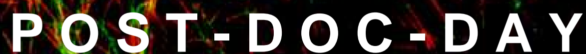

May 21, 2015
Schloss Au, Wädenswil

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y o u r
r e s e a r c h

L I F E S C I E N C E
U N I V E R S I T Y of Z U R I C H
S w i t z e r l a n d

Post-Doc-Day organizing committee:

Ulrike Rieder
Juan Miguel Escobar Restrepo
Richard Börner

Post-Doc-Day supporting Professors:

Nathan W. Luedtke, *Department of Chemistry*
Alex Hajnal, *Institute of Molecular Life Sciences*
Roland K.O. Sigel, *Department of Chemistry*

Cover: "Network"
Confocal microscopy image of fluorescently labeled DNA fibers. Courtesy of Ulrike Rieder

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Sessions

Opening Remarks
Ulrike Rieder & Prof. Bernhard Schmid

Bioengineering & Synthetic Biology
Chair: Juan Miguel Escobar Restrepo & Rashel V. Grindberg

Chemistry towards Biology
Chair: Ulrike Rieder & Elizabeth Cline

Signaling & Epigenetics
Chair: Juan Miguel Escobar Restrepo & Bisrat Tewhibe Woldemichael

Physics towards Biology
Chair: Richard Börner & Tommaso Fedele

Closing Remarks & Awards
Richard Börner & Prof. Roger Alberto

Dear Scientists!

It is a pleasure for us to welcome you to the 1st Post-Doc-Day in Life Sciences especially created for Postdoctoral Researchers.

The objective of this event is to encourage and to enable the interaction between different life science research communities and thus to promote academic research among postdoctoral scientists.

Within the Post-Doc-Day you will have the opportunity to enjoy about 60 scientific contributions as talks, flash poster or poster presentations. This allows you to actively spread your research in order to increase your research visibility and to establish collaborative research with other scientists.

We hope that you will broaden your scientific view and gain awareness of different disciplines. Take the chance to learn something new and to network. Especially, you should enjoy yourself and have a good time with your colleagues.

....explore new ideas, network, discuss and grow closer together...

Your Post-Doc-Day organizing committee
Ulli, Juan and Richard

08.00	Registration - Welcome Coffee - Mounting of Posters
09:00	*Opening:* Prof. Dr. Bernhard Schmid - Dean MNF
	1st session - Bioengineering & Synthetic Biology
09:10	***Plenary Lecture:*** **Dr. Andrea Degen** - EUrelations AG
09:45	Philipp R. Spycher - Radiopharmaceutical Science, PSI Villigen
10:00	Christoph Klenk - Biochemistry, UZH
10:15	Micheal C. Wilson - Microbiology, ETHZ
10:30	Coffee Break
	2nd session - Chemistry towards Biology
10:50	***Plenary Lecture:*** **Prof. Dario Neri** - Biomacromolecules, ETHZ
11:25	Alicia Dominguez-Martin - Chemistry, UZH
11:40	Danny Kowerko - Chemistry, UZH
11:55	Wittlin, Winterberger, Fuhrmann - VAUZ, UZH
12:10	Lunch Break
	3rd session - Signaling & Epigenetics
13:30	***Plenary Lecture:*** **Prof. Alex Hajnal** - Molecular Life Sciences, UZH
14:05	Lena Harder - Pediatric Oncology, KISPI-UZH
14:20	Lorenzo Borghi - Plant Biology, UZH
14:35	Victoria Green - Molecular Life Sciences, UZH
14:50	Flash Poster Presentations
15:10	Coffee Break and Poster Session
	4th session - Physics towards Biology
16:40	***Plenary Lecture:*** **Dr. Jonas Ries** - Cellular Nanoscopy, EMBL
17:15	Julien Boudet - Molecular Biology and Biophysics, ETHZ
17:30	Hidayet Günhan Akarcay - Applied Physics, UniBe
17:45	Jean-Nicolas Longchamp - Physics, UZH
18:00	Closing Remarks and Award Ceremony
18:10	Get Together Apéro

Schloss Au

The Post-Doc-Day 2015 takes place at Schloss Au, Wädenswil. The castle is a conference center for education, culture, and meetings located on an idyllic penisula at lake Zurich.

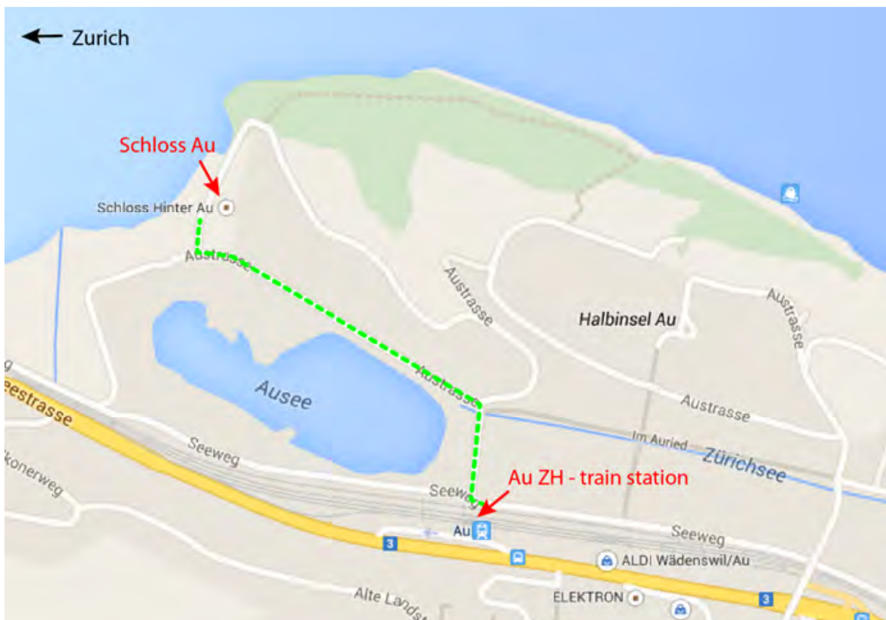
Travel to Schloss Au

The train S8 goes every 30 minutes from Zurich/Oerlikon or Pfäffikon / SZ to Au ZH. A short walk of approximately 10 minutes leads you to the conference center. During the summer months also ships stop at the ship-station penisula

Schedule:

from Zürich HB	07.38	from Au ZH	18.24 / 19.24
to Au ZH	08.10	to Zürich HB	18.53 / 19.53

Address:
Tagungszentrum Schloss Au
Hinter Au 1
8804 Au ZH



Funding for Science:
A permanent challenge

Dr. Andrea Degen

EUrelations AG, Switzerland

Learn all about EUrelations, funding opportunities and career advice services!

Andrea Degen is a medical doctor by professional training and gained experience as a Clinical Researcher, National Programme Manager, Project Manager and Business Developer for basic science projects all the way down towards application. She founded companies of which the Research Management Agency www.EUrelations.com is implementing up to the best her vision of a world wide support network for science. She is a dedicated team motivator and trainer especially for young scientists in Fundraising and Project Management to enable more effective and output oriented collaboration in research.

Targeting tumors with armed antibodies and small molecule drug conjugates: from the bench to the clinic

Prof. Dario Neri

Department of Chemistry and Applied Biosciences, ETHZ

Hear about Dario Neri's personal experience and about what can be learned from the literature. He will provide information for PostDocs who wish to be active in the pharmaceutical research field.

Dario Neri is a co-founder of Philogen (Swiss-Italian Biotech company), and Full Professor of Biomacromolecules at the Department of Chemistry and Applied Biosciences (ETHZ, Switzerland). Dario Neri studied Chemistry at the Scuola Normale Superiore of Pisa graduating in 1987. In 1992, he earned his doctorate in chemistry under Prof. Kurt Wüthrich at the Institute of Molecular Biology and Biophysics (ETHZ). His dissertation was awarded the ETH Zurich silver medal. With an EU Bridge Bursary, he spent four years at the Cambridge Centre for Protein Engineering, Medical Research Council, where he worked under the supervision of Sir Gregory Winter.

Systems genetics of the RAS/MAPK pathway in C. elegans: an unexpected function of monoamine oxidases

Prof. Alex Hajnal

Institute for Molecular Life Science, UZH

Professor Hajnal uses the *C. elegans* model organism as a gene discovery platform for cancer regulatory networks.

Alex Hajnal studied Biochemistry and Molecular Biology at the University of Zurich and completed his PhD studies at the division of Cancer Research (UZH). Afterwards, he spent four years as a Postdoctoral Fellow at the Department of Molecular Pharmacology and at the Department of Developmental Biology, Stanford University, USA. In 1997 he joined the Division of Cancer Research (UZH) as a Junior group leader and since 2007 he is Professor at the Institute of Molecular Life Sciences (UZH).

Superresolution microscopy of protein structures *in situ*

Dr. Jonas Ries

Cell Biology and Biophysics, EMBL Heidelberg

Jonas Ries is developing new methods for superresolution microscopy and applying them to biological questions.

Jonas Ries studied physics in Bremen and Konstanz and completed his PhD in Biophysics at the TU Dresden in the group of Petra Schwille, where he developed methods to measure dynamics in artificial and cellular membranes. As a Postdoctoral Fellow at the ETH Zurich in the group of Vahid Sandoghdar he established single-molecule localization based superresolution microscopy and developed an efficient and simple labeling scheme for this method. In 2012 he joined the EMBL in Heidelberg as a group leader.

T-1	Philipp Spycher	**P-9**	Elena Chiavacci
T-2	Christoph Klenk	**P-10**	Artur Yakimovich
T-3	Micheal C. Wilson	**P-11**	Sebastian Streb
T-4	Alicia Dominguez-Martin	**P-12**	Tommaso Fedele
T-5	Danny Kowerko	**P-13**	Nikolas Friedrich
T-6	Wittlin, Winterberger, Fuhrmann	**P-14**	Charlotte Soneson
T-7	Lena Harder	**P-15**	Harsha Kocherla
T-8	Lorenzo Borghi	**P-16**	Elizabeth Cline
T-9	Victoria Green	**P-17**	Nathalie Selevsek
T-10	Julien Boudet	**P-18**	Hidayet Günhan Akarcay
T-11	Hidayet Günhan Akarcay	**P-19**	Johann Wanek
T-12	Jean-Nicolas Longchamp	**P-20**	Silke Johannsen
FP-1	Julia Svozil	**P-21**	Elena Osto
FP-2	Alexa Burger	**P-22**	Melroy Miranda
FP-3	Arina Rybina	**P-23**	Helen Lindsay
FP-4	Kelsey Byers	**P-24**	László Demkó
FP-5	Yaroslav Sych	**P-25**	Felix Scholkmann
FP-6	Carsten Magnus	**P-26**	Jitin Bali
FP-7	Evelyn Lattmann	**P-27**	Margot Crucet
FP-9	Cornelia Eisenach	**P-28**	Konstantis Konidaris
FP-10	Asya Makhro	**P-29**	Saho Kobayashi
P-1	Rolf Baumberger	**P-30**	Anna Bogdanova
P-2	Stephanie Heinrich	**P-31**	Museer Ahmad Lone
P-3	Maria Hondele	**P-32**	Bithi Chatterjee
P-4	Athena Hoi Yee Chu	**P-33**	Anahita Rafiei
P-5	Rashel V. Grindberg	**P-34**	Daniel Hain
P-6	Steven Waldauer	**P-35**	Pablo Emiliano Tomatis
P-7	Kinga Rutowicz	**P-36**	Rosa Chiara Paolicelli
P-8	Bisrat Tewhibe Woldemichael		

Poster session Even Numbers: 15:10 - 15:55
Poster session Odd Numbers: 15:55 - 16:40

T-1

Controlled functional modifications of proteins using microbial transglutaminase

Philipp R. Spycher[a], Patrick Dennler[a], Laura K. Bailey[a], Eliane Fischer[a], Roger Schibli[a,b]

[a]*Center for Radiopharmaceutical Sciences ETH-PSI-USZ, OIPA 10A, Villigen PSI, Switzerland*
[b]*Institute of Pharmaceutical Sciences, ETH Zurich, Zurich Switzerland*
Email: philipp.spycher@psi.ch

Bioconjugation represents an important key tool in biotechnology and greatly advanced our understanding in many fundamental biological processes but also filled clinical pipelines with novel protein-conjugates for therapeutic applications. Sophisticated methods have thus been elaborated to conjugate the desired molecule to the protein of interest.[1] However, site-specific incorporation under physiological conditions, in a timely and reproducible manner yielding homogeneous conjugates still remains a major challenge. We have addressed this challenge and developed a method with which proteins can efficiently be functionalized under physiological conditions using the enzyme microbial transglutaminase (mTGase) yielding homogeneous constructs with exactly one functional moiety attached, e.g. a fluorescent dye suitable for fluorescence microscopy or a chelator applicable for SPECT-CT imaging.[2] The developed method does not require major genetic engineering of the target protein but solely needs a short terminal peptide tag (c-myc-tag) introduced for the mTGase, which, however, in many recombinant expression vectors is already conveniently pre-integrated serving to detect the expressed protein. Using this technology, we demonstrate that different therapeutically relevant protein scaffolds can be very efficiently functionalized yielding homogeneous constructs which then were applied to image organisms ranging from whole animals down to the single cell level. Furthermore, we immobilized these proteins in a controlled manner on solid as well as on artificial cell membrane surfaces both of which are relevant for biosensor applications. We believe that the developed technology opens up new possibilities to study protein behavior and dynamics from the single molecule (e.g. protein clustering) up to the whole organism level and to ultimately generate exciting insightful knowledge which will be directly applicable to elaborate novel therapeutic approaches.

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[2] P. Dennler, L. K. Bailey, P. R. Spycher, R. Schibli and E. Fischer, *ChemBioChem*, **2015** (accepted).

T-2

Directed Evolution of GPCRs for Improved Expression and Stability

Christoph Klenk[a], Janosch Ehrenmann[a], Marco Schütz[a], Andreas Plückthun[a]

[a]*Department of Biochemistry, University of Zurich, Zurich, Switzerland*
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Most G protein-coupled receptors (GPCRs) are difficult to express and exhibit low protein stability after solubilization. Thus, GPCRs remain one of the most challenging class of proteins for structural and biophysical studies in order to explore their molecular functions. To overcome these limitations, we have recently developed several methods for improving functional expression and simultaneous thermo-stabilization of GPCRs by directed evolution [1-5]. Using periplasmic expression of randomized receptors genes in *E. coli* and subsequent selection of highly expressing variants with fluorescent ligands and flow cytometry, key residues within a receptor sequence can be rapidly identified that are responsible for improved biophysical properties without greatly affecting the pharmacological features of the receptor. However, so far this technology was limited by the availability of small and specific fluorescent ligands for each receptor to be evolved. Here we present a novel system to evolve GPCRs for improved expression and stability without the need for specific fluorescent ligands. We have engineered a fluorescence-activating module based on Designed Ankyrin Repeat Proteins which specifically bind and activate a small membrane-impermeable fluorogen. When fused to the N-terminal domain of a GPCR, these modules are targeted to the periplasmic space upon correct integration of the receptor into the inner cell membrane. Selective permeabilization of the outer cell membrane and incorporation of the dye into the periplasmic space allows to directly measure functional receptor expression in *E. coli* by flow cytometry. Combining this technology with our directed evolution approach we were able to evolve variants of the rat neurotensin receptor 1 from highly diverse libraries without a specific ligand. With this generic approach, receptors with poor expression properties for which no or only ligands with poor pharmacokinetic properties are available can now readily be evolved for increased expression and protein stability.

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[2] I. Dodevski, A. Plückthun, *J. Mol. Biol.*, **2011,** *408,* 4
[3] K. M. Schlinkmann, M. Hillenbrand, et al., *J. Mol. Biol.*, **2012**, *422,* 3
[4] K. M. Schlinkmann, A. Honegger, et al., *Proc. Natl. Acad. Sci.*, **2012**, *109,* 25
[5] D. J. Scott, A. Plückthun, *J. Mol. Biol.*, **2013**, *425*, 3.

T-3

Natural Product Biosynthesis from Microbial Dark Matter

Micheal C. Wilson[a], Simon Burgener[a], Toshiyuki Wakimoto[b], Jörn Piel[a]

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[b]*Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan*
Email: wilsonm@ethz.ch

More than half of all bacterial phyla do not contain a single cultured representative and are functionally unknown. Metagenonic and single-cell genomic studies have shown that this microbial dark matter is an almost inexhaustible source of novel biochemical and enzymatic princples.1, 2 Marine sponges are a rich source of biologically active natural products and many harbor a vast diversity of microbial symbionts. Here we present metagenomic and single-cell genomic evidence that uncultivated sponge symbionts from a new candidate phylum have the capacity to produce numerous peptide and polyketide natural products. Many of the pathways discovered can be attributed to nearly all of the previously known sponge-derived metabolites from the sponge specimens investigated, revealing that the biosynthetic potential of these "Tectomicrobia" rivals some of the most prolific cultured bacterial producers.1 With these data in hand, we can now target these uncultivated natural product factories for novel drug candidates and biocatalysts.

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[2] C. Rinke, P. Schwientek, A. Sczyrba, N. N. Ivanova, I. J. Anderson, J. F. Cheng, A. Darling, S. Malfatti, B. K. Swan, E. A. Gies, J. A. Dodsworth, B. P. Hedlund, G. Tsiamis, S. M. Sievert, W. T. Liu, J. A. Eisen, S. J. Hallam, N. C. Kyrpides, R. Stepanauskas, E. M. Rubin, P. Hugenholtz and T. Woyke, *Nature*, **2013**, *499*, 431-437.

T-4

Dynamics and stability of the biologically relevant BCL2 RNA G-quadruplex structure

Alicia Dominguez-Martin[a] and Roland K.O. Sigel[a]

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Guanine quadruplexes (G4) are non-canonical secondary structures present in G-rich DNA and RNA sequences. In the presence of metal ions, these sequences fold into stable tetra-helical structures formed by the coplanar cyclic arrangement of four Hoogsteen-paired guanines. The resulting G-tetrads stack on top to each other building the final G4 structure [1]. The existence of a G4-forming sequence at the 5'-untranslated region (UTR) of the BCL2 mRNA, with the ability to module protein expression, has been reported [2]. The BCL2 proto-oncogene (B-cell Lymphoma/leukemia 2) encodes for a family of proteins involved in the apoptosis regulation via the mitochondria pathway [3]. Thus, the understanding of this biologically relevant RNA G4 sequence is of high interest for the anticancer therapy.
In the context of the native 5'-UTR, we synthesized and investigated the folding of the 22-mer BCL2 RNA G4-forming sequence (5'- $G_3CCGUG_4UG_3AGCUG_3$- 3') in the presence of potassium(I) ions. Our results from UV/Vis, CD and EMSA reveal the stabilization of mainly one G4 structure, defined as unimolecular, intramolecular and parallel-stranded. 1D and 2D [^{1}H,^{1}H]-NMR spectra confirmed the presence of two different but non-equally populated species in solution. Currently, mutational studies are being carried out to isolate one single G4 conformation in solution while further NMR investigations are devoted to the elucidation of the solution structure of this BCL2 G4 sequence.

Acknowledgments: Financial support by the Fundacion Ramon Areces and Forschungskredit (A.D.M.), the Swiss National Science Foundation (R.K.O.S.), the COST Action CM1105 (Swiss State Secr. Ed. Red. Innov. to R.K.O.S) and by the University of Zurich is gratefully acknowledged.

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T-5

A new view on structure formation in nucleic acids revealed by Single Molecule Spectroscopy (SMS) in combination with NMR (Nuclear Magnet Resonance)

Danny Kowerko[a], Sebastian König[a], Roland Sigel[a]

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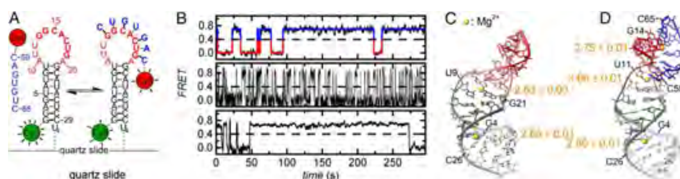
Single molecule fluorescence microscopy is one of the exciting techniques to study molecules and their interactions one molecule at a time. An intriguing phenomenon observed in a variety of DNA and RNA folding and bimolecular interactions is the kinetic heterogeneity averaged out in ensemble experiments. The physical or chemical mechanisms behind this are under debate for more than a decade. In this work, RNA interaction kinetics were studied by SMS, complementary to structural studies of the same RNA constructs by NMR. A potential working model was derived unambiguously linking heterogeneity phenomena to metal ion effects[1]. The role of specific Ca^{2+} and Mg^{2+} binding sites and their incomplete occupation in certain concentrations ranges are discussed as source causing heterogeneity and rugged free energy landscapes in RNA.
Further insights are given in the extension of this concept to 10 other metal ions of the so-called Irving-Williams series[2]. Methodical limits of SMS to study intra- and intermolecular kinetics at the single molecule level are discussed along with work in progress to overcome these limitations using nanostructured samples, so-called zero mode waveguides within a high-end and high-throughput DNA sequencer based on single molecule fluorescence.



Acknowledgments: This work was supported by the Forschungskredit of the University of Zurich (UZH).

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[2] S. L. B. König, D. Kowerko, M. Khier, R. K. O. Sigel, under review.

T-6

VAUZ - Academic Self-Administration - What for?

Christine Janine Wittlin - VAUZ Executive Manager
Wolfgang Fuhrmann - VAUZ Co-President
Georg Winterberger - VAUZ Co-President

Chris Wittlin, Wolfgang Fuhrmann, and Georg Winterberger stand at top of the association of the non-professorial academic staff of the University of Zurich (VAUZ). VAUZ - that means almost fifty years of academic self-administration and lobbying for the so-called Mittelbau. Their brief presentation is aimed at introducing mission and vision of VAUZ, and why it is important to be active at the university. Finally, they want to give an idea how to get involved as a Post-Doc at the UZH.

If you want to know more about VAUZ: http://www.vauz.uzh.ch/index.html.

T-7

Functional exploration of the leukemia niche under chemotherapy in the bone marrow

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The treatment of patients with acute lymphoblastic leukemia (ALL) is associated with significant long-term toxicity and salvage of refractory and relapsed ALL remains challenging1. Relapses are driven by the outgrowth of persistent residual leukemic cells that could not be completely eliminated by chemotherapy. To improve chemotherapy for ALL it is important to understand the reasons for resistance and to find strategies to overcome such resistance to decrease residual disease in patients. Since ALL cells are dependent on interactions with the microenvironment the characterization of specific signaling events between leukemic blasts and their niche cells may identify new therapeutic targets for chemotherapy2. Using our established xenotransplantation approach3, which faithfully recapitulates the human disease in immunodeficient mice, we have generated a model of minimal residual disease after chemotherapy in vivo. This model allows us to investigate the localization and architecture of the leukemic niche under therapeutic pressure by microscopy. We found that the residual leukemic cells primarily localize to sinusoidal regions in the distal metaphysis of the murine femur that are enriched for adipocytes. These data suggest that there are distinct regions within the bone marrow that protect leukemia cells from chemotherapy to enable survival and subsequent relapse. Our model provides the basis to dissect the cellular and molecular components of the crosstalk between the niche and leukemia in order to develop strategies to eliminate residual relapse driving leukemia cells.

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T-8

Ins and outs of directional hormone transport: the strigo-lactone case

Joëlle Sasse[a], Guo-Wei Liu[a], Sibu Simon[b], Christian Gübeli[a], Xi Cheng[c], Jiří Friml[b], Harro Bouwmeester[c], Enrico Martinoia[a], Lorenzo Borghi[a]

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[b]*Institute of Science and Technology Austria, Klosterneuburg, Austria*
[c]*Wageningen University, Wageningen, The Netherlands*
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Strigolactones (SLs) are carotenoid-derived hormones playing major roles both in plant development and plant-soil communication. In plants, SLs regulate shoot and root architecture. Once exuded from roots, SLs induce the germination of plant-parasitic weeds as well the onset of beneficial plant-fungal symbioses (mycorrhiza)[1].

The diverse functions regulated by SL suggested that its localization might be tightly controlled by cellular transporter(s). We recently discovered the strigolactone transporter PLEIOTROPIC DRUG RESISTANCE 1 (PDR1)[2], which is required for efficient mycorrhizal colonization and inhibition of lateral bud outgrowth. PDR1 exhibits a cell-type specific, asymmetric localization in different root tissues: in main root tips it is localized at the apical membrane of hypodermal cell; above the root tip, PDR1 is present in the outer-lateral membrane of hypodermal passage cells, gates for the entry of mycorrhizal fungi[3]. These results support the hypothesis that PDR1 polar localizations mediate shoot-ward strigolactone transport out of the root tip, where the hormone is synthetized, as well as localized exudation into the soil.

The de-regulation of PDR1 expression, and therefore of strigolactone transport, causes a plethora of effects related to nutrient uptake, root cell identity, plant architecture and plant-microbe interaction. These results make PDR1 a key-player in integrating signals from the rhizosphere with plant fitness.

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T-9

Genome-wide screen on rotavirus replication reveals viroplasm recruitment of low complexity host factors

Victoria A. Green[a] and Lucas Pelkmans[a]

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Rotaviruses are the leading cause of gastroenteritis in young children, responsible for significant morbidity and mortality worldwide. These non-enveloped, dsRNA viruses replicate in the cytoplasm of small intestinal cells, where protein/RNA-rich replication factories, termed viroplasms, are formed. Here, we performed a genome-wide, image-based, RNAi screen for host factors involved in early stages of rotavirus replication by detection of viral capsid protein VP6 in human colon carcinoma cells. Single-cell analysis revealed 1,000 candidate host factors that were further screened with three individual siRNAs and one esiRNA pool. We combined all datasets to calculate the probability of expression knockdown resulting in a perturbed infection phenotype (rank aggregation score). With this novel approach, we identified known rotavirus host factors, such as casein kinase 1α, along with many proteins not previously implicated in rotavirus infection. Unbiased, functional annotation enrichment analysis revealed that several of these newly identified host factors contain low complexity sequences, and have roles in alternative initiation of translation and RNA processing, with members of the latter category shown to undergo recruitment to viroplasms. Furthermore, the formation of liquid-unmixed stress granules and P bodies were disrupted in infected cells. We suggest a two-pronged model in which viral replication is enhanced by (1) disruption of endogenous RNA granules and phase separation of intrinsically disordered RNA processing proteins to liquid-unmixed viroplasms; and, (2) employment of an alternative translation initiation pathway to selectively enable viral protein synthesis.

T-10

NMR structural investigations on the functional primase domain of the pRN1 replication machinery

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Primases are single-stranded DNA dependent polymerases that synthesize RNA/DNA primers during replication. A primase, a DNA polymerase and a helicase compose the replication machinery of the archaeal plasmid pRN1[1]. The structure of the archaeal functional primase domain has been solved by X-ray crystallography [2,3] and it revealed an heteromeric structure with a catalytic prim/pol domain and a novel helix bundle domain.
We investigated the NMR structure of the functional pRN1 primase domain in complex with a single-stranded DNA template containing the GTG motif [4]. We showed that the prim/pol domain of this 38 kDa enzyme is not required for template binding. Intermolecular contacts detected exclusively between the helix bundle domain and the DNA led us to isolate specifically this structurally independent unit. Our results are compatible with a conformational switch between a template-bound open state and a closed active complex [3,5].
We solved the solution structures of the helix bundle domain in complex with the single-stranded DNA template and cofactors and we performed affinity measurements to confirm the importance of residues located in the helices 10 and 12 for the interaction with the GTG motif.
In association with functional assays, these novel transient structures will allow us to decipher the series of reactions required for replication initiation.

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T-11

Combination of optoacoustic imaging and diffuse optical tomography for high-resolution 3-D cerebral oxygenation map in preterm neonates

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Despite the improved survival rate of preterm infants, most of them suffer a lifetime brain damage caused by brain lesions. These brain injuries occur early after birth due to lack of oxygen supply in some parts of the brain[1]. To study this phenomenon, the so-called “fontanel”, which is the soft area on top of the infant’s head where there is a space between the bones of the skull, gives us access to the brain with state-of-the art techniques.

Our goal is to combine diffuse optical tomography (DOT), optoacoustic (OA) and ultrasound imaging into one multimodal instrument for morphological and functional mapping of brain. All the three techniques will be combined in a single hand-held probe, which will allow us to simultaneously obtain real-time structural and functional information.

First, a commercial device (Imagent, ISS, USA, www.iss.com) using modulated light source measures the absolute values of optical properties in tissue[2] and performs the optical tomography, yet with low spatial resolution. Next, a Monte Carlo program[3] uses the measured optical properties to calculate the photon fluence rate. Then, the calculated fluence rate is utilized for fluence compensation in optoacoustic imaging to obtain high-resolution absolute value of oxygenation[4] in the infant’s brain.

In this presentation I will show the results of measurements performed on tissue simulating phantoms that demonstrates the optical tomography procedure and its impact on the quantitative OA images.

Acknowledgments: This research was funded in part SwissTransMed platform “ONIRIUS”.

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T-12

Structural biology at the single particle level: Imaging tobacco mosaic virus by low-energy electron holography

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A milestone for structural biology would definitely be attained if methods and tools were available, that do away with averaging over an ensemble of molecules and thereby enable structural biology on a truly single molecule level. To obtain atomic resolution information about the structure of any individual biological molecule, entirely new concepts and technologies are needed. One approach of this kind is associated with the recent X-ray free electron laser (XFEL) projects. There are now strong indications that also in the XFEL experiments averaging over a large number of molecules will be inevitable in order to obtain images with a sufficiently high signal-to-noise ratio to enable numerical reconstruction of the diffraction pattern with atomic resolution. Our approach to structural biology at the single molecule level is motivated by the experimental evidences that electrons with a kinetic energy in the range of 50-250eV are harmless to biomolecules. Even after exposing fragile molecules like DNA or proteins to a total electron dose of $10^6 e^-/Å^2$, more than six orders of magnitude higher than the critical dose in transmission electron microscopy, no radiation damage could be observed. This, combined with the fact that the de Broglie wavelengths associated with this energy range is between 0.8 and 1.7Å, makes low-energy electron microscopy a candidate for structural biology at the single molecule level.

Here, we will report nanometer resolution imaging by means of low-energy electron holography of individual tobacco mosaic viruses (TMV) deposited onto ultraclean freestanding graphene. We will show that structural details arising from the helical structure of the viruses can be revealed and that the agreement between our images and an atomic model of TMV available from the protein database is remarkable. We will also describe our on-going efforts towards 2Å resolution by means of low-energy electron coherent diffraction imaging (CDI). By implementing a CDI experimental scheme for low-energy electrons, we could already image a 210nm freestanding region of graphene at 2.3Å resolution, revealing more than half a million of graphene unit cells at once.

FP-1

Uncover the mode of action of cis-NATs as translation en-hancers

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In recent years, research has revealed that a large proportion of the genome is transcribed, generating numerous non-coding RNAs. One important class, the natural antisense transcripts (NATs), target gene expression and play important roles in development and physiology. Cis-NATs are complementary to the transcripts from the protein-coding genes on the opposing DNA strand at the same locus and their expression usually results in reduced expression of the associated sense mRNA. However, recently a new feature was discovered where the expression of the cis-NAT to the rice gene *PHO1;2* did not change its mRNA levels, but led to translation enhancement (TE).
The main goal of this project is to specifically address the frequency and the mode of action of cis-NATs in controlling gene expression via TE in Arabidopsis and rice. We will mine existing and generate new transcript and protein datasets to uncover new links between expression of cis-NATs and increased translation of the cognate sense mRNAs and to identify novel cis-NATs with TE potential. In addition, we will probe the mode of action of cis-NATs as translational enhancers using ribosome footprinting and by identifying proteins interacting with cis-NAT$_{PHO1;2}$ and assessing their role in TE.

FP-2

Molecular mechanisms of chordoma formation and treatment

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Chordoma is a slow-growing tumor that arises from remnant cells of the notochord. Rarely, notochord cells can undergo an as-of-yet undefined malignant transformation forming a solid tumor mass and invade the surrounding bone. Therapeutic intervention remains difficult due to the lack of animal models hindering research on the molecular causes of chordoma formation and the development of therapeutic compounds.
We have recently developed the first animal chordoma model using zebrafish to study chordoma tumors and to interfere with chordoma development. Our zebrafish chordoma model bases on stable transgene-driven expression of HRASV12 in developing notochord cells. Extensive intra-notochord tumor formation is evident within 3-5 days of transgene expression. The zebrafish tumors share histological characteristics with human chordoma, as demonstrated by immunohistochemistry and electron microscopy. We have shown that the mTORC1 inhibitor rapamycin delays the onset of tumor formation in our zebrafish chordoma model and improves survival of tumor-bearing animals.
For the first time, our in vivo model enables now rapid testing of potential chordoma-causing candidate genes, pathways, and genetic modifiers, as well as the search for therapeutic agents for the treatment of this refractory cancer. We are using modular transgenic zebrafish techniques and the CRISPR-Cas9 system to drive pathways and genes that have been implicated in, but never causally tested for, chordoma initiation. This approach will instruct diagnostic sequencing and therapeutic avenues to target aberrantly activated signaling pathways in chordoma. Further, we will screen a targeted mini-library of small bioactives to target a range of molecular processes in zebrafish embryos during chordoma onset to gain insight into possible therapeutic pathway intervention.
Altogether, our expertise in early development and in vivo disease models provides a deeper insight into the therapeutically elusive chordoma onset. Our findings will be instructive in establishing diagnostic markers and therapeutic compounds for treating human chordoma.

FP-3

Characterization of cytoplasmic architecture in *S. pombe* and *Drosophila* embryo

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So far the cytoplasm, the space between organelles, was seen as a fluid, the physical and chemical properties of which are mainly controlled indirectly, by the complex cellular machinery providing cell type-specific architecture and function. However, recent studies revealed active modulation of cytoplasmic fluidity in bacteria [1]. In the much larger eukaryotic cells, heterogeneities in cytoplasmic fluidity were proposed to result from local differences in macromolecular crowding [2]. Other works hinted at a grid-like, poro-elastic network in the cytoplasm, which controls the physico-chemical dynamics of cellular components [3]. These findings raise the possibility of active regulation of the cytoplasmic environment also in eukaryotic cells.
During my Postdoctoral research in the laboratory of Damian Brunner I am going to further investigate this hypothesis based on new data showing the presence of a molecular machinery that can drastically increase cytoplasmic fluidity. In particular, the work will focus on characterization of physical properties and architecture of the cytoplasm in two types of eukaryotes: fission yeast (*S. pombe*) and fruit fly (*Drosophila*) embryo. Studies of the intracellular fluidity will primarily include quantitative tracking of intracellular fluorescent particles of different sizes and fluorescence correlation analysis.

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FP-4

Selection on floral volatiles in two species of Alpine orchids (*Gymnadenia*)

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Pollinator-mediated reproductive isolation is a major factor in driving the diversification of flowering plants. Studies of floral traits involved in reproductive isolation have focused nearly exclusively on visual signals, such as flower color. The role of less obvious signals, such as floral scent, has been studied in detail only recently[1]. The closely related species *Gymnadenia densiflora* and *Gymnadenia (Nigritella) rhellicani* (Orchidaceae) [2,3] appear to be largely reproductively isolated due to floral isolation[3]. Multiple floral traits are divergent in these two species, including color, inflorescence size and shape, nectar spur length, and floral scent. Both species emit a complex blend of floral volatiles, with limited overlap. Previous work has shown multiple floral volatiles found in these species to be important in pollinator neural response and behavior [4,5]. Using data from three field populations of *G. densiflora* and six populations of *G. rhellicani*, I will discuss the relationship between female fitness and individual floral volatiles in these species.

Acknowledgments: funding from a PLANT FELLOWS postdoctoral fellowship awarded to Byers and from the University of Zürich is gratefully acknowledged; we also thank K. Gross for field assistance.

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FP-5

Fiber-optical implants to measure calcium activity in the deep brain structures

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Genetically encoded calcium indicators are widely applied together with the two-photon microscopy to study *in vivo* neuronal circuit dynamics at a cellular resolution. However, the accessible depth for this kind of measurement is limited to approximately 1 mm due to light scattering in the brain tissue. We designed optical setup and miniaturized fiber implants which can record calcium activity by detecting fluorescence from the genetically encoded green calcium indicator (GCaMP 6m) in the deep brain areas.
Simultaneous measurement of voltage and calcium from population of neurons was done in the hippocampus. The optical fiber is combined with tetrodes (opto-tetrode), the total diameter of implant is less than 150 micron. The resulting structure is mounted on the micrometer translation driver and implanted in the deep cortical layers of the mouse. The mouse is trained for the texture discrimination task. During successive measurement sessions the opto-tetrode is moved in depth of the hippocampus. We rationalize combined voltage measurements from single neuronal units and optical fluorescence from the neuronal population and relate signals to decision making processes in the hippocampus.

FP-6

Assessing the propensity of HIV-1 to evolve antibody escape variants during free virus and cell-cell transmission

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The human immunodeficiency virus type 1 (HIV-1) can infect target cells via two distinct routes: (i) free virus transmission: free virions can enter and infect target cells and (ii) cell-cell transmission: virions can be transmitted directly from infected to uninfected cells via narrow range connections between cells, such as the virological synapse. Cell-cell transmission has been shown to be less sensitive to neutralizing antibodies. Here we investigate if this increases the chances of viral escape variants to evolve as a response to selective pressure induced by neutralizing antibodies.

To study whether one transmission route favors the occurrence of viral escape variants, we measured virus inhibition by broadly neutralizing antibodies (bnAbs) in experimental settings that either only allows for free virus or cell-cell transmission to occur. Based on the information on the inhibitory potential in the two transmission modes, we developed a model that allows us to compare the probabilities that an escape variant arises in either transmission setting for each antibody-virus pair measured. We further determined the mutant selection windows for bnAbs PGT121 and PG128 using a neutralization escape mutant and the matching wildtype virus.

We find strong evidence that the cell-cell transmission route serves as a rescue pathway for virus transmission because escape variants can evolve over substantially wider antibody concentration ranges via this route. Knowledge on which infection route is more prone to evolution of escape variants opens possibilities to tailor future intervention strategies that directly target both transmission routes reducing the chances for escape mutants to occur.

FP-7

Melanoma metastasis vs. cell invasion in *Caenorhabditis elegans*

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A critical step in metastasis formation by invasive tumor cells is the breaching of the basement membrane (BM), a natural barrier that separates sheets of cells. Besides its involvement in disease, BM breaching is essential for proper organ formation during normal development. Recent studies provide evidence that there are parallels between developmental and tumor cell invasion. One well-studied developmental invasion process is anchor cell invasion in *Caenorhabditis elegans*, where a specialized cell (anchor cell) in the uterus invades into the adjacent vulval epidermis. Because anchor cell invasion can easily be manipulated and observed, we use this system as a functional assay to characterize the *C. elegans* homologues of human genes, which are upregulated in invasive melanoma cells and during neural crest cell migration. From an initial list of 112 invasion-associated human genes we identified through our functional *C. elegans* screen 14 genes that showed an anchor cell invasion defect. Notably, three of the candidate genes encode cell cycle regulators and four genes are involved in protein sumoylation. Currently, the validated candidates are further studied using *in vitro* invasion assays with human melanoma cells (Boyden Chamber assays). Our long-term goal is to establish molecular markers predicting tumor invasiveness based on biopsies and to identify potential drug targets.

FP-9

ALMT4 - Vacuolar Channel Involved in Stomatal Closure

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Plant CO_2 and water relations are crucial for biomass production and are regulated by stomata. Stomatal aperture is regulated by a pair of guard cells, which swell to open and shrink to close a stoma. Swelling and shrinkage depend on the uptake and loss of osmotically active substances, mainly K^+, Cl^- and malate^{2-}. In this process solute transport in and out of the vacuole is of central importance. Although we know a lot about the regulation of solute fluxes across the plasma membrane our knowledge about the transporters and ion channels involved in mediating these solute fluxes across the vacuolar membrane is scarce. Proteins that are responsible for mediating malate^{2-} and Cl^- fluxes during stomatal opening and closure are members of the AtALMT family of *Arabidopsis thaliana*. These proteins are ion channels that have been shown to localise to the plant vacuolar membrane. Here we present data showing that heteromerisation of ALMT channels may be a regulatory mechanism to effectively 'shut-down' ALMT channels and vacuolar malate uptake during stomatal closure. Cell-biological and electrophysiological data reveal that two previously uncharacterised ALMT channels form oligo-heteromers. Both channels display inward malate currents, alone or when co-expressed. However, when incubated with the drought-stress hormone abscisic acid (ABA), the putative channel-heteromer shows a marked shift in half-activation voltage, towards negative cross-tonoplast voltages. Thus, malate current is significantly reduced at physiological cross-tonoplast potentials. ABA induces stomatal closure and is known to regulate conductances at the plasma membrane to induce solute loss and inhibit solute up-take. Interestingly, one of the ALMT channels' loss-of-function mutant displays decreased sensitivity to ABA-induced stomatal closure and increased water stress sensitivity. The mechanism that induces interaction or transduces the ABA signal to channel regulation is currently under investigation.

FP-10

Development of a new method for fast optical diagnosis of rare anemias

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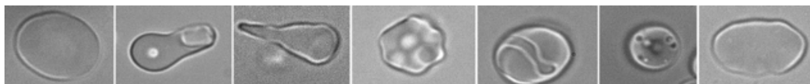
Rare hereditary anemia is a lifelong condition highly impacting the quality of life. This group of disorders is widespread in European population including Switzerland. However, the molecular causes of very few rare anemias can be identified using available clinically approved tools. Many patients are waiting for their diagnosis for years and splenectomy routinely performed to reduce red cell clearance often fails to increase RBC mass.
One of the goals of the European CoMMiTMenT consortium# is to select characteristic features of diseased red blood cells and design a battery of tests that can be used for quick identification of the possible causes of rare anemia using conventional bright field and fluorescent microscopy. Cell size, projection shape and cell surface microstructures in unstressed cells and the cells exposed to mechanical and chemical stimuli can be used to recognize membranopathies, hemoglobinopathies or enzymothathies. Using microfluidic chips designed by Epigem Ltd. we are able to detect changes in various cell parameters right after the exposure to osmotic stress, shear stress and evaluate drug resistance. Currently, a library of samples of patients with various forms of rare anemia is generated by the consortium members. These samples and the samples obtained from healthy humans are screened for responses to shear stress, calcium overload and other chemical stressors. The obtained images are analyzed by algorithms for recognition of specific fingerprints of rare forms of anemia created by the Arivis AG. These algorithms will then be implemented in a digital analysis module of a new diagnostic system called µCOSMOS.



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P-1

Environmental Shaped Transition in Monkey Flower *(Mimulus aurantiacus)*

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Diversity in gene activity forms the basis for phenotypic variation in populations, upon which environmental needs can act. The role of genetic variation as a basis for diversity is widely acknowledged. However, epigenetic variation - that is, changes in gene activity that are not associated with changes in DNA sequence - may substantially play an important role in the context of evolutionary biology[1]. In plants, epigenetic states are often heritable and stable, such that they can contribute to phenotypic variation and thus may alter a gene pool of a population. A possible role for epigenetics in evolution has been uncovered in the species *Mimulus aurantiacus* in Southern California. Flower phenotypes along an east west transect range from large, yellow, insect-pollinated flowers through orange flowers to small, red, bird-pollinated flowers. Until now, intermediate forms were attributed to recurrent hybridization at the subspecies level. However, by monitoring the flower phenotypes of these populations in field studies over the past 20 years, Rolf Baumberger observed that the transition in flower phenotype occurs during the lifespan of individual long-lived plants, thus ruling out a hybrid origin of intermediate forms. Further research has revealed that this transition bears the hallmark of an epigenetic transition. The small, red, bird-pollinated state is stable and heritable but reverts at frequencies of 1-2 %, much higher than that of genetic alterations. The phenotypic transition does not occur in plants grown under controlled laboratory conditions but only in the field, which suggests that environmental factors such as visiting hummingbirds are triggering this transition. As the transition from yellow to red flowered plants leads to a distinct and environmental shaped species, *M. aurantiacus* may become a classic example of how epigenetic changes play a role in affecting population structure and speciation in higher plants.

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P-2

Quantitative single molecule analysis of mRNA transport dynamics

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mRNA transport from the nucleus to the cytoplasm via nuclear pores is a fundamental and highly dynamic step in eukaryotic gene expression. All protein-coding mRNAs need to be faithfully exported out of the nucleus to allow for their translation[1]. Although factors involved in mRNA transport have been characterized, the lack of information on molecular mechanisms and dynamics of directional mRNA transport has hampered a comprehensive understanding of this essential process and its regulation[2].

To study the dynamics of mRNA transport through the nuclear pore we set up a state-of-the-art quantitative *in vivo* single molecule mRNA export assay (SiMEx) in budding yeast. This technique allows a functional characterization of individual factors in mRNA transport through single nuclear pores in unprecedented detail. In addition, the technique will be further developed to investigate the hypothesis that mRNAs are transported asymmetrically with their 5' end first through nuclear pore complexes, thus clarifying the importance of directional mRNA translocation across nuclear pores.

This project will shed light on the individual steps of nucleo-cytoplasmic mRNA transport in an unparalleled quantitative manner and provide key insight into a central part of the eukaryotic gene expression program.

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P-3

Analysis of mRNA turnover, with a special focus on the DEAD-box ATPase Dhh1

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Gene expression is tightly regulated, from transcription to m(essenger)RNA stability, but we still know very little about the signals and mechanisms that determine the fate of a cytosolic mRNA, and the abundance of the encoded protein. Regulation of mRNA activity occurs at multiple levels, including efficiency of translation, translational repression, and mRNA decay.

Many studies have started to elucidate how individual factors affect mRNA turnover, but the sequence of events and molecular mechanisms remain poorly understood. To better understand the life of an mRNA, I am setting up a time-resolved analysis of mRNP (mRNA-particle) protein composition using mass spectrometry, in order to identify regulatory `bifurcations' in mRNP composition.

P-bodies are mysterious cytosolic mRNP granules found in all eukaryotes that contain mRNA decay factors and translationally repressed mRNAs. However, their exact function and composition is still poorly characterized, in part at least, because they could not be isolated so far. P-body like granules also form in oocytes, neurons and aging cells where they sequester mRNAs in a stably repressed state. These mRNPs contain cell-specific proteins in complex with the DEAD-box ATPase Dhh1, a factor essential for translation repression and P-body formation in general. To better understand how Dhh1 contributes to translational repression and mRNA turnover, I am using a variety of biochemical and in the future also structural assays.

P-4

Genetic exchanges as the main driver of adaptation and genome architecture evolution

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Operon-like gene clusters[1], which are formed by functionally related but non-homologous genes, are found to play an important role in the production of structurally complex natural products. Understanding the formation of gene clusters will give insights in how novel metabolic functions emerge, leading to gene functional specialisation and eventually shape the genome.
I investigate the selective forces driving genomic changes brought about by adaptation and horizontal gene transfer using experimental evolution followed by whole genome sequencing and bioinformatics on *Escherischia coli* populations. Briefly, I evolved three different *E. coli* strains in novel carbon or nitrogen sources under conditions either permeates or inhibits foreign DNA exchanges with a collection of *E. coli* high-frequency recombination strains[2], which actively donate DNA to neighboring bacteria via conjugation.
This experimental regime provides a time series of evolving bacterial populations with accessible genomic sequence data for my investigation into how genomic incorporation of foreign DNA contributes to adaptation and new genome arrangement.

Acknowledgments: I would like to thank my mentors Professor Andreas Wagner and Dr Kathleen Sprouffske and the IEU for hosting me. We thank James Bourdon, Pablo Bryant, Karl Huwiler, Hanspeter Schöb, Rico Kunzmann, Thomas W. Baumann, Sibylle Hirsch, Bernhard Schmid, Beat Keller. Supported by the European Research Council.

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P-5

Single-cell genomics and transcriptomics: methods in sub-cellular gene expression analysis for biomedical applications

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Single-cell studies hold great promise in biology and medicine, offering new insights into cellular function, cell-cell communication, or cell response to external stimuli[1]. Genome-wide RNA analysis is a powerful tool for understanding gene expression differences in biological systems and has had a dramatic impact on a broad range of scientific fields[2]. In viral infections, the capacity of viruses to propagate from infected to uninfected cells is a critical factor for virulence. The mechanisms underlying viral infection and spreading are poorly understood, hindered by the reliance on bulk methods that fail to capture the full complexity of virus-cell interactions. We have developed FluidFM (a unique instrument that combines atomic force microscopy (AFM) with nanofluidic probes that reliably transfer femtoliter volumes while under direct microscopic visualization) into a multifunctional tool and versatile platform for single-cell manipulation and perturbation[3], providing a unique opportunity to investigate the molecular mechanisms underlying viral infection and spreading in human host cells. Here, we report our progress in developing subcellular gene expression analysis and single cell viral transcriptomics with next generation sequencing (Fig. 1).

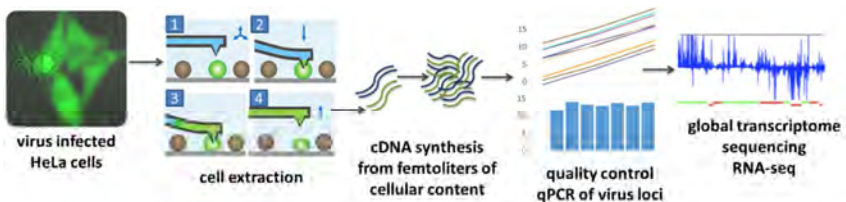

Figure 1. Workflow for subcellular expression analysis from single infected host cells.

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P-6

Effect of viscogens on the kinetic response of a photoperturbed allosteric protein

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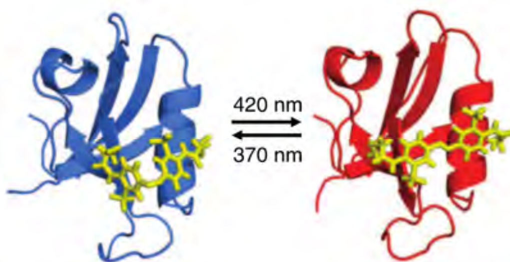

The interplay between internal and external friction in molecular dynamics and kinetics is an active topic of investigation in protein science. At what length and timescales does one dominate over the other, and how does one determine these limits experimentally? How does friction moderate or enable important properties such as allostery (intramolecular signaling) or protein function? By attaching a photoswitchable linker across the binding groove of a small protein, we are able to photo-initiate a conformational change that mimics an allosteric transition and observe the propagation directly with ultrafast pump-probe IR spectroscopy. We have found that this small perturbation initiates a global change that evolves in a surprising complex manner. Simulations have shown evidence that the solvent plays an integral part in these kinetics. To explore this further, we investigate the role of the solvent and external friction by observing the transition kinetics as a function of solvent viscosity[1,2].

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P-7

Regeneration potency in plants and cnidarians – what are the crucial chromatin factors which are responsible for it?

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Plants and Cnidarians share the remarkable ability to regenerate from dissociated cells. Although described more than a century ago and exploited for many years in horticulture and agriculture, the molecular basis of plant cellular plasticity remains largely enigmatic. Plant cells show a surprisingly dynamic nuclear organization of chromatin during development, responses to stress and plant regeneration. This is in stark contrast with most animals in which cellular plasticity is mostly lost *in vivo*, albeit can be induced *in vitro*. Although the correlative link was made several years ago, it is still not known whether nuclear organization is causative to cellular plasticity.
Epigenetic reprogramming and cellular plasticity are in the spotlight of life sciences and societal debates, yet research is conventionally bound to model organisms with a restricted potential of development. Plants and Cnidarians are, by contrast, ideal and unique systems to understand durable cellular plasticity.
The goal of this project is to answer the unresolved question of cellular plasticity from a new perspective - by identification of nuclear organization factors (NOFs) causally linked to it in plants and, plausibly, in Cnidarians. The originality of this project lies in combining the power of Arabidopsis molecular genetics with an automated microscopy-based screen and in transcending the kingdoms by exploring plant and cnidarian regeneration models.
Specifically, we are establishing a conditional, amiRNA-based screen for down-regulating target protein groups with known or predicted functions in nuclear organization; we will screen the mutant lines for alteration of well-described nuclear organisation events during trans-differentiation using a microscopy-based automated phenotyping platform. The candidate mutants will be described in details for their nuclear organization, transcriptome and epigenome profiles and their regeneration potency. The second aim will be a pilot study carried in collaboration with an expert lab in Geneva and will involve RNAi-mediated downregulation of candidate NOFs in Hydra and regeneration test.

Acknowledgments: Project supported by a grant from Switzerland through the Swiss Contribution to the enlarged European Union.

P-8

Regulation of microRNAs by protein phosphatase 1 in memory formation

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Emerging evidence suggests that microRNAs (miRNAs) contribute to the regulation of neuronal circuits involved in synaptic plasticity and memory formation. Our previous results have shown that protein phosphatase 1 (PP1) is a molecular constraint on memory formation. Inhibition of nuclear PP1 parallels the effect of training in a hippocampus-dependent memory task involving novel object recognition. We investigated whether PP1 exerts its regulatory effect on memory formation by modulating the expression of miRNAs. We conducted a deep-sequencing screen on mouse hippocampal miRNAs using a transgenic mouse model with improved memory performance resulting from inhibition of nuclear PP1 (NIPP1) in the adult brain. The results show that specific miRNAs are differentially expressed in NIPP1 mice, and that the same miRNAs are similarly differentially regulated after training in control mice. We further show that over-expression of these miRNAs in the mouse hippocampus enhances long-term memory. Importantly, PP1 regulates their activity-induced biogenesis, mainly through transcription-independent increase of the precursor miRNA (pre-miR). Our work provides new evidence for a role of protein phosphatases in the control of miRNAs involved in memory formation.

P-9

Reverse-genetics in zebrafish for TAR syndrome candidate genes

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The heart and the forelimbs serve completely distinct purposes in the human body plan, yet surprisingly during development they emerge together as bilateral field in the lateral plate mesoderm (LPM). The LPM also forms the remaining cardiovascular and blood cell fates, suggesting a common molecular program that drives the initial emergence of cardiovascular and limb cell fates. The tight connection of heart and forelimb development is evident in the phenotypes of several human congenital disorders that selectively affect both the cardiovascular system and upper extremities. A better molecular understanding of LPM patterning would provide substantial insights into the molecular basis of human heart-hand syndromes and the evolutionary basis of joint cardiovascular and limb formation.
Thrombocytopenia with absent radius (TAR syndrome) is a rare compound blood platelet and forelimb defect, with a subset of patients featuring also cardiac defects and mild skeletal abnormalities[1]. We hypothesize that TAR syndrome results from an early LPM defect. TAR syndrome links to 1q21.1 that harbors eleven genes, most of which remain uncharacterized for developmental functions. We have recently developed a rapid CRISPR-Cas9-based method to generate somatic-mutant zebrafish embryos to study loss-of-function phenotypes of candidate genes. Zebrafish are uniquely suited for rapid reverse-genetics studies due to their high fecundity, optically transparent embryos, and fast development. I now systematically silence all 1q21.1 genes, starting with *rbm8a* that is mutated in some TAR cases[2]. At 3 days of development, *rbm8a* targeting consistently caused pectoral fin hypoplasia, pericardial edema with blood circulation defects, and mild body axis perturbations (80-98% of embryos, n=320), all features reminiscent of human TAR syndrome phenotypes. CRISPR-Cas9-based phenotype screening in zebrafish embryos thus allows causative linking of the other TAR candidate genes with developmental phenotypes. We are now investigating the impact of *rbm8a* loss on overall LPM formation and cardiovascular target genes.

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P-10

A High-Throughput Analysis Framework for Virus-Cell Transmission and Clonal Cell Expansion

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Classical plaque [1,2] assay measures infection, or scores cell population features, infection propagation or spreading processes of infectious agents, but is limited by requirements of cell lysis, user-biased settings and low throughput. Here, we developed Plaque2.0, a broadly applicable, fluorescence microscopy-based high-throughput method to systematically mine patho-biological clonal cell features in live cells. The framework robustly extracts population features from virus-infected cells based on immuno-histochemistry, GFP transgene expressions, or RNA in situ hybridization signals. It yields multi-parametric measurements at single cell or multicellular object-based levels, such as infection density, intensity, area, shape or nearest neighbor data. It thereby distinguishes lytic and non-lytic replication in a variety of DNA and RNA virus infections, including adenovirus, herpes virus, rhinovirus, vaccinia virus, and can be used in time-resolved experiments to characterize infection phenotypes. Plaque2.0 also analyzes clonal growth of cancer cells, relevant for migration or metastatic invasion studies. Plaque2.0 bears relevance for virology and medical oncolysis and cancer communities, or vector production facilities in need of sensitive viral titration data.

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P-11

Establishing a universal method to predict protein complexes and generate protein interaction networks

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The era of whole genome sequencing has yielded unparalleled amounts of detailed genetic information. However, what genetic information cannot tell us is how these tens of thousands of proteins interact and work together in the cell. Many biological processes e.g. DNA replication, protein synthesis/degradation, primary and secondary metabolism require stable long term multimeric protein complexes. Unfortunately, our knowledge about their assembly is incomplete and a pastiche collection from several different species, often based on predictions. All attempts to define protein interactome maps required high investment of human resources and money.

Here, we show that with three basic native separation methods coupled to a proteomics approach, we can predict the subunit composition based on co-behavior of the proteins. Currently, we are able to define potential association in protein complexes for more than 2000 proteins in *Arabidopsis thaliana* and *Saccharomyces cerevisiae*. Surprisingly, the majority of the proteins assemble in multimeric long term complexes and the *in vivo* status of a protein in a monomeric form is rather exceptional.

The next goal is to develop a standard methodology which can be applied to any organism for which the genome is known with affordable cost for “standard” Laboratory to study protein complex composition and changes in their biological samples of interest.

P-12

HFO detection in intra-operative ECoG recordings: validation of a time-frequency based automatic detector

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Purpose: High frequency oscillations (HFOs) in the electrocorticogram (ECoG) have recently been shown by several centers to be a specific biomarker for epileptogenic tissue. Time consuming and subjective visual inspection prevents to benefit from the HFO information in intraoperative sessions. While several automatic HFO detectors have been proposed, we validate here our time-frequency based HFO detection algorithm (Burnos et al., 2014).

Methods: Pre-resection intra-operative ECoG (N=7 patients, 2xN recordings of 1 minute) was recorded at 2048 Hz in the UMC Utrecht and HFO events were marked visually. For each patient, we optimized the detector's parameters defining baseline and threshold on a first dataset (training), and validated it on the second dataset (test). The performance was quantified in terms of ROC (Receiving Operator Curve). Automatic detection of a visually marked event was considered as a True Positive (TP). A correctly undetected baseline interval was a True Negative (TN). False Positives (FP) were events detected but not marked visually, and False Negatives (FN) were undetected but visually marked events.

Results: In the ECoG with visually marked HFOs, we achieved high sensitivity and specificity (across patients TP rate>0.95, FP rate<0.01). The detector robustly identified baseline intervals (low FP rate). Optimal tuning of baseline and threshold parameters over 1 minute data emulated visual detection for each single patient (high TP rate). Interestingly, in the ECoG without visually marked HFOs, in test sessions the method provides very low FP rate (<0.01), corresponding to a very high specificity.

Conclusion: Our semi-supervised HFO detector rapidly and accurately emulates experienced visual HFO marking, which is currently considered the gold standard. It thereby holds promise for future clinical application.

P-13

DARPins as Entry Inhibitor Alternative to HIV-1 broadly neutralizing antibodies

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Development of drugs that share properties of HIV-1 broadly neutralizing antibodies (BNAbs), active against genetically divergent viral strains, is highly desirable. Here we utilize the Designed Ankyrin Repeat Protein (DARPin) technology to generate such inhibitors directed against the V3 loop of the HIV-1 envelope protein (env), essential for viral entry into target cells. DARPins, small synthetic binding proteins with high target affinities and specificities, have interesting properties in particular as they can recognize their target in a structure dependent manner[1].
DARPins were selected from 1st and 2nd generation libraries, the latter featuring additional randomized positions in the binding surface and a higher overall stability of the DARPin scaffold. The env subunit gp120 and a structurally arrested epitope mimetic of the V3 loop on gp120 were used as panning targets in ribosome display selection rounds. Resulting sub-libraries were screened by binding ELISA and pseudovirus neutralization assay.
Epitope focusing selection using the V3 mimetic proved to be successful. Interestingly, selected DARPins differ in neutralization breadth. As the panning targets were of subtype B origin, the neutralization activity of DARPins was mostly restricted to this subtype. This was improved using the 2nd generation DARPin library, from which we selected DARPin 13.2G10 with broad neutralization capacity. 13.2G10 neutralizes 62.5% of pseudoviruses out of 32 viruses from 5 different subtypes. In summary, the V3 mimetic proved to be a valuable tool to focus selection on this epitope and to derive a broadly active entry inhibitor. This result opens novel opportunities for selecting HIV-1 inhibitory DARPins specific for different epitopes.

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P-14

compcodeR - a benchmarking environment for RNAseq differential expression analysis

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Transcriptome profiling studies using RNAseq, with the goal of finding genes that are differentially expressed between conditions, are abundant in the current scientific literature and can be expected to become even more so as next-generation sequencing technology becomes cheaper and more accessible. Many differential expression approaches applicable to count matrices from RNAseq experiments have been proposed in the past five years, and at this point both users and developers of such methods would greatly benefit from objective and standardized benchmarking and characterization of new and existing approaches. Given the rapid movement of the field, static comparison papers quickly become outdated. compcodeR[1] (COMParison of COunt-based Differential Expression analysis methods with R) is a benchmarking R package that, in a few steps, lets the user evaluate and compare differential expression methods on synthetic or experimental data. The package comes with a large collection of pre-generated benchmarking data sets, but also with a function to generate new synthetic data sets with user-specified properties. It is also easy for developers to add their own method to the comparison. The comparison framework is not specific to the RNAseq context, and many of the evaluation metrics are relevant also for other types of data. All results and exact code and version details are stored for reproducibility.

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P-15

Biophysical Characterization of N-Terminal Domain of LptD (NLptD) and Its Interaction with an Antimicrobial Peptide by NMR

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Determining induced chemical shift changes in the protein-NMR spectra upon ligand binding is an efficient approach to identify protein-ligand interactions. Chemical shift mapping for proteins is generally based on the ^{1}H-^{15}N HSQC that yields a two-dimensional spectrum comprising one cross peak for each amide, with the exception of prolines. The distribution of signals or the chemical shifts of each residue in the HSQC spectrum depends on the nature of protein folding. The local conformational changes occurring in a protein as a result of ligand binding are translated as chemical shift changes in the HSQC spectrum. On assigning the peaks to the respective amino acids in a protein-NMR spectrum, interacting residues with the ligand can be identified revealing the binding site(s) in a protein. This approach is termed as structure-activity relationships (SAR) by NMR[1]. More specifically, we are interested in using this approach to study protein–drug interactions.

Our lab is specialized in designing novel peptidomimetics that act as antibiotics against Gram Negative bacteria. One (L27-11) of the several peptides designed was successful in showing potent antimicrobial activity against *Pseudomonas* sp[2]. The target for this peptide was identified as LptD, which is a β-barrel outer membrane protein playing a crucial role in lipopolysaccharide (LPS) transport to the outer membrane[3]. LptD comprises of a C-terminal β-barrel domain embedded within the outer membrane and an N-terminal domain (NLptD) located in the periplasm. Although we know that this peptide binds to LptD and blocks LPS transport to the outer membrane, establishing SARs will help explain the exact mode of action of the binding site of the antibiotic in LptD. My research involves biophysical characterization of the periplasmic domain NLptD by NMR and in identifying the chemical shift changes upon addition of L27-11.

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P-16

Photolabeling Studies of Polymyxin B in Gram-Negative Bacterium *Escherichia coli*

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The control of infectious diseases with antibiotics is a serious problem as the number of bacterial genera and species that have developed drug resistance is continually growing. The class of polymyxin antibiotics is often considered as a final option for treatment against multidrug resistant strains of bacteria. Despite the antimicrobial activity of polymyxins against Gram-negative bacteria being known for several decades, the mechanism of action leading to cell death is not yet fully understood. The antibiotic initially binds to lipopolysaccharide (LPS) exposed at the cell surface, followed by a key "self-promoted uptake" across the outer membrane (OM) where the antibiotic crosses the asymmetric LPS-phospholipid bilayer before it is transported to the periplasm. Once in the periplasm, the antibiotic finally targets and disrupts the bacterial phospholipid inner membrane [1-2]. Our lab began exploring the mechanism of action of polymyxins under the hypothesis that these antibiotics may interact with proteins in the OM, resulting in their self-promoted uptake and permeabilizing effects. A photoprobe based on Polymyxin B has been designed in our lab that retains potent antimicrobial activity [3]. Initial photolabelling experiments with Escherichia coli ATCC25922 have shown that several OM proteins are photolabeled. Further detailed proteomic studies to identify these photolabeled proteins could aid in better understanding the mechanism of action of this family of antibiotics.

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P-17

Functional Genomics Center Zurich: The Core Lab of the Zurich Life Science Community

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The Functional Genomics Center Zurich (FGCZ) is a joint state-of-the-art research and training facility of the ETH Zurich and the University of Zurich. With the latest technologies and key expertise for Omics research, the FGCZ carries out research projects and technology development in collaboration with the Zurich Life Science research community.

New analytical approaches in the areas of genomics, transcriptomics, proteomic and metabolomics are being developed to answer the variety of biological questions. Using ultra-high throughput sequencing systems and mass spectrometry-based technologies, molecular measurements at DNA, RNA, protein and metabolite levels can be investigated for the elucidation of biological and cellular systems.

P-18

Determining the optical effective attenuation coefficient of tissues from opto-acoustic measurements

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The non-invasive nature optoacoustic (OA) imaging, together with its ability to provide high spatial resolution, makes OA imaging a modality of choice for medical diagnostics, whereby highly absorbing chromophores in tissue (e.g., blood vessels) can be visualized. Over the past couple of years, we have been investigating different methods to perform so-called "spectral fluence compensation" on OA images, so that quantitative analysis can be achieved (e.g., calculating blood oxygenation levels) [1]. The 3D light fluence distribution needed for the compensation can be determined in various manners: in this contribution, we present an approach that consists in retrieving the spatially averaged optical effective attenuation coefficient μ_{eff} of the tissue under investigation, based solely on OA measurements, without the need of external near-infrared spectroscopy devices (as it is the case in common practice) [2,3]. The principle consists in using "multiple-illumination optoacoustic sensing", i.e., measuring the absorbed energy density at some regions of interest, for different positions of the illuminating laser source. The μ_{eff} is determined by fitting the measured data to an appropriate light diffusion model. Here, we present the theoretical work we have undertaken [4] to (i) introduce the appropriate light diffusion model in the case of a "semi-infinite tissue sample" and (ii) to extend the latter to more realistic tissue samples with arbitrarily irregular geometries (e.g., in the brain, breast, forearm etc.), where the boundaries can significantly affect the propagation of the light. Further, we investigated the limits of applicability of our approach with respect to the "excess absorption" of the chromophores, to ensure the validity of the primary assumption behind the "fluence compensation": that is, the measured optoacoustic signal is proportional to the background fluence in the sample.

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P-19

Radiation risk of fragmented DNA in dry, wet and frozen subjects following CT exposure: A Geant4-DNA study

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Ancient remains represent increasingly a valuable database for imaging and molecular studies to investigate pathological bone changes or infectious diseases in DNA dataset. Consequently, the major concern of investigators using X-ray imaging is focused on DNA degradation in a subsequent PCR procedure. Previous studies revealed [1] that the radiation risk decreases dramatically with decreasing DNA fragment size in dry subjects. Here, the radiation risk of CT imaging of wet and frozen subjects using the "**Ge**ometry **an**d **T**racking Toolkit" (Geant4-DNA, CERN & European Space Agency) was determined.

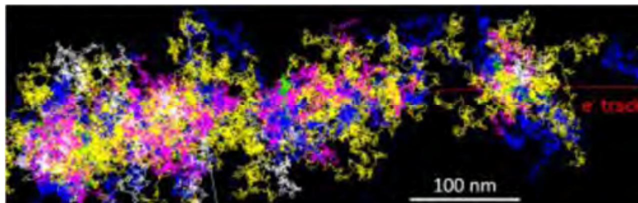

Figure 1: Screenshot showing the simulation of an electron track in water (red, E_{kin} = 20 keV) and the produced chemical species, namely H• (white), OH• (magenta), H_2O_2 (green), H_3O (yellow), H_2 (white) and e_{aq} (blue) in a phantom cell.

For the risk estimation, the possible number of damaged fragments was calculated by multiplying the diffusion volume (DV) of all chemical species VDV = 0.056 µm3 in Fig. 1 with the DNA fragment density (FD) [2] ρFD = 153 fragments/µm3 at 1054 bps. The radiation risk of fragmented DNA in wet subjects was p = 1.5×10-6. A value which lies between the radiation risk of living subjects p ≈ 10-3 [3] and dry subjects p ≈ 1×10-12 at 150 bps [1]. Note the radiation risk of frozen subjects is an ongoing re-search task.

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P-20

Intercalation properties $Ru(bpy)_2(dppz)]^{2+}$ into Hg(II) modified DNA

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Metal-mediated base pairs represent a powerful tool for the site-specific functionalization of nucleic acids with metal ions.[1] Such modified nucleic acids are expected to exhibit enhanced conducting properties which are a prerequisite for the application of nucleic acids as nanomaterial in electronic devices.[2] We want to probe the charge transfer in Hg(II)-modified nucleic acids by luminescence quenching experiments using metallointercalators as a donor-acceptor couple.[3] The well-known "DNA light-switch" complex $[Ru(bpy)_2(dppz)]^{2+}$ will be the electron donor that upon photoexcitation transfers an electron through the metal-modified nucleic acid toward the acceptor.[4] Even though a wealth of information regarding fluorescent $[Ru(L)_2dppz]^{2+}$ complex interactions with DNA is available in literature, the different intercalative binding modes and their emergence are still poorly understood.[5]
In this study we investigate the intercalation behaviour of rac-, Δ-, Λ-$[Ru(bpy)_2(dppz)]^{2+}$ into a Hg(II) modified DNA and its natural counterparts by absorption and fluorescence spectroscopy. Comparison of the data indicate that the observed differences derive rather from small structural changes of the DNA than from the electronic influence of the bound Hg(II) ions.

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P-21

Endothelial protective properties of HDL improve early after RYGB but not after diet treatment: the cardiometabolic benefits of RYGB beyond body weight loss

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Background: Roux-en-Y gastric bypass (RYGB reduces cardiovascular risk and mortality. **Purpose:** This study aimed to assess the effect of RYGB on high density lipoprotein cholesterol (HDL-C) concentration and endothelial-protective properties after RYGB in comparison with a hypocaloric diet.
Methods: Thirty-two obese patients with a body mass index (BMI) > 35 kg/m2 underwent RYGB and 30 followed a diet treatment. HDL was isolated before and 6 months after RYGB or diet. In endothelial cells stimulated with HDL, we quantified nitric oxide (NO) production by DAF-2 fluorescence and paraoxonase-1 (PON-1) antioxidant activity. Total cholesterol, low density lipoprotein (LDL-C), HDL-cholesterol (HDL-C), and triglycerides (TG) as well as glucose plasma fasting levels were measured.
Results: At baseline there was no significant difference in mean BW and BMI between the surgical (10 men and 22 women) and the diet (11 men and 19 women) patients (119.9 Kg; 42kg/m2 vs. 110.9 kg; 37.4 kg/m2, respectively, p=ns). After 6 months, the BW reduction was about 25.9 kg for the RYGB and 11 kg for diet group (P < 0.0001). At 6 months BW and BMI were not different between the 2 groups (94kg; 33 kg/m2 after RYGB and 99kg; 33.4 kg/m2 after diet, p=ns). In both groups, there was a significant reduction in mean total cholesterol and TG concentrations; instead, LDL-C concentration was significant reduced only after RYGB. Interestingly, a significant increase in HDL-C concentrations was observed after both interventions (1.25±0.5 0 vs 1.38±0.40mmol/L HDL-C after RYGB and 1.15±0.25 vs 1.33±0.33mmol/L after diet). Surprisingly, HDL-stimulated NO production and HDL-associated PON-1 activity, which were impaired at D0, increased at 6 months only after RYGB. Fasting glucose levels improved in both groups and were not different at 6 months between the 2 interventions.
Conclusions: Our study shows that although the lipid profile significantly improved after diet and RYGB induced BW loss, endothelial protective HDL properties were restored only in the surgery group even if the patients were still obese; this suggests that a certain level of body weight is not sufficient or critical per se to improve the protective properties of HDL, unless accompanied by yet unknown surgery-specific beneficial effects.

P-22

Loss of SIRT5 protects against atherosclerosis through an increase in reverse cholesterol transport

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Background: Atherosclerosis is a chronic progressive disease characterized by structural changes in the intima due to lipid deposition leading to plaque formation. Reverse cholesterol transport, a term used to describe cholesterol efflux to HDL particles is known to protect against atherosclerosis. Mice lacking SIRT5 have been shown to have higher plasma levels of HDL-cholesterol when fed a high-fat diet compared to wild-type mice.

Aim: We postulated that loss of SIRT5 in macrophages would protect against atherosclerosis through an increase in reverse cholesterol transport and reduced foam cell formation.

Methods & Results: *In vitro* experiments were performed using murine macrophage cell line RAW 264.7 cells. SIRT5 knockdown in RAW 264.7 macrophage cells causes an increase in ABCA1 gene expression with a concomitant increase in protein expression while, SIRT5 overexpression in RAW 264.7 reduces ABCA1 protein and gene expression. In line with this, ABCA1 expression is prevented with cycloheximide treatment upon SIRT5 knockdown, suggesting that SIRT5 transcriptionally regulates ABCA1 expression. Interestingly, SIRT5 knockdown in RAW 264.7 cells also causes a reduction in Dil-oxLDL uptake and foam cell formation along with a reduction in reactive oxygen species (ROS) generation.

Conclusion: Our findings identify ABCA1 as a novel downstream target of SIRT5 activity and highlight SIRT5 inhibition as a potential anti-atherosclerotic strategy.

P-23

Analysing the CRISPR-Cas9 mutation spectrum with crispRvariants

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The CRISPR-Cas9 system is an efficient method of introducing mutations into genomic DNA. A guide RNA directs nuclease activity to a 20 nucleotide target region, resulting in efficient mutagenesis. To realise the enormous potential of CRISPR-Cas9 genome editing, experimental conditions must be optimised to maximise mutation efficiency and eliminate off-target effects. This in turn requires that mutation frequencies, locations, and off-target effects are precisely quantified. Dedicated tools for visualising and quantifying CRISPR-induced mutations are currently lacking, despite many available tools for designing guide RNAs. To address this gap, we developed an R-based software package called crispRvariants that summarizes the spectrum of introduced variants, facilitating the exploration of mutagenesis patterns and understanding of genotype-to-phenotype correlations. crispRvariants provides tools for counting insertion/deletion variant allele combinations with respect to the nuclease cleavage site, interfacing with existing variant effect prediction tools and visualising variants and chimeric read alignments. crispRvariants works with a variety of experimental designs, from Sanger sequencing to high-throughput amplicon sequencing. With this toolkit, we aim to assist researchers in characterising the efficiency of their mutagenesis system using standard and high-throughput sequencing methods, assessing off-target effects, and ultimately understanding how the mutations are induced.

P-24

Towards engineered neuronal networks – from realization to modeling

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Understanding how the human brain stores and processes information is undoubtedly one of the grand challenges of this century. Despite of the vast amount of technical possibilities we still have very little understanding (and especially consensus) about e.g. learning, which might be partially due to the lack of well-defined, small "study networks" of real neurons that can be reproducibly and quantitatively analyzed at a few or single neuron level over extended periods of time. Realizing such bottom-up systems on multi-electrode arrays (MEA) makes a way to investigate the dynamics of functional neural networks systematically as a function of the network structure.

In order to gain insight into the information processing and neurocomputation of small networks, we realize neuronal systems with simple but controlled topology, study and model how the activity changes as a function of it, then explore the effect of directional connections and how these networks react to different stimuli. We start the experimental characterization of signal propagation between small groups of neurons with or without directional connections by measuring the delay times between the neuronal activities of the different groups. When changing the network topology from simple linear feed-forward type to circular ones including loops we expect to observe activity dependent systematic changes in the delay times due to long-term potentiation (memory effect).

By creating reproducible neuronal networks with well-defined topology, our vision is to bridge the gap between micro and macro, the molecular or few cell level and whole brain experiments. With the results, we aim to provide insights into the role of neuronal and synaptic properties such as synapse development, plasticity and connectivity, and extract the algorithms that support information processing and neurocomputation in brain tissue.

P-25

Short-time effects of colored light exposure on human body and brain physiology – a multimodal functional near-infrared spectroscopy study

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Although humans are constantly exposed to different light conditions, the short and long-term effects of colored light exposure on body and brain physiology are not yet fully investigated. Therefore, the aim was to measure reactions of the brain and body onto short-term colored light exposures with different colors (red, blue, green). Cerebral hemodynamics and oxygenation were measured using optical neuroimaging, i.e. functional near-infrared spectroscopy (fNIRS). We assessed cardiovascular, respiratory and skin biophysical parameters representing systemic physiology. **Fig. 1** depicts changes during blue light exposure in brain and body physiology exemplarily for one subject. Significant *subject*-specific as well as *color*-specific changes were observed. Implications for future fNIRS studies will be discussed.

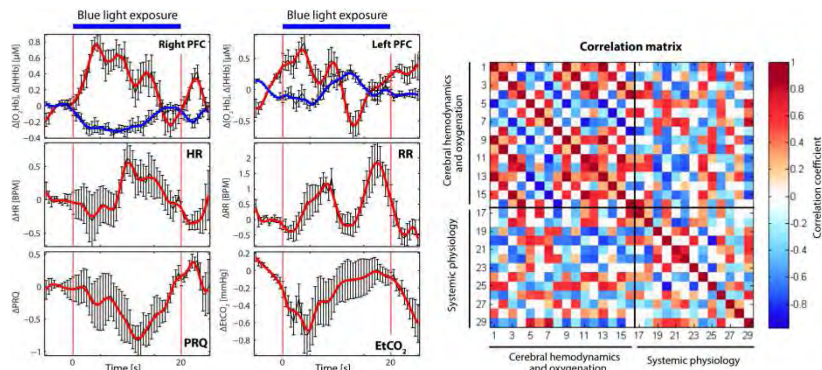

Fig. 1 Overview of changes in cerebral hemodynamics/oxygenation and systemic physiology (HR: heart rate, RR: respiration rate, PRQ: pulse-respiration quotient, $EtCO_2$: end-tidal CO_2) exemplarily for one subject. The right subfigure depicts the correlation matrix, i.e. the correlation coefficient between brain and systemic physiology signals.

P-26

Systems Biology of Beta-Amyloid production: Implications for Alzheimer's disease

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Objective: Alzheimer's disease (AD) is a memory disorder affecting mainly the elderly, for which the exact cause(s) are unknown. AD is divided into two categories, early onset and late onset. Both of which contain amyloid plaques and NFTs as the ultimate diagnostic marker. However we think, both forms of AD differ on molecular level. While early-onset AD is caused due to mutations in APP or presenilins, the molecular etiology of the late-onset AD, remains largely undiscovered. Currently we don't understand exactly how late-onset AD is caused, but we are aware that several factors can contribute to its genesis. It is these various genetic factors we are exploring using systems biology approaches.
Methods: To identify novel genes and pathways that regulate the amyloidogenic processing of APP and thereby could influence AD, we first developed and optimized a multiplexing screeening to detect sAPPβ and Aβ. This multiplexing assay allows us to immediately categorize hits into β-regulating or γ-regulating genes/drugs thereby giving us mechanistic insight of the process. We used this platform to screen various families of genes that could influence APP processing. To Identify and elucidate various key regulators of APP processing we used gene-silencing strategies using small interfering RNAs (siRNA) and performed genome wide screen of gene families. We also complemented our siRNA screens with activity inhibiting small molecule inhibitor screen.
Results: We identified a large set of kinases as regulators of APP processing. We also found that signaling networks regulate Aβ metabolism. Our results reveal that many genes regulate APP processing and many signaling networks and pathways regulate Aβ metabolism. We provide experimental validation of AD being a multifactorial syndrome rather than a single disease. Moreover, we believe that systems biology approaches will play a crucial role in our future understanding of networks involved in AD pathogenesis.
Conclusion: Elucidating novel mechanisms underlying APP processing give a clearer picture of AD and increase the number of protein targets available for potential drug development. Our results reveal many such genes and provide experimental validation of AD being a multifactorial disease.

P-27

Endothelial Function in Rheumatoid Arthritis

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Chronic inflammatory diseases such as rheumatoid arthritis (RA) are not only associated with pain and joint destruction, but also with increased cardiovascular (CV) risk beyond that of classical risk factors. Rheumatoid arthritis patients independent on all other risk factors (diabetes mellitus, obesity, smoking, etc.) are exposed to a high risk of cardiovascular complications. In patients with RA endothelium-dependent responses are impaired even in the absence or in presence of only few CV risk factors suggesting the RA involves alteration in endothelium[1]. However, RA molecular mechanisms causing endothelial dysfunction are poorly understood. hTNF alpha transgenic mice (Line 3647 and Line TG 197) at the age of 4 and 8 weeks, develop a stronger and milder form of arthritis, respectively[2]. The aim of the present study is first to confirm endothelial function impairment in these animal models and to address molecular mechanisms which lead to it. Furthermore, we aim to assess the effect of anti TNF alpha treatment on disease progression and potential to restore endothelial function in rheumatoid arthritis-impaired endothelium. The mechanisms of endothelial dysfunction in RA are of interest, both to understand the molecular mechanisms of endothelial and vascular dysfunction as well as for the search of novel therapeutics in RA to improve not only symptoms (i.e. joint pain, immobility etc.), but also outcome (reduction in major adverse cardiac events).

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P-28

Metal-Oxime Complexes as Potent OP-Inhibited Acetylcholinesterase Reactivators

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Acetylcholinesterase (AChE) is a serine hydrolase whose principal role is the termination of nerve impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine. AChE is irreversibly inhibited by organophosphorus compounds (OPs),[1] including toxic insecticides and lethal chemical warfare agents. OPs exert their acute toxicity through full inhibition of AChE, by forming covalently attached phosphorus conjugates with the hydroxyl group of a serine residue in the enzyme's active site.[2] Phosphylated AChE can be reactivated by strong nucleophilic agents such as the family of cationic pyridinium oximes.[3] The reactivator is able to cleave the covalent bond between the OP and AChE, restoring the enzyme activity; this reactivation process proceeds *via* a nucleophilic attack of the oximate anion on the P-O bond (Fig. 1). Under this context, a series of Zn(II)/pyridyloxime complexes has been previously synthesized and studied as AChE reactivators.[4] In this study, the reactivation potency of metal-oxime complexes with diverse structural characteristics has been tested *in vitro* and the results have been evaluated in the light of structure activity relationships.

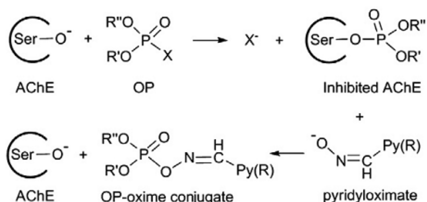

Fig. 1. The general reaction of inhibition of AChE by OPs and its reactivation by a pyridyloximate anion.[4]

Acknowledgments: KFK gratefully acknowledges the Swiss Federal Commission for a postdoctoral scholarship.

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P-29

Mismatch repair in recombination and oxidative damage response

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Mismatch repair (MMR) proteins play a key role in the processing of DNA base-base mismatches and insertion/deletion loops (IDLs) arising during DNA replication. MMR malfunction is associated with increased mutagenesis and carcinogenesis. The principal MMR factors are the mismatch recognition factors MutS (heterodimer of MSH2/MSH6) and MutS(heterodimer of MSH2/MSH3)[1]. Once activated, they recruit the downstream repair factors including MutL(MLH1/PMS2 heterodimer) for further repair processing[1]. However, MMR has additional, non-canonical roles, for example in the control of recombination fidelity, oxidative DNA damage response or antibody diversification[2]. We set out to study the molecular pathways involving MMR proteins in the hyper-recombinogenic chicken DT40 cell line. Interestingly, DT40 MMR mutants including $MSH3^{-}$, $MSH6^{-/-}$, $MSH2^{-/-}$, $MLH1^{-/-/-}$ and $PMS2^{-/-}$ are all slow-growing[3]. We hypothesised that this phenotype may be associated with illegitimate recombination events triggered by oxidative damage in MMR-deficient cells.

To address the involvement of oxidative damage in the slow-growing phenotype, we incubated the cells under hypoxic conditions (1% O_2). As we failed to observe a noticeable change, we are currently testing the hypoxic effect of MMR mutants in the sensitivity to DNA damaging agents (H_2O_2, Olaparib; parp inhibitor) and the foci formation of Rad51, a protein that plays a key role in recombination.

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P-30

Rare anemias from the technological perspective

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CoMMiTMenT stands for the “Combined Molecular Microscopy for Therapy and Personalized Medication in Rare Anaemias Treatments”. Together we aim to develop and new diagnostic system µCOSMOS combining optofluidic microscopy and scanning ion conductance microscopy for assessment parameters of individual red blood cells in patients' blood samples. Never before individual cells of patients with idiopathic hereditary anemia were sampled and probed for their response to shear stress, oxidation, osmotic stress. Calcium permeability and ion conductance as well as membrane topology can be assessed at the molecular resolution. These measurements will be performed using micromolar volumes of blood using the novel miniaturized technologies Optofluidic Microscopy-based Cell Sorting (OMiCS) and Scanning Ion Conductance Microscopy (SICM). A set of microfluidic chip-based modules is developed for rapid probing of cells for their responses to stress stimuli. The device will accumulate information on various forms of rare anemia and “learn” to recognize the new forms emerging. The prototype of the machine will be tested in two hematological clinics and two research labs working in red cell research. It will be used to assess the progress in a clinical trial in which memantine, a blocker of NMDA receptors, will be used to treat a small cohort of patients with sickle cell disease at the Division of Hematology of the University Hospital in Zurich. For more details and updates visit our project’s web-page: http://www.rare-anaemia.eu/

Acknowledgments: Project is funded from the EU Seventh Framework Programme for research, technological development and demonstration. Grant agreement No 602121.

P-31

Structural and Functional Characterization of Serine Palmitoyl Transferase in Mammals

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Objective:
Regulatory and signaling mechanisms that govern SPT function have been the focus of several recent studies, primarily in yeast cells. However, initial findings from the cultured mammalian cells suggest that mammalian SPT is subject to differential and complex regulations. Moreover, mechanistic changes leading to an increased activity or the shift in substrate preference of the SPT enzyme have not been worked out yet. In particular the factors which lead to the formation of neurotoxic 1-deoxySLs which are associated with peripheral neuropathies (HSAN1 or diabetic sensory poly neuropathy) is not understood. Here, we aim to identify the protein interactome of the functional SPT complex to determine the regulatory factors of the SPT holoenzyme and to characterize the biological relevance of the interactions in vivo.

Methods:
We are using genetically modified KO-cell lines, in combination with fluorescent tagged proteins and FRET as well as co-immunoprecipitation studies followed by mass spectrometry to elucidate SPT subunit interactions. Furthermore we challenge cells with pharmacologically and biologically stimuli to modulate sphingolipid synthesis to investigate the effect on the molecular and enzymatic properties of the SPT complex.

Results:
Methods to immuno-purify the SPT enzyme by targeting individual subunits have been established. Preliminary data show that specific mutations in certain SPT subunits influence regulation and alter the overall SPT activity and product spectrum.

Conclusions:
SPT activity is subject to complex regulations which might be mediated by direct modifications of subunits and the association and dissociation with the inhibitory and activating proteins.

P-32

Epstein-Barr Virus infection results in CD8$^+$ T cell dysfunction in a humanized mouse model

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Epstein-Barr virus (EBV) infection and EBV-associated malignancies are underrepresented fields of research despite affecting millions of people worldwide. While EBV infection mainly occurs asymptomatically in children in the third world due to earlier viral exposure, people in the Western world often contract EBV in their second decade of life or later as a severe primary infection called infectious mononucleosis (IM) that is slow to resolve and poses significant health risks. EBV$^+$ individuals are at increased risk of malignancies such as Hodgkin's lymphoma and post-transplant lymphoproliferative disease. Poor understanding of the in vivo pathogenesis of EBV and the lack of treatments available present a significant unmet medical need, mainly due to the strict human tropism of this virus. EBV-specific CD8$^+$ T cells from IM patient samples appear to express PD-1, a receptor that has been associated with poor immune control by T cells in a phenomenon known as exhaustion. We therefore wondered whether T cell exhaustion plays a role in EBV pathogenesis in vivo. We have examined human CD8$^+$ T cell exhaustion in EBV infection of NOD/scid/γ-chain deficient mice that have been reconstituted with human CD34$^+$ cells. These animals reconstitute most major human immune compartments. In response to EBV infection, they exhibit viral loads and expand CD8$^+$ T cells. Interestingly, CD8$^+$ T cells in infected animals upregulate multiple surface inhibitory receptors that have been associated with T cell differentiation or dysfunction, including PD-1, BTLA, KLRG1, 2B4, and Tim-3. Despite this, proinflammatory cytokine levels are elevated and dose dependent in response to EBV, indicating that T cells retain functionality despite the presence of inhibitory receptors. Efforts to determine the role of these receptors in EBV infection and tumorigenesis are currently underway.

P-33

Establishment of human neoplasia in mice

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A fundamental understanding of many biological processes in humans has stemmed from experimental studies in animal models, particularly in rodents. Using these models will elucidate not only key aspects of the development and regulation of the hematopoietic and immune systems but also allow both, research on the underlying pathophysiology of neoplastic diseases and preclinical predictive evaluation of new therapies.
Our recent studies take benefit of already in use but also newly developed mouse models and using these models we were able to demonstrate the followings: 1) the presence of ABL/BCR fusion protein simultaneously with BCR/ABL oncogene alters the phenotype of the leukemia disease from a CML-like disease to ALL-like disease in mouse. This effect seems to be related to the activation of p53 and GADD45a and the subsequent alteration in the hematopoietic stem cell targeted by BCR/ABL alone and could probably leads to the insensitivity of CML and ALL stem cell to the treatment; 2) a novel kinase inhibitor (PF114) could be evaluated and we were able to show that in BCR/ABL or BCR/ABL-T315I-driven syngeneic murine leukemia as well as in xenograft models of primary Ph+ leukemia harboring the T315I, PF-114 significantly prolonged survival to a similar extent as the third generation kinase inhibitor, Ponatinib. Currently we developed the 3rd generation of humanized mice upon demonstration that these mice are more suitable for the development and function of healthy human hematopoiesis, we now are evaluating the potential of these models to sustain primary human myeloid cell malignancies as e.g. myeloproliferative diseases, chronic myeloid leukemia, and Langerhans cell histiocytosis.

P-34

Tissue specific regulation of cell competition in development and ageing - Identifying regulators of the "*flower*-code"

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Vivid cell interactions in developing and adolescent tissues of our body are omnipresent and essential for the maintenance of our health. A process which allows cells to compare their fitness and elimination of unfit members of the cell population is called cell competition. Cell competition can improve the quality of a tissue or organ over time, preventing accumulation of unfit or damaged cells. In addition, cell competition is actively used in development to eliminate unwanted cells or define compartment borders as publications from our lab showed in the definition of the eye disk and maintenance of the stem cell niche in *Drosophila* ovaries. An essential component of the cell competition mechanism is the selective expression of different isoforms of the *flower* (*fwe*) gene. While ubiquitous expression of fwe^{UBI} is found in many tissues fwe^{Lose} isoforms are specifically upregulated in suboptimal cells that are soon after committing suicide by apoptosis. In this project we are defining the process of isoform selection with the aim to identify upstream regulators of *flower* and to explain tissue specific variations of the "flower code".

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P-35

Improving heterologous expression of functional eukaryotic integral membrane proteins in bacteria

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Integral membrane proteins (IMPs) have essential biological functions, constituting 30% of all proteins in humans. Most IMPs are inherently difficult to study, because of complications in obtaining adequate amounts of stable and functional proteins. Apart from a few exceptions, IMPs need to be heterologously overexpressed, typically in microbial hosts, such as *Escherichia coli*, *Lactococcus* or yeasts; mammalian or insect cells; or cell-free systems, yet to various degrees of success.

Our laboratory has investigated the expression of G protein-coupled receptors (GPCRs; the largest IMP family in the human genome and one of the most important class of drug targets in pharmaceutical discovery) in various hosts. Using *Escherichia coli* as the first choice for heterologous protein expression, our group has developed directed protein evolution methods, a functional selection strategy specifically for GPCRs and a novel technology termed CHESS (cellular high-throughput encapsulation, solubilization and screening) in order to generate stable and well expressing protein variants that retain functionality.

Nevertheless, frequently IMPs overexpressed in bacteria are toxic for the host. Unfortunately, neither the biosynthetic pathway nor the structural requirements on the protein are understood well.

We are currently studying how *E. coli* genes could regulate the heterologous production of eukaryotic IMPs, using GPCRs as a model system. In this way, we want to understand the details of the biosynthetic pathway and the influence of molecular structures.

P-36

Identification of LOAD susceptibility genes as modulators of Aß phagocytosis and degradation in microglia

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Mutations in many familial AD-associated genes are known to affect the production of the longer Aβ42 variant that preferentially accumulates in plaques. In the case of late-onset AD (LOAD), which accounts for more than 95% of cases, several genes are associated with increased risk of the disease (AlzGene database, containing about 600 risk genes for AD). However, our lab has recently shown that LOAD susceptibility genes do not specifically alter the Aβ42/40 ratios and suggest that these genes probably contribute to AD through distinct mechanisms. We hypothesize that these polymorphisms could also affect the clearance mechanisms in non-neuronal cells, including microglia. This opens the exciting possibility that the AlzGenes could affect microglia (the major phagocytes of the brain), thereby modulating their mechanisms of Aβ uptake and clearance.

Methods: To study the role of LOAD susceptibility genes on microglial function, RNAi-mediated silencing of 10 AlzGenes was performed in a microglial cell line, in a loss of function approach. In addition, 5 random genes, implicated in other neurodegenerative diseases, were used as control. The functional consequences of the selective knockdown were evaluated in respect to Aβ uptake from the extracellular space, intracellular Aβ degradation and lysosome function.

Result: From the phagocytic screen of the AlzGenes, along with control genes, we identified a new hit as a critical modulator of Aβ clearance, suggesting that loss of function mutations in these gene could promote the phagocytic activity, and vice versa, gain of function mutations could impair the mechanisms of Aβ clearance microglia-mediated.

Conclusion: Our preliminary findings support the hypothesis that LOAD could be predominantly caused by dysregulation in Aβ clearance, rather than by abnormal Aβ production, and that microglia cells could be responsible for such impaired mechanism. Genes likely to selectively modulate microglia phagocytic activity could thereby represent candidate players, conferring the risk for developing AD.

Swiss scientific society for developing countries: a concept of relationship

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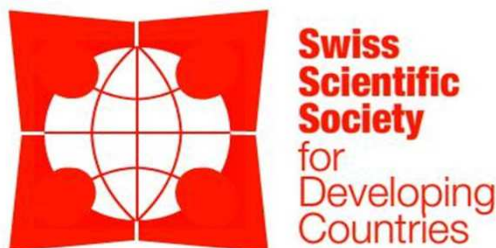

Cultural setup is varied from country to country and nation to nation, but the ability to adapt successfully to the new cultural setup may pave the way toward the development of cultural intelligence. To overcome differences may require to equip our personality with the ability to learn, exchange thoughts, and to have a constructive dream. Adaptation process can be accelerated if we effectively utilize our cultural diversity. This can be done through a unified body or society where people with common goals can collectively work to satisfy their values. Narrowing the gap between developed and developing countries is considered to be of prime interest. In order to be effective and productive we need to establish a neutral body where members are influenced by developed countries intellectuality and developing countries' personality. For this reason we opted to establish Swiss scientific society for developing countries (SSSDC) as a neutral, non-profit organization. With this organization we are able to promote communications, research, education and build trust, resolve conflict, and minimize the probability of misunderstanding between their partners. This allows achieving a better understanding that may assist in narrowing gaps and promote/strengthening relationship between Switzerland and developing countries. For more details about the Society, visit www.swisssdc.ch or email to info@swisssdc.ch

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