinical development are the fibrates (that target PPARα), thiazolidinediones (that target PPARγ), PPARβ/δ agonists, bile acids and bile acid derivatives (that target both the TGR5 and FXR), and resveratrol and SRT1720 (which activate SIRT1).

Selected publications


Non-invasive imaging of a bile acid reporter mouse.
IBI - Institute of Bioengineering

Barrandon Lab

Head of Lab (PI) - http://ldcs.epfl.ch/

Introduction
The joint chair (EPFL/Unil-CHUV) of Stem Cell Dynamics is involved in fundamental and translational stem cell research.

Keywords
Stem cell, morphogenesis, micro-environment, plasticity, reprogramming, cell and gene therapy.

Results obtained in 2008
The laboratory of Stem Cell Dynamics at EPFL and Experimental Surgery at the CHUV has three main objectives: firstly, to understand the relationship between stem/progenitors cells of stratified epithelia, secondly to understand the impact of the environment on stem cell behavior and thirdly to comprehend stem cell engraftment. All projects ultimately aim at improving cell and gene therapy using epithelial stem/progenitor cells. Seven PhD’s were successfully defended in 2008, and the laboratory has been named a partner in two stem cell consortia of the EEC 7th framework program, aiming at the fundamentals of stem cells (EuroSyStem) and stem cell therapy (OptiStem).

Maintenance of organ function relies on the same basic mechanisms involved during morphogenesis; in those tissues and organs undergoing extensive remodeling, there are cells termed stem cells that are responsible for long-term renewal, tissue regeneration and repair. Stem cells have two fundamental properties, the capacity to self-renew and to generate a differentiated progeny for an extended period of time (theoretically for a lifetime). Within tissue stem cells, the skin is privileged because its stem cells (epithelial and mesenchymal) can be extensively cultivated and cloned, genetically manipulated and transplanted in laboratory animals, but also in human. We have demonstrated that all stratified epithelia of the rat, independent of their primary germ line origin (e.g. the endodermal esophagus or the ectodermal cornea), contain clonogenic stem cells that can respond to skin morphogenetic signals by forming epidermis, sebaceous glands and functional hair follicles in serial transplantation. Furthermore, we have demonstrated that the thymus, which has a unique 3D structure that does not resemble that of a simple or stratified epithelium, contains a population of clonogenic epithelial cells with astonishing capabilities. These clonogenic cells maintain a thymic identity in vitro or in a reconstituted thymus in vivo, but adopt the fate of bona fide multi-potent stem cells of the hair follicle when exposed to skin morphogenetic signals by forming epidermis, sebaceous glands and functional hair follicles in serial transplantation. Furthermore, we have demonstrated that the thymus, which has a unique 3D structure that does not resemble that of a simple or stratified epithelium, contains a population of clonogenic epithelial cells with astonishing capabilities. These clonogenic cells maintain a thymic identity in vitro or in a reconstituted thymus in vivo, but adopt the fate of bona fide multi-potent stem cells of the hair follicle when exposed to skin morphogenetic signals, a property maintained in serial transplantation. Gene profiling experiments have demonstrated that several transcription factors important for thymic identity were either down regulated or silenced in thymic epithelial cells recovered from skin. This clearly represents a crossing of lineage boundaries, an increase in potency and the demonstration that adult stem/progenitor cells can be robustly reprogrammed by micro-environmental cues. Squamous epithelia, like the skin, the cornea or the esophagus,
are exquisitely located the interface of the body with the external world and are particularly exposed to environmental hazards. Yet little is known on how their stem/progenitor cells adjust to small micro-environmental changes. We have demonstrated that 0.5 degree Celsius (36.5 to 37°C) impacts gene expression in cultured human keratinocyte stem cells and have identified mTOR as a transcriptional modulator to balance environmental changes. Finally, engraftment is the quintessence of stem cell behavior as it draws on all stem cell basic functions, i.e. homing, attachment, migration, proliferation, fate choice, renewal, differentiation and death. In a normal situation, these decisions are tightly controlled and influenced by the micro-environment (the niche). In therapy, the micro-environment may be diseased, damaged by the preconditioning treatment, or even completely missing as in third degree burns or limbal deficiency. Hence, transplanted stem cells have to adapt to an environment that is far from ideal, if not hostile. Surprisingly, little is known on engraftment that remains more a lottery than a scientifically controlled process. We have demonstrated that transplanted stem cells respond to adverse conditions by favoring differentiation rather than self-renewal or death. We are now using state-of-the art architecture, informatics and visualization technology to construct models that will allow us to virtually manipulate stem cell behavior and predict the consequences on organ function using the skin, the thymus and the cornea as model systems.

Selected publications


Introduction
We use advanced molecular modeling techniques combined with high-performance computing to investigate biological systems, in particular their function emerging from structure. Our main targets are bacterial and viral systems and their mechanism of resistance towards natural and clinical drugs. We develop new multi-scale schemes and models to extend the power of current molecular simulations to tackle problems such as the assembly of large macromolecular complexes and the design of remedies for pathogenic infections.

Keywords
Computational biophysics, biochemistry, and structural biology; bacteria and viruses; multi-scale molecular simulations; macro-molecular assembly; protein and drug design; high-performance computing.

Results obtained in 2008
In the past years, computational structural biology has greatly improved our knowledge of biological function at the molecular level, shedding light on issues that are often experimentally inaccessible. We used state-of-the-art molecular simulations in synergy with experimental inputs to understand the function of biological complexes from bacteria and viruses.

In particular, in 2008 we focused on the M2 channel from the influenza A virus, which is one of the major targets for the development of new antiviral drugs. M2 conducts protons to the viral interior permitting the uncoating of the viral RNA and fusion of the viral envelope with the endosomal bilayer. Adamantane drugs (e.g. amantadine) block the proton conduction and have been used for the treatment and prophylaxis of influenza A infections. However, in recent years several strains (including H5N1 avian flu and recent H1N1 swine flu) have rapidly developed a broad resistance against these drugs. Based on the first X-ray structure of the M2 channel, molecular simulations suggested that protons are conducted through a transporter-like mechanism, in which the protein alternates between two different conformations. The transporter-like mechanism is consistent with the known properties of the M2, including its relatively low rate of proton flux and its strong rectifying behavior. Importantly, this mechanism has in turn important implications for the binding mode of adamantane drugs. We found, in fact, that amantadine under both low- and high-pH conditions is stable inside the lumen of the channel (Figure 1), close to the most relevant mutations occurring in resistant strains. These results can provide new hints for the design and development of new blockers with improved efficacy [Proc. Natl. Acad. Sci. USA, 106(4), 1069].

Using the same computational approach we studied the injectisome or needle complex from Yersinia enterocolitica, which allows pathogenic or symbiotic bacteria to inject effector proteins across eukaryotic cell membranes (a process called type III secretion). In particular, we investigated the mechanism by which a specific regulator protein, YscP controls the length of the injectisome needle. The correlation between the size of YscP protein as modeled in its extended form and the observed needle length reinforced the hypothesis that YscP acts as a molecular ruler, and hinted that the secondary structure of YscP influences needle length. The simulated lengths, when the YscP helical content is preserved, correlated strikingly with the measured needle length, thus supporting the idea that the functional ruler has a helical structure [Mol. Microbiol., 71(3), 692].

Finally, it still remains very difficult by using current simulation techniques to reach dimensions and dynamical scales that are significant to most of the biological processes both in vitro and in vivo. Thus, we are currently engaged in the development of novel schemes and models to enable a more consistent overlap of quantities one can derived from the computational and experimental setting. We are building
a new coarse-grained description of proteins, nucleic acids and membranes in molecular simulations, which will allow us to tackle complex problems such as protein-ligand recognition and protein-protein interactions in large macromolecular networks with unprecedented sampling power and accuracy. This framework will also permit to efficiently integrate experimental inputs to guide the computational modeling of the biological systems of interest [J. Chem. Theory Comput., 4(8), 1378].

Selected publications


Figure 1. M2 channel from influenza A virus. The transmembrane portion of M2 homo-tetramer helical bundle (shown in orange cartoons) is embedded in the membrane bilayer (in licorice representation) and solvated by water. The M2 proton channel is blocked in this configuration - taken from an actual molecular simulation trajectory - by the anti-viral drug amantadine (cyan scaffold) [Proc. Natl. Acad. Sci. USA, 106(4), 1069].
**Introduction**

Gene regulatory networks play a vital role in the development and function of multicellular organisms since they control the spatio-temporal expression of genes. Consequently, deregulation of these networks has been implicated in several diseases including cancer, diabetes and neurodegenerative pathologies. The interactions between genes and their respective regulatory transcription factors (TFs) that form the basis of gene regulatory networks have however been poorly characterized. The overall goal of our laboratory is to reverse engineer and model the gene regulatory networks that control metazoan development and function to make predictions on how specific regulatory networks will behave under different physiological or pathological conditions.

**Keywords**

Systems biology, gene regulatory network, transcription, quantitative genetics, mouse, drosophila, yeast, genetic engineering

**Results obtained in 2008**

A first step toward the comprehensive identification of gene regulatory networks is the large-scale mapping of protein-DNA interactions. The latter can be identified using either TF- or gene-centred approaches. The former focuses on TFs and aims at identifying their gene targets (e.g. chromatin immunoprecipitation (ChIP)). The latter uses regulatory elements as starting point and aims at determining the identity of interacting TFs. An example of a gene-centred method is the high-throughput yeast one-hybrid (Y1H) assay that we previously developed to detect interactions between TFs and C. elegans gene promoters (Deplancke et al. Genome Res., 2004; Cell, 2006). A key break-through in the development of this patented method was the generation of a screening library that solely consists of C. elegans TF open reading frames (ORFs), which significantly increases the efficiency of Y1H screens for this model organism.

To enable the mapping of protein-DNA interactions in mouse and Drosophila model organisms using our Y1H system, we spent considerable efforts in the last year to clone the respective TF ORFs. Using bioinformatic analyses and manual curation, we first determined that the Drosophila and mouse genomes encode respectively 755 and 1576 TFs. As of today, we have completed the Gateway-based cloning of 95% of all Drosophila TF ORFs (Figure 1) as well as 70% of all mouse TF ORFs. Pending successful sequence validation, TF ORFs will be transferred to a versatile panel of destination vectors which we are currently generating for various downstream applications including in vitro transcription translation, viral transduction, and yeast one- and two-hybrid assays. In parallel, we have developed a highly integrated liquid handling platform that enables the fully automated, matrix-based (one-on-one) screening of relevant regulatory elements in 384-well instead of the manual 96-well plate format that is used today. The latter platform will significantly increase the overall throughput and efficiency of Y1H screens and thus enable large-scale protein-DNA interaction mapping in complex organisms encoding a large number of TFs such as humans and mice.

**Selected publications**


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**Figure 1. Drosophila TF overview.** TFs are classified into families according to their DNA-binding domain composition. TFs with multiple DNA-binding domains were counted only once. Families with less than five members were classified as ‘other’. "PCR" (red) means that these TF ORFs were PCR’d, but not yet cloned into a Gateway (GW)-compatible Entry Vector. "PCR+GW+Seq" (blue) indicates that these clones were PCR’d, Gateway cloned and sequence verified.
Introduction
We design novel materials for investigation of basic cell biological phenomena such as stem cell self-renewal and differentiation and applications in medicine such as drug delivery, regenerative medicine, and vaccination. We focus on examples where novel materials are necessary to solve the problem, thus working at the interface between materials science and biology.

Keywords
Biomaterials, tissue engineering, protein engineering, drug and gene delivery, vaccines

Results obtained in 2008
Regenerative medicine: The laboratory made exciting advances in engineering matrix-bound morphogens for conjugation in biomaterial matrices for tissue repair and regeneration: IGF-1 variants for use in smooth muscle, VEGF-A variants for inducing angiogenesis, fibronectin variants for supporting stem cell differentiation in bone repair, and FGF-18 variants for cartilage repair. We have discovered that a domain of fibronectin displays very promiscuous binding to a very large number of growth factors, and we are exploring its use as a very generic tool in tissue engineering. We explored synthetic materials as matrices in embryonic stem cell self-renewal, and demonstrated that signalling through four specific integrins is critical in this process.

Drug and gene delivery: Chemists, biologists and bioengineers collaborated to push our approaches with block copolymers forward in delivering small molecule drugs (such as paclitaxel in cancer, cyclosporine A in immunosuppression) and gene-based drugs (both siRNA and plasmid DNA in vitro and in vivo). Polymers have been developed to carry these diverse payloads.
Vaccines and immuno-therapeutics: In collaboration with the Laboratory for Mechanobiology and Morphogenesis (Prof. M.A. Swartz), the laboratory demonstrated that nanoparticles can be used as a vaccine platform for targeting cells in the lymph nodes. This, combined with advanced design of the polymeric nanoparticle surface, may enable a new generation of vaccines, highly stable and very economical, for use in both the developing and the developed world. The team has demonstrated that ultra-small particles, smaller than biological particles, can be swept into the lymphatics within a few minutes of injection, drain to the lymph nodes, and are collected there for antigen presentation. Novel approaches for vaccination of the oral and airway mucosae have been demonstrated.

**Selected publications**


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**Penetration of 20 nm nanoparticle vaccines (red) into the mucosa of the nasal sinus in the mouse (nuclei in blue).**
Introduction
Tissue homeostasis and regeneration are critically dependent on a limited number of adult stem cells, their self-renewal capability and their commitment to become specialized cells. Due to these unique properties, stem cells hold enormous potential for the treatment of many diseases. However, despite extensive research on elucidating molecular stem cell regulation, significant hurdles need to be overcome before stem cells can be used for therapy. Arguably one of the greatest challenges is controlling stem cell behavior outside of the body, as this would, for example, allow expanding them to sufficient numbers. Adult stem cells reside in specialized niches, comprised of complex mixtures of extracellular cues delivered by support cells in close proximity. Niches protect stem cells from rapid differentiation and regulate the delicate balance between self-renewal and differentiation. The underlying mechanisms remain poorly defined in mammals, mainly because of the difficulties in manipulating these intricate micro-environments in vivo.

To address how micro-environmental signals control the behavior of adult stem cells, we develop innovative bioengineering strategies that allow us to biochemically and structurally deconstruct in vivo adult stem cell niches, and reconstruct them in vitro. These well-defined artificial stem cell niches are applied in the lab to decipher adult stem cell regulation. We expect that some of these technologies have the potential to be translated into clinical settings, mainly because of the difficulties in manipulating these intricate micro-environments in vivo.

Results obtained in 2008
We have developed novel stem cell culture technologies that allow fate changes of individual stem cells to be monitored in vitro, under near-physiologic conditions and in real time. These artificial niches were fabricated from ‘smart’ poly(ethylene glycol) (PEG) hydrogels that allow key biochemical characteristics of adult stem cell niches to be mimicked and the physiological niche complexity deconstructed into a smaller, experimentally amenable number of distinct signalling interactions. Moreover, because many adult stem cell populations are inherently heterogeneous and current state-of-the-art culture techniques do not permit efficient dynamic analyses of fates of large numbers of single cells, 2D and 3D hydrogel patterning techniques were developed that allow to confine and array single stem cells for high-throughput experimentation. In order to mimic cell-cell interactions typical of niches without the complexity of co-culture, we have for example developed a novel protein micro-patterning method for hydrogels allowing to expose confined stem cells to site-selectively tethered protein cues, singly or in combination.

These artificial niches were utilized to explore the fate of individual mouse hematopoietic stem cells (HSC) as well as neural stem/progenitor cells. For example, time-lapse microscopy of several thousand single HSC cultured in micro-well arrays over several days, combined with subsequent image analyses allowed growth kinetics of selected populations to be statistically analyzed. Retrospective transplantation experiments in mice were performed in order to correlate proliferation kinetics with self-renewal function. A pronounced difference in cell division kinetics, that is predictive of their in vivo blood reconstitution potential, was observed when we compared the behavior of standard multipotent progenitors (LKS phenotype) with long-term repopulating HSC (LKS-CD150+). Testing of ca. 20 putative soluble HSC regulatory proteins, including Wnt and Notch ligands, as well as surface-tethered cell-cell adhesion proteins such as Cadherins, allowed identification of factors that dictate distinct HSC proliferation kinetics and in vivo functions. Ongoing experiments are...
geared towards the identification of the role of these niche factors in directing the symmetry of stem cell divisions. To address this question, we have developed microfluidic chips (see figure) that allow the automated separation of dividing stem cells, allowing to track the genealogical relationship between developing progeny over multiple generations (unpublished).

We strongly believe that the systematic ‘deconstruction’ of a stem cell niche may thus serve as a broadly applicable paradigm for defining and reconstructing artificial niches to accelerate the transition of stem cell biology to the clinic.

Selected publications


Introduction
Cancer metastasis, lymphedema, lipid transport, and immune cell function all depend on lymphatic function or dysfunction, and are all tied to interstitial fluid balance and transport. Despite its importance, the regulatory biology of lymphatic function is poorly understood. Furthermore, lymphatic drug delivery holds great potential because of localized targeting of lymph nodes, where one might target metastasized cancer cells, deliver imaging agents, or deliver immuno-modulatory drugs to lymph node-resident antigen presenting cells. Our lab aims to elucidate and exploit the integrated physiology and biology of interstitial and lymphatic transport – how it is actively regulated by cells, how it affects cancer metastasis and immune cell trafficking, and how it can be exploited for drug delivery – using interdisciplinary in vivo, in vitro, and silico approaches. In doing so, we aim to both describe new fundamental mechanisms of lymphatic and interstitial flow mechanobiology and develop new design principles for tissue engineering, immuno-modulatory therapeutics, and drug delivery.

Keywords:
Lymphatic biology, cancer metastasis, biological transport, interstitial flow, tissue engineering, cell engineering, lymphangiogenesis, immuno-modulation, mechanobiology, lymph node

Results obtained in 2008
In 2008, we made headway in several areas of lymphatic biology related to immunology and tumor micro-environment, and also in immuno-therapeutics targeting lymphatics.

In tumor-lymphatic interactions, we described a novel relationship between lymphatics and invasive tumor cells. We showed that tumor expression of the lymphatic growth factor VEGF-C and the lymphoid homing receptor CCR7 act synergistically to promote their invasion towards lymphatics. First, VEGF-C increased lymphatic secretion of CCL21, driving CCR7-dependent tumor chemo-invasion towards lymphatics. Second, VEGF-C acted in an autocrine fashion to increase tumor invasiveness by increasing the proteolytic activity and motility of tumor cells in a 3D matrix. These findings bridge the pro-metastatic functions of CCR7 and VEGF-C in tumors, and demonstrate that beyond lymphangiogenesis, VEGF-C promotes tumor invasion towards lymphatics by both autocrine and CCR7-dependent paracrine signaling mechanisms (Issa et al, Cancer Res).

CCL21, which again is a critical chemokine for leukocyte and tumor cell homing to lymphatics, is also strongly regulated by flow, both in the lymph node stroma and in lymphatic endothelium; these findings are described in two manuscripts (Tomei et al, and Miteva et al, both under review). In lymphatic endothelium, flow also regulates leukocyte adhesion molecules and ultimately dendritic cell transmigration. Since increased fluid flow is the most immediate response to tissue injury or some inflammatory events, these findings suggest that lymphatics use fluid flow as perhaps a first cue of injury or inflammation.
CCL21 is not only important for chemo-attraction to lymphatics. In 2008, we discovered that invasive tumor cells can also secrete CCL21 to start a chain of events that drive lymphoid-like changes in the tumor micro-environment where naive T cells can become educated in the highly tolerogenic micro-environment of the tumor (Shields et al, in press). This 'lymph node mimicry' may be one mechanism whereby tumors escape host immunity.

In another aspect of lymphatic biology, we developed a new in vitro model of the intestinal lacteal, the specialized lymphatic vessel in the small intestine that is responsible for transporting dietary lipids (Dixon et al, in press). We are currently using the model to determine how lymphatic endothelium actively regulates lipid transport, which will ultimately be important for designing strategies of targeting lymphatics.

Finally, together with the Laboratory for Regenerative Medicine and Pharmacobiology, we expanded our work in exploiting the phenomenon of interstitial flow to target immuno-therapeutics to the lymphatics. We have built up our team to focus on a number of applications with relevant collaborators, including vaccines against cancer (Merck-Serono), Lassa virus (Stefan Kunz), and tuberculosis (John McKinney), and are currently evaluating several different strategies.

Selected publications

A Issa, TX Le, AN Shoushtari, JD Shields, and MA Swartz (Jan 1 2009). VEGF-C and CCL21 in tumor cell-lymphatic crosstalk promote invasive phenotype. Cancer Res. 69:349-57


Dendritic cell (covered with green dots=CD11c) transmigrating across a lymphatic endothelium through the cell cytoplasm. (Blue=cell nuclei)
Introduction
The major goal of the Laboratory of Cellular Biotechnology is the development of novel and/or improved tools for gene (DNA) transfer to cultured mammalian cells and subsequent high level expression of recombinant proteins from such cells in scalable production systems (bioreactors). This work has become important since mammalian cells are now considered the most versatile and productive system for the manufacture of recombinant proteins for pharmaceutical applications.

Keywords:
Recombinant protein expression, mammalian cell culture, bioreactor, bioprocess control, gene transfer, DNA integration, micro-injection, stable cell line development, tissue engineering, orbital shaking

Results obtained in 2008
Research at the LBTC is situated on the crossroads between biology and engineering, and it addresses the expression of recombinant proteins from suspension cultures of mammalian cells, which is the major approach to therapeutic protein production in modern biotechnology industry. We are investigating three major thematic areas: (i) gene delivery and transient gene expression, (ii) orbital shaking technology for the cultivation of animal cells in suspension, and (iii) novel methods of gene transfer and integration for the generation of high-producing stable cell lines. The main results obtained in 2008 are summarized below.

1. Gene delivery and transient gene expression. We have studied the cellular uptake and disassembly of PEI-DNA complexes in mammalian cells, and by combining our knowledge of cellular metabolism in suspension cultures with our most recent results we were able to dramatically increase recombinant protein productivity from transiently transfected cells. We achieved the highest titers ever reported for a transiently expressed recombinant antibody (1 g/L) in mammalian cells. This was obtained in an orbitally shaken, non-instrumented cultivation system, and is now being upscaled to 100 and 1'000 Liters (see below).

2. The orbitally shaken bioreactor technology for mammalian cell cultivation, designed in our lab, has been scaled-up to 1’000 L with a custom-made research bioreactor. This and other orbitally shaken cylindrical vessels (with nominal volumes from 50 mL to 250 L) are being extensively studied in order to characterize the hydrodynamics of this type of agitated systems. For these studies we have formed collaborations with the Professor Alfio Quarteroni (Chair of Modelling and Scientific Computing and Dr. Mohamed Farhat of the Laboratory of Hydraulic Machines) to study the fluid dynamics in orbitally shaken bioreactors.

3. Gene transfer and integration strategies for the generation of high-producing cell lines. Stable cell line development has had an important impact on biotechnology. Methods have been constantly innovated over time, as the general knowl-
edge of the molecular mechanisms of gene integration have become better understood. Our research has focused on one cell line, CHO, which is the most widely used cell line in the biotech industry. We have investigated in collaboration with researchers at the CHUV in Lausanne the cytogenetics of CHO-derived stable cell lines generated using different DNA delivery techniques, and extensively analysed the clonal stability of these cell lines over time. We have also investigated micro-injection as a tool for DNA delivery for cell line generation, which allows quantitative delivery of plasmid DNA inside a single cell. We are also investigating biological gene delivery for cell line generation using lentiviral vectors and transposons.

Overall, our research provided useful insights for understanding cell cultivation in suspension, gene integration and protein expression. These studies are of general interest in biotechnology (gene therapy, protein production, industrial biotechnology). Through our research we also provide innovative tools for industrial applications and development.

Selected publications


Comparison of mixing time in the same glass cylindrical container either orbitally shaken (140 rpm) or stirred with a pitch blade impeller (150 rpm). An acid/base reaction was carried out in the presence of two pH indicators, and the time to achieve full mixing in every part of the reactor was analysed with ImageJ software. The artificial color intensity gradient indicates the time to reach the final state (longer time = poor mixing).
IBI - Co-affiliated Research Group - School of Engineering (STI)

Aminian Lab

Head of Lab (PI) - http://lam.epfl.ch/

Introduction
We design medical devices and wearable systems to characterize human mobility and locomotion in daily conditions. Based on these instruments, we provide objective clinical metrics for diagnosis and outcome evaluation of treatments.

Keywords:
Biomechanics, sport, inertial sensors, wearable systems, outcome evaluation, orthopaedics engineering, long-term monitoring, daily activity

Results obtained in 2008
Orthopaedics engineering and Sport Biomechanics:
During the past two decades, numerous studies were conducted to analyze the change of knee joint function after anterior cruciate ligament (ACL) rupture and reconstruction. Most of these studies considered only a few steps of level walking and analyze the function during particular gait events. We proposed an ambulatory system based on inertial sensors to measure 3D angle of the knee joint over long distance walking outside a laboratory and quantified the improvement of 3D knee joint angle in 10 ACL-deficient patients before and two times after reconstructive surgery during various daily activities.

The choice of the ideal outcome measure to assess shoulder pathology remains a complex issue. We analysed shoulder function during daily activity to answer this dilemma. We designed a new body worn system including inertial sensors and EMG electrodes and performed long term monitoring in 10 patients before and after operation, and in 10 healthy participants. A new method to detect external loading of shoulder during lab activity was devised.

The kinematics of foot-ankle complex and plantar pressure distribution was studied in healthy subject and patients with osteoarthritis. We have proposed new foot segmentation and described multi-segment foot kinematics with a bone-embedded anatomical frame definition. The results provide new metrics based on joint kinematics, spatio-temporal gait features and plantar distribution for outcome evaluation of ankle osteoarthritis treatments.

In sport, the biomechanics of alpine skiing and ski jump was studied. We finalized the design of a wearable system composed of 6 body worn inertial sensors modules recording kinematics signals at 200Hz. For ski jump, an algorithm extracts precise timing information (error< 50ms compared to camera based motion capture) and reconstructed the segment orientation (error< 3.2deg).

Fall prevention in elderly: Investigating the manifestations of frailty from its first signs in the youngest old are essential to determine the role of fear of falling as a potential modifier of the relation between gait and balance performance. The assessment of fear of falling as a predictor of the incident frailty was achieved using gait and balance data recorded on more than 750 elderly subjects (+65 years old) using an ambulatory system based on inertial sensors.

Neurology and movement disorders: In collaboration with Oregon Health Science University, we devised an instrumented “Up and Go test” (iTUG) which quantify accurately several balance features during sit-stand and stand-sit transfer, walking and turning. Our findings show that though the standard test did not detect abnormalities in the early-to-mid stage in patients with Parkinson Disease (PD), our system shows significantly lower amplitude in peak arm swing velocity on the more affected side, average turning velocity, cadence and peak trunk rotation velocity. These iTUG metrics were also correlated with the UPDRS Motor Scale. The iTUG test is sensitive to untreated PD and could potentially detect progression of PD and response to symptomatic and disease-modifying treatments.

Physical activity and Quality of Life: Physical activ-
ity (PA) decreases with age especially with regards to leisure-time physical activity. Most data were derived from subjective questionnaire-based studies, which concentrate on exercise and leisure activities, neglecting spontaneous activities. In our study with older adults, PA was captured on seven consecutive days in 44 community-dwelling older adults (80.75 + 4.05 years). The mean time of walking and the mean “time on feet” of the group was 10.2 hours (+ 3.5 hours) and 35.1 hours (+ 9.43 hours), respectively, for the duration of seven consecutive days. We could demonstrate that the measurement of PA of only one day could describe the mean time of activity (i.e. standing and walking) for the whole group, but not for a single person.

Selected publications
A. Salarian, H. Russmann, F. Vingerhoets, P. R. Burkhard, K Aminian, (2007), Ambulatory monitoring of physical activities in patients with Parkinson’s disease, IEEE Transactions on Biomedical Engineering, 2296-2299
Introduction
The visualization and characterization of biologically relevant molecules and activities inside living cells continues to transform cell biology into a truly quantitative science. However, despite the spectacular achievements in some areas of cell biology, further progress will depend increasingly on the development of new (fluorescent) sensors and chemical probes to target and characterize these activities. Our research addresses this need by developing and applying chemical approaches to observe and manipulate protein function in living cells.

Keywords:
Chemical Biology, protein engineering, sensors

Results obtained in 2008
The main focus of my group was to use our expertise in organic chemistry and protein engineering to develop new tools for studying protein functions in living cells. In the following paragraphs, we briefly describe some of our achievements.

The visualization of complex cellular processes involving multiple proteins requires the use of spectroscopically distinguishable fluorescent reporters. We have previously introduced the SNAP-tag as a general tool for the specific labeling of SNAP-tag fusion proteins in living cells. The SNAP-tag is derived from the human DNA repair protein O6-alkylguanine-DNA alkyltransferase (AGT) and can be covalently labeled in living cells using O6-benzylguanine (BG) derivatives bearing a chemical probe. In 2008 we reported the generation of a novel AGT-based tag, named CLIP-tag, which reacts specifically with O2-benzylcytosine (BC) derivatives (Gautier et al). Since SNAP-tag and CLIP-tag possess orthogonal substrate specificities, SNAP and CLIP fusion proteins can be labeled simultaneously and specifically with different molecular probes in living cells. We furthermore showed for the first time simultaneous pulse-chase experiments to visualize different generations of two different proteins in one sample. Comments on this method were published in the journals Cell and ACS Chemical Biology.

We also introduced a method to measure Ca2+ concentrations in defined locations in living cells that is based on linking the Ca2+-sensitive dye Indo-1 to SNAP-tag fusion proteins. Fluorescence spectroscopy of SNAP-Indo-1 conjugates, in vitro, showed that the conjugates retained the Ca2+-sensing ability of Indo-1. In a proof-of-principle experiment, local Ca2+ sensing was demonstrated in cultured primary muscle cells of mice expressing a nucleus-localized SNAP-tag fusion. Ca2+ concentrations inside nuclei of resting cells were measured by SEER (shifted excitation and emission ratioing) of confocal microscopic images of fluorescence. After permeabilizing the plasma membrane, changes in the bathing solution induced corresponding changes in nuclear [Ca2+] that were readily detected and used for a preliminary calibration of the technique. This work thus demonstrated the synthesis and application of SNAP-tag-based Ca2+ indicators that combine the spatial specificity of genetically encoded calcium indicators with the advantageous spectroscopic properties of synthetic indicators.

Finally, we introduced a method for sensing protein proximities and interactions in living cells through the selective and covalent crosslinking of SNAP-tag fusion proteins (Lermercier et al.).

Selected publications
Helen M. O’Hare, Rosario Durán, Carlos Cerveñansky, Marco Bellinzoni, Anne Marie Wehenkel, Otto Pritsch, Gonzalo Obal, Jens Baumgartner, Jérome Vialaret, Kai Johnsson, Pedro M. Alzari, “Regulation of glutamate metabolism by protein kinases in mycobacteria” Molecular Microbiology, 70, 1408-23 (2008)


Christopher Chidley, Katarzyna Mosiewicz, Kai Johnsson “A designed protein for the specific and covalent hetero-crossjigation of biomolecules” Bioconjugate Chemistry, 19, 1753-6 (2008)

Arnaud Gautier, Alexandre Juillerat, Christian Heinis, Ivan


Double pulse-chase labeling of cell wall proteins of the budding yeast Saccharomyces cerevisiae
IBI - Co-affiliated Research Group - School of Engineering (STI)

Maerkl Lab

Head of Lab (PI) - http://lbnc.epfl.ch

Introduction
We are interested and developing and applying state-of-the-art microfluidic devices to challenging problems in systems biology. Specifically, we are interested in characterizing the dynamics of large and complex systems, such as protein expression dynamics in yeast. We approach the problem from the ground up by characterizing the biophysics of molecular interactions as well as from the top down by measuring protein expression dynamics of over 60% of all proteins present in yeast on the single cell level.

Keywords:
Microfluidics, systems biology, yeast, transcriptional regulatory networks, protein dynamics

Results obtained in 2008
In January of 2008, Prof. Maerkl established the Laboratory of Biological Network Characterization (LBNC) at the EPFL; since then the laboratory has grown to a total size of 14 people. The LBNC is principally interested in developing highly integrated microfluidic devices and apply these to pertinent problems in biology. Of particular interest to the lab at the moment is systems biology, which has the potential to benefit tremendously from the development of novel, high-throughput technologies. Last year Prof. Maerkl was awarded a grant from the Swiss systems biology initiative SystemsX.ch. Prof. Maerkl is the lead principal investigator of DynamiX, one of the eight approved SystemsX.ch RTD projects in 2008. DynamiX aims to characterize the dynamics of transcriptional regulatory and protein expression networks in the budding yeast S. cerevisiae, both on the molecular and single cell level. Nano-Tera is funding a project on the technology development front with the goal of developing software programmable microfluidic devices. Currently, all fluidic devices are designed for a specific task, and thus can’t be applied to a variety of even closely related applications. With the next generation of software programmable devices, it will be possible to apply a single microfluidic device design to any number of applications, by on-the-fly, software based, re-configuration of the fluidic system.

In 2008 and early 2009, Prof. Maerkl published three research articles and one review. An article published in Nature Biotechnology, describes the first microfluidic based drug discovery assay, which resulted in the discovery of a potential anti-viral drug compound against Hepatitis C. A similar platform was used to synthesize protein on-chip in high-throughput and test these proteins for binary interactions (Nature Methods 2009).

Selected publications


This image depicts a photograph of a highly-integrated microfluidic circuit containing over 2000 micromechanical valves on a footprint the size of a postage stamp. The device was used to screen enzyme mutants evolved through directed evolution.
Introduction
LBO has four fields of research: tissue engineering of musculo-skeletal tissues, implant and joints biomechanics, drug delivery system and mechno-biology. A combination of biomechanical and biological approaches is used to describe and understand the different clinical problems of interest such as bone loss following total joint arthroplasty, arthritis or intervertebral disc degeneration. Based on these analysis, original solutions are developed such as fetal cell therapy, a scaffold with high mechanical properties or an orthopaedic implant used as a drug delivery system.

A strong collaboration exists between the LBO and the “Département de l’Appareil Locomoteur (DAL)” at the CHUV allowing a translation of research results to clinical applications. A detailed 2008 Report activity of the LBO can be downloaded at: lbo.epfl.ch

Keywords:
Biomechanics, tissue engineering, biomaterials, orthopaedic implant

Results obtained in 2008
A one year evaluation in sheep of the developed bone scaffold showed that this scaffold is well integrated and support bone formation. This positive study represents the last stage before a clinical study can be initiated. In parallel, biomechanical stimulations on the scaffold inserted in rat condyle, situation mimicking a clinical application, demonstrated the positive effect of this mechanical loading.

The study of viscoelastic behavior of intervertebral disc and the corresponding dissipation process has been initiated. The impact of this dissipation phenomena on cell behavior will now be evaluated as we hypothesize that this aspect is central in the production of an extracellular matrix in soft tissues.

A numerical/biomechanical analysis of the developed scaffold to be used in total knee arthroplasty has been done and demonstrated that this new approach is feasible. The design of a new revision total knee arthroplasty is ongoing using this result. Finally, an ex vivo evaluation of the effect of micromotion on human bone sample highlighted the importance of the OPG/RANKL pathway in the resorption of bone around an orthopedic implant.

Selected publications


*Cartoon of the developed deformation µcalorimeter used to evaluate the dissipative phenomena in soft tissue and their impact on cell behavior.*
Introduction
The Optics Laboratory is mainly active in optofluidic devices, imaging applications with nanoparticles markers and nonlinear optics.

Keywords:
Optofluidics, nanoparticles, nonlinear optics

Results obtained in 2008

Optofluidics:
Fluids are used to synthesize novel optical systems. The optical properties of fluids can be modified by chemical synthesis relatively easily and the insertion of fluids in the optical path specifies or adapts the functionality of the optical system. One of the approaches we are pursuing is the integration of microfluidic circuits with photonic structures that contain voids into which fluids are injected. Another approach is the use of colloidal solutions of nanoparticles. Electrical fields or light beams redistribute the nanoparticles and modify the optical properties of the structure. Liquid dyes injected into microfluidic chips provide the optical gain necessary for building a dye laser on a chip. We develop molecular photonics for lab-on-a-chip applications, where the light source is integrated for imaging or sensing. We develop lasers, based on fluids and organic dyes. The gain is in the fluid and is optically pumped. We develop lasers, based on fluids and organic dyes. The gain is in the fluid and is optically pumped. The fabrication is a critical point, to this end we have developed PDMS fabrication capabilities and nano-lithography using the electron beam.

Imaging applications with nanoparticles as markers:
Second harmonic generation (SHG) from nanoparticles is opening new types of imaging applications. Those nanoparticles can be used as markers to label biological cells and allow for better detection techniques. In contrast to fluorescent markers such as green fluorescent protein (GFP) or quantum dots (QD), both limited by photobleaching, Second Harmonic Radiation Imaging Probe (SHRIMP) emits a stable signal over a long time owing to the lack of real energy state transition. The femtosecond response allows for observing fast dynamic process, such as protein conformation. Moreover the coherent nature of SHG enables us to capture three-dimensional (3D) information in single frame by recording hologram at the doubled optical frequency. We worked with BaTiO3 and LiNbO3 nanoparticles as well as KNbO3 nanowires.

Nonlinear optics:
We are studying theoretical and experimental nonlinear propagation of femtosecond pulses in 3-D media. The nonlinearity produces very distinctive diffraction patterns such as self focusing and solitary waves (waves that remain stable as the beam propagates). The research is mainly on nonlinear optics with evanescent waves, filament formation, modulation and transverse instabilities, non-paraxial nonlinear optics with emphasis in subwavelength imaging and nonlinear wave propagation in fluidic devices.

Selected publications


Introduction
The research of the Laboratory of Nanoscale Biology focuses on developing tools and probes for single-molecule biophysics. The group uses optical tweezers, AFM, single-molecule fluorescence, and nano-fabricated structures to study biomolecular systems and advance new nanotechnology. Current experimental work in our lab focuses on two interconnecting areas:

Nanofabricated probes and platforms for single-molecule biophysics experiments
Including nanofabricated SHG nanocylinders, solid-state nanopores, local nanoelectrodes for molecular sensing and sequencing

Local probe studies of single biomolecules
For example RNA polymerase, DNA binding proteins, membrane proteins such G protein–coupled receptors (GPCRs)

Keywords:
GPCRs, solid-state nanopores, single molecule, DNA sequencing, nanoelectrodes

Results obtained in 2008
We have developed a versatile tool by marrying solid-state nanopores with optical tweezers. With our technique it is now possible to directly probe not only the biopolymer translocation but also to do direct quantitative measurements of the forces on a biopolymer molecule inserted in the nanopore as a function of the applied potential and to probe and characterize the thermodynamic and kinetic landscape of biomolecules. The widespread use of optical tweezers in single molecule biophysics suggests their use to control sample presentation to a pore or channel, reduce polymer propagation speeds without impairing ionic currents, and to repeatedly characterize one biopolymer. We have fabricated 2 nm solid state pores, Fig.1.a), by using a TEM at CIME. We use fabricated pores as platforms for force spectroscopy and molecular sensing. Specifically, we use nanopore based force spectroscopy to understand dynamics of transcription, how RNAP translocation is applied in the molecular and mechanistic terms and how DNA responds to local application of the force.

Our laboratory made exciting advances in nanofabrication of functionalized quartz nanocylinders, Fig.1.b, suitable for optical manipulation and possibly second harmonic generation. These quartz nanostructures are trappable and can be used as probes in single-molecule experiments. Nanolithography and subsequent dry etching allow us to tailor nanostructure geometry and functionalize only one end of each cylinder for specific attachment to a DNA molecule or proteins. We plan to compare SHG efficiency of quartz nano-cylinders with KNbO3 nanowires. If we obtain adequate signal, we will proceed and find strategies to produce nano-cones with best suited geometry and use quartz as a reference material for other materials. Should the signal be inadequate, we will apply the same approach to other crystals as a replacement for quartz such as LiNDO3, CdS, CdTe, GaAs and ZnS. All mentioned materials can be easily purchased as wafers with well defined polarization axis and have an order of magnitude higher SHG efficiency.

We are also interested to identify GPCRs microdomains, real proximity, diffusion and trafficking of the two signaling components G protein and G protein coupled receptor. A large body of evidence indicates that GPCR signal transduction events occur in the cell via plasma membrane receptors localized in signaling microdomain. Super-resolution microscopy techniques can resolve the localized molecules with a nanometer resolution and therefore could offer very important information about these biological processes. The established PALM (Photoactivatable light microscopy) setup makes it possible to acquire enough single molecule positions to assemble a probability density plot of a single protein species of endogenous expression levels at a resolution of 20 - 25 nm, which is ~10 times higher than for conventional fluorescence microscopy. Our aim is to establish a
microscope setup where two protein species can be analyzed simultaneously at high speeds in live specimens. In PALM, the number of photons dictates the resolution and leads to a fundamental trade-off between spatial and temporal resolution. Our aim is to provide technical improvements that will facilitate simpler, faster and more reliable analyses of the spatiotemporal relations.

Selected publications


(*) equally contributing first author,

Figure 1. a) Optical and TEM images of produced nanopores. One of proposed innovative solutions to the challenge of identifying each base using transverse tunneling currents is to form base-specific hydrogen bonds between chemically modified metal electrodes and the nucleobases in the molecule while attachment to the trapped bead allows control over translocation speed. Scale bars, 50 µm, 100nm and 5 nm b) SEM image of quartz nanocylinders The quartz posts fractured evenly at their bases in a consistent manner. A crystalline quartz cylinder is designed to trap with its extraordinary axis perpendicular to the propagation direction of the trapping laser. Its bottom surface is chemically functionalized for attachment to DNA. Scale bars, 2 µm.
IBI - Co-affiliated Research Group-School of Engineering

Stergiopulos Lab

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STI

Introduction

The Laboratory of Hemodynamics and Cardiovascular Technology (LHTC) focuses on the relation between blood flow and the development, progression and regression of cardiovascular disease. Development of vascular implants and non-invasive or mini-invasive technologies for the diagnosis and treatment of cardiovascular disease is also a major objective.

Keywords:
Cardiovascular mechanics, hemodynamics, arterial remodelling, cerebrovascular disease, vascular prostheses, active implants.

Results obtained in 2008

Hemodynamics

With advancing age arteries stiffen, reducing arterial compliance and leading to the development of systolic hypertension and to a substantial increase in pulse pressure. An augmented pulse pressure can be a predictor of the development of hypertension, which has been linked to several cardiovascular diseases including atherosclerosis, and to pathologies such as diabetes and renal dysfunction. We tested the hypothesis that reduced wall compliance induces pulse-pressure-mediated changes in arterial wall metabolism and remodelling, using an in vitro artery culture system. Control arteries were exposed to a pulse shear stress (6 +/- 3 dynes/cm²) combined with a pulse pressure of 80 +/- 10 mm Hg, yielding a physiological cyclic stretch of 4-5%. A reduced compliance group was also studied. Reduced compliance caused a decreased contraction capacity induced by norepinephrine (NE), and this was associated with lower levels of alpha-smooth muscle cell-actin (alpha-SMC-actin) and desmin protein expressions. Arteries exposed to a reduced cyclic stretch exhibited a higher level of matrix metalloproteinase-2 (MMP-2) expression activity as well as an increase in Ki67 expression, thereby suggesting that matrix degradation and cellular proliferation had been initiated. The findings underlined the importance of cyclic stretch in the maintenance of a differentiated and fully functional phenotype of vascular SMCs, as well as in the regulation of migratory properties, proliferation, and matrix turnover.

We analyzed the major determinants of pressure transfer from a peripheral artery (clinical measurement site) to the aorta and we found out that the most influential parameter is the local wave speed. This way let us improve or personalize the transfer function characteristics as applied on a "per patient" basis.

Vascular Mechanics

We studied alterations in histology and biomechanical properties of the human cerebral arteries with ageing. We showed that ageing affects structural morphology and the mechanical properties of intracranial arteries. In contrast to main systemic arteries, intima and media thicken while outer diameter remains relatively constant with age, leading to concentric hypertrophy. The structural morphology of elastin changed from a fiber network oriented primarily in the circumferential direction to a more heterogeneously oriented fiber mesh, especially at the intima. Biomechanically, cerebral arteries stiffen with age and lose compliance in the elastin-dominated regime. Elastin, becomes dysfunctional with aging.

Conclusions

Elastin loses its functionality in cerebral arteries with aging, leading to stiffer less compliant arteries. The area fraction of elastin remained, however, fairly constant. The loss of functionality may thus be attributed to fragmentation and structural reorganization of elastin occurring with age.

We developed a theoretical model for the prediction of wall remodelling in response to sustained changes in blood flow. Stress-driven geometrical remodelling and shear stress-driven elastin remodelling laws were applied to yield the remodelling rate equations describing the evolution of geometry and material properties. Plausible results in good agreement with experimental data show that the model, despite its simplicity, provides an interesting theoretical basis for studying wall remodelling dynamics.
Selected publications


Tsamis A and Stergiopulos N. Arterial remodeling in response to hypertension using a constituent-based model.


Analysis of flow inside a stented intracranial aneurysm, using a direct CFD as well as a much less computationally-intensive porous media approach
**GHI - Global Health Institute**

The Global Health Institute has been created to contribute to the understanding, diagnosis, prevention and treatment of infectious diseases, which still claim 18 million lives each year and account for half of the deaths in the developing world. The GHI is currently composed of 5 groups, whose activities already reflect the Institute’s future ambitions. Basic mechanisms of host-pathogen interactions and innate immunity towards pathogens are being studied using multidisciplinary approaches. Crucial world health issues, like tuberculosis and HIV/AIDS, are being tackled. These include understanding, and hopefully counteracting, the persistence of Mycobacterium tuberculosis, the causative agent of tuberculosis, or designing drugs to treat this disease. Mechanisms of HIV infection and use of this virus in gene therapy approaches are also the subject of intense research. Further recruitment is planned, in particular of talented young researchers, to extend these efforts to other pathogenic bacteria and viruses. Intensification of current research themes by including novel technological approaches is also a priority. [http://ghi.epfl.ch](http://ghi.epfl.ch)
Introduction
A multidisciplinary approach is being taken to tackle major public health problems such as tuberculosis (TB) and leprosy with particular emphasis on the discovery of new TB drugs.

Keywords
Microbial Pathogenesis, TB drug discovery, signal transduction, protein secretion, phylogeography of leprosy.

Results obtained in 2008

TB Drug Discovery
Our lab (UPCOL) is leading a major effort to discover new drugs for the treatment of TB as part of the New Medicines for Tuberculosis Project, NM4TB, funded by the European Commission. In 2008, we were particularly successful and identified and characterized the 1,3-benzothiazin-4-ones (BTZ), a new class of anti-mycobacterial agents that kill Mycobacterium tuberculosis in vitro, ex vivo and in murine models of TB. Using genetics and biochemistry, the enzyme decaprenylphosphoryl-β-D-ribose 2′-epimerase was identified as a major BTZ target. Inhibition of this enzymatic activity abolishes formation of decaprenylphosphoryl arabinose, a key precursor required for the synthesis of the cell-wall arabinans, thus provoking cell lysis and bacterial death. The most advanced compound, BTZ043 is a candidate for inclusion in combination therapies for both drug-sensitive and extensively-drug resistant TB.

Signal transduction, phosphorelays and cell wall biosynthesis in M. tuberculosis
There is mounting evidence that cell growth and elongation is controlled by a phosphorelay involving serine-threonine protein kinases (STPK) and phosphatases, and forkhead-associated proteins that recognize phospho-threonine residues. We are intensively investigating the biological function of PknB, and trying to identify the ligand for this essential receptor kinase, a potential therapeutic target for new TB drugs. Downstream signalling partners have been identified and their physiological roles are being uncovered.

Protein secretion and pathogenicity
The ESX-1 protein secretion system is the major virulence determinant operating in M. tuberculosis and has been lost by the vaccine strains M. bovis BCG and M. microti. ESX-1 is required for the export of small helical-hairpin proteins belonging to the ESAT-6 family as well as other effector proteins of unknown function. ESX-1 mediates host cell entry of tubercle bacilli and triggers inter-cell spread. We are using an integrated approach to establish the organization, architecture, structure and function of this ATP-driven secretory apparatus and consider that it represents an attractive target for chemical biology and drug discovery.

A regulatory map of the M. tuberculosis genome
We have adopted an integrated approach to studying gene regulation by using chromatin-immunoprecipitation of DNA-binding proteins in conjunction with high density oligonucleotide-based micro-arrays or high-throughput sequencing to map the genome. Using this approach we demonstrated that the BlaI repressor controls expression of five separate genomic loci that respond to beta-lactam antibiotics. Regulatory information is being incorporated into Tuberculist, the genome server dedicated to M. tuberculosis http://tuberculist.epfl.ch/, for which we are the official curators.

Phylogeography of leprosy
Despite the massive and highly successful implementation of multi-drug therapy by the World Health Organisation, leprosy remains a serious public health
problem in several countries probably due to our inability to identify infectious cases early enough. One of our goals is the development of an epidemiological tool to monitor transmission of the disease. This uses comparative genomics, particularly SNP (single nucleotide polymorphism) analysis of patient isolates, to monitor the phylogeography of leprosy.

Selected publications


Rapid drug susceptibility testing of M. tuberculosis.
Introduction
Our group studies the molecular basis of host defense responses to microbial infection using Drosophila as a model system. Analysing how insects combat microbial infection may improve our understanding of infectious processes and innate host defense in vertebrates.

Keywords
Innate immunity, host-pathogen interactions, insect, genetic

Results obtained in 2008
Microbial infections are characterized by a continual, dramatic interplay between pathogen and host: pathogens exploit an array of host cell functions during infection and hosts respond with efficient immune responses. Drosophila provides a powerful model system for dissecting innate host defense mechanisms. In response to an immune challenge, the fat body of this insect produces a battery of small peptides with antifungal and antibacterial activities. Two signaling pathways, Imd and Toll, control the expression of antimicrobial peptide encoding genes in Drosophila. These pathways exhibit striking similarities with the Toll-Like Receptors and Tumor Necrosis Factor α cascades that regulate NF-κB activity in vertebrates (Lemaitre and Hoffmann 2007; Leulier and Lemaitre 2008).

1) The systemic immune response
Our group employs genetic screens to identify novel factors regulating the immune response of Drosophila. These studies extend our understanding of how the Toll and Imd pathways activate immune responses, as well as determine how the host recognizes and distinguishes between different microbial pathogens. A previous micro-array analysis, which systematically identified Drosophila immune-regulated genes, has launched several post-genomic analyses with the goal of characterizing new genes required for combating infection. This approach led to our recent characterization of two immune-responsive serpins (Serpin 27A and Serpin 28D), several serine proteases with important roles in melanization (an arthropod specific immune defense) or Toll pathway activation, the identification of the novel clotting factor Fondue, and the description of Pims, a negative regulator of the Imd pathway (Scherfer et al., 2006 and 2008, Lhocine et al., 2008, Tang et al., 2006, 2008).

2) Drosophila intestinal response to bacterial infection: Activation of host defense and stem cell proliferation
Recently, we identified bacterial and fungal pathogens that infect Drosophila through a natural route of ingestion. These pathogens have a high degree of specificity, suggesting that these species have evolved mechanisms for exploiting Drosophila as a host. To study these mechanisms, we are now using molecular and genetic approaches to identify microbial virulence factors as well as their targets in Drosophila (Muniz et al. 2007). In addition, we have also begun to analyze the local immune response of the Drosophila gut. The gut is the major interface between microbes and their animal hosts and constitutes the main entry route for pathogens. As a consequence gut cells must be armed with efficient immune defenses to combat invasion and colonization by pathogens. However, the gut also harbors a microbiota, with potentially beneficial effects for the host, which must be tolerated without a chronic, and harmful, immune response. However, in spite of growing interest in gut mucosal immunity, very little is known about the immune response of the Drosophila gut. One of our major goals is to increase our understanding of Drosophila gut immunity to a level comparable with systemic immunity within the next five years (Grant SNS 2007, ERC Advanced Grant 2008). Recently, we reported a comprehensive analysis of the gene expression changes that occur in the Drosophila gut following infection by the bacterium Erwinia carotovora (Buchon et al., 2009). This study revealed that immune responses in the gut are regulated by the Imd and JAK-STAT pathways, but not the Toll pathway. Ingestion of bacteria had a dramatic impact on the physiology of the gut that included modulation of stress response.

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and increased stem cell proliferation and epithelial renewal. Our data suggests that gut homeostasis is maintained through a balance between cell damage, due to the collateral effects of bacteria killing, and epithelial repair by stem cell division. We believe that the Drosophila gut provides a powerful model to study the integration of stress and immunity with pathways associated with stem cell.

**Selected publications**


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**Bacterial infection promotes Intestinal stem cell proliferation in Drosophila**

In Drosophila, ingestion of the Gram-negative bacterium Erwinia carotovora provokes a massive increase in epithelial renewal via increased intestinal stem cell proliferation and differentiation. Domains of cell proliferation in the Drosophila midgut are revealed by the expression of an Escargot-GFP reporter. Nuclei are stained in DAPI (blue).
Introduction
Most bacterial infections come and go in a matter of days or weeks, but tuberculosis (TB) resists elimination by the immune system and persists for the lifetime of the host. TB is also notoriously difficult to cure with antibiotics. In the Laboratory of Bacteriology (LBAC), we study the mechanisms of tuberculosis persistence in the face of host immunity and antibiotics. This work has generated new insights into the biology of bacterial persistence and new tools for developing and evaluating antimicrobial agents.

Keywords
Microbiology, bacteriology, mycobacterium tuberculosis, infectious disease, persistence, counter-immunity, carbon metabolism, antibiotic tolerance, growth control, biosafety Level 3 (BSL3); time-lapse fluorescence microscopy; micro-electromechanical systems (MEMS); micro-fluidic systems; computational modeling; mass spectrometry

Results obtained in 2008
Most bacterial infections come and go in a matter of days or weeks, but tuberculosis (TB) persists for the lifetime of the host. Although the live-attenuated Bacille Calmette Guérin (BCG) vaccine has been widely administered since the 1920s, the protective efficacy of BCG in clinical trials has been highly variable and generally poor. Although effective anti-TB therapy has been available since the 1950s, successful treatment requires administration of multiple antibiotics for six months or longer, a regimen that most TB patients fail to complete unless closely supervised. In the Laboratory of Bacteriology (LBAC), we study the persistence mechanisms that underpin the recalcitrance of the TB bacillus (Mycobacterium tuberculosis) to host immunity and antimicrobial therapy. Our research is focused in four areas:

1) COUNTER-IMMUNITY.
The host immune response is usually sufficient to contain M. tuberculosis infection, but not to eradicate infection. Our goal is to identify the strategies that M. tuberculosis deploys to counter host immunity in order to establish and maintain a lifelong infection. In the past year, we identified a surface receptor signal transduction pathway that is essential for bacterial resistance to the mammalian acquired immune response. In future, we will explore the mechanistic basis of this pathway’s role in immune evasion.

2) IN VIVO METABOLISM.
Central metabolism has recently emerged as an attractive target for antimicrobial drug discovery. Our goal is to identify the metabolic pathways that are essential for M. tuberculosis growth and persistence during infection of the mammalian host. In the past year, in collaboration with investigators in EPFL’s School of Basic Sciences (Hatzimanikatis lab), we constructed the first thermodynamically constrained genome-scale computational model of M. tuberculosis metabolism. The model has uncovered some surprising new insights into the metabolic capabilities and vulnerabilities of M. tuberculosis. In future, we will carry out wetlab experiments to test the predictions generated by our metabolic model.

3) ANTIBIOTIC TOLERANCE.
TB is notoriously difficult to treat with antibiotics due to the recalcitrance of a subpopulation of persisting bacteria. Our goal is to elucidate the epigenetic basis of cell-to-cell variation in antibiotic sensitivity and persistence. In the past year, we tested the traditional (but unproven) concept that persistence is due to a subpopulation of dormant bacteria. Using time-lapse fluorescence microscopy in conjunction with micro-fluidic systems, we demonstrated that this traditional explanation is incorrect. We further showed that bacterial persistence in the face of is-
niazid, a frontline anti-TB drug, is due to apparently stochastic cell-to-cell variation in the expression of an antibiotic resistance determinant. In future, we will carry out further experiments to explore the generalizability of this new concept of bacterial persistence.

(4) GROWTH CONTROL.

M. tuberculosis is also notorious for its slow intrinsic growth rate (population doubling time, 20-24 hours). Our goals are to understand why M. tuberculosis grows so slowly, to identify the physiologic adaptations that are required for slow growth, and to measure the scaling of physiological processes with growth kinetics. In the past year, we discovered that mycobacteria display extreme cell-to-cell variation in their fundamental growth parameters. These include the rate of biomass increase, the time between cell divisions, the size at cell birth, the size at cell division, and the symmetry of division. These findings challenge the assumption that a cell’s phenotype is a predictable outcome of its genotype and its environment. In future, we will explore the mechanistic basis of this remarkable non-genetic phenotypic heterogeneity.

Our EXPERIMENTAL TOOLS include molecular genetics, biochemistry, tissue culture and animal infection models, computational modeling, mass spectrometry, micro-fluidic and micro-electromechanical systems, and time-lapse fluorescence microscopy. In collaboration with investigators in EPFL’s School of Engineering (Maerkl, Renaud, and Unser labs), we are developing new tools to analyze the time evolution of bacterial phenotypes at single-cell resolution.

Selected publications


Introduction
Retroelements constitute important evolutionary forces for the genome of higher organisms, yet their uncontrolled spread, whether from endogenous loci or within the context of viral infections, can cause diseases such as cancer, hepatitis and AIDS. Correspondingly, a variety of host-encoded activities limit this process, belonging to a line of defense commonly called intrinsic or innate immunity, which notably contributes to taming endogenous retroelements and to restricting the cross-species transmission of retroviruses. Our work aims at characterizing the relationship between retroelements and their hosts. Recently, this has led us to investigate more broadly how epigenetic mechanisms shape the expression of mammalian genomes.

Keywords:
Molecular virology, HIV, hepatitis B virus, endogenous retroelements, innate immunity, lentiviral vectors, mammalian genetics, epigenetics, KRAB zinc finger proteins, KAP1, transcriptional repression

Results obtained in 2008
Of the delicate equilibrium between retroelements and higher organisms
Retroelements not only comprise deadly human pathogens such as human immunodeficiency and hepatitis B viruses (HIV and HBV, respectively) but they also account for almost half of the DNA contained in the genome of higher organisms. Sophisticated mechanisms are thus engaged in their control, which include transcriptional silencing by DNA methylation, post-transcriptional silencing by RNA interference and inhibition by cellular factors such as polynucleotide cytidine deaminases (CDAs) of the APOBEC family or tripartite motif proteins such as TRIM5α. We previously demonstrated that APOBEC proteins can be broadly active anti-retroviral factors that act by lethally editing the nascent HIV minus strand DNA product of reverse transcription, and that they can also block HBV. Our recent work has explored the molecular mechanisms of action of these antivirals, asking in which physiological framework they act as effectors of innate immunity against HIV and HBV, and how they contribute to the control of endogenous retroelements. Our ongoing structure-function analyses of APOBEC- and TRIM5α-mediated restriction dissect both the modalities by which these antivirals recognize their targets and the cellular factors that regulate their functions.

Of KRAB zinc finger proteins, KAP1, and the epigenetic control of gene expression
The human genome contains more than four hundred genes coding for KRAB (Krüppel-associated box) zinc finger proteins (KRAB-ZFPs), and comparative sequence analyses indicate that this family of epigenetic repressors is vertebrate-specific. While large variations between organisms suggest a role in speciation, few KRAB-ZFPs gene targets have been unequivocally identified and the physiological roles of these regulators remain essentially unknown. Nevertheless, extensive in vitro molecular analyses have revealed that their conserved KRAB domain recruits the KAP1 (KRAB-associated protein 1) corepressor (also known as TRIM28, TIF1β or KRIP-1), which in turn serves as a scaffold for a multi-molecular histone- and DNA-modifying complex that silences transcription by triggering the formation of heterochromatin.

We previously reported that KRAB/KAP1 promotes de novo promoter methylation during the first few days of mouse embryogenesis. This corroborates the ear-
ly embryonic lethality of the KAP1 knockout mouse, and suggests that KAP1 partakes in the wave of DNA methylation characteristic of this developmental period, which notably mediates the transcriptional repression of endogenous retroelements. We are currently investigating this hypothesis and studying the genomic modalities of KRAB/KAP1 epigenetic regulation. In parallel, we have started a systematic examination of the role of KAP1 and KRAB-ZFPs in somatic tissues. We found that KAP1 is expressed at high levels and necessary for KRAB-mediated repression in mature neurons of the mouse brain. Correspondingly, we discovered that mice deleted for KAP1 in the adult forebrain exhibit heightened levels of anxiety-like and exploratory activity and stress-induced alterations in spatial learning and memory. In the hippocampus of these animals, a small number of genes are deregulated, including some imprinted genes, and chromatin analyses of their promoters reveal decreased histone 3 K9-trimethylation and increased histone 3 and histone 4 acetylation, consistent with KAP1 loss. This suggests a model in which the tethering of KAP1-associated chromatin remodeling factors via KRAB-ZFPs epigenetically controls gene expression in the hippocampus, thereby conditioning responses to behavioral stress. Our ongoing work similarly indicates that KAP1 is essential for embryonic stem cell renewal and acts at multiple levels to control hematopoietic homeostasis.

Selected publications


Stress-controlling protein KAP1 in the hippocampus of a mouse brain (immunofluorescence microscopy)
Introduction
The effort of laboratory is focused on understanding the molecular and cellular mode of action of bacterial protein toxins, such as pore-forming toxins or anthrax toxin, which are major determinants of human infectious diseases. We have recently broadened these studies to understanding the physiological role and the cell biology of the anthrax toxin receptors and their partner proteins. Our work lies at the frontier of cell biology and cellular microbiology and bacterial toxins provide us with a powerful tool to study basic cellular processes, in addition to their role as virulence factors.

Keywords:
Bacterial virulence factors, toxins, anthrax, pore-forming toxins, cellular microbiology, cell biology, structure of membrane proteins, systemic hyalinosi

Results obtained in 2008
Pore-forming toxin aerolysin
Aerolysin is produced by Aeromonas species as a precursor protein called proaerolysin, which bears a 40 residues C-terminal peptide that must be processed for the toxin to form heptamers, the form competent for pore formation. Our work over the last year has been focused on three main aspects: understanding the role of the C-terminal peptide, reconstituting the aerolysin pore in 2 dimensional crystals that diffract well with the aim of solving the structure by electron crystallography (coll. With A. Engel, Basel) and finally understanding how cells respond to pore formation and how they attempt to repair the damage. We will here restrict ourselves to the first aspect. As could be expected, we found that the pro-peptide of aerolysin inhibits the oligomerization process and thus acts as a chaperone by preventing premature assembly of the oligomer. Interestingly however we found that the peptide also plays an essential role in the initial folding of the protein, acting as an bona-fide chaperone despite its C-terminal location. A collaboration has been initiated with the group of M. Dal Perarro (EPFL IBI) to combine our experimental approach with molecular dynamics analysis to get a better understanding of the chaperone role of the peptide.

Anthrax toxin and its receptors
The anthrax toxin is composed of three polypeptide chains: the protective antigen (PA), the lethal factor (LF) and the edema factor (EF). LF is a zinc dependent metalloprotease that cleaves all MAP kinase kinases ; EF is a calmodulin dependent adenylyl cyclase that is responsible for the edema observed in anthrax patients ; PA has no enzymatic activity and is involved in escorting EF and LF to the cytoplasm. Toxicity is strictly dependent upon the delivery of the enzymatic subunits to the cytoplasm and thus much of our attention has been focused on understanding the molecular mechanisms that mediate toxin uptake and cytoplasmic delivery. These events are largely dependent on the anthrax toxin receptors (ATRs), of which there are two: capillary morphogenesis gene 2 (CMG2) and tumor endothelial marker 8 (TEM8). Very little is known about the structure and physiological functions of these proteins. We could show that the ectodomains of ATRs become glycosylated in the endoplasmic reticulum and undergo formation of 3 disulfide bridges and that both these post-translational modifications are required for efficient exist from the ER.

It has recently been proposed, and subsequently challenged by two groups, that endocytosis of the anthrax toxin depends not only on ATRs but also the transmembrane protein LRP6 (low-density lipoprotein receptor-related protein 6), well known for its role in the canonical Wnt signaling pathway. We could show that LRP6 modulates anthrax toxin endocytosis, but is not absolutely required. More specifically, we found that anthrax toxin triggers LRP6 tyrosine phosphorylation, its redistribution at the cell surface, subsequent endocytosis and degradation in lysosomes, demonstrating that it is part of the toxin-ATR complex. More interestingly, we found that ATRs regulated the cellular levels of LRP6: RNAi against or overexpression of ATRs led to a drastic post-translational down regulation of LRP6. As a consequence, RNAi against CMG2 led to a strong decrease in Wnt
signaling. Since the physiological role of ATRs is not act as a toxin receptor but to interact with the extracellular matrix, these findings raise the interesting possibility that, through the ATR-LRP6 interaction, adhesion to the extracellular matrix could locally control Wnt-signaling.

As a first attempt towards understanding the physiological roles of ATRs, we investigated the effect of silencing these genes in zebrafish, in collaboration with the lab of M. Gonzalez-Gaitan (Univ. Geneva). Unexpectedly, we found that silencing CMG2 led to a convergent extension phenotype. More specifically, CMG2 appeared to be a positive regulator of the non-canonical Wnt signaling pathway. Interestingly, we found that silencing LRP6 had a similar effect illustrating that LRP6 not only plays a role in canonical but also non-canonical Wnt signaling in zebrafish. These studies are on going.

**Selected publications**


**ISREC - Swiss Institute for Experimental Cancer Research**

The ISREC has, for over 40 years of its existence as an independent institute, been devoted to cancer-related basic research, before being integrated as an institute into the EPFL School of Life Sciences. With the renewal of a substantial part of its scientific staff in the recent past, its research focus has shifted to areas including genome stability, cell proliferation and differentiation, and the role of developmental pathways in tumorigenesis and tumor progression. The ISREC Foundation ([www.isrec.ch](http://www.isrec.ch)) continues to raise and provide resources that are primarily aimed at supporting projects with a potential for diagnostic or therapeutic innovation - 'http://isrec.epfl.ch’

The ISREC is also leading house of a National Center of Competence in Research (NCCR) in molecular oncology, a network research program launched by the Swiss National Science Foundation. The program focuses on questions relating to the interaction of tumors with their microenvironment. Its explicit goal is to support projects at the interface to the clinic - ‘http://www.nccr-oncology.ch’
Introduction
The Wnt pathway regulates critical processes during embryonic development and adult tissue renewal, and aberrant activation of this pathway is associated with colorectal and other cancers. Our group has been focusing on the phenotypic characterization of mouse Wnt pathway mutants in which the transcriptional co-activators Bcl9 and Bcl9l, and Pygo1/2 were conditionally ablated. These Wnt pathway components were discovered in Drosophila where they are essential for canonical Wnt signaling. Bcl9 proteins act as linkers to tether Pygo to the β-catenin-Tcf activation complex (Kramps et al., Cell 109, 47-60, 2002).

Keywords
Mouse mutants of Wnt signaling components, tumor cell differentiation

Results obtained in 2008
Phenotypic characterization of mice lacking Bcl9/9l
To elucidate the role of Bc9 and Pygo proteins during embryonic development and adult tissue homeostasis in the mouse, we generated loxP-flanked alleles of the four relevant genes (Bcl9/9l and Pygo1/2) and derived several conditionally mutant strains. Our recent work has focused on the following two projects:

Role of Bc9/9l in homeostasis and regeneration of adult intestinal epithelium.
In the intestinal epithelium, locally confined Wnt signals regulate and compartmentalize cell proliferation and differentiation along the crypt villus axis. In contrast to other Wnt pathway mutants, ablation of Bc9/9l in the adult intestinal epithelium did not result in any detectable anomaly and had no effect on cell proliferation and lineage determination. However, mutant mice proved highly susceptible to the irritant dextrane sulfate sodium (DSS) and displayed epithelial lesions that were markedly more extended as compared to wild-type mice (see Figure), likely due to impaired epithelial regeneration during wound healing. Transcriptional profiles of epithelium microdissected at different time points of DSS treatment revealed few informative gene expression differences.

While these findings imply that in the mouse Bc9/9l are by and large not obligatory for Wnt-mediated gene regulation, some of the observed differences in gene expression indicate that these proteins may selectively regulate a subset of Wnt target genes.

Role of Bc9/9l in tumor development.
Constitutive activation of Wnt/β-catenin signaling is an initiating event in the development of colorectal carcinomas in humans. To assess the role of Bc9 proteins in colorectal tumorigenesis, colon tumors were induced in mice through exposure to the mutagen N,N'-dimethylhydrazine (DMH) and subsequent challenge with DSS. Unexpectedly, wild-type and Bc9/9l mutants developed tumors to the same extent and tumors had comparable morphology. Wnt signaling, as assessed by expression level of the Wnt target gene Axin2, was activated to a similar extent in wild-type and mutant tumors. Transcriptional profiles of micro-dissected tumor tissue, however, revealed major differences, pointing again to a likely role of Bc9/9l in regulating a selective set of Wnt target genes that may control a subprogram of Wnt-regulated cell functions. Interestingly, some transcriptional signatures observed in our colon cancer model that are affected by the loss of Bc9/9l function overlap with gene expression patterns that correlate with poor outcome in human cancers.
**Outlook**

In view of these recent findings our efforts are now largely focusing on characterizing the subset of Bcl9/9l-regulated genes both in the epithelial regeneration and in the tumor model, and on investigating the functional implications notably in tumor progression and dissemination. In vivo models are being adapted to assess the role of Bcl9/9l-regulated genes in tumor invasion and dissemination, and new in vitro assays are being developed that recapitulate the observed transcriptional profile changes, as a basis for addressing the mechanisms of Bcl9/9l-mediated gene selective transcriptional regulation.

**Selected publications**


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**Immunohistochemistry using a Bcl9 specific antibody on A) wild-type and B) Bcl9/9l deleted mouse colon adenocarcinomas confirming complete loss of Bcl9 expression in the mutant tumor epithelium.**

**Gene expression changes between wild-type (WT) and Bcl9/9l mutant (KO) colon adenocarcinomas revealing substantial transcriptional differences and pointing to a role of Bcl9/9l in a subprogram of Wnt regulated cell functions.**
Introduction
The cellular response to DNA damage is a crucial factor in tumour formation. Our work uses a virus (adeno-associated virus, AAV) as a biological probe to study DNA damage signalling pathways in cells. We found that AAV infection triggers a damage response that can lead to death of p53-defective tumour cells. AAV therefore provides a unique opportunity to study this response without actually damaging the cellular DNA. Understanding the tumour-suppressive activity of AAV may lead to novel approaches to cancer therapy.

Keywords
Adeno-associated virus, cancer, DNA damage response, cell cycle, cell death

Results obtained in 2008
Cell death by mitotic catastrophe can take place in the absence of apoptosis
Cell death in mitosis, known as mitotic catastrophe, is a process that occurs as a result of centrosome overduplication and absence of proper cell cycle checkpoints. Mitotic catastrophe often takes place in conjunction with apoptosis, but it is still unclear whether it consists of a subtype of programmed cell death or if it requires apoptotic factors. We have now found that mitotic catastrophe can occur in the absence of apoptosis. To induce mitotic cell death, we used UV-inactivated AAV which has been shown to induce a DNA damage response, and subsequent cell death, without affecting the integrity of the host genome. p53-deficient osteosarcoma cells (U2OSp53DD) lack the G1 checkpoint and respond to AAV infection through a transient G2 arrest. Infected U2OSp53DD cells died via mitotic catastrophe shown by formation of multiple spindle poles and micronucleated cells. These cells showed no signs of chromosome condensation or DNA fragmentation. Moreover, cell death was also shown to be independent of caspases, AIF and the autophagy marker LC3B. Breast tumour cells MCF-7, which are deficient in caspase-3, were also sensitive to AAV infection. On the other hand, glioma cells M059K that have a functional G1 checkpoint reacted differently to the virus and died via apoptosis. Together, these data indicate that, in the absence of a functional G1 checkpoint, mitotic catastrophe can occur as a result of mechanical damage and not as a consequence of a suicide signal.

The replication of adeno-associated virus in the presence or absence of viral helper functions
AAV’s life cycle in vitro is strongly dependent not only on cellular factors, but also on helper functions provided by other viruses, for example adenovirus, herpes virus and, to a certain extent, papillomavirus (HPV). Although AAVs are wide-spread in the population, no disease is yet associated with them. Accordingly, AAV is attractive as a vector for gene therapy studies. We have undertaken a systematic study of AAV growth in presence of helper activities and in differentiating skin keratinocytes. We used three systems of cultured keratinocytes and showed that in each case in vitro differentiation was achieved. In two of these, the cells were from HPV-induced lesions. We found that AAV replicates efficiently in differentiating monolayer foreskin keratinocytes in the presence but not in the absence of adenovirus. Although HPV can provide certain helper functions, it is less efficient as a helper than adenovirus. U2OS osteosarcoma cells are particularly well infected by AAV. These cells are semi-permissive for AAV growth and induction of a DNA damage response stimulates AAV genome amplification and the appearance of capsid protein. However, this is at a relatively low level and we conclude that although these alternative helper systems can contribute to the natural history of AAV, the presence of an efficient helper virus seems likely to be needed for AAV to persist in the human population.

Control of the cell cycle by the adeno-associated virus Rep78 protein
The AAV Rep78 protein has been found to block the cell cycle at several stages. To induce this...
arrest, Rep78 affects several proteins implicated in the regulation of the cell cycle, such as the family of the cell division cycle 25 (Cdc25) phosphatases. Part of our work focuses on the question of whether Rep78 exerts its effect through interaction with Cdc25B. We showed earlier that Rep78 interacts with Cdc25A and inactivates it. As Cdc25B has an equally important role in cell cycle regulation, we investigated the interaction between Rep78 and Cdc25B. 3xFlag tagged proteins were used. The results showed that 3xNt-Flag-Rep78 and 3xCt-Flag-Rep78 interact with Cdc25B at both endogenous and overexpressed levels. Cdc25B is nuclear most of the time, but migrates to the cytoplasm at the end of the G2 phase to activate proteins needed for entry into mitosis. Overexpressed Cdc25B, contrary to the endogenous protein, is predominantly cytoplasmic. The expression of Rep78, which is nuclear, leads to the relocation of Cdc25B to the nucleus. Our model is that Rep78 interacts with Cdc25B and thus retains it in the nucleus. This work establishes a novel mechanism in which sequestration of Cdc25B in the nucleus prevents it from activating the proteins in the cytoplasm needed to give the entry signal into mitosis, so contributing to the G2/M arrest induced by Rep78. Cdc25B phosphatase assays using phosphorylated Cdk1 as substrate showed that Rep78 binding also inhibits this function. The 3x-Flag-Rep78 was also used to find new interacting partners for Rep78, focussing on proteins used by AAV to replicate its genome or integrate it specifically into chromosome 19. DNA polymerases α and δ and PCNA, but not RPA, were found to interact. Flag-Rep78 was also found to bind ATR. If this interaction inactivates ATR, it could explain our unexpected earlier finding that the nicks made in DNA by Rep78 do not activate ATR.

**Selected Publications**


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*Rep78 (red) sequesters Cdc25B (green) in the nucleus (left panel), except if the Rep78 bears a nuclear localisation signal mutation (right panel). The bar represents 10μm.*
Introduction
Breast cancer strikes one out of eight women in Switzerland. A woman’s risk to get breast cancer is linked to her lifetime exposure to endogenous and exogenous hormones. While early pregnancies have a protective effect, breast cancer risk increases with the number of menstrual cycles a woman experiences prior to her first pregnancy. Hormones also influence the course of the disease. We study how hormones control the breast in vivo, in particular the mechanisms by which they elicit cell proliferation and changes in structure of the breast tissue, to gain insights into the genesis of the disease and to develop new preventive and therapeutic strategies.

The use of mutant mice to study breast development
The mouse mammary gland provides a unique experimental system to study in vivo how systemic hormones impinge on molecular determinants of development in the breast and how deregulation of these pathways leads to tumorigenesis. The mammary gland being the only organ to undergo most of its development after birth allows for extensive experimental manipulation. In young female mice, the part of the inguinal mammary glands that contains the epithelial tree can be surgically removed creating a «cleared fat pad». When epithelial tissue or primary cells are engrafted into a cleared fat pads, they will repopulate it and form a well-organized mammary gland, which responds to all hormonal stimuli (see scheme 1).

We have made extensive use of this model to define the influence of estrogens, progesterone and prolactin signaling on the branching of the milk duct system and the formation of the secretory pouches and to study how these hormones control developmental signaling pathways in the breast (summarized in scheme 2).

Scheme 1: Mammary gland reconstitution: In the 3-week-old female mouse the ductal tree growing out from the nipple has only partially penetrated the mammary fat pad (left panel). It can be surgically removed leaving behind a cleared fat pad. Primary mammary epithelial cells injected into this fat pad can form new ducts that grow out to populate the fat pad (right panels).

Scheme 2: Schematic representation of mammary gland development (black) and our current working model of how various factors control different morphogenetic steps (color) based on our previous work.

Hormones exert control in the breast tissue by regulating interactions between different cells
We have shown that a subset of cells in the mammary gland, which have the receptors for estrogens and progesterone act as «sensor cells» and translate the hormonal stimulus into local signals which they pass on to different neighboring cells. As a result, cells in close vicinity to the «sensor cells» proliferate.
Selected publications


Reviews:


Scheme 3: Model of estrogen-induced proliferation : estrogens bind to the estrogen receptor in the sensor cell. In response, the sensor cell secretes the growth factor amphiregulin that acts on stromal cells. In response to an unknown signal from the stroma, cells next to the sensor cells, divide.
Introduction

Through biochemical and genetic analysis, our lab investigates how stem cells communicate with their micro-environment in the mouse embryo to coordinate the development of different tissues according to their position, and how the molecules involved can become oncogenic.

Keywords

Stem cells and lineage differentiation; inductive tissue interactions in development and cancer; TGFβ signaling and trafficking; morphogen gradients

Results obtained in 2008

Background:

The allocation of pluripotent cells to distinct germ layers and the establishment of antero-posterior and left-right asymmetries are orchestrated by the TGFβ-related precursor protein Nodal, its proprotein convertases Furin and Pace4, and the glycosylinositolphosphate-anchored coreceptors Cripto and Cryptic of the EGF-CFC family. Cleavage of Nodal leads to the activation and nuclear translocation of Smad2 and Smad3 transcription factors to regulate target genes. Nodal signaling sustains trophoblast cells in the extra-embryonic ectoderm and prevents precocious neural differentiation of pluripotent progenitors in the inner cell mass of the implanted blastocyst. During gastrulation, however, Nodal induces endoderm and mesodermal formation. To elucidate how such disparate activities of Nodal might be regulated, we explored when and where the proteases Furin and Pace4 activate the Nodal precursor in the embryo, and how this cleavage influences the subcellular trafficking and signaling of Nodal in endocytic compartments. Furthermore, we identified Bicaudal-C as a novel factor which confines Nodal signaling to the left side to specify asymmetry of the visceral situs.

1) Cripto binds the Nodal precursor and its convertases to spatially couple processing and signaling.

Complexes of the signaling receptor Alk4 with an EGF-CFC coreceptor such as Cripto mediate Nodal signal transduction by phosphorylating the transcription factors Smad2 and-3 (Fig. 2). We found that Cripto captures Nodal already during exocytosis, in part by tethering its prosegment. Cripto thus promotes the localization or stability of Nodal precursor in lipid rafts and stimulates endocytosis in membrane carriers marked by GFP-Flotillin. Co-immunoprecipitation assays revealed that Cripto also binds Furin and PACE4. To determine whether Nodal must mature in a complex, we mapped a Cripto-interacting region (CIR) in the prosegment of the Nodal precursor and we showed that mutation of this motif in the Nodal precursor inhibited signaling. These results suggest a new role for Cripto as a protease receptor that localizes Nodal processing to specific membrane microdomains (Blanchet et al., 2008a).

Why does the localization of Nodal processing by Cripto enhance Smad2,3 signaling? Possibly, Cripto increases the affinity of Nodal for Alk4, but this has never been demonstrated. As an alternative, we tested whether Cripto facilitates uptake or retention of processed Nodal in signaling endosomes. Labeling of endocytosed Nodal by antibody uptake in transfected cells revealed that processed Nodal after uptake in uncoated membrane invaginations is delivered to early endosomes irrespective of the presence or absence of Cripto. However, Cripto attenuated the sequestration of Nodal from the endosome-limiting membrane to intralumenal vesicles. Furthermore, mutant forms of Cripto which still bind Nodal yet fail to inhibit intralumenal sorting were unable to stimulate Nodal signaling, indicating that localization at the limiting membrane is essential. Together, these observations suggest a model whereby Cripto guides Nodal trafficking to couple proteolytic processing and signaling in target cells. By contrast, Cripto-independent processing may serve to sequester excess Nodal in multi-vesicular endosomes for delivery to lysosomes (Fig. 1).

2) Role of Smad-independent Cripto signaling

In cultured cells, Cripto has been shown to also activate MAPK. To test whether Smad-independent Cripto signaling plays a role during development, our collaborator, Dr. Minchiotti, targeted the Cripto locus by homologous recombination in ES cells to substitute F78 by an Alanine mutation that inhibits Smad
signaling. Interestingly, the F78A mutant Cripto induced gastrulation movements, even though the expression of known Smad2,3-dependent Nodal target genes was inhibited (D’Andrea et al. 2008). These results suggest a novel function for Smad2-independent Cripto signaling in vivo.

3) Identification of the RNA-binding protein Bicaudal-C as a regulator of left-right axis formation and renal morphogenesis

Studies in Xenopus indicated that Nodal signaling during gastrulation may depend on Bicaudal-C, an RNA binding protein previously found to control the localization of the posterior determinant Oskar in Drosophila oocytes. By contrast, mutations in mouse Bicc are associated with polycystic kidney disease. By generating a targeted deletion in ES cells, we confirmed a role for Bicaudal-C during renal morphogenesis. In addition, the targeted mutation randomized the sidedness of Nodal signaling during left-right axis formation. Video microscopy and scanning electron microscopy analysis revealed that loss of Bicaudal-C disrupts the planar alignment of node cilia and the cilia-dependent flow that is required to bias Nodal signaling to the left side.

Selected Publications


Figure 1. Cripto stimulates Smad2,3 signaling by localizing Nodal during proteolytic maturation and endosomal trafficking. A) Localization of Nodal (red) in early endosomes marked by GFP-Rab4 (green) in COS1 cells transfected without (top) or with (bottom) Cripto. B) Left: The GPI-anchored EGF-CFC proteoglycan Cripto binds the prosegment (pro) and the mature region (mat) of the Nodal precursor and one of the proprotein convertases (PC) Furin or PACE4 to assemble a processing complex at the plasma membrane. Cripto thus facilitates Nodal uptake in Flotillin carriers (green bar) and subsequent access to SARA-positive platforms at the limiting membrane of early endosomes marked by EEA1, Rab5 and Rab4. The Nodal receptors Acrv1b and Acrv2 are retained by the Nodal/Cripto complex in early endosomes for signaling until their delivery to lysosomes by Dpr2. Right: Independently of EGF-CFC proteins, the Nodal precursor also interacts with soluble PCs and unidentified cell surface proteins (X) and signaling receptors. However, in the absence of Cripto, Nodal is internalized in unidentified membrane microdomains (grey bar) and sequestered on intralumenal vesicles (ILV) of early endosomes for delivery to lysosomes, so that it cannot couple to the cytoplasmic signal transduction machinery. Center: In the absence of proteolytic maturation, Nodal still binds to receptors at the cell surface, but endocytosis is diminished (thin arrow) and limited to microdomains lacking Flotillin, suggesting that it probably involves Caveolin (red bar) or unknown carriers (not shown). After Constam, 2009a.
Introduction
The aim of this research is to understand how genes are regulated during mammalian embryonic development. We are particularly interested to study the relationships that exist between genomic organization (e.g. gene topology) and the control of transcriptional activity, by using one of the Hox gene locus as a paradigm.

Keywords
Embryos, development, evolution, transcription, epigenetic regulation, Hox gene clusters

Main results obtained in 2008

SystemsHox.ch; an in vivo System Approach to Hox Genes Regulation in Vertebrates. The aim of this research programme is to understand the relationships between genomic topology and the control of transcription, using the HoxD locus as a paradigm. We take a system approach using the mouse, combining tools of genetics, evolutionary genomics and biochemistry to try and model various modes of large-scale gene regulations occurring during development. The results will tell us about the mechanistic bases of global gene regulation and how such regulation has evolved, by integrating these mechanisms into the evolutionary contexts of the respective structures. The approach makes use of a unique collection of mutants at this locus along with both transcript profiling and quantitation, ChIP of several proteins (modifications thereof-) indicative of chromatin states, chromosome conformation capture (4C) and transgenesis after phylogenetic footprint. To keep datasets within reasonable scales and allow for their integration into a single model structures, we focus on a 2 Mb large DNA interval centered around the HoxD locus, which contains range of conserved non-coding DNA sequences and includes a gene-rich island, flanked by two large gene-deserts bordered again by gene-rich regions. The aim is to conduct systematic analyses of these multiple parameters in various embryonic tissues, at different times where and when Hox genes are required. The large datasets generated (various points for transcript quantification, profiling, chromatin modifications, 4C, from ranges of different tissues at various developmental times, from as many as 20 mutant strains) will feed mechanistic models accounting for the modalities of these regulations. Models will be compared to one another to try and reconstitute the evolution of these types of regulation. Such a combination of genetics, biochemistry and modeling, carried out on a locus where global gene function is already well worked out, will give us a comprehensive view of the underlying regulatory mechanisms, with a high heuristic value.

Selected publications
Zacchetti G, Duboule D, Zakany J. Hox gene function in...


*Transcript profile for the posterior HoxD cluster in developing digits (E12.5), using reverse-transcribed total RNA and hybridization on high-resolution tiling arrays*
**Introduction**

We are interested in understanding fundamental cell division processes that are critical for genome integrity. To this end, we utilize a unique combination of genetic, functional genomic, cell biological and live imaging approaches in the embryo of the nematode Caenorhabditis elegans as well as in human cells in culture.

**Keywords**

Cell biology, developmental biology, centrosome duplication, asymmetric cell division, C. elegans

**Results Obtained in 2008**

Our research focuses on three beautiful fundamental cell division processes that are critical for genome integrity: centrosome duplication, asymmetric cell division and cell division timing. We will restrict the description hereafter to the former two processes.

**Centrosome duplication.** The centrosome is the principal microtubule-organizing center of animal cells and comprises two microtubule-based centrioles surrounded by peri-centriolar material. Just like the genetic material, the centrosome must duplicate once per cell cycle to ensure genome integrity. Formation of a single pro-centriole next to each centriole is key for initiating the entire centrosome duplication cycle. Together with other laboratories, we have identified five proteins required for pro-centriole formation in C. elegans: the kinase ZYG-1, as well as the coiled-coil proteins SAS-4, SAS-5, SAS-6 and SPD-2. We established that pro-centriole formation is an orderly assembly process in which these proteins are recruited in a step-wise fashion. More recently, we found that the kinase ZYG-1 exerts its function in part by phosphorylating SAS-6. This modification somehow imparts a conformational change on SAS-6 that is critical for initiating pro-centriole formation. Except for SAS-5, the five proteins identified as being essential for pro-centriole formation in C. elegans have relatives in other metazoans. For instance, we found that a protein related to SAS-6 is present in every species with centrioles, and also that HsSAS-6 is essential for pro-centriole formation in human cells. More recently, we established that the SAS-4-related protein CPAP is similarly required for centrosome duplication in human cells (see Figure). Interestingly in addition, we found that over-expression of CPAP results in the formation of overly long centrioles, together indicating that CPAP levels are critical for proper pro-centriole formation and elongation. Overall, such observations indicate that lessons learned in C. elegans are applicable to other species and thus will have a significant impact on our understanding of genome integrity in human cells.

**Asymmetric cell division.** Asymmetric division is crucial for generating cell diversity, both during development and in stem cell lineages. Accurate spindle positioning is critical for proper asymmetric cell division. Together with the work of other laboratories, our findings support a model for spindle positioning in C. elegans whereby two Gα proteins recruit the GoLoco protein GPR-1/2 and the coiled-coil protein LIN-5 to the cell cortex to generate force on the plus-end of astral microtubules. The LIN-5/GPR-1/2/Gα complex serves in turn to recruit the minus-end directed microtubule motor dynein to the cell cortex. Together with microtubule depolymerization, dynein activity then allows pulling forces to be exerted along astral microtubules, which ensures proper spindle positioning. More recently, we found that the adaptor protein clathrin is an important modulator of pulling forces during spindle positioning in C. elegans embryos, presumably because it impinges on the distribution of Gβγ subunits, which act as negative regulators of force generation. Given that Gα proteins and their associated partners, including Gβγ, have been implicated in asymmetric cell division across metazoan evolution, we expect the mechanisms characterized in C. elegans to be of broad significance.
Selected publications:


Human cells during mitosis fixed and stained with antibodies against α-tubulin (green, which mark the microtubules of the mitotic spindle), Centrin-3 (red, which mark the pro-centrioles and centrioles) and counterstained with a DNA dye (blue). This figure illustrates that depletion of the SAS-4-related protein CPAP results in the assembly of a monopolar spindle with a single centriole (right, compare to bipolar spindle with four centrioles on the left).
Introduction
Our research addresses how the pancreas normally develops during embryogenesis, how this can be used to generate replacement cells of use in diabetes therapy and how these mechanisms are reactivated during pancreas cancer progression.

Keywords
Development, embryo, gut, pancreas, diabetes, endoderm, Wnt, patterning, beta-cell, chick, mouse

Results Obtained in 2008
Our work was focussed on several stages of pancreas development in vertebrates.

Organizing endoderm organs along the body axis
We had previously shown that the pancreas is induced by signals from the mesoderm in contact with endoderm and that graded levels of Fgf4 are necessary to form posterior foregut, mid- and hindgut and accurately maintain the boundaries between different digestive tract organs. We recently showed that increasing levels of retinoic acid along the antero-posterior axis are also necessary to form these regions (Bayha et al., 2009). We are pursuing similar experiments with the Wnt pathway.

Regulation of pancreas organogenesis: role of the bHLH transcription factor Ptf1a in pancreas progenitor maintenance
Signaling activity from the mesoderm induces pancreatic genes at a given position along the A-P axis. The transcription factors Pdx1 and Ptf1a maintain pancreas progenitors (see figure). At a later stage Ptf1a becomes restricted to exocrine cells (see figure). We compared the transcriptome of early pancreas progenitors lacking Ptf1a to that of wild type progenitors. Our experiments identified the molecular mechanisms by which Ptf1a maintains pancreas progenitors. Ptf1a blocks the expression of intestinal genes while activating a network of transcription factors of known importance in pancreas progenitors. In addition, we identified new targets of Ptf1a that we are studying functionally.

Regulation of pancreas organogenesis: role of the bHLH transcription factor Neurogenin 3 (Ngn3) in endocrine cell differentiation
In addition to exocrine cells, the pancreas gives rise to multiple endocrine cells whose main function is the regulation of glucose homeostasis. The transcription factor Ngn3 is absolutely necessary to generate endocrine cells. All pancreatic endocrine cells, producing glucagon, insulin, somatostatin or PP, differentiate from Pdx1+ progenitors that transiently express Neurogenin 3 (see figure). We showed that pancreas progenitors, similar to retinal or cortical progenitors, go through competence states that each allow the generation of a subset of endocrine cell types (see figure) (Johansson et al., 2007). We further showed that the progenitors acquire the competence to generate late-born cells in a mechanism that is intrinsic to the epithelium. Ongoing experiments aim at understanding whether the change in competence is cell autonomous and what its molecular basis is. This transgenic line was also used to identify new targets of Ngn3 that we are studying functionally.

Significance of this work for diabetes and pancreatic cancer
Although the function of the pancreas in controlling glucose homeostasis is compensated by insulin injection in diabetic patients, the physiological effects are inexact and too variable. Among approaches that are currently being explored to find a cure for diabetes are the isolation and propagation of embryonic or adult stem cells that can be engineered to produce endocrine hormones and then transplanted to patients. Our experiments are aimed at identifying the critical cellular transcription factors and signaling molecules that are sufficient to transform cells...
into pancreas and β cells. Collaborations within the context of the European 6th framework project BetaCellTherapy allow us to test these ideas by introducing transcription factors in stem cells or exposing these cells to signalling molecules in vivo or in vitro to force their differentiation into β cells (Grapin-Botton, 2008). To assist diabetes therapy, we contributed to developing new imaging techniques of the islets of Langerhans (Villiger et al., 2009).

In addition, pre-cancerous and cancerous cells often reactivate the expression of developmental genes. In pancreatic carcinoma many developmental genes are reactivated. Ongoing work on developmental genes may give a better understanding of pancreas cancer development and may point to new therapeutic targets.

Selected publications:


Pancreas progenitors are induced at a specific location in the embryo, as shown with a mouse transgenic line expressing the green fluorescent protein in the pancreas primordium. These progenitors progressively differentiate into endocrine or exocrine progenitors and subsequently into 4 types of endocrine cells expressing specific hormones. Endocrine cells (green and red) migrate out of the epithelium made of progenitors (white) as shown with the green arrows and further aggregate into islets of Langerhans.
Introduction
The last years of cancer research have established the concept of cancer stem cells (CSC) as a sub-population of cells within a tumor entirely responsible for long-term tumor growth. This has aroused expectations that targeting specifically these cancer stem cells would allow effective tumor eradication. The focus of attention is now moving to discover molecular signals which are essential to sustain such CSC.

Keywords
Stem cells, cancer stem cells, stem cell niches, Wnt signaling, metastatic colonization

Results Obtained in 2008
A variety of developmental signaling pathways such as the Wnt, Notch, Hedgehog, BMP, FGF, IGF and TGFβ pathway are known to influence stem cell self-renewal and differentiation in normal tissues. We are only beginning to understand the roles these pathways play in different cancer stem cell populations. For example, the Hedgehog pathway has long been implicated in various cancers and is active in CSC of multiple myeloma and brain tumors. Here, blocking of hedgehog signaling can eliminate CSC and prevent tumor outgrowth. In contrast, increased BMP signaling was shown to promote CSC differentiation, and treatment with BMPs can block glioblastoma formation in an experimental model.

We have worked for several years now on the Wnt signaling pathway which is implicated in many steps during embryonic development and in tissue homeostasis in the adult organism. Activation of the canonical Wnt pathway is prominent in bronchioalveolar stem cells of the lung which multiply in response to Wnt ligands. However, this pathway can also do the opposite and induce differentiation of stem cells. For example in mesenchymal stem cells, Wnt ligands promote lineage commitment. We and others have demonstrated that in some organs such as the intestine the canonical Wnt pathway, i.e. β-catenin mediated signaling, is essential for the maintenance of tissue-specific stem cells. Accordingly, activating mutations in the pathway have been suggested to drive tumorigenesis by expanding the stem cell pool. This indicates that the ability of our body to regenerate and to repair tissue damage may come at a price that is increased risk of cancer formation.

In organs like the intestine, targeting this pathway therefore appears difficult, since the normal organ function may be affected when Wnt signalling is blocked. In the skin, the prospect of targeting Wnt signaling to affect tumor formation appears more favorable. We have shown recently, that cutaneous squamous cell carcinomas contain CSCs which depend on β-catenin signaling in order to sustain their stemness. Deletion of β-catenin in established tumors was sufficient to provoke complete tumor regression which was initiated by a rapid loss of CSCs. This was followed by a cease of proliferation and terminal differentiation of all tumor cells. Importantly, the normal stem cells of the skin do not rely on Wnt signals for their maintenance. These bulge stem cells are maintained in the absence of Wnt signals which makes this pathway a very interesting target to treat skin cancers.

For the skin, we also now provided direct evidence that normal stem cells are the direct target for oncogenic mutations and can very efficiently produce tumors. We used lineage tracing to show a direct derivation of cancer stem cells from such normal stem cells (see picture). A transgenic mouse strain was generated using the keratin15 promoter, which is active specifically in skin stem cells, to drive expression of a tamoxifen-inducible variant of cre-recombinase. This allowed timely controlled labeling of skin stem cell progeny in combination with a cre-inducible lacZ reporter allele. After induction of the cre by tamoxifen injection for five days, we found locally restricted expression of β-galactosidase in the bulge as expected. Since we aimed to access the direct contribution of these cells to tumor formation, we initiated tumorigenesis the next day by treatment with DMBA which induces mutations before these stem cells have enough time to produce progeny. After several weeks of TPA mediated tumor promotion,
we could isolate lacZ+ tumors. This clearly demonstrates that tumors directly derive from normal skin stem cells which are the target of mutation to produce cancer stem cells.

We also have shown last year that deletion of the central Wnt signal transmitters β- and γ-catenin does not disturb hematopoietic function. It has therefore to be postulated that Wnt signaling indirectly affects hematopoietic stem cells by altering the mesenchymal niche for these cells. This has raised expectations that targeting the Wnt pathway might be an option in the treatment of leukemia as several lines of evidence have implicated over-activation of the pathway in these tumors. For instance, ectopic expression of γ catenin or LEF-1 has been linked to acute myeloid leukemias, and over-expression of Wnt ligands has been reported in lymphoid leukemia and multiple myelomas. However, partially blocking Wnt signal transduction by deletion of β-catenin turned out not to be sufficient to prevent tumorigenesis in an experimental model of BCR-ABL induced leukemia but only shifted the phenotype from a chronic myeloid towards an acute lymphocytic disease. We are currently developing a treatment regimen along these lines with the aim to achieve complete block of Wnt signal transduction in vivo. This is done initially in a mouse model of chronic myeloid leukemia and we will extend these experiments to human xenograft models of the same disease. We hope that this will allow us developing a new opportunity for leukemia therapy.

Selected Publications:


Introduction
We study newly derived mice that lack the gene for the iron storage protein ferritin H. Mice that cannot store iron in the liver show strong signs of oxidative liver damage. Moreover, mice that have lost ferritin H in the intestine absorb more iron from food than normal mice. These studies are important for the understanding of the hereditary disease of iron overload and of anemia.

Keywords
Conditional knock-out mice for ferritin H, oxidative cell damage, iron physiology, mRNA degradation, RNA-protein interactions

Results Obtained in 2008
Analysis of ferritin H knock-out mice
Iron is an essential metal for life and at the same time a hazard since, in its free form, it catalyzes the formation of hydroxyl radicals, which are a cause of cell damage and mutations in DNA. Therefore, body iron absorption from nutrients and free iron in cells are delicately controlled in order to avoid the excess or deprivation of iron. In the human population, an excess of iron is observed in the hereditary disease of hemochromatosis, which is accompanied by tissue damage in liver, heart and pancreas. It can be the cause of liver cancer. Anemia due to insufficient body iron is, on the other hand, a frequent problem in developing countries and can affect the immune system. We study the role of ferritin in the regulation of iron physiology. Ferritin, a complex composed of ferritin H and L chains, stores excess free iron and thereby protects cells against radical formation. We have generated mouse strains with a conditional deletion of the ferritin H gene in adult animals using the Cre lox strategy.

Ten days after an Mx-Cre induced ferritin H deletion, mice lost their cellular iron stores in the liver, spleen and bone marrow indicating the requirement of ferritin H in iron deposition. Serum iron and transferrin saturation were slightly increased and correlated with a 2-fold increased liver hepcidin 1 mRNA and a reduced duodenal DcytB mRNA level. Under normal iron regimen, deleted mice survived for 2 years without a visible disadvantage. However, mice fed on a high iron diet prior to ferritin H deletion suffered from severe liver damage. Similarly, ferritin H deleted mouse embryonic fibroblasts showed rapid cell death after exposure to iron salt in the medium. This was reversed by wildtype ferritin H, but not by a ferritin H mutant lacking ferroxidase activity. Cell death was preceded by an increase in cytoplasmic free iron, reactive oxygen species, and mitochondrial depolarization.

Parallel studies were initiated to analyze tissue-specific ferritin H gene deletion in hepatocytes, intestine, B lymphocytes, and heart. Deletion of ferritin H in hepatocytes alone showed no signs of severe liver damage, probably because macrophages can capture the iron liberated from deleted hepatocytes. Mice with an intestine-specific deletion of ferritin H showed a phenotype that resembles hemochromatosis. Mice accumulated too much iron in the liver over time. This was observed in spite of a 2-fold induction of hepcidin 1 mRNA. mRNAs of iron transport proteins DMT1 and Dcytb were down-regulated. We conclude that ferritin plays an important role in the regulation of iron absorption. This is a change in the current paradigm as hepcidin 1 was previously thought to be sufficient. It corroborates data from the 1940’s on iron physiology (Figure). Ferritin deletion in the heart provoked fibrosis indicating damage of heart muscle cells. In collaboration with Professor Pedrazzini at the CHUV, Lausanne, we have measured parameters of heart physiology and see that the mice are strongly affected. Deletion of ferritin H by CD19-Cre provoked a diminished number of mature B lymphocytes. The precise cause for this decrease is currently being investigated.
Mechanisms of rapid mRNA degradation
In parallel, we study rapid mRNA degradation as it occurs in a large number of mRNAs that harbor instability elements, such as AU-rich sequences, in their 3’ untranslated regions. We have devised methods to enrich RNA-protein complexes from cells or tissues by immuno-precipitation. These are combined with micro-array analysis, high-throughput sequencing and identification of target genes under various physiological conditions.

Selected Publications:

![Figure: New model for intestinal iron absorption. A) Iron is absorbed by enterocytes from nutrients and exported through ferroportin into the blood stream. Iron-loaded transferrin signals the body-level of iron to the liver that responds by secreting hepcidin 1, which in turn reduces iron export by binding and internalizing ferroportin. B) When iron is increased, more hepcidin 1 is secreted and ferroportin further down-regulated. It provokes a transient increase of the labile iron pool in enterocytes and induction of ferritin that sequesters this free iron. Concomitantly, iron import is diminished by reduced DMT1 and Dcytb mRNA expression. C) When ferritin H is deleted in the intestine, free iron cannot be sequestered and is continuously exported, leading to liver iron loading, in spite of increased hepcidin 1 activity and down-regulation of ferroportin, DMT1 and Dcytb](image-url)
Introduction
Telomeres are the physical ends of eukaryotic chromosomes. They function as guardians of genome stability, cellular clocks and tumor suppressors. Our laboratory studies telomere structure and maintenance in human cells and in the yeast Saccharomyces cerevisiae in order to gain a detailed understanding of the function of these fascinating structures. This may allow manipulation of telomere function in tumors in the future.

Keywords
Telomeres, telomerase, TERRA, genome stability

Results Obtained in 2008
Telomerase is regulated at individual chromosome ends to mediate telomere length homeostasis. In humans, telomere length is set in most tissues early in embryogenesis as telomerase is repressed later in life. Short telomeres that accumulate upon excessive number of cell division cycles induce cellular senescence and this is thought to counteract growth of pre-malignant lesions in our body. Most cancers re-express telomerase to overcome this growth barrier. Our work during 2008 concentrated on the question of how the telomerase enzyme is regulated at chromosome ends. In addition, we extended our studies on telomeric repeat containing RNA (TERRA), a large non-coding RNA whose functions may include heterochromatin assembly at the telomere and the regulation of telomerase.

Regulation of telomerase at chromosome ends
Telomerase is a cellular reverse that counteracts telomere shortening that occurs due to incomplete DNA end replication and nucleolytic processing. Telomerase extends chromosome 3' ends by iterative reverse transcription of a small region of its tightly associated telomerase RNA moiety. Telomerase activity is regulated at individual chromosome ends by telomere binding factors. Using single telomere extension analysis, we demonstrated in the past in yeast that telomerase exhibits an increasing preference for telomeres as their lengths decline. We also identified that the DNA checkpoint kinase Tel1 (ortholog of human ATM) and Tbf1p, a protein that binds sub-telomeric sequence, activate telomerase at short telomeres.

Recruitment and activation of telomerase at chromosome ends is not well understood in complex eukaryotes including humans. Therefore, we developed assays to measure association of human telomerase with chromosome ends by chromatin immunoprecipitation, and our collaborators from the Terns-lab (University of Georgia) could for the first time detect human telomerase at chromosome ends by fluorescence in situ hybridization. Through our analyses, we identified that Cajal bodies, subnuclear structures implicated in ribonucleoprotein maturation are critical for telomerase activation at telomeres. Furthermore, through down-regulation of telomere binding proteins by RNA interference, we identified proteins that mediate the recruitment of human telomerase to chromosome ends.

Telomeric repeat containing RNA (TERRA)
Telomeres consist of simple repetitive DNA repeats and a large set of specialized proteins that are crucial for telomere function. We recently discovered in eutherian mammals with TERRA a large non-coding (nc) RNA, which forms an integral part of telomeric heterochromatin (see figure). TERRA molecules are heterogeneous in length from ~100 to >10'000 nucleotides. TERRA association with chromatin is negatively regulated by RNA surveillance. Some of the RNA surveillance factors, including EST1A, physically interact with telomerase. Thus, we suspect that TERRA may be a key regulator of the enzymatic activities that assure telomere replication and length homeostasis.

We now demonstrate that telomeres are also transcribed in S. cerevisiae by RNA polymerase II. Thus, telomere transcription is conserved in different kingdoms of the eukaryotic domain. The yeast system will also accelerate dissection of TERRA function in
the future. We already learned that yeast TERRA is polyadenylated and regulated by the 5’ to 3’ exonuclease, Rat1p. Rat1-deficient cells accumulate TERRA and harbor short telomeres because of impairment in telomerase-mediated telomere elongation. Over-expression of RNaseH overcomes telomere elongation defects in rat1-1 cells, indicating that RNA/DNA hybrids inhibit telomerase function at chromosome ends in these mutants. Thus, telomeric transcription combined with Rat1p-dependent TERRA degradation is important for regulating telomerase in yeast.

Selected Publications


**TERRA Biogenesis, telomere association and displacement from telomeres.**

TERRA is a RNA PolII transcript whose transcription initiates within the sub-telomeric sequences and proceeds into the telomeric tract. A fraction of TERRA is polyadenylated via the canonical poly (A) polymerase, Pap1. TERRA co-localizes with telomeres and at least a portion of TERRA is bound to telomeres through base-pairing with telomeric DNA. Undefined RNA-protein interactions may also tether TERRA to telomeric DNA. The 5’ to 3’ exonuclease, Rat1, degrades TERRA. The poly (A) polymerase, Trf4, also contributes to TERRA degradation. The NMD factors UPF1, SMG1 and EST1A/SMG6 all contribute to TERRA removal from telomeres. Inhibition of any of these factors results in both more and brighter TERRA foci. RNaseH over-expression also reduces cellular TERRA levels when Rat1 function is impaired.
Introduction

Life on earth is organized in temporal rhythms of 24 hours that follow the earth rotational frequency. These rhythms are manifestations of organism’s internal metronome called the circadian oscillator or clock. This clock drives periodic behavior and physiology in most living organisms, through a complex network of feedback control mechanisms acting at the transcriptional and post-transcriptional levels. Clock dysfunction or misalignment can lead to many health conditions such as sleep deprivation or increased susceptibility to cancer. The behavioral clock has remarkable precision properties that allow it to tick unperturbed for weeks. In our lab, we tackle two main problems in circadian biology: (i) the study of transcription regulatory networks that drive expression patterns at all phases during the cycles in a tissue-specific manner. This project applies and develops a combination of bioinformatics and experimental methods; (ii) we investigate the factors that contribute to oscillator precision using mathematical modeling and analysis of time series data. With these approaches we hope to understand mechanisms and consequences of temporal organization at the cellular and organism level.

Keywords
Bioinformatics, circadian biology, gene regulation, fluctuations, modeling, systems biology

Results Obtained in 2008

Circadian gene regulation. Our group recently identified using comparative genomics a highly conserved transcriptional regulatory element (termed the E1-E2 motif) bound by the main circadian transcription activator hetero-dimer CLOCK/BMAL1. This sequence element was found to regulate circadian genes in mammals, birds, frogs, fish, flies, mosquito and honey bee. Beyond these species, notably in the nematodes, no orthologous sequences could be found. The E1-E2 element was found both near core clock genes, and also in the vicinity or within genes involved in mediating clock output functions such as detoxification in the liver. We started extending this work along two main directions. The first consists in experimental validation of the found enhancer using both in vitro binding assays (EMSA) and luciferase reporter assays in NIH3T3 fibroblast cells. While we have made significant progress on the in vitro analyses, showing that two CLOCK/BMAL1 heterodimers bind the E1E2 element with high cooperativity, we also started making the luciferase reporter constructs to be tested in Lumicycle experiments. Secondly, we are extending the bioinformatics studies to include a much broader class of binding elements to explain phase specific circadian gene expression profile in liver (Figure 1). Preliminary analysis shows that our methodologies are able to recover most of the described active circadian transcription factors, while also finding a set of novel putative circadian regulators.

Modeling period length and precision of circadian oscillators. In collaboration with the Schibler Lab (U Geneva), we studied circadian oscillators under reduced transcription rates experimentally and theoretically. One of the key and counter-intuitive finding was that period length is shortened under reduced transcription, a property that was consistent with simulation studies of delayed feedback oscillators, such as Goodwin models. Oscillator precision, or the rate at which population of circadian oscillators desynchronize due to noise, is another key and physiologically relevant property of circadian clocks. To study this problem, we developed novel analytical and computational approaches to assess precision in common circadian oscillator models. The current is to identify molecular processes that consistently affect oscillator precision across a library of common circadian oscillator models, and eventually design perturbation experiments to validate these predictions.

Bioinformatics and signal processing of Ultra High Throughput Sequencing (UHTS) data. We are involved in several collaboration that use Solexa sequencing to study circadian transcriptional regula-
tion and other mechanisms. In this context we have developed a signal processing method to improve the base calling algorithms, hence allowing to recover a higher fraction of tags that can be mapped to the genome. This method will also be instrumental as part of our efforts to analyze chromatin immunoprecipitation data that we are currently generating in circadian time course experiments. Briefly, Solexa/Illumina short-read ultra-high throughput DNA sequencing technology produces millions of short tags (up to 36 bases) by parallel sequencing-by-synthesis of DNA colonies. The processing and statistical analysis of such high-throughput data poses new challenges; currently a fair proportion of the tags are routinely discarded due to an inability to match them to a reference sequence, thereby reducing the effective throughput of the technology. We proposed a novel base-calling algorithm, using model-based clustering and probability theory to identify ambiguous bases and code them with IUPAC symbols. We also select optimal sub-tags using a score based on information content to remove uncertain bases towards the ends of the reads. We show that the method improves genome coverage and number of usable tags as compared with Solexa’s data processing pipeline by an average of 15%. An R package (Rolexa) is provided which allows fast and accurate base calling of Solexa’s fluorescence intensity files and the production of informative diagnostic plots.

Selected Publications


Figure 1. Phase (x-axis) enrichment profiles of binding sites for transcription regulators in TRANSFAC (y-axis) are inferred from mRNA times series data in mouse liver. Most phases are represented (left). Clusters of regulators are separated according to peak time (phase). E.g. E-box motifs peak around ZT10, as expected from CLOCK/BMAL1 activity.
Introduction
Our group is interested in the molecular mechanisms controlling stem cell maintenance, lineage commitment and differentiation in self-renewing systems such as the hematopoietic system, the skin and the gut. The basic principle of self-renewing tissues is that they constantly produce cells from a stem cell reservoir that gives rise to proliferating transient amplifying cells, which subsequently differentiate and migrate to the correct compartment. These processes have to be under stringent control to ensure life-long homeostasis. In recent years, a substantial body of evidence has accumulated to support the notion that signaling pathways known to be important during embryonic development (such as e.g. Shh, Wnt and Notch) play important roles in regulating self-renewing tissues. Moreover, the same pathways are often deregulated during tumorigenesis due to mutations of key elements of these pathways. The general concept is that a better understanding of the mechanisms controlling stem maintenance versus differentiation may lead to the identification of novel therapeutic targets, as well as improving strategies for influencing these players during tumorigenesis. Currently, attention is being focused on the evolutionarily conserved Notch signaling pathway, which plays pleiotropic roles in different self-renewing tissues and cancer.

Keywords
Notch, stem and progenitor cells, self-renewing tissues, differentiation, cancer, genetic mouse models

Results Obtained in 2008
Notch signaling in T cell development and leukemia. Using conditional gene targeting strategies we have established an essential role for Notch1 in specifying the T cell lineage. More recently, we explored the ability of the Notch ligands Delta1 (DL1) and Delta4 (DL4) to induce T cell lineage commitment and/or maturation in vitro and in vivo from BM progenitors. Collectively our results establish a hierarchy of Notch:Delta interactions in which Notch1:DL4 exhibits the greatest capacity to induce and support T cell development suggesting that DL4 might be the physiological ligand within the thymus. This hypothesis was confirmed in vivo by generating mice in which DL4 could be specifically inactivated in thymic epithelial cells (TECs). Loss of DL4 in TECs led to a complete block in T cell development coupled with the ectopic appearance of immature B cells in the thymus. These immature B cells were phenotypically indistinguishable from those developing in the thymus of conditional Notch1 mutant mice. Collectively, our results demonstrate that DL4 is the essential and non-redundant Notch1 ligand responsible for T cell lineage commitment within the thymus (Figure1).

Another aspect of our work focuses on the role of Notch in T cell leukemia. aberrant Notch1 signaling within the hematopoietic system results in the development of acute lymphoblastic T cell leukemia in mice and humans. Thus Notch1 functions as oncogene in the hematopoietic system. We are currently investigating the molecular mechanisms by which uncontrolled Notch1 signaling exerts its oncogenic functions.

Notch signaling in corneal repair and skin
In contrast to the previously established role of Notch1 as an oncogene and lineage specifier in the hematopoietic system, we unexpectedly identified novel roles for Notch1 in the cornea and the skin. Inducible ablation of Notch1 in the skin results in the development of acute lymphoblastic T cell leukemia in mice and humans. Thus Notch1 functions as oncogene in the hematopoietic system. We are currently investigating the molecular mechanisms by which uncontrolled Notch1 signaling exerts its oncogenic functions.

Notch1: lineage specifier, oncogene, tumor suppressor and progenitor gate-keeper
Notch signaling in T cell development and leukemia. Using conditional gene targeting strategies we have established an essential role for Notch1 in specifying the T cell lineage. More recently, we explored the ability of the Notch ligands Delta1 (DL1) and Delta4 (DL4) to induce T cell lineage commitment and/or maturation in vitro and in vivo from BM progenitors. Collectively our results establish a hierarchy of Notch:Delta interactions in which Notch1:DL4 exhibits the greatest capacity to induce and support
Moreover we established a tumor suppressor function of Notch1 in the skin. We are currently investigating the roles of additional Notch receptor and ligand family members within the epidermis and skin appendages under normal and cancer promoting situations.

**Notch signaling is essential for the maintenance of intestinal progenitor cells**

Aberrant Wnt signaling in the intestine is one of the most frequent events during the development of colorectal cancer. The inhibitory interaction between Notch and Wnt signaling in our skin studies prompted us to investigate Notch function in the gastrointestinal tract. If Notch signaling plays a similar role in the gut as in the skin, then mice in which Notch signaling is inhibited are expected to develop tumors as a consequence of increased β-catenin mediated Wnt signaling. Surprisingly, conditional inactivation of the CSL (which mediates Notch signaling of all receptors) within the crypt compartment results in the complete loss of transient amplifying cells followed by their conversion into mucus secreting goblet cells. Thus in the intestine Notch functions as progenitor gate-keeper, and seems to cooperate with Wnt signaling in order to maintain the undifferentiated proliferative crypt compartment.

More recently we assessed the crucial role of individual Notch receptors and the mechanism by which they maintain intestinal crypt progenitor cells by using a series of inducible gut-specific Notch mutant mice. We found that Notch1 and Notch2 receptors function redundantly in the gut, as only simultaneous loss of both receptors results in complete conversion of proliferating crypt progenitors into post-mitotic goblet cells. This conversion correlates with the loss of Hes1 expression and derepression of the cyclin dependent kinase (CDK) inhibitors p27Kip1 and p57Kip2. We also found that the Notch effector Hes1 in wild-type crypt progenitor cells occupies the promoter of both CDK inhibitor genes. Thus, our results indicate that Notch-mediated Hes1 expression contributes to the maintenance of the proliferative crypt compartment of the small intestine by transcriptionally repressing two CDK inhibitors.

**Selected Publications**


Benz, C., Martins VC., Radtke, F., and Bleul, C. The stream of precursors that colonizes the thymus proceeds selectively through the early T lineage precursor stage of T cell development. (2008) *J. Exp. Med.*; May 12, 1187-1199


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**Figure 1. Cross talk between thymic epithelia cells expressing Delta 4 and uncommitted Notch1 expressing bone marrow progenitors is essential to specify T cells in the thymus.** A. EM picture from the thymus showing the close interaction between immature thymocytes and thymic epithelial cells (Picture: Willem van Ewjik). B. LacZ staining on thymic sections derived from Delta 4 lacZ knock-in mice. The Delta 4 driven lacZ expression is preferentially confined to the cortex of the thymus (blue staining). C. Schematic illustration of T cell fate specification. Delta 4 mediated Notch1 signaling is essential for the generation of T cells. In absence of Delta-like 4 incoming BM progenitors adopt a B cell fate by default. Fluorometric analysis of thymocytes derived from wild type (upper graph) and mice in which Delta-like 4 was specifically inactivated within thymic epithelial cells (lower graph). Total thymocytes were either stained with anti-CD4 and anti-CD8 antibodies (specific for T cells) or with anti-B220 and anti-IgM antibodies (specific for B cells).
Introduction
New cells arise by division of a mother cell to produce two daughters. The events of the cell division cycle must be executed with high temporal and spatial fidelity: errors during cell division contribute to the onset of many diseases, notably cancer. Our work uses a simple model system to study how the events of the cell cycle are regulated and coordinated. The goal of our studies is to understand how mitosis and cytokinesis are coordinated to assure faithful transmission of the genome from a cell to its daughters.

Keywords
Cell cycle, cytokinesis, signal transduction, Schizosaccharomyces pombe, yeast.

Results Obtained in 2008
We use the well-established, genetically-tractable fission yeast model system. Cytokinesis in S. pombe begins at mitotic entry, when the site of division is defined by formation of the acto-myosin ring (CAR) at the cell cortex. Contraction of the CAR and formation of the division septum is triggered at the end of mitosis by the spindle pole body (SPB) associated septation initiation network (SIN) proteins. Loss of SIN signalling results in failure to undergo cytokinesis and the generation of multi-nucleate cells. In contrast, ectopic activation of the SIN can trigger formation of the contractile ring, followed by cytokinesis, at any stage of the cell cycle. Thus, regulation of the SIN is crucial for proper coordination of cytokinesis with other cell cycle events. Three aspects of our work are discussed briefly below.

The creation and analysis of an analogue-sensitive allele of the master cell cycle regulator cdc2p.
The protein kinase cdc2p is the master regulator of cell cycle progression in the fission yeast Schizosaccharomyces pombe. It is required both for entry into mitosis and onset of DNA replication. Cdc2p must be inactivated to permit exit from mitosis and cytokinesis. To study the role of cdc2p in greater detail, we created a cdc2 allele sensitive to an inhibitory ATP analogue. We examined the effect of inhibiting cdc2p on the regulation of the SIN. We found that inactivation of cdc2p in a mitotically arrested cell promoted both the asymmetric recruitment of SIN proteins to the spindle poles and the recruitment of the most downstream SIN components and beta-(1,3) glucan synthase to the contractile ring. Thus, we conclude that inactivation of cdc2p is sufficient to activate the SIN and promote cytokinesis. (See Dischinger et al.)

This work was carried out in collaboration with Professor Paulson and Professor Xie of the University of Wisconsin at Oshkosh, USA.

A novel role for the SIN in assembly of the contractile ring
Numerous previous studies had shown that components of the contractile ring (CAR) associated with the medial region of the cell in SIN mutants, leading to the dogma that the SIN was not required for CAR assembly, but only for its maintenance in checkpoint arrested cells, and its contraction at the end of mitosis. However, this created a paradox, as it had previously been shown that ectopic activation of the SIN
in interphase-arrested cells could trigger cytokinesis. We therefore examined assembly of the CAR in SIN mutants, filming cells that had GFP-tagged CAR components. We found that though CAR components assembled in the medial region upon entry into mitosis, they failed to condense into a homogeneous structure. We also showed that ectopic activation of the SIN in interphase cells bypasses the spatial cue provided by mid1p. Genetic analysis further demonstrated that no ring assembly was observed in cells lacking both mid1p and SIN function, thereby suggesting that the SIN and the mid1p cooperate to mediate CAR assembly. Furthermore, we found that recruitment of the CAR component cdc15p, which previous studies have shown is a key element in CAR assembly, is compromised in SIN mutants. In summary, these studies uncovered a novel role for the SIN early in mitosis, which may be effected in the cytoplasm, rather than at the SPB. (see Hachet and Simanis)

**Selected Publications**


Introduction
Pigment cells are responsible for hair graying, albinism, vitiligo, and melanoma. We use these cells to study gene regulation, development and interplay between stem and differentiating cells. The transgenic facility provides support for producing genetically modified mice for basic research.

Keywords
Melanocyte, melanoma, pigmentation, transgenic, knockout, mouse

Results Obtained in 2008
Melanocyte development
Melanocytes differentiate from pluripotent neural crest cells in early embryogenesis in the mouse, migrate along the dorsolateral pathway and subsequently proliferate through the dermis horizontally to the ventral region. By mid-gestation, melanocytes exit from the dermis and invade into the epidermis to finally be located in the skin and the hair follicles. Many genes are implicated in specific aspects of melanocyte/melanoblast differentiation and more than 280 loci are currently known to affect pigmentation in the mouse.

Hair graying is a natural process which occurs with aging. This change is caused by the gradual decrease of pigmentation that occurs when melanin ceases to be produced in the hair root, for example due to a loss of melanocytes. Among the large variety of genetic loci in the mouse which are implicated in coat color, some as Mitf or Bcl2 encode for genes involved in maintenance of melanocytes and melanocyte stem cells. We have addressed the role of the Notch signaling pathway as well as of the c-Myc oncogene in melanocyte development and homeostasis, and have demonstrated that both genes / pathways bring about precocious hair graying if affected. Disruption of the Notch pathway by inactivating Notch1 and Notch2, or RbpJk in the melanocyte lineage resulted in a dosage-dependent precocious

Transgenic service
The introduction of genetic material into the germ line of mammals is one of the major experimental achievements developed in the last three decades, and genetically-engineered mice represent a powerful tool in today’s biomedical research. The transgenic facility at the EPFL has performed micro-injection of DNA constructs into the pronucleus which were either derived from plasmid vectors or injected as supercoiled BAC DNA. The service also offers expertise and discussion regarding vector design and analysis of mice. Furthermore, the facility was and is involved in the rederivation of mouse lines which are received from outside.
Selected Publications


Introduction
Computational cancer genomics

Genome sequencing projects and new high-throughput technologies are producing large amounts of biological data that are potentially relevant to cancer. Our group contributes to the analysis of these data by developing new methods and database resources related to genome structure and gene regulation. In addition, we use our methods to carry out question-driven bio-informatics research focusing on transcriptional control mechanisms distorted in cancer cells.

Keywords
Computational genomics, sequence analysis methods, transcriptional regulation, ChIP-Seq data analysis

Results Obtained in 2008
Computers have become indispensable tools for accessing, visualizing, analyzing, managing, and publishing biological data. Over the last decade, high-throughput sequencing and functional genomics projects have generated unprecedented quantities of biological data which can neither manually be processed, nor comprehensively inspected by eye. The emergence of bioinformatics as a research field in its own right is a response to this development.

Cancer can be considered a gene regulatory disease. Normal regulation of genes permits the development and maintenance of a healthy human being. Abnormal regulation leads to various diseases. Cancer cells are maintained in a specific pathological state by gene regulatory circuits. Transcription factors are key elements of such circuits in that they control the expression of other genes, while themselves are being regulated by the products of genes. Our research aims at an understanding of transcriptional regulatory mechanisms, in particular those which are affected by genetic lesions that cause cancer.

Characterization of transcription factor binding sites
Transcriptional control mechanisms in higher organisms are poorly understood. We are, for instance, still not able to predict the effects of genetic variations and somatic mutations in gene regulatory regions. This is particularly regrettable because presently, we have the technology to detect such mutations on a genome-wide scale. A major bottleneck in gene regulatory bioinformatics is sequence-based prediction of transcription factor binding sites. Over the years, our group has devised several bioinformatics-inspired strategies to produce accurate binding site models for transcription factor binding sites, including the SAGE/SELEX technique developed in collaboration with Nicolas Mermod at the University of Lausanne. Responding to recent technology developments, we started to develop computational methods for the interpretation of protein binding micro-array (PBM) data. In parallel, we adapted computational protocols for analyzing in vitro SAGE/SELEX sequences to make them applicable to in vivo binding sequences defined by ChIP-Seq experiments.

Analysis of breast cancer gene expression data sets
Gene expression profiling has become a standard technique for molecular characterization of cancer tissues. By comparing large numbers of expression profiles together with corresponding clinical data, one hopes to improve tumor classification, which in turn could help choosing the optimal treatment for a patient. However, the relatively small number of samples from a single study has so far limited the success of this approach. One could possibly overcome this limitation by merging data from different studies. Our group has considerable experience with merging gene expression data thanks to the development of the CleanEx database, an early integration effort in this field. We recently carried out a systematic evaluation of various data integration methods using breast cancer survival prediction as a test problem to measure the benefits from merging. This systematic study serendipitously led to the
discovery of a new survival gene, CYB5D1, which encodes a member of the Cytochrome b5 family.

**Analysis of chromatin immunoprecipitation data**

The recently introduced genome-wide chromatin immunoprecipitation assays (ChIP-chip and ChIP-seq) provide a detailed view of the chromatin conformation of a whole genome, opening new perspectives for studying gene regulation. For instance, the comparison of histone modification profiles from different cell types constitutes a new way of analyzing differential gene activity, centering on the activity status of gene regulatory regions rather than mRNA abundance (Fig 1). Over the last year we have thus developed a basic set of tools for analyzing ChIP-Seq data, including a peak-finding and a segmentation algorithm.

**Collaborative projects with experimentalists**

With Nicolas Mermod (University of Lausanne) we have analyzed ChIP-Seq data for transcription factor CTF/NFI in mouse embryonic fibroblasts. As a major result, we demonstrated that the binding specificity of this protein is identical in vitro and in vivo. With Walter Reith (University of Geneva) we characterized the complete set of target loci of the regulatory protein CIITA using ChIP-chip data (Krawczyk et al. 2008). With Didier Trono (EPFL) we started to analyze a large collection of retroviral integration sites generated with a gene trap system. The goal of this study is to identify genomic features upstream of the integration site, which favor or prevent KAP1-mediated silencing.

**Development of Bioinformatics Resources**

Our group has developed a number of public databases and software resources over the last two decades including the Eukaryotic Promoter Database EPD (Schmid et al. 2007), CleanEx, the HTPSELEX database (Jagannathan et al. 2007) and the Signal Search Analysis (SSA) software for DNA motif discovery and characterization. During the year 2008, we added the ChIP-Seq tools to our collection of web-accessible bioinformatics resources (ccg.vital-it.ch/chipseq).

**Selected Publications**


![Figure 1. Chromatin conformation of gene control regions in different cell types.](image)

This figure is based on public ChIP-Seq data from (Mikkelsen et al., Nature 448, 553-560, 2007).
Introduction
The goal of my laboratory is to understand the genetic of vision, from the development of the eye until late in life, in order to develop better diagnostic tools and new therapies.

Keywords:
Gene identification, cell signalling, animal models, mouse, zebrafish, vision, eye, development, gene therapy, degenerative disorders, cataract, retinitis pigmentosa, glaucoma, age-related macular degeneration

Results obtained in 2008
In order to understand the genetic of vision, we use various approaches to identify new genes and to analyze their role in the development or the death of ocular cells. Eye development follows a complex program during which activating and inhibiting signals cohabit. For example, an excess of retinal cells is produced before apoptosis is turned on in order to fine tune the final number of photoreceptors. This process is under the control of many genes and various intra- and intercellular pathways. We have shown that a similar program involving Bcl-2 related proteins is turned on in one of the mouse models of Leber’s congenital amaurosis (Cottet et al. 2008). Our goal is now to understand all the details of this program in order to develop new therapeutic approaches by modulating these pathways using small cell-penetrating peptides or full proteins.

Human syndromes with eye malformation are observed in clinical settings. They may be due to spontaneous or inherited mutations in genes expressed in the eye. We have collected the DNA from members of numerous families with blindness disorders and have embarked on identifying all these genes. This is done by linkage analysis using SNP microarray and large-scale sequencing. Recently, we identified LAMP2 and PROM1 as responsible for a specific blindness disorder (Schorderet et al., 2007; Yang et al., 2008) and Nkx5-3 as the gene responsible for the human oculoauricular syndrome (MIM# 612109) (Schorderet et al., 2008, Nichini et al., 2008). We are now continuing this work in assessing the role of the latter gene on the development of the eye, brain and bones of the face by overexpressing or inhibiting its expression in the zebrafish, an animal model particular suitable for developmental studies.

For many blinding diseases, accession to affected tissues is crucial for the characterization of the physiopathology involved. In blinding diseases, availability of human retinas is poor and it is important to develop animal models. In my lab, we are using mouse and zebrafish for the study of eye development (Boisset et al., 2008; Bustamante et al., 2008, Escher et al., 2008). Zebrafish have a number of advantages over other animal models, including external fertilization and development, transparency of the eggs and easy access to them. Zebrafish eggs can be used to many studies, from investigating the role of specific genes to mass screening of active compounds.

Selected publications


Zebrafish overexpressing Nkx5-3: the development of the eye is perturbed. Depending on the level of Nkx5-3, microphthalmia, severe microphthalmia or anophthalmia can occur.
External Adjunct Professor

Marcel Tanner

http://www.sti.ch

Research Keywords
Epidemiology, public health, vaccines and drug resistance

STI and the EPFL School of Life Sciences are currently developing joint research projects on malaria and mycobacterial infections. In the field of malaria, an initial focus will be on the analysis of protein-protein interactions to elucidate complex functions and host-pathogen interactions. As for mycobacterial infections, first steps to investigate Mycobacterium ulcerans infection (Buruli ulcer) will be taken.

In addition, the STI and the GHI plan to establish a STI satellite laboratory at EPFL to bring together complementary expertise in research on infectious diseases and the development of new diagnostics, drugs and vaccines.
Welcome To Our New Research Groups!

ISREC - Swiss Institute For Experimental Cancer Research

Douglas Hanahan
Director of ISREC
Full Professor
Merck Serono Chair for Oncology

Former Home Institution
University of California San Francisco, USA

EPFL School of Life Sciences since July 2009

Research Keywords
Pathways of multistep tumorigenesis, genetically engineered mouse models of human cancer, mechanisms of angiogenesis, invasion, and metastasis, experimental cancer therapeutics

IBI - Institute of Bioengineering/Co-Affiliated Faculty from the School Of Engineering

Carlotta Guiducci
Tenure Track Assistant Professor,
Chair Swiss-Up in Engineering

Former Home Institution
University of Bologna, Italy

EPFL School of Life Sciences since February 2009

Research Keywords
Biosensors, integrated systems, miniaturized detection of biomolecules & interaction with living matter

Jose del Rocio Millan
Tenure Track Associate Professor,
Deftech Foundation Chair in Non-Invasive Brain-Machine Interface

Former Home Institution
Research Center of the European Commission, Ispra
Idiap Research Institute, Martigny, Switzerland
Visiting Scholar, Universities Stanford and Berkeley

EPFL School of Life Sciences since October 2008

Research Keywords
Statistical machine learning, brain-computer interfaces, adaptive robotics, human-robot interaction, neuroscience

GHI - Global Health Institute

Melanie Blokesch
Tenure Track Assistant Professor

Former Home Institution
Stanford University, California, USA

EPFL School of Life Sciences since May 2009

Research Keywords
Evolution of pathogens, horizontal gene transfer, environmental reservoirs, bacterial signal transduction, metabolic pathways, single cell expression analysis

BMI - Brain Mind Institute

Darren Moore
Tenure Track Assistant Professor

Former Home Institution
John Hopkins University School of Medicine, USA

EPFL School of Life Sciences since December 2008

Research Keywords
Parkinson’s disease, neuro-degeneration, transgenic, genetics, Parkinsonism, mouse model, cell biology, mutation, pathology
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