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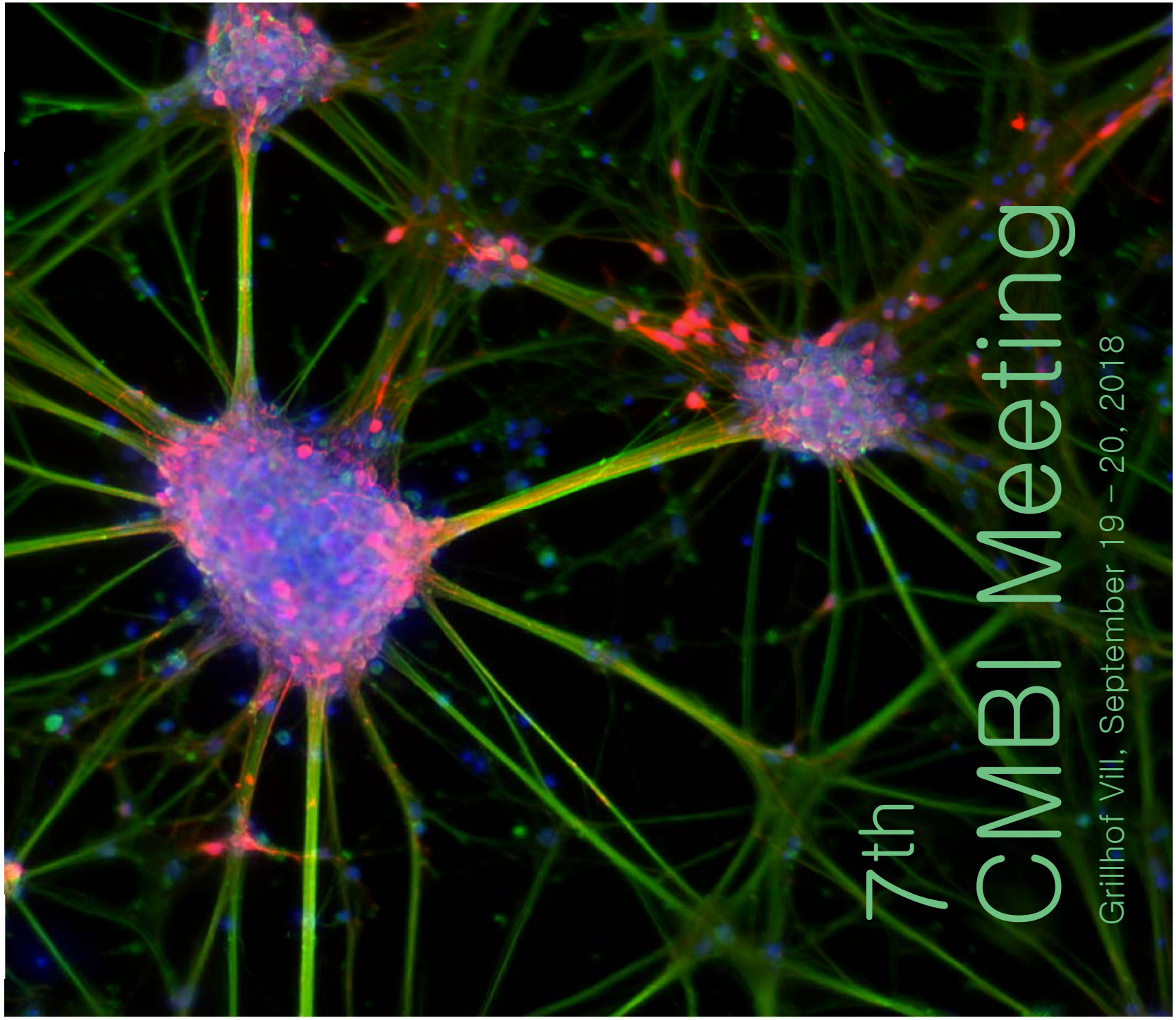

7th CMBI Meeting

Grillhof Vill, September 19 – 20, 2018

CMBI Center for Molecular Biosciences Innsbruck

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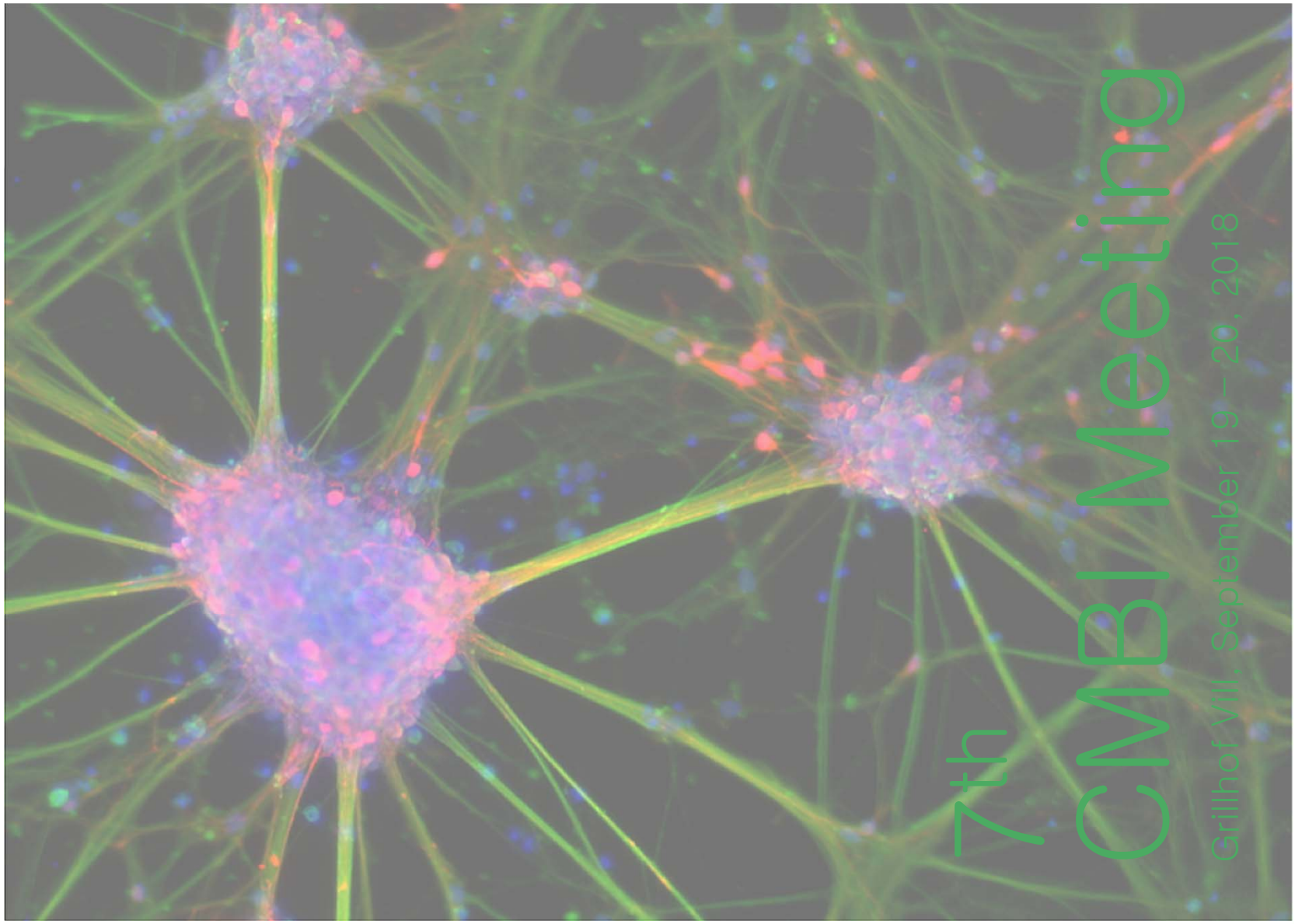
Program



7th CMBI Meeting

Grillhof VIII, September 19 – 20, 2018

Organizers: Bert Hobmayer, Ronald Micura, Jörg Striessnig

Meeting office: Gabi Reiter, CCB, University of Innsbruck
Innrain 80–82, 6020 Innsbruck
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CMBI Center for Molecular Biosciences Innsbruck

Wednesday, September 19, 2018

9:00 – 9:15	Opening remarks
9:15 – 10:15	Plenary lecture **Zlatko Trajanoski** Division of Bioinformatics, Medical University of Innsbruck ***Computing and probing cancer immunity***
10:15 – 10:45	Break
	Session 1: Signaling mechanisms Chair: Peter Ladurner Short talks
10:45 – 11:00	*Uncovering the Molecular Basis for the Roles of Gpr161 during Hedgehog Signalling* **Philipp Tschaikner**, Department of Molecular Biology
11:00 – 11:15	*Repression through Wnt signaling effectors* **Ute Rothbächer,** Department of Zoology
11:15 – 11:30	*Activation of β-catenin/Tcf signaling identifies Myc2 as the Hydra orthologue of the human MYC oncoprotein* **Markus Hartl,** Department of Biochemistry
11:30 – 11:45	*Different modes of FGF signaling regulation in zebrafish (Danio rerio) by the transcriptional factor Mixl1 downstream of Nodal signaling* **Patrick Fischer**, Department of Molecular Biology
11:45 – 12:00	*Regulation of Proliferation and Differentiation of Human Adipogenic Stromal/Progenitor Cells by Sprouty1* **Markus Mandl**, Institute for Biomedical Aging Research
12:00 – 13:45	Lunch

Wednesday, September 19, 2018

	Session 2: Methods and applications — Chair: Maren Podewitz Short talks
13:45 – 14:00	*Mouse Brain Tissue Imaging Using Desorption Electrospray Ionization (DESI) Mass Spectrometry* **Christina Meisenbichler,** Department of Organic Chemistry
14:00 – 14:15	*Studies towards the Total Synthesis of Mitrephorone A* **Lara Weisheit,** Department of Organic Chemistry
14:15 – 14:30	*Supercritical Fluid Chromatography – an alternative for natural products analysis?* **Markus Ganzera,** Institute of Pharmacy, Department of Pharmacognosy
14:30 – 14:45	*A Novel Mechanism for Temporary Bioadhesion* **Julia Wunderer,** Department of Zoology
14:45 – 15:00	*The Use of Vibrational Spectroscopy in Natural Product and Bio-Analysis: Current and Future Directions* **Christian Huck,** Department of Analytical Chemistry and Radiochemistry
15:00 – 15:30	Break
15:30 – 17:00	Poster session (uneven numbers – 1, 3, 5, ...)
17:00 – 17:30	Introduction lecture **Martina Höckner** Department of Zoology, University of Innsbruck ***Molecular responses to environmental stress in invertebrates***
17:30	Informal get together & dinner

Thursday, September 20, 2018

8:30 – 9:30	Plenary lecture **Hashim M Al-Hashimi** Department of Biochemistry, Duke University Medical Center, Durham North Carolina, USA ***Nucleic acid dynamics and the infidelity of information transfer across the central dogma of molecular biology***
	Session 3: RNA and protein structure-dynamics Chair: Christoph Kreutz Short talks
9:30 – 9:45	*Noncovalent RNA-ligand interactions probed by mass spectrometry* **Jovana Vušurović,** Department of Organic Chemistry
9:45 – 10:00	*Investigation on the structural and dynamic influence of 2-Methyladenosine and Dihydrouridine in tRNA* **Johannes Kremser,** Department of Organic Chemistry
10:00 – 10:15	*Atom-specific Mutagenesis Reveals Structural and Catalytic Roles for an Active-Site Adenosine and Hydrated Mg^{2+} in Pistol Ribozymes* **Christoph Falschlunger,** Department of Organic Chemistry
10:15 – 10:30	*Ferroptosis as new approach in cancer treatment – impact of iron salophene complexes* **Daniel Bäcker,** Institute of Pharmacy, Department of Pharmaceutical Chemistry
10:30 – 10:45	*The macromolecular architecture of PKA: interlinking metabolic pathways and cancer proliferation* **Andreas Feichtner,** Department of Biochemistry
10:45 – 11:15	Break
11:15 – 12:30	Poster session (even numbers – 2, 4, 6, ...)

Thursday, September 20, 2018

	Session 4: Neuron disorder and disease Short talks	Chair: Simone Sartori
12:30 – 12:45	*Role of L-type voltage-gated calcium channel Cav1.3 in the formation of persistent fear extinction-memory* **Anupam Sah,** Institute of Pharmacy, Department of Pharmacology and Toxicology	
12:45 – 13:00	*Morphological implications of the Neuroretina in Multiple System Atrophy (MSA): Does the Pathology of MSA manifest in Plp-α-SYN mice?* **Kathrin Kähler,** Institute of Pharmacy, Department of Pharmacology and Toxicology	
13:00 – 13:15	*Metabolic shift in Alzheimer-derived induced neurons* **Larissa Traxler,** Department of Molecular Biology	
13:15 – 13:30	*A Protein Interaction Linking two Diseases: Characterization of the Neurofibromin Spred1 interface* **Sebastian Führer,** Department of Organic Chemistry and Division of Biological Chemistry, MUI	
13:30 – 13:45	Award session and closing of the symposium	

Posters

P1*	*Generation of Neurons from Patient-Derived Cells to Model Network-Connectivity and the Metabolome in Schizophrenia* Fenkart G, Kruszewski K, Günther K, Mertens J & Edenhofer F
P2*	*Gain-of-function mutation of canonical Wnt signaling pathway promotes proliferation of human neural stem cells and aberrant neuronal differentiation* Strasser F, Rizzi S, Edenhofer F
P3*	*Profiling and functional modulation of voltage-gated calcium channels human stem cell-derived neurons* Marta Suárez-Cubero, Anna Hausruckinger, Katharina Kruszewski, Gerald Obermair, Frank Edenhofer
P4*	*Action of ß-Catenin in head- and foot-specific differentiation in regenerating Hydra polyps* Belinda Artes, Bert Hobmayer
P5	*Biological Adhesion of Flatworms* Pjeta R, Wunderer J, Salvenmoser W, Lindner H, Ladurner P
P6*	*Bioadhesion in the Ascidian Ciona intestinalis and the adhesive secreting collocytes* Zeng F, Wunderer J, Salvenmoser W, Hess MW, Ladurner P and Rothbächer U
P7*	*tBHP-induced senescence: a model to study molecular mechanisms of extrinsic skin aging* Sophia Wedel, Maria Cavinato, Pidder Jansen-Dürr
P8*	*In vivo monitoring of intracellular Ca^{2+} dynamics in the pancreatic beta cells of zebrafish* Reka Lorincz, Chris Emfinger, Andrea Walcher, Michael Giolai, Claudia Krautgasser, Robin Kimmel, Maria S. Remedi, Colin Nichols,, Dirk Meyer
P9	*Smoothened: 650 million years at the cilium* Dominik Regele, Greta Ebnicher, Philipp Tschaikner, Eduard Renfer, Bert Hobmayer, Ulrich Technau, Pia Aanstad
P10	*Hydrogen Peroxide under Hypoxia/Reoxygenation in Zebrafish Fibroblasts* Valentina Dikova, Julia Vorhauser, Bernd Pelster and Adolf Michael Sandbichler
P11*	*Functional characterization of $CD24^+$ and $CD24^-$ adipose stem/progenitor cells* Florian Hatzmann, Asim Ejaz, G. Jan Wiegers, Monika Mattesich, Marit E. Zwierzina, and Werner Zwerschke
P12	*Biochemical function of the tumor suppressor BASP1 in human cancer* Hartl M, Puglisi K, Raffeiner P, Bister K

Posters

P13*	*Function and architecture of the macromolecular Gpr161:PKA complex* Mayrhofer JE, Röck R, Bachmann V, Sunahara RK, Taylor SS, Michel Bouvier, Stefan E
P14*	*Surveillance of drug-driven BRAF conformations and interactions* Röck R, Mayrhofer JE, Torres-Quesada O, Enzler F, Raffeiner A, Huber R, Taylor SS, Troppmair J, Stefan E
P15*	*Novel Protein Kinase A substrates: cAMP oscillations regulate RNA-binding and ciliogenesis* Torres-Quesada O, Enzler F, Fuchs E, Micura R, Feliciello A, Stefan E
P16*	*FT-ICR MS and CAD as potential tools for the discovery of metal binding sites in RNA* Calderisi G, Breuker K.
P17*	*The effect of fixed-charge modifications on histone peptide fragmentation by ECD* Matthias Halper and Kathrin Breuker
P18*	*Developing Isotope Depletion Mass Spectrometry for the Analysis of Proteins* Michael Palasser, Kelly J. Gallagher, Sam Hughes, David P.A. Kilgour, and David J. Clarke
P19*	*Electron Attachment to OTfU: a novel potential radiosensitizer* Arthur-Baidoo E, Ameixa J, Meißner R, Makurat S, Kozak W, Ferreira da Silva F, Rak J, Denifl S
P20*	*Effectiveness of different isolated nitroimidazoles upon low energy electron interactions* Meißner R, Lochmann C, Feketeová L, Limão-Vieira P, Denifl S
P21	*A new pathway of Chlorophyll Breakdown in Fern* Theresia Erhart, Stefan Vergeiner, Bernhard Kräutler, Thomas Müller
P22	*Hydrogenobyric acid - the metal free ligand of B_{12}* C. Kieninger, J. A. Baker, F. J. Widner, A. D. Lawrence, E. Deery, K. Wurst, M. J. Warren, B. Kräutler
P23*	*Total Synthesis of Salimabromide: A Tetracyclic Polyketide from a Marine Myxobacterium* Schmid M, Grossmann AS, Wurst K, Magauer T
P24*	*Synthesis and incorporation of deazaadenosine nucleotides in RNA and their impact on RNA backbone cleavage* Elisabeth Fuchs, Kathrin Breuker, Ronald Micura

Posters

P25*	*SHAPE probing pictures Mg^{2+}-dependent folding of small self-cleaving ribozymes* Catherina Gasser, Jennifer Gebetsberger, Manuel Gebetsberger and Ronald Micura
P26*	*Synthesis of modified nucleoside phosphoramidites for atom-specific mutagenesis in small nucleolytic ribozymes* Maximilian Himmelstoß, Christoph Falschlunger, Ronald Micura
P27*	*Towards picturing of the ribosome–mRNA cap recognition of trypanosomatids* Josef Leiter, Ronald Micura
P28*	*Structure-based mutational analysis of the twister-sister ribozyme and implications on the cleavage mechanism* Elisabeth Mairhofer, Luqian Zheng, Marianna Teplova, Dinshaw J. Patel, Aiming Ren, Ronald Micura
P29*	*Structure-activity relationship of chemically modified ligands for gene regulation of engineered $preQ_1$ riboswitches in E.coli* Neuner E, Frener M, Lusser A, Micura R
P30*	*A site-specific TEMPO-labeling protocol via derivatization of functionally tethered oligonucleotides (FTOs)* Erharter K, Kreutz CH
P31*	*Resolving structural and functional properties of the pistol ribozyme by solution NMR spectroscopy* Juen M, Kreutz C
P32*	*Studying sparsely populated conformational states in RNA combining chemical synthesis and solution NMR spectroscopy* Felix Nussbaumer, Elisabeth Strebitzer, Johannes Kremser, Martin Tollinger, Christoph Kreutz
P33*	*Secondary structure probing of the Hatchet ribozyme by site-specific ^{15}N-labeling and solution NMR-spectroscopy* Plangger R, Kreutz C
P34*	*Do modifications of apple and hazelnut allergens with natural components alter their immunological behavior?* Unterhauser J, Führer S, Eidelpes R, Ahammer L, Meisenbichler C, Nothegger B, Covaciu C, Cova V, Letschka T, Eisendle K, Reider N, Müller T, Tollinger M
P35	*Kinetical studies on Gold-NHC-complexes: Insight into the mechanism of ligand scrambling reactions* Sina K. Götzfried, Caroline M. Gallati, Monika Cziferszky, Maren Podewitz, Klaus Wurst, Klaus R. Liedl, Ronald Gust
P36	*Investigations of the antiestrogenic profile exerted by bivalent estrogen receptor ligands* C. Kalchschmid, A. Knox, B. Kircher, C. Manzl, R. Gust

Posters

P37	*A new approach to overcome resistance in CML therapy: combination of imatinib and PPARγ modulators* Anna M. Schoepf, Stefan Salcher, Petra Obexer, Ronald Gust
P38	*Zeise's salt derivatives with Aspirin substructures: synthesis and biological evaluation* Alexander Weninger, Daniel Baecker, Victoria Obermoser, Dorothea Egger, Klaus Wurst and Ronald Gust
P39	*Peptide-based hydrogels as extended-release system for opioid analgesics* Dumitrascuta M, Martin C, Ben Haddou T, Edith O, Aquilino L, Bucher D, Hernot S, Schmidhammer H, Ballet S, Spetea M
P40	*Modulation of the kappa-opioid receptor by HS666 produces antiseizure/anticonvulsant effects without liability for aversion in mice* Erli F, Guerrieri E, Agostinho A, Schmidhammer H, Schwarzer C, Spetea M
P41	*The neuropeptidergic PACAP/PAC1 receptor system modulates behavioral and neuroendocrine stress reactions of rats within different forebrain areas* Veronica Fontebasso, Magali Basille, David Vaudry, Nicolas Singewald, Karl Ebner
P42	*Gating defects induced by a CACNA1D gain-of-function mutation linked to autism spectrum disorder* Laura Guarina, Nadja T. Hofer, Maryam S. Hashemi, Andrea Marcantoni, Nadine J. Ortner, Emilio Carbone, Jörg Striessnig
P43*	*Subtype-selective inhibitors of voltage-gated Cav2.3 Ca^{2+} channels to treat CNS disorders* Anita Siller, Toni Schneider, Jörg Striessnig, Nadine J. Ortner
P44	*Voltage-gated Calcium channels in retinal rod bipolar cells* Seitter, H., Kilicarslan, I., Tschugg, B., Obermair, G.J. and Koschak, A.
P45*	*Determination of the absolute configuration of sesquiterpenes from roots of Ferula hezarlalehezarica using electronic circular dichroism* Mostafa Alilou, Stefan Schwaiger, Hermann Stuppner
P46*	*Phytochemical and analytical study on Urceola rosea leaves* Hieu Nguyen Ngoc, Duc Trong Nghiem, Thi Linh Giang Pham, Hermann Stuppner, Markus Ganzera

*) Participation in poster prize competition

Uncovering the Molecular Basis for the Roles of Gpr161 during Hedgehog Signalling

Philipp Tschaikner[1,3], Dominik Regele[1,3], Johanna Mayrhofer[2,3], Stephan Geley[4], Eduard Stefan[2,3], Pia Aanstad[1,3]

Affiliations:
1: Institute of Molecular Biology, University of Innsbruck
2: Institute of Biochemistry, University of Innsbruck
3: Center for Molecular Biosciences Innsbruck
4: Division of Molecular Pathophysiology, Innsbruck Medical University

The Hedgehog (Hh) signalling pathway plays essential roles in cell fate specification and cell proliferation during embryonic development, and in adult tissue homeostasis, but how Hh signal transduction is regulated remains only poorly understood. The orphan G-protein-coupled receptor Gpr161 localizes to primary cilia, and has been suggested to act as a major negative regulator of the vertebrate Hedgehog signalling (Hh) pathway, by activating adenylate cyclase and Protein Kinase A (PKA), which antagonises Hh signalling. We have previously shown that Gpr161 can act as a selective, high-affinity anchoring protein of PKA, recruiting PKA to the primary cilium. However, the significance of this AKAP role of Gpr161 in Hh signalling remains unclear. In mouse, Gpr161 is essential for embryonic development and was linked to the development of severe forms of Hh-driven cancers such as medulloblastoma.
Here we present a novel genetic loss-of-function model of Gpr161 in zebrafish, generated by CRISPR/Cas9-mediated knock-out of both zebrafish *gpr161* paralogues.
Zebrafish *gpr161* mutants show that the role of Gpr161 as a negative regulator of Hh signalling is conserved among vertebrates, as they display many hallmarks of aberrant Hh signalling such as severe defects during craniofacial development, patterning of the brain and the eye. On a molecular level we show that Hh target genes are strongly expanded, and that the number Hh-dependent cell types, such as the superficial slow fibers of the myotome, are largely increased in *gpr161* mutants. However not all Hh-responsive processes are equally affected which suggests distinct tissue- and context specific roles for Gpr161 during embryogenesis.
This newly created zebrafish line will help to complete the understanding of the consequences of a loss of Gpr161, while rescue experiments with different mutant forms of Gpr161 promise to be a useful tool to investigate the distinct roles of Gpr161 on a cell biological level.

Repression through Wnt signaling effectors

Ritter B[1], Kari W[1], Prantl, V[1], Oda-Ishii I[2], Satou Y[2], Rothbächer U [1]

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[2]Department of Zoology, Kyoto University, Japan

We have recently discovered in the tunicate *Ciona intestinalis,* a novel mode of direct transcriptional repression by the Wnt signaling effectors TCF/ß-catenin that lacks canonical TCF DNA binding motifs but rather involves GATA binding sites [1] The resulting opposite transcriptional target gene activation in daughters of asymmetrically dividing precursors assures mutual exclusiveness and robustness of binary cell fates in the segregating tissues. The repressive mechanism, we have described in both, *Ciona* and *C. elegans*, are similar regarding the formation of a repressive complex between TCF/ß-catenin and another transcription factor to be repressed, i.e. Ref-2 in nematode neural precursors [2] and Gata.a in ascidian early blastomeres [3]. Notably, Gata.a binding to its target sites weakened in the presence of both, Tcf7 and ß-catenin, however, a direct competitive DNA binding was not considered likely as of EMSA or genomewide ChIP assays.
By *ex vivo* DNA pulldown and reporter gene activity assays on representative sets of GATA site variants and within different target genes we monitored for previously observed repressive differences. We now refine the initial model in a qualitative and quantitative way concerning the signature around the target GATA binding sites and concerning the role of Tcf7 at certain types of GATA sites. In addition we are working on the isolation of the repressive co-factor complex using GATA binding site pulldowns followed by Mass Spectrometry. Overall, we aim towards an advanced repression model that can more generally explain for how ubiquitous transcription factors are variably restricted by Wnt effectors to activate (or not) selected repertoires of target genes in defined regions of the developing embryo, a process likely important in stemness or cancer.

Acknowledgement**:** This work was supported by the Austrian Academy of Sciences (ÖAW DOC 23593) and the Nachwuchsförderung University Innsbruck, Austria.

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[2] Murgan, S, Kari, W, Rothbächer, U, Iche-Torres, M, Melenec, P, Hobert, O and Bertrand, V. *Developmetal Cell* **33**, 737-745 (2015).
[3] Oda-Ishii I, Kubo A, Kari W, Suzuki N, Rothbächer U, Satou Y. *PLoS Genetics* **6**, e1006045 (2016).

Activation of β catenin/Tcf signaling identifies Myc2 as the Hydra orthologue of the human MYC oncoprotein

Hartl M[1,4], Glasauer S[5], Gufler S[2,4], Raffeiner A[1,4], Puglisi K[1,4], Breuker K[3,4], Bister K[1,4], Hobmayer B[2,4]
Institutes of [1]Biochemistry, [2]Zoology, and [3]Organic Chemistry; [4]Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Austria; [5]University of Zurich, Switzerland (present address)

The c-Myc protein (MYC) represents a transcription factor with oncogenic potential controlling fundamental cellular processes. Genes homologous to the c-*myc* protooncogene (*myc1*, *myc2*) have been recently identified and characterized in the early diploblastic cnidarian *Hydra* [1-4]. The ancestral *Hydra* Myc1 and Myc2 proteins display the principal design, biochemical properties, and basic oncogenic potential similar to their vertebrate derivatives, suggesting that important MYC functions arose very early in metazoan evolution. MYC is part of a transcription factor network that is regulated by several upstream acting pathways implicated in oncogenesis and development [5, 6].

One of these signaling cascades is the Wnt/β-catenin/Tcf axis driving cell differentiation processes and organismal patterning, but also tumorigenic processes, where this signaling pathway ends in aberrant transcriptional activation of the c-*myc* (*MYC*) gene e.g. in colon cancer [7]. Here we show that similar to Myc1 and Myc2, the ancestral *Hydra* β-catenin has intrinsic oncogenic potential.

Furthermore, stimulation of β-catenin signaling or β-catenin overexpression in *Hydra* is accompanied by specific downregulation of *myc1* at mRNA and protein levels. The *myc1* and *myc2* promoter regions contain consensus binding sites for the transcription factor Tcf. *Hydra* Tcf binds to both regulatory regions but the *myc1* promoter is specifically repressed in the presence of ectopic *Hydra* β-catenin/Tcf. Hence, *Hydra myc1* is a negative Wnt signaling target, in contrast to the vertebrate *MYC* genes. *myc2* on the other hand is not suppressed by ectopic β-catenin in *Hydra* and presumably represents the structural and functional *MYC* orthologue. The close connection between Wnt signaling proteins and the *Hydra myc1* and *myc2* genes suggests that principal connections between this signaling pathway and MYC have emerged very early in evolution.

This work was supported by the Austrian Science Fund (FWF) grants P23652 and 20734.

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[2] Ambrosone A, Marchesano V, Tino A, Hobmayer B, Tortiglione C. *PLoS One* 7:e30660 (2012).
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Different modes of FGF signaling regulation in zebrafish (Danio rerio) by the transcriptional factor Mixl1 downstream of Nodal signaling

Fischer P[1], Huang A[1,3], Pacho F[1], Regele D[1], Chen H[1], Rieder D[2], Meyer D[1]
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The gastrulation and hence formation of the three germ layers – endoderm, mesoderm and ectoderm – is an important step in the development from single cell to multicellular organism. Mainly TGF-beta/Nodal, FGF (fibroblast growth factor) and BMP (bone morphogenetic protein) signaling pathways are required for the patterning. A failure causes severe defects up to the death of the animal.

Nodal signaling is needed for endoderm and mesoderm formation whereas FGF signal blocks endoderm and forces mesoderm. Both pathways are cross linked but less is known about the mechanisms behind it.

In this study we focus on the role of Nodal-regulated homeodomain transcriptional factor Mixl1. Former studies showed that endoderm formation is blocked in *mixl1* mutants but can be rescued by downregulation of FGF signaling. In micro-arrays and RT-qPCR studies we find *fgf3* and *fgf4* – two genes encoding for ligands of FGF signaling – highly up-regulated in *mixl1* mutants. We decided to create new loss of function mutants of *fgf3* and *fgf4* via TALEN or CRISPR/Cas9 technique to examine the role of both for mesendoderm formation. The mutations were sufficient to enhance endoderm formation in *mixl1* mutants. As the rescue was not 100% the regulation of *fgf3* and *fgf4* expression could not be the only joint between Nodal and FGF signaling.

Indeed, besides this mode of cross talk we find the FGF signaling inhibitor *dusp6* downregulated in *mixl1* mutants. Re-injection of *dusp6*-mRNA in *mixl1* mutants was also able to enhance endoderm development. We could show via ChIP data, RT-qPCR analyses and *in-situ* hybridizations that *dusp6* is a direct target of Mixl1.

Our findings present a new regulatory connection between Nodal and FGF signaling via Mixl1. We propose a model in which at one hand Mixl1 leads to the expression of *dusp6* causing down-regulation of FGF signaling and on the other hand to the downregulation of *fgf3* and *fgf4* in a yet unknown mechanism. In *mixl1* mutants expression of *dusp6* is impaired and FGF signaling is up-regulated. An additional loss of *fgf3* and/or *fgf4* can partly attenuate the resulted block of endoderm formation.

Regulation of Proliferation and Differentiation of Human Adipogenic Stromal/Progenitor Cells by Sprouty1

Markus Mandl[1], Sonja Wagner[1], Monika Mattesich[2] and
Werner Zwerschke[1]

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[2]Department of Plastic and Reconstructive Surgery, Innsbruck Medical University, Innsbruck, Austria

Sprouty1 (*SPRY1*) is a negative regulator of mitogen-activated protein kinase (MAPK) signalling and expressed in a tissue specific manner. Sprouty1 expression is stimulated by growth factors which bind to receptor tyrosine kinases (RTK) and trigger the MAPK cascade. Thus, Sprouty1 induction constitutes a negative feedback loop. Recent studies revealed a major role of this protein in stem cell maintenance and aging. However, the importance of Sprouty1 in human adipose stem cells is unknown.
To address the role of Sprouty1 in adipogenesis we use an *ex vivo* model of primary human adipogenic stromal/progenitor cells (ASCs). ASCs are propagated in low mitogenic medium or stimulated to differentiate into mature adipocytes by addition of appropriate hormones.
Sprouty1 knockdown augmented MAPK signaling in proliferating ASCs upon treatment with growth factors. Sprouty1 loss-of-function was accompanied by an anti-proliferative effect and triggered cellular senescence.
Monitoring of Sprouty1 during adipogenic differentiation revealed an increase of mRNA expression until day 3 after induction of adipogenesis while Sprouty1 protein levels were decreased at the same time point. Silencing of Sprouty1 impaired adipogenic differentiation whereas overexpression showed less pronounced effects. In order to confirm these findings, we recently employed a CRISPR/Cas9 approach which revealed similar results.
Taken together, this ongoing project indicates a novel regulatory role of Sprouty1 in adipose tissue physiology.

Acknowledgement: This project is supported by SWAROVSKI.

Mouse Brain Tissue Imaging Using Desorption Electrospray Ionization (DESI) Mass Spectrometry

Meisenbichler C[1], Sealey M[2], Konrat R[2] and Müller T[1]

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[2]Department for Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna, Austria

Mass spectrometry imaging (MSI) has emerged as a powerful technique in biological sciences. In MSI, the chemical identity of compounds present in tissue sections is investigated as a function of spatial distribution.
Desorption electrospray ionization mass spectrometry (DESI-MS) is an ambient mass spectrometry technique that allows direct sampling of surfaces with no or little sample pre-treatment in the open air. [1,2]
We investigated the use of DESI mass spectrometry imaging (DESI-MSI) as an analytical tool for lipid profiling and imaging of mouse brain tissue embedded in a matrix of water-soluble glycols and resins for cryostat sectioning. The interfering OCT (optimum cutting temperature) matrix was removed by performing a washing step prior to DESI-MSI on a Thermo LTQ ion trap mass spectrometer equipped with a lab-built 2D DESI source. Capillary size, angles and distances between sprayer, sample and mass spectrometer inlet, as well as solvent composition and solvent flow rate were optimized to increase the lateral resolution and to obtain high quality ion images. Data were processed using open source software packages. Results will be discussed.

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The Use of Vibrational Spectroscopy in Natural Product and Bio-Analysis: Current and Future Directions

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The field of molecular vibrational spectroscopy applied to natural product and bio-analysis is further developing very dynamically. Whereas traditional separation and mass spectrometric (MS) techniques offer analytical investigations with high selectivity and sensitivity, vibrational spectroscopy benefits from the short analyses times, non-invasiveness and the possibility to screen for chemical and physical properties simultaneously. Furthermore, chemometrical univariate and multivariate data treatment enables efficient spectral interpretation and the establishment of sufficient calibration/validation models. Advanced quantum chemical approaches can further support the challenge of band assignment, especially in case of complex overlapping peak patterns. Near-infrared (NIR, 4.000-10.000 cm^{-1}), attenuated total reflection (ATR, 400-4.000 cm^{-1}) and Raman spectroscopy have been demonstrated being very efficient for even complex qualitative and quantitative attempts in combination with selective reference analytical methods. Qualitative attempts comprise analysing, e.g., species and in some cases also origin, quantitative analysing chemical and physical parameters. Two-dimensional correlation spectroscopy (2D-COS) has been developed towards a powerful analysis tool for monitoring the dynamics of a spectrometer system [1]. The miniaturization of spectrometers is a highly demanding trend, enabling to carry out investigations at any independent place. Imaging and mapping spectroscopic attempts (MIR, NIR, Raman) enable high-resolution analysis of potent ingredients down to approximately 4 µm and 1 µm, respectively [2]. This contribution highlights recent advances of molecular spectroscopy in natural product and bioanalytical research. The latest technical developments will be discussed followed by several selected applications in food, medicinal plant and cancer analysis. Their limits and advantages over traditional methods will be critically evaluated to point out the future trends.

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Mouse Brain Tissue Imaging Using Desorption Electrospray Ionization (DESI) Mass Spectrometry

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Mass spectrometry imaging (MSI) has emerged as a powerful technique in biological sciences. In MSI, the chemical identity of compounds present in tissue sections is investigated as a function of spatial distribution.
Desorption electrospray ionization mass spectrometry (DESI-MS) is an ambient mass spectrometry technique that allows direct sampling of surfaces with no or little sample pre-treatment in the open air. [1,2]
We investigated the use of DESI mass spectrometry imaging (DESI-MSI) as an analytical tool for lipid profiling and imaging of mouse brain tissue embedded in a matrix of water-soluble glycols and resins for cryostat sectioning. The interfering OCT (optimum cutting temperature) matrix was removed by performing a washing step prior to DESI-MSI on a Thermo LTQ ion trap mass spectrometer equipped with a lab-built 2D DESI source. Capillary size, angles and distances between sprayer, sample and mass spectrometer inlet, as well as solvent composition and solvent flow rate were optimized to increase the lateral resolution and to obtain high quality ion images. Data were processed using open source software packages. Results will be discussed.

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Supercritical Fluid Chromatography – an alternative for natural products analysis?

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The supercritical stage of a substance is reached above its critical temperature and pressure, the resulting fluid shows liquid like density and the viscosity of a gas. It has high solvating power but creates less backpressure, so that it is considered as an ideal mobile phase for chromatography.
The potential of SFC for natural products analysis is highlighted in this presentation. Based on successfully conducted studies in our laboratory it will be shown that the separation of diverse compound classes is possible with high speed, separation efficiency and ease. After a short introduction to the technique itself, a few selected applications describing the analysis of alkaloids in *Cinchona* bark [1], anthraquinones in rhubarb [2], and isoflavones in different medicinal plants [3] will indicate the potential of SFC. Exemplarily, the latter describes the baseline separation of nine compounds (aglyca and glycosides) in less than 8 min, method validation showed excellent determination coefficients higher than 0.999 and a maximum on-column detection limit of 0.2 ng (injected sample volume was 1 µl). Like for the other SFC assays presented, the target analytes could be quantified in plant extracts with excellent precision (intra-day variation ≤ 2.1%) and repeatability (σrel ≤ 2.6%). In terms of technical performance there are no differences to state of the art techniques like UPLC; however, the use of an environmental friendly, "green" mobile phase renders SFC unique and preferable actually.

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A Novel Mechanism for Temporary Bioadhesion

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Biological adhesion is widely spread among the animal kingdom. Adhesives secreted by marine invertebrates show remarkable performances and provide great potential for biomedical and industrial applications. Whilst permanent adhesive systems of mussels, barnacles, and annelids have been investigated into detail over the last couple of years, nothing is known about the adhesive substances used for temporary adhesion of flatworms.The marine flatworm Macrostomum lignano, features a simple adhesive system, which allows the animal to attach and release several times within a minute. We could show that attachment of M. lignano relies on the secretion of only two large adhesive proteins, Macrostomum lignano adhesive protein 1 (Mlig-ap1) and 2 (Mlig-ap2). The expression of Mlig-ap1/2 in the adhesive gland cells has been shown with in situ hybridization. Antibody- and Lectin staining revealed their spatially restricted secretion as footprints. RNAi knock-down experiments proved the essential function of the two proteins in adhesion. The glycosylated Mlig-ap2 attaches to the substrate and Mlig-ap1 connects Mlig-ap2 with the glycocalyx of the animal. Notably, we demonstrate that negatively charged sugars inhibit attachment and animals cannot adhere to hydrophilic surfaces. Positively charged molecules, on the other hand, inhibit the natural release. In summary, we propose the first model for flatworm temporary adhesion. Our findings lead to a better understanding of an effective reversible adhesive system with great biomimetic potential for the future.

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Noncovalent RNA-ligand interactions probed by mass spectrometry

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Interactions of ribonucleic acids (RNA) with other RNAs, proteins, and small molecules play a key role in many biological processes. Ligand binding to RNA frequently involves salt bridges (SB) between deprotonated phosphodiester moieties and protonated, basic functionalities such as the guanidinium group of arginine (of, e.g., HIV-1 tat and Rev proteins) or the amine groups of aminoglycosides (e.g., paromomycin). We have previously shown that the electrostatic interactions between TAR RNA and a peptide comprising the arginine-rich binding region of tat protein are sufficiently strong in the gas phase to survive phosphodiester backbone cleavage of RNA by collisionally activated dissociation (CAD), thus allowing its use for probing tat binding sites in TAR RNA by mass spectrometry (MS).[1] Here we address the question of how many and what types of interactions are required to stabilize the binding interface of RNA-ligand complexes. As model systems for our study on the stability of noncovalent interactions, we used seven 8 nt RNAs of differing sequence and six dipeptides (GR, VR, DR ER, KR, and RR), a tripeptide (NGR), and the fixed charge ligand tetramethylammonium. All experiments were performed on a 7 Tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an electrospray ionization source. RNA-ligand complexes were electrosprayed from 1 µM RNA and 100 µM ligand solutions in 1:1 H_2O/CH_3OH at pH ~7.5.
For tetramethylammonium and RR, CAD of the RNA-ligand complexes produced fragments from phosphodiester backbone bond cleavage (with and without ligand attached) but no free RNA, indicating preservation of the noncovalent intermolecular interactions at energies that were sufficiently high to cleave covalent backbone bonds. For all other RNA-peptide complexes, both covalent and noncovalent bond dissociation was observed. The branching ratio between these two channels increased with the number of possible intermolecular hydrogen bonds (HB). We attribute the increase in stability of the RNA-ligand complexes to stabilization of salt bridge structures by HB networks that prevent proton transfer (PT). In the absence of such stabilization, PT from protonated ligand to deprotonated RNA converts SB into far weaker HB interactions.[2,3] E_{50} values for noncovalent and covalent bond dissociation from energy-resolved experiments allowed us to quantify the number of HB interactions required for SB stabilization during binding site mapping by CAD.

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Investigation on the structural and dynamic influence of 2-Methyladenosine and Dihydrouridine in tRNA

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Post-transcriptional modified nucleotides play crucial roles to fine-tune structural and dynamic features tRNAs. In many cases, however, the molecular details how these modifiers alter the folding landscape of RNA remain elusive.
In this research project, we want to shed light on the physicochemical properties and functions of 2-methyladenosine (m^2A) and dihydrouridine (DHU) within tRNAs. The synthesis of stable isotope labeled phosphoramidite building blocks of both modifications for RNA solid phase synthesis as well as their incorporation into oligonucleotides was achieved. These precursors were incorporated into target RNAs to investigate base pairing properties and internal dynamics via NMR spectroscopy. In case of m^2A, a modification prevalent at position 37 in iso-acceptor tRNAs [1], NMR data revealed a Watson-Crick like base pair with in a 35 nucleotide (nt) long hairpin. The solution state WC like base pairing is additionally supported by crystallographic studies.
In contrast, DHU, one of the most common RNA modification in all three domains of life, tends to disrupt helix formation due to its elevated conformational flexibility and the loss of planarity through saturation of the double bond between C5 and C6 [2]. DHU's role as a flexible hot-spot was confirmed by NMR spectroscopy on a 15 nt long hairpin mimicing the D-arm in a S. pombe initiator $tRNA^{Met}$. By the incorporation of a 5,5,6-D_3-6-^{13}C-DHU as well as 1'-^{13}C-DHU residue we could demonstrate motional dynamics both in the nucleobase and the sugar moiety, with a clear preference of the C2'-endo sugar pucker.

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Atom-specific Mutagenesis Reveals Structural and Catalytic Roles for an Active-Site Adenosine and Hydrated Mg^{2+} in Pistol Ribozymes

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The discovery of twister, twister sister, pistol and hatchet self-cleaving ribozymes by comparative genomic analysis has reinvigorated research to explore the chemical strategies RNA utilizes to catalyze chemical reactions.[1] We solved the crystal structure of a pre-catalytic state of the pistol RNA motif that reveals a guanosine (G40) and an adenosine (A32) close to the G53–U54 cleavage site.[2] While the N1 of G40 is within 3.4 Å of the modeled G53 2′-OH group that attacks the scissile phosphate, thus suggesting a direct role in general acid–base catalysis, the function of A32 has been less clear. We now present evidence from atom-specific mutagenesis that neither the N1 nor N3 base positions of A32 are involved in catalysis. By contrast, the ribose 2′-OH of A32 seems crucial for the proper positioning of G40 through an H-bond network that involves G42 as a bridging unit between A32 and G40. We also found that disruption of the inner-sphere coordination of the active-site Mg^{2+} cation to N7 of G33 makes the ribozyme drastically slower. A mechanistic proposal is suggested, with A32 playing a structural role and hydrated Mg^{2+} playing a catalytic role in cleavage.[3] This scenario is further supported by our recent structure that pictures the post-catalytic state of the pistol ribozyme at 2.9 Å resolution.

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Ferroptosis as new approach in cancer treatment – impact of iron salophene complexes

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Ferroptosis appears as a promising strategy for cancer therapy because it is associated with tumorigenesis. Moreover, activation of regulated cell death reveals a fundamental and beneficial approach for cancer treatment. [1]

Ferroptosis is an iron-dependent form of non-apoptotic cell death, which results in the accumulation of reactive oxygen species (ROS). Catalysis of the lipid peroxidation and the consequent arising of damaging radicals is feasible by redox-active iron ions according to the Fenton's reaction. [2] Thus, sufficient availability of cellular iron is essential, whereas uptake is obtained by binding of ferrous compounds to transferrin and subsequent endocytosis via the transferrin receptor 1. [3]

Iron complexes bearing Schiff-base ligands such as the representative [*N,N'*-bis(salicylidene)-1,2-phenylenediamine]chloridoiron(III) [Fe(III)salophenCl], are considered to cause their effects by induction of ferroptosis. In order to optimize its pharmacological profile, various derivatives of [Fe(III)salopheneCl] were synthesized and tested for anti-leukemic effects using different leukemic cell lines.

The compounds concentration-dependently reduced the proliferation and the metabolic activity of all examined cell lines. Furthermore, they induced late apoptosis / necrosis and the generation of ROS. The cellular effects of the complexes were completely abolished upon simultaneous administration of the necrosis or ferroptosis inhibitors Necrostatin-1 or Ferrostatin-1, respectively.
The involvement of ferroptosis in the mode of action is unique since it is yet described as effect of $Fe^{2+/3+}$ ions. To exclude iron release from the complexes, stability was studied under physiological conditions on the example of [Fe(III)salophenCl], whereby no degradation even in the presence of Ferrostatin-1 or the iron chelator deferoxamine took place. Moreover, the extent of [Fe(III)salophenCl] to bind apo-transferrin was quantified and revealed a high degree of binding, thus indicating a transferrin-mediated uptake.
In conclusion, induction of ferroptosis can be suggested as a new approach for anti-leukemic treatment as demonstrated with the present iron salophene complexes.

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The macromolecular architecture of PKA: interlinking metabolic pathways and cancer proliferation

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Cellular membrane receptors sense and convert the vast array of extracellular input signals and transmit information through intracellular signaling circuits [1]. To spatiotemporally control the flow of information in the cell, central signaling nodes are inevitable. Hereby, diverse kinase scaffolding proteins play leading roles by interlinking receptors and intracellular effectors. Deregulation in kinase pathways can lead to the etiology and progression of cancer. Activating mutations in the Gα-protein lead to cAMP-dependent PKA activation which has recently been shown to be deregulated in colon and glioblastoma cancers [2, 3]. We set out to determine the phosphoproteomic composition of macromolecular PKA complexes in diseased human cells and tissues [4]. We hypothesize that elucidation of whole kinase interaction networks will help to explain the molecular basis for deregulated kinase activities. First, we performed affinity isolations along with a subtractive phosphoproteomic approach to establish a PKA protein interaction network [5]. Therefore we affinity-isolated PKA complexes from patient derived colon cancer cell lines and human Glioblastoma tissue biopsies. Second, we used MS/MS analyses to identify enriched phosphoproteins, and bioinformatic data analyses to unveil proliferation relevant substrates. Third, we classified key regulators of metabolic pathways such as glycolysis and lactate metabolism which we now analyse in the context of pharmacological perturbation and cancer cell proliferation.

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Role of L-type voltage-gated calcium channel Cav1.3 in the formation of persistent fear extinction-memory

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Extinction-based exposure therapy is used to treat anxiety- and trauma-related disorders; however, there is the need to improve its limited efficacy in individuals with impaired fear extinction learning and to promote greater protection against return-of-fear phenomena. L-type voltage-gated calcium channels (LTCCs) have been implicated in both the formation and the reduction of fear through Pavlovian fear conditioning and extinction respectively. We and others have previously shown that dihydropyridine (DHP) LTCC antagonists, such as nifedipine impair the extinction of conditioned fear without interfering with its acquisition. Cav1.2 and Cav1.3 are predominant LTCCs in the mammalian brain, but their individual contribution to fear memory formation/extinction is unknown since no isoform-selective DHP blockers are available. Identification of the LTCC isoforms involved in fear learning and in particular extinction is an essential basis to reveal their role as potential drug targets for the treatment in extinction impaired individuals. In order to elucidate the role of isoform specific contribution in fear and extinction we used a unique mouse model expressing DHP-insensitive Cav1.2 LTCCs (Cav1.2DHP(-/-) mice) to address this question. In the present study we selectively activated the Cav1.3 channel by systemically administering BayK-8644 (Ca2+ channel activator) in the Cav1.2DHP-/- mice. Our findings reveal that select activation of Cav1.3 channels following fear conditioning enhanced long-term fear memory in the Cav1.2DHP-/- mice. Different to fear learning, select activation of Cav1.3 channel prior to extinction training had no effect on extinction learning. Activating the Cav1.3 channel following extinction training even impaired extinction consolidation in the Cav1.2 DHP-/- mice. Based on these findings, we next selectively blocked the Cav1.3 channel following extinction training using nifedipine in Cav1.2DHP-/- mice and observed facilitation of extinction memory consolidation together with long-term fear inhibition. Together our findings suggest that distinct Cav1.3 mediated mechanisms are being recruited for acquisition and extinction of fear memories, respectively. Interestingly, if confirmed in additional experiments, blocking of Cav1.3 channels could be used as a neuroenhancer strategy to augment the effect of exposure based therapy without the risk of alleviating fear memories while on treatment. Based on our findings we are currently investigating whether selective inhibition of Cav1.3 can normalize aberrant extinction learning capacity in an animal model that displays impaired fear extinction acquisition and extinction consolidation.

Morphological implications of the Neuroretina in Multiple System Atrophy (MSA): Does the Pathology of MSA manifest in Plp-α-SYN mice?

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Neurodegenerative diseases like Parkinson's disease (PD) and Multiple System Atrophy (MSA) have been shown to exhibit physiological and morphological neuronal abnormalities. These abnormalities can be related to exceeding aggregation of α-synuclein (α-SYN), a 140aa presynaptic protein that exerts toxic function in case of dysregulation and has been shown to be the disease hallmark of PD and MSA in the brain. Both PD and MSA are associated with a variety of visual symptoms and a potential role of the retina as a biomarker for the progression of PD is being discussed. This hypothesis is also supported by recently published data that have reported the number of retinal ganglion cells (RGCs) to be significantly reduced in MSA patients' peripheral retinae. To investigate whether changes can also be observed in a transgenic mouse line, we aimed at studying retinal changes in homozygous transgenic mice overexpressing human α-SYN under the proteolipid protein (PLP)-promoter (PLP-α-SYN) compared to wild type (WT) animals of two different age groups (two months (adult), one year (aged)). Immunohistochemical analyses on retinal wholemounts using RBPMS showed a significantly reduced RGC density in the peripheral MSA retina of adult animals, a finding that is in line with data from MSA patients (Mendoza-Santiesteban et al., 2017). Neither the microglial marker Iba1 nor glial fibrillary acidic protein (GFAP) expression was upregulated, leading to the assumption that no neuroinflammatory events occur in adult MSA mouse retinas. We observed distinct immunoreactivity of human α-SYN in different retinal cell layers of PLP-α-SYN. This is remarkable as the PLP promotor driving the α-SYN expression in oligodendrocytes was reported to be inactive in the retina. PLP expression stopped at the optic nerve/retina junction, where we observed a colocalization with α-SYN. Notably there was one cell type distinctly stained in the central, but even more in the peripheral part of the inner retina that we identified to be rod bipolar cells. Additionally, aged mice exhibited even stronger signals for human α-SYN and showed enhanced GFAP expression in the peripheral retina of MSA as compared to WT mice, which might reflect the stronger impairment of the retina as the condition progresses. Taken together our findings clearly implicate an impairment of retinal neurons in the PLP-α-SYN MSA model, which may reflect the recent findings in MSA patients.

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Metabolic shift in Alzheimer-derived induced neurons

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Aging is the major risk factor for neurodegenerative diseases like Alzheimer's Disease (AD). Nevertheless, non-human as well as human iPSC-based models of AD do not recapitulate human aging and often utilize external stressors to complement for aging. In contrast to iPSCs, induced neurons (iNs) maintain the key features of aging during their conversion from fibroblasts such as nuclear pore and mitochondrial dysfunction, which might play essential roles in brain aging and the onset of neurodegenerative diseases. Therefore, iNs represent an attractive patient-specific human neuronal model system for studying age-related neurodegenerative diseases such as Alzheimer's disease. In this project, we generate iNs from a large set of fibroblasts derived from sporadic AD patients and age-matched controls. Transcriptomic RNA-Seq data from 24 patients and controls showed that genes and splice variants involved in glycolysis are upregulated in AD patients, while genes involved in mitochondrial oxidative phosphorylation as well as neuronal terminal differentiation and maturity were found repressed. These data are indicative of a metabolic switch in AD patient-derived iNs, that might be connected to a slightly dedifferentiated neuronal phenotype. Metabolome data and first functional assessment supports our transcriptional data as we could show an increase in glycolysis and ROS associated changes in AD.

A Protein Interaction Linking two Diseases: Characterization of the Neurofibromin Spred1 interface

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Neurofibromatosis type I (NF1) and Legius syndrome are rare inherited diseases which share mild symptoms like skin fold freckling and café-au-lait spots, whereas an increased risk to develop severe tumors of the peripheral and central nervous system is only associated with NF1. The *NF1* gene, mutated in NF1, encodes the 320 kDa neurofibromin protein, whereas Legius syndrome is caused by mutations in the *SPRED1* gene encoding the 50 kDa sprouty-related EVH1 domain-containing protein 1 (Spred1). Both proteins function as negative regulators of the Ras/MAPK signaling pathway, where neurofibromin acts as a Ras specific GTPase-activating protein (Ras-GAP) and Spred1 as a neurofibromin recruitment factor. The N-terminal Ena/VASP Homology1 (EVH1) domain of Spred1 binds to cytosolic neurofibromin and the complex relocates to the membrane dependent on the Spred1 C-terminal sprouty-related domain.
In a previous study, we mapped the Spred binding site to the GAP-related domain of neurofibromin (NF1-GAP) and identified the GAP_{ex} subdomain as being critical for EVH1 binding. Here we present results from a current approach where we apply heteronuclear NMR spectroscopy in order to characterize the protein contact sites in more detail. So far, we have obtained resonance assignments of the wild type Spred1(EVH1) domain and of a Legius syndrome patient derived mutated variant (Thr102Arg). Introduction of the mutation weakens the interaction between neurofibromin and Spred significantly but does not alter the topology previously determined for EVH1 domains. Thus, this mutant form is suitable for NMR titration experiments and we have identified residues of Spred1 which are involved in NF1-GAP binding. NMR spin relaxation experiments allowed the identification of flexible regions in the EVH1 domain which may be regulatory elements in cellular protein contacts. In a second approach, we minimize the binding region in NF1-GAP to short peptides with the aim to get structural information of a small complex representing the interacting regions between neurofibromin and Spred1.
Our data support a basic understanding of molecular mechanisms involving the Ras regulatroy proteins neurofibromin and Spred1 in rare human cancer-related diseases.

Molecular responses to environmental stress in invertebrates

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Organisms are frequently exposed to environmental stress, which is defined as an action, agent, or condition that impairs the structure or function of a biological system. Therefore, molecular stress response mechanisms have evolved ranging from the activation of gene expression to metabolic adaptations. Adverse effects, coping strategies, and regulatory mechanisms have been analyzed mainly in response to cadmium, a carcinogenic heavy metal, which is one of the main sources of soil pollution. Because of their soil dwelling lifestyle and important ecological role in terrestrial ecosystems, earthworms have been used as model organisms. Detoxification mechanisms, the interplay with the immune system and adapted metabolism regarding the provision of energy will be presented herein.

Computing and probing cancer immunity

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Recent breakthroughs in cancer immunotherapy and decreasing costs of high-throughput technologies sparked intensive research into tumour-immune cell interactions using genomic tools. However, the wealth of the generated data and the added complexity pose considerable challenges and require computational tools to process, analyse and visualise the data. Recently, a number of tools have been developed and used to effectively mine tumour immunologic and genomic data and provide novel mechanistic insights. In this talk I will first review discuss computational genomics tools for mining cancer genomic data and extracting immunological parameters. I will focus on higher-level analyses of NGS data including quantification of tumour-infiltrating lymphocytes (TILs), identification of tumour antigens and T cell receptor (TCR) profiling. Additionally, I will address the major challenges in the field and ongoing efforts to tackle them.

In the second part I will show results generated using state-of-the-art computational tools addressing several prevailing questions in cancer immunology including: estimation of the TIL landscape, identification of determinants of tumor immunogenicity, and immunoediting that tumors undergo during progression or as a consequence of targeting the PD-1/PD-L1 axis. Finally, I will propose a novel approach based on perturbation biology of patient-derived organoids and mathematical modeling for the identification of a mechanistic rationale for combination immunotherapies in colorectal cancer.

Nucleic acid dynamics and the infidelity of information transfer across the central dogma of molecular biology

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Tautomeric and anionic Watson-Crick-like mismatches play important roles in replication and translation errors through mechanisms that are not fully understood. Using NMR relaxation dispersion, we resolved a sequence-dependent kinetic network connecting G•T/U wobbles with three distinct Watson-Crick mismatches consisting of two rapidly exchanging tautomeric species (G^{enol}•T/U⇌G•T^{enol}/U^{enol}; population <0.4%) and one anionic species (G•T^{-}/U^{-}; population ≈0.001% at neutral pH). Inserting the sequence-dependent tautomerization/ionization step into a minimal kinetic mechanism for correct incorporation during replication following initial nucleotide binding leads to accurate predictions of dG•dT misincorporation probability across different polymerases, pH conditions, and for a chemically modified nucleotide, and provides mechanisms for sequence-dependent misincorporation. Our results indicate that the energetic penalty for tautomerization/ionization accounts for ≈10^{-2}-10^{-3}–fold discrimination against misincorporation, which proceeds primarily via tautomeric dG^{enol}•dT and dG•dT^{enol} with contributions from anionic dG•dT^{-} dominating at pH ≥8.4 or for some mutagenic nucleotides.

P1

Generation of Neurons from Patient-Derived Cells to Model Network-Connectivity and the Metabolome in Schizophrenia

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SPIN – Signal processing in neurons

Schizophrenia (SCZ) is a devastating psychiatric disorder that appears in late adolescence with a prevalence of 0.5-1% worldwide. SCZ is characterized by a loss of contact with reality and a disruption of thought, perception, mood and movement [1]. Studies on monozygotic (MZ) twins have revealed that, although sharing the same genome, around 50% of SZ patients have a non-affected twin, suggesting an environmental contribution to the disease [2]. Investigations on the metabolome in plasma of SZ patients have shown that aberrations in biosynthetic pathways may contribute to the mechanisms of the disease [3]. Cellular reprogramming of patients' cells into pluripotent stem (iPS) cells or induced neurons (iN) provide a promising cell source to study the patient specific metabolome and connectivity of neurons in SCZ. Using the iPS cell-based approach, diminished neuronal connectivity, decreased neurite number, PSD95-protein levels and glutamate receptor expression were revealed in neurons differentiated from iPS cells [4]. However, a sub-type specific detailed analysis of the connectome and metabolome is lacking.
Here, we perform metabolic analyses of cortical neurons [5] of SCZ patients and controls to gain a comprehensive unbiased insight into the metabolic components of the SCZ brain. Pilot experiments with undifferentiated neural stem cells will be shown using bioinformatics analysis of metabolites. Neuronal connectivity in CamKII expressing glutamatergic neurons is assayed using monosynaptic spread of a rabies virus deleted in G. In a further step, CamKII is replaced by the dopamine receptors 1 and 4 to reach a necessary specificity of the assay. Preliminary results will show the regulation of CamKII during the development of the brain and expression of dopaminergic receptors in neurons derived from neural stem cells.

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P2

Gain-of-function mutation of canonical Wnt signaling pathway promotes proliferation of human neural stem cells and aberrant neuronal differentiation

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The three identified Wnt signaling pathways are essential signal transduction pathways in embryonic development, involved in the regulation of cell fate specification, proliferation, cell migration and axis formation. It has been previously shown that activation of Wnt-signaling accelerates proliferation and supports maintenance of pluripotency in pluripotent stem cells. Moreover, it has been demonstrated that depletion of c-Myc and N-Myc, the major Wnt target genes, causes biosynthetic dormancy in pluripotent stem cells, in which cells keep their differentiation potential, but stop proliferation [1].
This phenotype appears to be conserved in hematopoetic stem cells, however, the role of Wnt/b-catenin signaling in neural stem cells (NSC) is poorly investigated. Here we assess the impact of canonical Wnt signaling on NSC proliferation by generating a functional knockout of the b-catenin-destruction-complex component APC employing CRISPR/Cas genome editing technology in human induced NSCs [2]. For control we use pharmacological inhibition of Wnt-signaling by small molecules in mutant and control cells. We used a double gRNA strategy to induce two double strand breaks in the APC-gene, resulting in a 64 bp deletion and frameshift. Transfection of NSCs with Nucleofector™ technology resulted in generation and isolation of 12 mutant clones, 3 of which are currently under investigation.
We show that loss of function mutation in the APC gene seems sufficient to sustain self renewal in NSCs in Wnt-agonist-free conditions indicating essential role for Wnt signaling in NSCs. Differentiation analyses of mutated clones reveal aberrant neuronal differatioin capability as compared to control cells. Presentation will include detailed analyses on Wnt-target genes
A deeper understanding of Wnt-signaling in NSCs might allow for more stable cultivation and improvement of (existing) differentiation protocols for use in disease modelling and on the way to therapeutic use of patient-specific neural stem cells.

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P3

Profiling and functional modulation of voltage-gated calcium channels human stem cell-derived neurons

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Voltage-gated calcium channels (CaVs) are involved in the regulation of various neuronal functions, such as neurotransmitter secretion, postsynaptic signal integration and neuronal plasticity. CaV dysfunction is implicated in the pathophysiology of neurological diseases in humans, such as schizophrenia, epilepsy and Alzheimer's disease (AD). Current research mainly depends on the analyses of heterologous expression systems or primary animal neurons that are far from being ideal to correlate with the physiology of human CNS. Recent progress in cellular reprogramming enables for the virtually unlimited derivation of patient-specific neural cells from readily accessible tissues including skin and blood. Here we set out to determine a comprehensive mRNA expression profiling of CaV subunits in iPS-derived neural stem cells (NSCs) and the neuronal progeny derived thereof. Moreover, we aim to investigate to which extent the modulation of CaV subunits has an impact on the synaptogenesis. iPS-derived NSCs were differentiated into neurons for up to 10 weeks and CaV subunit expression was quantified using real-time qPCR. This analysis revealed upregulation of L-type CaV subunits 1.2 and 1.3 from 2 weeks on whereas mRNA encoding CaV1.1 and CaV1.2 was poorly detectable. Moreover, we found CaVa2d1 highly expressed throughout all stages of differentiation as compared to about 10-fold lower expression of a2d2 and a2d3, respectively. The a2d4 subunit was hardly detectable in NSC-derived neural progeny. In order to assess CaV impact on synaptic plasticity we transiently overexpressed *CACNA2D1* and *CACNA2D2* in iPS-derived neurons. Preliminary results indicate an increase in spine and growth cone formation as well as enhanced neuronal branching turned out to be enhanced. These results suggest that iPS-derived neurons serve as reliable model to study CaV function in human cells.

P4

Action of ß-Catenin in head- and foot-specific differentiation in regenerating *Hydra* polyps

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Maintaining the self renewal capacity of a stem cell or initiating the differentiation of a cell are fundamental mechanisms in regeneration processes and need to be orchestrated precisely. In the ancestral freshwater polyp *Hydra* the canonical Wnt pathway is known to initiate head organizer formation, to play a significant role in positional information along the single body axis, and to act in the pre-patterning phase in head regenerates. ß-Catenin was shown to act as early response gene within the first 30 minutes after decapitation. Here, we present data using the small molecule inhibitor iCRT14, which is able to inhibit ß-Catenin-Tcf interaction in the nucleus and thereby down-regulates canonical Wnt signaling. Treatment of multi-headed ß-Catenin transgenic animals with iCRT14 results in a rescue towards a normal wild type phenotype. Treatment of regenerates shows that both head and foot regeneration depend on nuclear ß-Catenin activity and that a block of ß-Catenin responsive transcription represses the terminal differentiation of head- and foot-specific cells. Furthermore, ß-Catenin and ß-Catenin induced target gene expression remains upregulated in head and foot regenerates when ß-Catenin responsive transcription is inhibited by iCRT14. We therefore propose, that a gene regulatory ß-Catenin activity is required for a transition from pre-patterning to position-specific differentiation. In addition, the spatiotemporal differences of transcriptional and translational profiles of the head-specific factor HyBra1 provide evidence that a translational control mechanism is involved at the onset of a position-dependent differentiation program.

P5

Biological Adhesion of Flatworms

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The flatworm adhesive system consists of few to hundreds adhesive organs, composed of three cell types - an adhesive gland cell which secretes the glue, a releasing gland cell which is secreting the detachment component, and an anchor cell, a modified epidermal cell with long enforced microvilli for structural stabilization. Flatworms occur in diverse habitats from marine to freshwater environments, in calm waters but also wave exposed beaches. Certain parasitic flatworm taxa use adhesive pads with secretions to attach to the host. We aim identifying novel proteins that are involved in biological adhesion and release in 20 diverse flatworm species using transcriptomics, differential gene expression, in situ hybridization screening, RNA interference, antibody- and Lectin staining, combined with Mass Spectrometry and light - and electron microscopy. The selected species cover the major taxa of the Platyhelminthes from marine, brackish, and freshwater habitats as well as parasitic species. Furthermore, the selection includes species occurring in low tide regions, in high energy beaches, in lakes, in calm freshwater environments, on animals or plants. We have established a laboratory model system for the proseriate flatworm species *Minona ileanea* and identified the respective adhesive proteins. We hypothesize that flatworms have evolved adaptations to meet the requirements for adhesion in the respective environment.

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P6

Bioadhesion in the Ascidian *Ciona intestinalis* and the adhesive secreting collocytes

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Marine bioadhesion research has increasingly inspired the design of biomimetics for tissue compatible glues in the medical field and antifouling compounds for shipping and aquaculture. Tunicates (ascidians) are not only important, because of their phylogenetic position, being the sister group to vertebrates, but also ecologically they are one of the major biofoulers massively populating artificial surfaces like ship hulls causing serious damage. In recent years, it therefore became of great interest to understand how bio-adhesion is functioning in ascidians. We use the ascidian *Ciona intestinalis* as model organism to study the settlement and adhesive properties of their larvae. Recent research demonstrates that larval adhesion organs (papillae) of *Ciona intestinalis* mainly consist of glue secreting collocytes, papillary neurons and supporting cells [1]. However, the exact secretion mechanism, and the molecules involved are poorly understood. We have performed serial-section electron microscopy and confocal imaging of the *Ciona* papillae using various marker combinations to precisely distinguish papillar cell types, define their exact numbers and describe their subcellular and molecular characteristics [2]. Furthermore, we successfully established CRISPR/Cas9 towards the loss of function analyses of papillae associated genes. Finally, we plan to biochemically analyse (Protein pulldown, Mass-spectrometry) candidate adhesion proteins in parallel to their functional analysis. Our findings contribute to the understanding of how adhesive secreting cells develop and function in tunicates which has general implications for developmental biology, cell differentiation, evolution and bioadhesion.

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P7

tBHP-induced senescence: a model to study molecular mechanisms of extrinsic skin aging

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Skin aging is influenced by intrinsic factors and extrinsic factors, like UV light, tobacco smoke and air pollution. The molecular mechanisms that underlie the changes of the skin's integrity and appearance upon exposition to these extrinsic factors are scarcely understood. Consequently, it is of great importance to develop new models that help us to improve our understanding of this matter. We have established a model for studying the mechanisms of extrinsic skin aging by treating human dermal fibroblasts and reconstructed human skin equivalents with eight sublethal doses of the organic peroxide *tert*-butyl-hydroperoxide (tBHP). The oxidative stress injury and depletion of antioxidant defense resulting from tBHP treatment are similar to the effects of tobacco smoke and air pollution on skin. Furthermore, tBHP is used as a plastic hardener in the plastic industry and is emitted into the environment itself. We were able to show that tBHP treatment led to proliferative arrest, increase in senescence-associated β-galactosidase activity and to changes in morphology in human dermal fibroblasts. Cell cycle progression was inhibited by activation of p53 and pRB pathways. Additionally, we found that exposure to the organic peroxide increased intracellular ROS, persisting in the cells even after the phase of treatment ended. RNAseq results provided a broad spectrum of different genes, significantly up- or downregulated upon administration of tBHP and senescence-induction. These genes are mainly known to be involved in apoptosis resistance, extracellular matrix (ECM) remodeling or are part of the ECM, leading the way for further experiments and providing a deeper insight into molecular mechanisms triggered by oxidative stress inducing extrinsic skin aging factors. In human skin equivalents treatment with tBHP reduced the granular layer and increased thickness of *stratum corneum*, which suggested that tBHP also had impact on a more complex skin aging model.

P8

In vivo monitoring of intracellular Ca^{2+} dynamics in the pancreatic beta cells of zebrafish

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Understanding the biological function of gene variants associated with increased Type 2 Diabetes (T2D) risk requires test systems for monitoring their effect on endocrine cell function and insulin dynamics. Studying the response of pancreatic islet cells to different stimulation is important for understanding cell function in healthy and disease states. Here, we introduce transgenic zebrafish expressing genetically-encoded *in vivo* sensor for addressing such question in native pancreatic islet of living animals. Using a zebrafish line encoding a membrane localized beta-cell specific GCaMP6s Ca^{2+} sensor, we demonstrate *in vivo* and *ex vivo* assays to examine Ca^{2+} fluxing in intact islets or in individual beta cells in response to glucose, free fatty acid or amino acid challenges. Wild-type zebrafish pancreatic beta cells show rapid fluorescence signal alteration of GCaMP6s sensor in response to intraperitoneal injection of D-glucose to zebrafish larvae. Fluorescence signal alteration was not detected neither before injection of the component, nor after injection of L-glucose. $Ca_V1.2$ channel mutant in combination with the GCaMP6s imaging line serve as a prove of principle to assure the physiological usefulness of GCaMP6s sensor in relation with disease phenotypes. Furthermore, *in vivo* imaging and *ex vivo* physiological studies done on islets from Cav1.2 mutant will help to determine requirements of these channels in glucose-induced insulin secretion. This approach to monitor the intracellular Ca^{2+} dynamics in the pancreatic beta cells for the first time in native pancreas, *in vivo* will expand the understanding of beta cell physiological function and the disease state.

P9

Smoothened: 650 million years at the cilium

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Hedgehog (Hh) signalling plays essential roles in embryonic development and adult tissue homeostasis. It controls cell specification, proliferation and migration during embryogenesis, and in the development and progression of several human cancers. Although the core components of the Hh pathway are conserved across the eumetazoans, differences in signalling mechanisms have been identified. A defining feature of vertebrate Hh signalling is the compartmentalisation of the pathway components to the primary cilium. Defects in ciliogenesis lead to impaired Hh signalling, and key components like Smoothened (Smo), the Hh signal transducer protein, requires ciliary localisation to function correctly. In contrast, Hh signalling in *Drosophila melanogaster* does not require cilia, suggesting that the connection between Hh signalling and cilia arose in the vertebrate lineage.

To investigate whether the role of primary cilia in Hh signalling is limited to vertebrates, we isolated the *Smo* homologue from the sea anemone *Nematostella vectensis*. *Nematostella* belongs to the Cnidarians, which split off from the bilaterian lineage about 650 million years ago, and is the most basal animal possessing all core components of the Hh pathway. We show that, despite extensive sequence divergence, *Nematostella* Smo localises to motile cilia in *Nematostella*, and can also localise to primary cilia of zebrafish and mammalian cells, suggesting that the involvement of cilia is a basal feature of Hh signalling. Further, we show that Nv Smo can activate the vertebrate Hh signalling pathway, and partially compensate for loss of Smo function in zebrafish mutant embryos. The differences observed in downstream effects upon overexpression of vertebrate and *Nematostella* Smo will allow us to dissect the molecular mechanisms of vertebrate Smo activity.

P10

Hydrogen Peroxide under Hypoxia/Reoxygenation in Zebrafish Fibroblasts

Valentina Dikova, Julia Vorhauser, Bernd Pelster and Adolf Michael Sandbichler

Cellular response to hypoxia is a multifaceted process including physiological and molecular events. Especially the involvement of reactive oxygen species (ROS) under low oxygen conditions is still under debate ranging from being an important hypoxia signaling molecule to being a major toxic byproduct of restricted cellular respiration. This discrepancy may arise from the difficulty of measuring individual intracellular ROS species and the short-lived nature of ROS due to the conversion activity of different redox enzymes. Therefore, the ability to follow specific ROS levels, especially peroxide levels, in real-time in live cells is a prerequisite to unravel some of the hypoxia- and reoxygenation-induced events.
This study applied roGFP2-Orp1, a pH-insensitive ratiometric fluorescent protein biosensor for peroxide, to follow H_2O_2 emergence in a live-cell imaging setup. The sensor was transfected in a zebrafish embryonic fibroblast cell line (Z3) and was measured in a live-cell imaging setup (Cellasic Onix, MerckMillipore) with a laser scanning microscope. Additionally we measured oxygen consumption rate (OCR) and extracellular acidification rate to align metabolic responses with intracellular redox behavior. Different than expected, two-hour intermittent hypoxia led to a decrease in H_2O_2 in mitochondria. Using several mitochondrial inhibitors which all reduced OCR we could show that for this mitochondrial H_2O_2 decrease to occur under hypoxia, (a) no functional mitochondrial oxidative phosphorylation (OXPHOS) is necessary, (b) mitochondrial uncoupling does weaken the response and (c) diphenyleniodinium (DPI) omits the response. Since DPI also inhibits the pentose phosphate pathway which generates reducing potential in response to ROS stress the source for the observed H_2O_2 decrease under hypoxia appears to be upstream of OXPHOS. This is supported by the observation that effective detoxification of H_2O_2 is dependent on glycolysis.

P11

Functional characterization of CD24$^+$ and CD24$^-$ adipose stem/progenitor cells

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White adipose tissue (WAT) is not only a major energy storage depot, but also an endocrine gland [1]. Adipose derived stem/progenitor cells (ASCs) are essential for adipose tissue regeneration und homeostasis [2]. These cells can be isolated from the stromal vascular fraction (SVF) of adipose tissue. The SVF is a heterogeneous cell population that consists of pericytes, endothelial cells, hematopoietic cells, and several other cell types besides ASCs [3]. A set of cell surface proteins can be employed to characterize the immunophenotype of ASCs [4]. However, the functions of these proteins in ASCs remain to be elucidated. The glycophasphatidylinositol-linked cell surface receptor CD24, that is expressed by various stem cell populations and thought to play a role in cell proliferation and differentiation, is one interesting candidate for a functional cell surface marker of ASCs [5]. We isolated CD45$^-$/CD31$^-$/DLK1$^-$/CD34$^+$/CD24$^+$ and CD45$^-$/CD31$^-$/DLK1$^-$/CD34$^+$/CD24$^-$ ASC subpopulations and present data on the functional characterization of these cells.

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P12

Biochemical function of the tumor suppressor BASP1 in human cancer

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The MYC protein is a transcription factor with oncogenic potential converting multiple cellular signals into a broad transcriptional response, thereby controlling the expression of numerous genes important for cell proliferation, metabolism, differentiation, and apoptosis. Constitutive activation of MYC leads to neoplastic cell transformation and tumorigenesis. MYC is a major cancer driver, and elevated MYC protein levels are present in 60-70% of all human tumors.
We have previously shown that the signaling protein and transcriptional co-repressor BASP1 is specifically downregulated by the viral *MYC* oncogene. Moreover, we discovered that BASP1 interferes with viral MYC-induced cell transformation [1]. Recent reports confirmed BASP1 suppression, and malignant growth inhibition by ectopic BASP1 in several human tumor cells including breast cancer or leukemia [2-4].
Mutational analyses had revealed that the myristoylated eleven-amino acid BASP1 effector domain, which interacts with the intracellular calcium sensor calmodulin (CaM), is sufficient for the inhibitory function of BASP1. Recently, we found that CaM also interacts with MYC in a calcium-dependent manner, and that ectopic CaM increases MYC's transactivation and transformation potential [5]. Here we show that BASP1 sequesters CaM from oncogenic MYC. Mutational analysis of the highly conserved BASP1 effector domain revealed that CaM binding capacity and transformation inhibition potential correlate with each other. Experiments to test if BASP-1-mediated MYC inhibition is reversible by excess CaM are currently performed. Because BASP1 elicits tumor suppressor activity in human cancer cells [2-4], small compounds or peptides based on the BASP1 effector domain structure could be tested and applied to expand the spectrum of therapeutic approaches for treating malignant tumors in which MYC is aberrantly activated.

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P13

Function and architecture of the macromolecular Gpr161:PKA complex

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Scaffolding proteins organize the information flow from activated G protein-coupled receptors (GPCRs) to intracellular effector cascades both spatially and temporally. By this means, signaling scaffolds, such as A-kinase anchoring proteins (AKAPs), compartmentalize kinase activity and ensure substrate selectivity [1, 2]. Using a phospho-proteomics approach we identified an unanticipated physical and functional connection between protein kinase A (PKA) and GPCR signalling. We showed that the orphan GPCR Gpr161 is a PKA substrate and that it has an AKAP motif embedded in its cytoplasmic C-terminal tail to function as selective and high-affinity scaffolding protein for PKA type I holoenzymes [3]. The AKAP function and the phosphorylation status of Gpr161 is relevant for recruiting type I PKA complexes to the primary cilium. There, in this ancient cell compartment, the cAMP-sensing Gpr161:PKA signalosome is positioned at the crossroad of Hedgehog and cAMP signalling. Currently we generate chimeric GPCR proteins consisting of the transmembrane regions of the beta-adrenergic receptor and the carboxy terminus of Gpr161 to determine the structural details of this unique macromolecular complex. We aim to purify the full-length GPCR:PKA complex in the presence of selective agonists/antagonists to initiate Cryo-electron microscopy analyses. Our ultimate goal is to visualize the architecture of this dynamic signalosome in the context of GPCR/kinase activation and G protein coupling. To gain a better understanding of the GPR161 biology we further plan to use a combination of fluorescence and bioluminescence resonance energy transfer (Nano-BRET) analysis [4] to directly monitor the dynamics of the GPR161:PKA activation and use this approach to screen for the so far unknown GPR161 ligand.

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P14

Surveillance of drug-driven BRAF conformations and interactions

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Oncogenic mutations in RAS GTPases or BRAF kinases deregulate the integrity of their binary protein-protein interactions (PPIs) leading to hyper-activation of MAP-kinase signaling. Tumor formation originates from a panel of oncogenic BRAF mutations, which diminish the auto-inhibitory kinase conformation and promote RAS-uncoupled proliferative RAF-MEK-ERK signaling [1,2,3].
We have engineered luciferase-based protein-fragment complementation assays to systematically track BRAF kinase conformations (KinCon reporter) and interactions (PPI reporter) which are controlled by GTP-loading of H/N/K-RAS, cancer mutations, or clinical BRAF inhibitor (BRAFi) binding [4]. The full-length kinase is the basis for an extendable cell-based reporter platform which enables both noninvasive recordings of (i) open (active) and closed (inactive) BRAF conformations and (ii) transformations of binary GTPase:kinase interactions following dose- and time-dependent exposure to lead molecules. We demonstrate the suitability of the KinCon reporter to implicate various BRAF mutations to systematically profile and compare BRAFi target specificity and efficacy directly in the living cell. Notably, we show that effective BRAFi such as vemurafenib, dabrafenib, encorafenib, and PLX8394 differ in shifting the open conformation of patient-mutations harboring BRAF KinCon reporters to the more closed inactive state. The unexpected consequence is that the specific BRAFi engagement with the kinase ATP-binding pocket caused an allosteric boosting effect exclusively on mutated/oncogenic RAS:BRAF complexes. We propose that BRAFi driven transformations of binary RAS:RAF interactions contribute to the incidence of paradoxical MAP-kinase activation and drug resistance as observed in BRAF-mutant tumors. We anticipate that integration and extension of the RAF reporter toolbox will foster and accelerate the rational design of more effective kinase drugs in the context of personalized medicine and combinational kinase inhibitor drug therapy.

Acknowledgements: This work is supported by the Austrian Science fund FWF (P22608, P27606, P30441, SFB-F44).

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P15

Novel Protein Kinase A substrates: cAMP oscillations regulate RNA-binding and ciliogenesis

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Phosphorylation drives the propagation of signals generated in response to hormones and growth factors in living cells. Cyclic adenosine monophosphate (cAMP) is the most prominent second messenger involved in key biological functions. In mammals, most of the effects elicited by cAMP are mediated by activation of the protein kinase A (PKA) leading to phosphorylation of a variety of cellular substrates related to proliferation, differentiation, survival and metabolism [1].
In a phospho-proteomics screen for PKA substrates and interaction partners [2], we have identified unexpected functional connections between the kinase pathway with the *TATA binding-protein-associated factor 2N* (TAF15) [3] and the *NIMA-related kinase 10* (NEK10) [4]. We identified and confirmed that both TAF15 and NEK10 are phosphorylated by PKA [5].
We show that NEK10 is essential for ciliogenesis. PKA phosphorylation primes NEK10 for CHIP-mediated ubiquitination and proteolysis resulting in cilia resorption [5]. Moreover our findings highlight a pericentriolar kinase signalosome that efficiently links the cAMP cascade with the ubiquitin proteasome system, thereby controlling essential aspects of ciliogenesis [6].
In case of TAF15, we currently perform cross-linking and immunoprecipitation experiments (iCLIP) to identify TAF15 and ZnF-bound RNA species which are regulated by PKA phosphorylation. In the first experiments we show that aminoacid substitutions in the phosphorylation site change global TAF15 RNA-binding properties indicating that it might regulate molecular interactions between TAF15 and its RNA targets. We set out to identify critical RNA species to analyze the feasible impact of PKA phosphorylation on physiological and pathological TAF15 functions.

This work was supported by the Alfonso Martín Escudero Foundation, the Tirolean Science Fund TWF (AP712003) and the Austrian Science fund FWF (P22608, P27606, P30441 and SFB-F44).

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P16

FT-ICR MS and CAD as potential tools for the discovery of metal binding sites in RNA

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It was previous shown that noncovalent interactions of nucleic acids with ligands such as proteins and small molecules can be detected by electrospray ionization mass spectrometry (ESI-MS) [1-3]. More recently, we demonstrated that collisionally activated dissociation (CAD) of RNA-peptide complexes can provide complete sequence information and locate ligand binding sites by producing fragments from phosphodiester backbone bond cleavage while maintaining the electrostatic interactions between the peptide and the RNA [3]. Thus native top down mass spectrometry is a promising tool for the exploration of RNA-ligand interactions [3-4]. Bivalent transition- and alkaline-earth-metal ions are another class of ligands that play an important role in folding and function of ribonucleic acids [5-6]. However, the structural elucidation of nucleic acids in complexes with metal ions has so far been limited to NMR and X-ray techniques. Here we have explored the potential of native top down MS using CAD for the characterization of RNA-metal ion interactions. Co(II) was selected as model ion because of its simple isotope pattern and its strict binding geometry.

Preliminary Data
We found that Co(II) forms very strong complexes in the with various hairpin RNAs containing tetraloops of different stability. CAD studies on a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer revealed that Co(II) binds to all nucleotides in the tetraloop region with high specificity but does not dependent on nucleobase indentity. Moreover, Co(II) was found to bind to a hairpin loop that completely lacks any nucleobases, indicating that Co(II) preferentially binds to the phosphodiester moieties in the loop region. Our studies illustrate the potential of transition metal ion binding site mapping by FT-ICR MS for RNA secondary structure characterization. In future studies, we will extend this concept to larger, biologically relevant RNAs with more intricate secondary structural motifs.

Methods
Experiments were performed on a 7 Tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an ESI source and a hexapole ion cell floated with Argon gas for CAD. Solutions in 50:50 H_2O:CH_3OH: with 15 nt RNA sequences (2 µM), Co(II)acetylacetonate (4 µM) and 2.5 M ammonium acetate were electrosprayed at 1.5 µL/min in negative ion mode.

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P17

The effect of fixed-charge modifications on histone peptide fragmentation by ECD

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Introduction
Posttranslational modifications (PTMs) of proteins play an important role in many biochemical processes, including protein activity, turnover rates, localization within the cell, interaction with other proteins or small molecules, and ultimately, human disease. Mass spectrometry (MS) is ideally suited for the detailed characterization of modified proteins such as histones as it can identify, localize, and potentially quantify all PTMs. However, quantitation of PTMs by MS requires a solid understanding of how they affect ionization by electrospray ionization (ESI) and fragmentation by electron capture dissociation (ECD).

Methods
Experiments were performed on a 7 Tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an ESI source and a hollow dispenser cathode for ECD. Model peptides (26 aa) were partially hydrolyzed in 0.5 vol% trifluoroacetic acid (TFA) at pH ~1 and 99 ºC for 2 h. The hydrolyzed peptides were electrosprayed from 5 µM solutions in 1:1 CH_3OH/H_2O.

Preliminary data
Various histone H3 peptide forms, i.e., peptides with the same canonical amino acid sequence but different methylation sites and extent, including mono-, di-, and trimethylation, were studied by ESI and ECD. For this propose, model peptide (26 aa) ions were isolated at charge states 3+ and 4+ and dissociated by ECD. Lysine trimethylation (fixed charge) was found to have an effect on the site-specific yield at charge state 3+ whereas higher charge states of the different modified peptides showed no significant differences in ionization and fragment ion yields.
To eliminate possible interferences of multiple arginine residues that could mask individual effects of methylation, the histone peptides (26 aa) were hydrolyzed in TFA into shorter peptides (e.g., 13 aa from cleavage between ~GG~). Experiments with and without vibrational ion activation prior to ECD were performed to probe peptide ion compactness in the gas phase and to reveal information about internal solvation of charged sites.

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P30087 to KB

P18

Developing Isotope Depletion Mass Spectrometry for the Analysis of Proteins

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Introduction
A major challenge in protein mass spectrometry (MS) is heterogeneity in signal due to the naturally occurring isotopes of the elements, which limits signal-to-noise ratio and thus sensitivity, such that the monoisotopic peak is often undetectable. Overall, the mass spectrum becomes difficult to analyse, and these problems grow with the size of the protein. A possible solution to overcoming the limitations associated with isotope heterogeneity is the depletion of rare stable isotopes.

Methods
Recombinant encapsulated ferritin (EncFtn, 13.2 kDa) and bovine carbonic anhydrase (BCA, 29.3 kDa) were expressed in M9 minimal media. For the production of isotopically depleted (ID) protein, the only carbon source was ^{13}C depleted glucose (99.9% ^{12}C) and the only nitrogen source was ^{15}N depleted ammonium sulfate (99.99% ^{14}N). MS-experiments were performed on a 12 T FT-ICR. Samples were ionized using a TriVersa Nanomate nanoelectrospray robot. Top-down MS experiments were implemented using collision activated dissociation (CAD) and electron capture dissociation (ECD). Analysis and data processing was carried out using DataAnalysis and Autovectis software.

Preliminary data
The mass spectra of isotopically depleted (ID) proteins showed far fewer and far higher signals than those of the corresponding isotopically normal (IN) spectra. The monoisotopic peak was either the most abundant or a highly abundant peak, which increased the mass assignment accuracy. Moreover, the narrow isotope patterns of the ID proteins reduced overlap of isotope distributions of different ions. This facilitates the characterization of posttranslational modifications, improves isolation capabilities in top-down MS, and allows for faster measurements. Characterization of ID instead of IN proteins by top-down MS assigned up to three times more fragments. As predicted by theoretical calculation, the effects were greater for the 29.3 kDa than for the 13.2 kDa protein. For ID BCA, more fragments were observed in an ECD spectrum with 20 scans compared to that of IN BCA with 200 scans. To further assess potential benefits of isotope depletion, protein deamidations, which are more challenging to detect by MS as protein size increases, were studied. Using isotope depletion, it was possible to detect and quantify deamidations and unambiguously assign the site of deamidation even in a mixture of 75% non-deamidated protein.

P19

Electron Attachment to OTfU: a novel potential radiosensitizer

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The search for highly efficient radiosensitizers with less toxicity has received increased attention for years. Radiosensitizers are clinically known to increase the efficacy of radiotherapy by significantly enhancing the damage to radioresistant hypoxic cells and other tumor cells [1],[2]. The interaction of high-energy radiation with biological tissues during radiotherapy yields low-energy secondary electrons (1-20 eV) which leads to biologically induced damages including DNA strand breaks through dissociative electron attachment (DEA) [3]. DEA involves an electron capture resulting in an initial temporary negative ion (TNI) followed by subsequent bond dissociations into a negatively charged ion and neutral fragment(s). In the present study we investigated DEA to 5-triflouromethanesulfonyl-uracil (OTfU), which is suggested as a novel radiosensitizer [4]. The compound represents a derivative of uracil substituted with a triflate (OTf) group at the C_5-position (see Fig. 1). We report a variety of fragmentation pathways due to simple bond or complex multi-bond cleavages upon DEA in the gas phase. The most favorable DEA channel corresponds to the formation of the OTf^- as well as the reactive uracil-5-yl radical by the cleavage of the O–C_5. The latter reactions are mainly driven by the large electron affinity of the triflate anion (5.5 eV). Quantum chemical calculations using the M06-2X/aug-cc-pvtz functional/basis sets were performed and are compared with the experimental obtained results. Ultimately, this study endorses OTfU as a potential radiosensitizer for therapeutic applications.

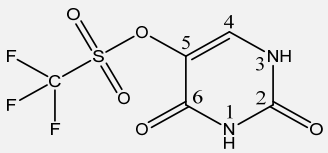

Fig. 1. *Chemical structure of 5-trifluoromethanesulfonyl-uracil.*

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P20

Effectiveness of different isolated nitroimidazoles upon low energy electron interactions

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In radiotherapy, patients are irradiated with high energetic particles. These pass through the human body interacting with the tissue. Within this process, low energetic electrons (LEEs) are emitted by the molecular structures (e.g. water radiolysis) [1]. Those LEEs with typical energies between 0 to 30eV can cause DNA damage and thus, cell death in form of single- and double-strand breaks [2,3]. To enhance the ratio of cell death of malignant cells towards healthy cells, radiosensitisers can be administered. These accumulate in tumour cells deprived of oxygen (hypoxic cancer cells) and increase electron interactions within them and thus indirect DNA damage.

The physical process underlying both direct DNA strand breaks and interactions with radiosensitisers is mainly dissociative electron attachment (DEA). The electron is captured by the molecular constituents (e.g. nucleobase, radiosensitiser) forming a transient negative ion. This can either stabilise to the charged parent anion or decompose into (charged) fragments. By this process highly reactive radicals may form which can induce DNA damage.

A wide range of radiosensitisers already exists. For the present study, the focus lies on nitroimidazoles with different hydrocarbon side chains. Nitroimidazole itself is based on an imidazole ring structure which is a building block of e.g. amino acids and thus omnipresent. The radiosensitisers studied are the bare nitroimidazole [4,5], nimorazole, which is in use for head and neck cancer in Denmark [6], and misonidazole which has been extensively tested but failed clinical trials.

The present study reveals the effectiveness of those isolated nitroimidazoles upon low energy electron interactions. Using a mass spectrometer in cross beam setup [7], single collisions between the compound and an electron were studied. While electron attachment to nimorazole predominantly gives rise to the parent anion with remarkably high cross sections, nitroimidazole and misonidazole show a rich fragmentation pattern, forming charged fragments and radicals such as NO and NO_2^-.

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P21

A new pathway of Chlorophyll Breakdown in Fern

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The autumnal degradation of the chlorophylls and the resulting discoloration of leaves is part of one of the most intriguing natural phenomena of life on earth. However, in the late 1980s when biosynthesis and biochemistry of chlorophyll (Chl) were already extensively studied, chlorophyll degradation in higher plants still remained a puzzle.

In the meantime, the structures of a range of tetrapyrrolic products of Chl breakdown (phyllobilins) were characterized and a series of the enzymes involved in Chl breakdown were identified.[1] These studies have dealt with the degradation of Chl in angiosperms, where natural Chl breakdown follows the PaO/phyllobilin pathway, or in algae.[1,2] Hence, products of Chl breakdown have so far not been identified in gymnosperms, ferns, or mosses.[3]

Here we present an investigation of Chl catabolites from a fern, which suggest a strikingly divergent path of Chl breakdown in this branch of the vascular plants.

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Hydrogenobyric acid - the metal free ligand of B_{12}

P22

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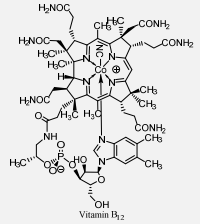
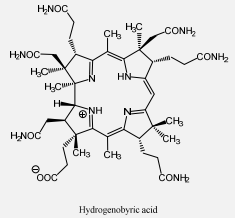
Natural metal free corrinoids have not previously been subject to a full structural characterization. Fischli and Eschenmoser were the first to develop a synthetic route to metal-free corrins in the late 60's [1] and a few years later Edmond and Hodgkin solved the X-ray crystal structure of the hydrochloride of this heptamethyl corrin compound. [2]


Vitamin B_{12}


Hydrogenobyric acid

In our studies we have prepared hydrogenobyric acid (Hby) using a genetically modified *Escherichia coli* strain. Here we describe the isolation of crystalline pure Hby and report its structure in solution and in the single crystal. The structure of Hby is compared with those of the metal free model corrin [2], the corrinoids coenzyme B_{12}, [3] cob(II)alamin [4] and the rhodium analogue of coenzyme B_{12}, adenosylrhodibalamin. [5]

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P23

Total Synthesis of Salimabromide: A Tetracyclic Polyketide from a Marine Myxobacterium

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Myxobacteria of terrestrial origin produce an abundance of structurally complex secondary metabolites with notable biological activities. Marine myxobacteria, on the other hand, have eluded their cultivation and isolation on many occasions and constitute a largely unexplored treasure trove of bioactive molecules.[1] Salimabromide is an antibiotic polyketide that was previously isolated from the obligate marine myxobacterium Enhygromyxa salina, and its densely functionalized and conformationally rigid tetracyclic framework is unprecedented in nature.[2] We achieved the first chemical synthesis of the target structure by employing a series of well-orchestrated, robust transformations, highlighted by an acid-promoted, powerful Wagner–Meerwein rearrangement/Friedel–Crafts cyclization sequence to forge the two adjacent quaternary carbon centers embedded in the tetrahydronaphthalene. A high-yielding ketiminium mediated [2+2]-cycloaddition was also utilized for the simultaneous construction of the remaining three stereocenters. The overall sequence benefits from a series of practical transformations that can be also conducted on large scale.

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P24

Synthesis and incorporation of deazaadenosine nucleotides in RNA and their impact on RNA backbone cleavage

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Atomic mutagenesis is an essential tool to illuminate structure-function relations of RNAs such as ribozymes, where selectively replacing individual atoms of highly conserved nucleotides allows the investigation of their impact on cleavage activity and potential roles, e.g. as acid-base catalysts or metal ion coordination sites.[1,2] While cleavage in ribozymes occurs at specific locations within the RNA[1], phosphordiester backbone cleavage in the gas phase during collision activated dissociation (CAD) takes place throughout the RNA sequence. Interestingly, previous studies showed, that phosphodiester backbone cleavage of $(M+nH)^{n+}$ on the 5'-end of an adenosine is facilitated, where cleavage in the gas phase is thought to be critically supported by hydrogen-bonding of the protonated N-3 of the adenosine moiety[3]. In this work, we studied the impact of 1-deazaadenosine (c^1A), 3-deazaadenosine (c^3A), 1,3-dideazaadenosine (c^1c^3A) and 7-deazaadenosine (c^7A) on the mechanism of RNA phosphodiester backbone cleavage based on electrospray ionization (ESI) mass spectrometric methods in CAD of $(M+nH)^{n+}$ and $(M-nH)^{n-}$ ions. Our results support the mechanistic proposal of Riml *et al.* 2015 [3] and, moreover, show that backbone cleavage on the 5'-end next to adenosine is facilitated at lower pH.

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P25

SHAPE probing pictures Mg^{2+}-dependent folding of small self-cleaving ribozymes

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Self-cleaving ribozymes are biologically relevant RNA molecules, which catalyze site-specific cleavage of the phosphodiester backbone [1-3]. Gathering knowledge of their three-dimensional structures is critical toward an in-depth understanding of their function and chemical mechanism. Equally important is collecting information on the folding process and the inherent dynamics of a ribozyme fold. Over the past years, *S*elective 2'-*H*ydroxyl *A*cylation analyzed by *P*rimer *E*xtension (SHAPE) [4,5] turned out to be a significant tool to probe secondary and tertiary interactions of diverse RNA species at the single nucleotide level under varying environmental conditions. Small self-cleaving ribozymes, however, have not been investigated by this method so far. Here, we describe SHAPE probing of pre-catalytic folds of the recently discovered ribozyme classes twister, twister-sister (TS), pistol and hatchet [6]. The study has implications on Mg^{2+}-dependent folding and reveals potentially dynamic residues of these ribozymes that are otherwise difficult to identify. For twister, TS and pistol ribozymes the new findings are discussed in the light of their crystal structures, and in case of twister also with respect to a smFRET folding analysis. For the hatchet ribozyme where an atomic resolution structure is not yet available, the SHAPE data challenge the proposed secondary structure model and point at selected residues and putative long-distance interactions that appear crucial for structure formation and cleavage activity.

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P26

Synthesis of modified nucleoside phosphoramidites for atom-specific mutagenesis in small nucleolytic ribozymes

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Ribozymes are biologically relevant RNA molecules that are capable of catalyzing chemical reactions such as site-specific cleavage of their own phosphodiester backbone. Four catalytic strategies are believed to be required, two of which most commonly utilize nitrogen atoms in the vicinity of the cleavage site for acid/base catalysis [1]. Atom-specific mutagenesis allows to determine the effect of single atoms regarding cleavage activity. Hence, appropriate modified nucleosides and their corresponding phosphoramidite building blocks for chemical RNA synthesis are needed[2]. In this work, we introduce synthesis pathways to 3-deazaadenosine (c^3A) and 1,3-dideazaadenosine (c^1c^3A) phosphoramidites, starting from readily affordable compounds. These nucleoside building blocks are readily incorporated into RNA by solid-phase synthesis to probe the catalytic activity of recently discovered ribozyme classes. Thus, they contribute substantially towards a mechanistic understanding of ribozyme catalysis.

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posters

P27

Towards picturing of the ribosome–mRNA cap recognition of trypanosomatids

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To provide a thorough basis for the comprehensive understanding of ribosome–mRNA cap recognition, it is essential to picture the process in a spatial and temporal manner. The method of cryo electron microscopy should enable the observation of the whole cap-recognition and the generation of the much-needed structural information in high resolution[1]. To this end, the trypanosomatide cap-4 structure is of special interest because these organisms are an early form of eukaryotes with interesting features in their metabolism[2]. This cap-structure is composed of five distinct, highly methylated nucleosides and possesses the typical 5'-5'-triphosphate bridge between the first and the second nucleoside[2]. To observe the cap-recognition as unadulterated as possible, one challenge is to create access to chemically synthesized caps or suitable mimics thereof. Equally challenging is the integration of the synthetic caps into longer RNAs as needed for assembly of the corresponding ribosomal complexes for cryo electron microscopy. Here, we present the syntheses of nucleoside phosphoramidite building blocks, their incorporation into short RNAs, and first results on their enzymatic ligation to larger trypanosomatid mRNA cap-4 –like probes.

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P28

Structure-based mutational analysis of the twister-sister ribozyme and implications on the cleavage mechanism

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The twister-sister RNA motif belongs to a group of four recently discovered [1] self-cleaving ribozymes that catalyze cleavage of the intramolecular phosphodiester bond in a site-specific manner [2].The discovery of the new ribozymes has sparked a widespread interest towards an in-depth understanding of the cleavage mechanism of these catalytic RNAs. Here, we present structure-guided mutational analyses based on our observed crystal structure of the twister-sister ribozyme [3]. Eleven conserved and spatially separated loop nucleotides are brought into close proximity at the C-A cleavage site. Comprehensive studies including cleavage assays on key base substitutions, different ribose mutations, and Mn^{2+} for Mg^{2+} replacements in the twister-sister construct, have revealed that the interactions between a guanine (i.e. G5) and a hydrated Mg^{2+} with the non-bridging phosphate oxygens at the cleavage site are important for the cleavage activity. To gain further insight into the cleavage mechanism of the twister-sister ribozyme, in particular the involvement of G5, mutational studies with chemically modified guanosines are envisaged. To this end, we have focused on 3-deaza-guanine because of the shifted pKa value of its N1-H group compared to the native counterpart [4]. Due to the expected altered interaction between NH-1 of G5 with the non-bridging oxygen of the scissile phosphate, a decrease in cleavage rate is expected, and should provide evidence for the mechanistic impact of G5 on phosphodiester cleavage. Here, we will delineate a synthetic path towards 3-deaza-guanosine building blocks for RNA solid-phase synthesis.

Acknowledgement: This work was supported by Austrian Science Fund FWF (P27947).

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P29

Structure-activity relationship of chemically modified ligands for gene regulation of engineered $preQ_1$ riboswitches in *E.coli*

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Riboswitches are *cis*-acting gene regulatory elements mainly occurring in the 5´-untranslated region of bacterial mRNA. They generally consist of a highly conserved aptamer domain that specifically binds a small metabolite and an expression platform that undergoes a conformational change upon ligand recognition.[1] Subsequently this leads to the control of either transcription or translation without the need for a protein cofactor. So far, three distinct riboswitch classes specific for the guanine derivative 7-aminomethyl-7-deazaguanine ($preQ_1$) are known.[2] $PreQ_1$ naturally occurs in prokaryotes as the biosynthetic precursor for the modified nucleoside queuosine which is found in the wobble position for certain tRNAs.[3] Here, we investigate the influence of tethered modifications at the 7-deaza-7-aminomethyl functionality of $preQ_1$ on the gene regulation of natural riboswitch motives in $preQ_1$ deficient *E.Coli* cells. The system we developed allows a rapid analysis of structure-function relationships and the dynamic control of gene expression as well as the dose-dependency and responsiveness of “non-natural” $preQ_1$ derivatives.

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P30

A site-specific TEMPO-labeling protocol via derivatization of functionally tethered oligonucleotides (FTOs)

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In this work, we present a reliable new strategy applicable for the site-specific attachment of paramagnetic centers within nucleic acids to meet the demand of high sample amounts for NMR spectroscopic investigations.
To this end, a 5-trifluoroacetamido-propargyl uridine phosphoramidite building block was synthesized and site-specifically incorporated during the RNA chain elongation on an automated synthesizer using standard solid phase synthesis. The subsequent work-up procedure yields a functionally tethered oligonucleotide (FTO) with a propargyl amine moiety. This amino functionality was used to attach a paramagnetic center by treating the RNA with an electrophilic 4-thioisocyanato-TEMPO reagent. Almost quantitative yields were obtained in the derivatization reaction making the approach suitable for NMR spectroscopic applications relying on high sample amounts.
The introduction of the paramagnetic TEMPO radical sets the stage for PRE-NMR experiments giving valuable information on structure and dynamics in biomolecules. Such measurements have proven to be a powerful tool in the solution structure determination and for the investigation of dynamics within transitions between a sparsely populated high energy- and a global minimum state.
The novel methodology was tested on an 18 nt RNA hairpin – a substrate for the ribosomal N6-adenosine methyltransferase RlmJ. Exploratory NMR and EPR experiments suggest that the approach will be useful to map the RNA binding site on the RlmJ protein.

P31

Resolving structural and functional properties of the pistol ribozyme by solution NMR spectroscopy

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Recently, Roland R. Breaker and his co-workers reported on a new class of self-cleaving ribozymes found by a bioinformatics search strategy. In detail, they characterized three new RNA sequences called twister sister, hatchet and pistol ribozyme [1]. In the last years, the pistol ribozyme was in the focus of intensive research in our group with a special interest in its structure and the catalytic mechanism. The env25 pistol ribozyme sequence that was used for the X-ray crystal structure determination by Patel and co-workers [2], was optimized to improve the NMR spectra quality. After several rounds of re-design, cleavage assays were performed to ensure that the mutant is still a catalytically active self-cleaving ribozyme. Then, our aim was to get a more detailed insight into the folding landscape of the mutant.
Therefore, relaxation dispersion (RD) NMR spectroscopic methods have been applied to the pistol ribozyme complex. In detail, R1ρ RD, CPMG RD and chemical exchange saturation transfer (CEST) experiments were measured. With these powerful methods, a deeper understanding of functional dynamics could be gained. We have been able to localize three “dynamic hotspots” within the ribozyme complex inherently linked to its catalytic activity.
Further NMR experiments to localize magnesium binding sites in this particular ribozyme were performed. Thereby, an on-going discussion on the functional role of divalent metal ions in the cleavage process could be addressed. Finally, we started to elucidate the solution structure of the pistol ribozyme complex.
Combining all the NMR data will give us novel insights into the folding landscape and the functional dynamics of the pistol ribozyme.

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P32

Studying sparsely populated conformational states in RNA combining chemical synthesis and solution NMR spectroscopy

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Using chemical synthesis and solution NMR spectroscopy, RNA structural ensembles including a major ground state and minor populated excited states can be studied at atomic resolution.
On this poster, the chemical synthesis of a 2,8-$^{13}C_2$-adenosine building block is presented to introduce isolated ^{13}C-^{1}H-spin topologies into a target RNA to probe structural ensembles via NMR spectroscopy. The 2,8-$^{13}C_2$-adenosine 2’-*O*-TBDMS-phosphoramidite building block was incorporated via solid phase synthesis into two target RNAs- a 9 kDa and a 15 kDa construct derived from the epsilon RNA element of the duck Hepatitis B Virus. The resonances of the isotope labelled adenosines of the 9 kDa 28 nt sequence could be mapped to the full-length 53 nt construct. The isolated NMR active nuclei pairs were used to probe for low populated excited/ground states via ^{13}C-Carr-Purcell-Meiboom-Gill (CPMG)-relaxation dispersion NMR spectroscopy. The ^{13}C-CPMG relaxation dispersion experiment recapitulated a unfolding event occurring on the millisecond time scale was found in the upper stem in-line with earlier observations. This unpaired conformational state is presumed to be important for the binding of the epsilon reverse transcriptase (RT) enzyme. Thus, a full description of an RNA’s folding landscape helps to obtain a deeper understanding of its function, as these high energy conformational states often represent functionally important intermediates involved in (un)folding or ribozyme catalysis.

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P33

Secondary structure probing of the Hatchet ribozyme by site-specific ^{15}N-labeling and solution NMR-spectroscopy

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Recently Ronald Breaker et al. [1] discovered four new classes of catalytic active ribozymes using bioinformatics search strategy. For some of these novel ribozymes high resolution structures are available, but for the hatchet ribozyme structural information is rare limited to a secondary structure proposal. By using solid phase synthesis and site specific ^{15}N-labeled RNA phosphoramidites building blocks we were able to examine the base pairing pattern of the hatchet ribozyme with state of the art NMR experiments. We could confirm some of the postulated secondary structure features, other substructures could not be observed but we found strong Watson-Crick like base pair interactions in postulated single stranded regions. Even if we are able to study atom- and site-specific labeled RNA constructs with sizes up to 86 nucleotides, one of the main focus points was the hairpin (P1) closest to the cleavage site which seem to be quite flexible in the full-length construct. Currently, a rigorous NMR study is carried out to establish a full secondary structure proposal for the hatchet ribozyme, a crucial pre-requisite for a full high resolution 3D structure elucidation.

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P34

Do modifications of apple and hazelnut allergens with natural components alter their immunological behavior?

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Allergies represent a serious and potentially life threatening health problem, affecting a significant part of the population. In industrialized countries, up to 40% of the population are allergic to one or more allergen sources. One particularly wide-spread allergen source is plant food, which can cause allergic reactions ranging from mild clinical symptoms such as itching and scratching to fatal symptoms such as rhinitis, asthma and anaphylaxis.
In central and northern Europe, allergic reactions to certain kinds of fruits and vegetables are highly prevalent. This is the result of initial allergic sensitization to birch pollen, followed by subsequent development of allergic reactions to apples, hazelnut, plums, peaches and more. From a molecular perspective, allergic cross-reactions arise due to the presence of proteins in these fruits that are similar in their three-dimensional structure to the sensitizing protein in birch pollen, which belongs to the pathogenesis-related protein class 10 (PR-10). To understand the immunological cross-reactivity on a molecular basis, it is thus necessary and critical to determine the three-dimensional structures of these proteins at high resolution.
Recently, we have obtained complete resonance assignments of the major apple allergen Mal d 1.0101 [1] and other isoforms of the Mal d 1 cluster, along with the major hazelnut allergen Cor a 1.0401. A NMR-derived structure of Mal d 1.0101 [2] was published and structure models of the other isoforms were established. They all show a highly conserved fold consisting of a seven-stranded antiparallel b-sheet, which is interrupted by two short a-helices and closed by a long C-terminal a-helix.
In addition, recent immunological data indicate that other food constituents, like vitamin C or phenolic compounds, can affect the allergenicity of these proteins, possibly due to chemical modification of the protein surface of the allergen itself. [3,4]
After incubation of Mal d 1 isoforms with vitamin C or polyphenols (oxidized by polyphenol oxidase, PPO) mass spectrometric analysis showed an increase in mass for Mal d 1.01 isoforms, indicating a modification of the protein.
Polyphenolic compounds can also be found in hazelnut, and could therefore also modify Cor a 1 allergens.
To determine if these modifications also occur naturally in apple fruits or hazelnuts, several attempts were made to extract Mal d 1 or Cor a 1. For allergen extraction mild conditions were used in order to leave potential modifications intact. [5]

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P35

Kinetical studies on Gold-NHC-complexes: Insight into the mechanism of ligand scrambling reactions

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Au(I)-complexes are widely used for example in medicinal[1] and catalytic chemistry[2]. The exchange of the ligands is well known for decades, but the mechanism behind is still unknown. Different journals published proposed mechanism for ligand scrambling reactions.[3]
The ligand exchange reactions are not only of interest for the solution chemistry of gold complexes, but also for the understanding of the mode of action under physiological conditions. To get insight into these reactions, studies using High Pressure Liquid Chromatography (HPLC), Liquid Chromatography Mass Spectroscopy (LC-MS) and Density Functional Theory (DFT) calculations were performed. As an example, the Au(I)-N-heterocyclic carbene (NHC) complex bromido[(3-ethyl-4-(4-methoxyphenyl)-5-(6-methoxypyridin-3-yl)-1-propyl)imidazol-2-ylidene]gold(I) **1** (Figure 1) was incubated in various solvents. Its transformation to the bis[(3-ethyl-4-(4-methoxyphenyl)-5-(6-methoxypyridin-3-yl)-1-propyl)imidazol-2-ylidene]Au^{I} cation (**2**) (Figure 1) as well as exchange of the bromide in complex **1** in the presence of halide salts at various concentrations and temperatures was detected. For the first time it was possible to detect the ligand exchange reactions via HPLC. The LC-MS analysis revealed two novel intermediates, which were considered to participate in the reaction mechanism. The DFT calculation confirmed the energetic favourable constitution.

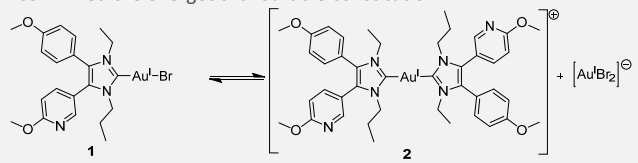

*Figure 1: Forming of complex **2** by ligand scrambling reaction.*

In conclusion, we found a strategy to verify the reaction mechanism of the ligand scrambling reactions of Au(I)-NHC complexes and we are able to give an insight into the ligand exchange. These findings could allow an interpretation of the effects in in medicinal chemistry and can also be used for the design of further novel gold-drugs.

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P36

Investigations of the antiestrogenic profile exerted by bivalent estrogen receptor ligands

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Background:
In recent years new approaches to inhibit the estrogen receptor (ER) have been investigated. Bivalent ligand binding, both inter- and intramolecular, represents a promising strategy for more effective antiestrogenic therapy [1]. Besides the ligand binding domain (LBD), blocking the coactivator binding site (CBS) is a further possibility to prevent dimerization and estrogen action [2]. Guided by structure-based ligand design and assisted by molecular modelling, bivalent ligands tethered by varying spacers have been synthesized. Hereby, a new perspective of antihormonal therapy may be provided.

Aims:
This study presents the evaluation of the cytotoxicity, the binding affinity to the ER, the antiestrogenic properties and the ER downregulation caused by the newly synthesized compounds.

Methods:
The determination of cell growth inhibition was performed in ER-alpha positive MCF-7 and ER negative MDA-MB-231 human breast cancer cells via crystal violet staining. The inhibition of the transactivation was evaluated using a luciferase based assay in transient transfected U2OS cells. The relative binding affinity was tested in a competitive experiment exerting radiolabelled estradiol on an isolated recombinant human ER. Downregulation of the ER was examined by western blot technology.

Results:
The bivalent ligands featured binding affinities in the low nanomolar range. The spacer length and the chemical scaffold that blocked the CBS determined the extent of cytotoxic potential, ER downregulation and transactivation interference.

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P37

A new approach to overcome resistance in CML therapy: combination of imatinib and PPARγ modulators

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Tyrosine Kinase Inhibitors (TKIs) represent an excellent example for targeted cancer therapy. TKIs are already well established in case of Chronic Myeloid Leukemia (CML) treatment. However, therapy remains associated with limitations since deep molecular response is hardly reached and drug resistances occur. [1]
Imatinib, the commonly used first generation TKI, was intensely examined to overcome resistance and benefits were observed in combination with other therapeutics, including Peroxisome Proliferator-Activated Receptor gamma (PPARγ) ligands [2]. Investigations of the most compatible representative pioglitazone combined with imatinib revealed the gradually elimination of the residual CML stem cell pool. [1]
Telmisartan, a partial PPARγ agonist, was thoroughly studied concerning its effect in cancer therapy as well. Our group showed for the first time that telmisartan, combined with imatinib, has an even better effect on treatment of resistant CML cells compared to the full agonist pioglitazone. Based on these findings syntheses of new PPARγ modulators and their biological evaluation were aimed, whereby telmisartan served as lead and was modified in structure-activity relationship.
Syntheses were performed following various reaction pathways. The activation of PPARγ in COS-7 cells was analyzed employing a Dual Luciferase Assay. Drug efficacy on imatinib resistant CML cells was evaluated inducing apoptosis in combination with imatinib. The compounds themselves did not affect cytotoxicity. However, combined with imatinib some of them displayed advantageous effects towards resistant CML cells.
In conclusion, the investigations clearly demonstrate that resistance towards imatinib might be circumvented upon combining with PPARγ modulators. Apoptosis was induced in resistant cells treated with the TKI and the synthesized compounds. Thus, such a combination is promising and can be suggested as new approach for treatment of resistant CML cells.

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P38

Zeise's salt derivatives with Aspirin substructures: synthesis and biological evaluation

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The development of novel biologically active organometallic compounds bearing an acetylsalicylic acid (aspirin, ASA) substructure led to the synthesis of analogical Zeise-type salts (see Figure 1) that accordingly inhibit cyclooxygenase (COX) enzymes. In *in vitro* studies, conducted earlier, it could be shown that Zeise's salt itself is pharmacologically active and a very potent COX inhibitor, whereas potassium tetrachloridoplatinate(II) and Cisplatin caused no effect on the enzyme activity at comparable concentrations. [1,2]
In order to determine the influence of the length of the alkyl chain between the platinum(II) center and the ASA moiety, compounds with increasing number of methylene groups (*n* = 1: Pt-Propene-ASA, *n* = 2: Pt-Butene-ASA, *n* = 3: Pt-Pentene-ASA, *n* = 4: Pt-Hexene-ASA; see Figure 1) were synthesized and fully characterized by 1D and 2D NMR and ESI-MS experiments. For the propene derivative structural elucidation by X-ray crystallography was possible.
Prior to evaluation of biological activity, the complexes were investigated regarding their stability in different media, such as water, physiological sodium chloride, and phosphate buffered saline. For this purpose, an analytical method that was based on capillary electrophoresis (CE) was established. In contrast to HPLC analysis, it was possible to separate the platinum complex from the free ligand. Pt-Propene-ASA showed a half-live of about 30 min, whereas the other complexes degraded with half-lives of around 50 to 70 h, indicating a strongly increased stability.
All of the compounds were tested for their COX inhibitory potential. In general, complexes with longer alkyl chains caused higher inhibition of COX enzymes and the inhibitory potential towards COX enzymes was enhanced when compared to Zeise's salt.
The growth inhibitory effects of the synthesized complexes were investigated *in vitro* against the colon carcinoma (HT-29) and the breast cancer (MCF-7) cells. The IC_{50} values of the new derivatives ranged from 30 to 50 µM, whereas neither Zeise's salt itself nor ASA showed any antiproliferative activity at the used concentrations.
In conclusion, it could be shown that complexes derived from Zeise's salt are a potential class of low-toxic COX inhibitors. Stable complexes can be obtained by simple elongation of the alkyl length between the ASA and the Zeise moiety. [3]

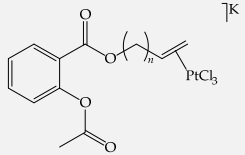

Figure 1. Zeise's salt derivatives with acetylsalicylic acid substructure.
n = 1: Pt-Propene-ASA; *n* = 2: Pt-Butene-ASA;
n = 3: Pt-Pentene-ASA; *n* = 4: Pt-Hexene-ASA;

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Peptide-based hydrogels as extended-release system for opioid analgesics

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Central goals in chronic pain control are to provide analgesia of adequate efficacy and duration. Current pharmacotherapy with opioids offers unsatisfactory long-term solutions, due to serious side effects associated with the chronic administration of opioids. One long-standing focus of drug discovery is the pursuit for innovative opioid-based therapies that can maintain consistent circulatory drug levels, pain relief with improved safety profile, and better patient compliance. Injectable peptide-based hydrogels represent highly attractive controlled drug-delivery systems due to biocompatibility and biodegradability. Furthermore, lower dosage and frequency of administration are possible, resulting in improved drug efficacy, while reducing the risk of side effects. Herein, peptide-based hydrogels were designed as extended-release (ER) drug delivery carriers of opioid drugs (morphine and 14-methoxymetopon) and opioid peptides. In this study, we report on the in vitro and in vivo characterization of two different ER formulations: (i) the analgesic drug is encapsulated within the hydrogel network ('co-formulation'), and (ii) the analgesic pharmacophore is covalently linked to the hydrogelator, resulting in an analgesic hydrogel conjugate ('biogel'). Competition radioligand binding, [^{35}S]GTPγS functional and proteolytic stability assays were used for in vitro characterization. In vivo studies included antinociception assessment in a model of acute thermal nociception and in vivo stability (SPECT/CT imaging) after subcutaneous (s.c.) administration in mice. First, sustained and significant antinociceptive effects (up to 3-4 days) in the tail-flick test was observed after s.c. injection of co-formulations of morphine or 14-methoxymetopon with hydrogelators. Second, peptidomimetic opioid ligands bound with high affinity to the mu-opioid receptor, and were potent and full agonists at the mu-opioid receptor in vitro. Antinociceptive efficacy with prolonged action was also demonstrated for co-formulations of active mu-opioid peptides and hydrogelators and biogels after s.c. administration to mice. Regarding the biogel strategy, an in vitro study of the proteolytic stability of biogels in human plasma established the biodegradability of conjugates and allowed identification of main metabolites responsible for the biological activity. In vivo, hydrogelators showed a slow degradation profile (SPECT/CT imaging) in mice, with around 20% of the hydrogel still present 72 hours after s.c. injection. In conclusion, two peptide-based hydrogel strategies were deployed to achieve effective and prolonged antinociception. These results establish the potential of such peptide-based hydrogels as efficient systems for the controlled-delivery of opioids, and opens an avenue for new strategies to treat chronic pain.

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Modulation of the kappa-opioid receptor by HS666 produces antiseizure/anticonvulsant effects without liability for aversion in mice

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Epilepsy is one of the most frequent neurological diseases (1-2% prevalence worldwide), with mesial temporal lobe epilepsy (mTLE) being one of the most severe types of epilepsy. The high incidence of resistance and numerous side effects of currently available drugs highlight the need of new mechanism-based and safer treatment strategies. Preclinical and clinical evidence established the importance of endogenous dynorphin, and its primary target, the kappa-opioid receptor (KOR) in epileptogenesis and seizure control. Modulation of the dynorphin/KOR system emerges as a prominent avenue in the pursuit of novel therapies for epilepsy [1]. Along with promising antiepileptic effects of full KOR agonists, dysphoria and sedation limit their potential clinical use. A behavioral study on the antiseizure/anticonvulsant efficacy and potential for adverse effects of a selective KOR partial agonist, HS666 [2], after systemic intraperitoneal (i.p.) administration in mice is presented. HS666 was selected based on its exciting in vitro and in vivo profiles at the KOR [2-5]. We recently reported that HS666 display biased signaling towards G protein activation in vitro [3]. Using high-throughput phosphoproteomics in mouse brain, it was recently demonstrated that HS666 elicited differential dynamic phosphorylation of synaptic proteins as compared to the prototypical KOR agonist U50,488 [5]. Pentylenetetrazole (PTZ)-induced seizures were used to model acute seizures in prodynorphin-knockout mice. The intrahippocampal injection of kainic acid (KA) in C57BL/6N mice was used as a model for drug-resistant TLE. Conditioned place aversion and locomotor activity were assessed in C57BL/6N mice. Intraperitoneal administration of HS666 showed dose-dependent (0.3-10 mg/kg) and significant increase in the threshold for PTZ-induced seizures in prodynorphin-knockout mice, and reduced paroxysmal activity in the murine KA model. The central site of action and specific KOR-mediated antiseizure/anticonvulsant actions were demonstrated using selective KOR antagonists, 5'-GNTI (intracisternal administration) and nor-binaltorphimine (i.p. administration). Additionally, HS666 did not induce aversion or affected locomotor activity of mice after i.p. administration at therapeutic doses. These findings indicate that the KOR partial agonist HS666 has the prerequisite pharmacological characteristics of an effective drug in experimental epilepsy, by activating central KOR to produce antiseizure/anticonvulsant effects without liability for typical KOR-mediated adverse effects such as aversion, and locomotor impairment.

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The neuropeptidergic PACAP/PAC1 receptor system modulates behavioral and neuroendocrine stress reactions of rats within different forebrain areas

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Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is a neuropeptide with neurotransmitter/neuromodulator, properties that has been implicated in the regulation of emotional processes such as stress and anxiety reactions. However, despite the evidence of an implication of PACAP in stress mechanisms, there has been no direct functional evidence for an action of endogenous PACAP in distinct forebrain area on stress responses under ethologically relevant conditions. The aim of the present study was to investigate the role of the PACAP/PAC1 receptor system on neuroendocrine and behavioral stress reactions. We administered a PACAP agonist (PACAP-38) ICV and bilaterally into the PVN, LS or BNST of male Sprague-Dawley rats (200-250g at surgery) and tested animals in a stress behavioral task such as the modified forced swim test with simultaneous stress hormone measurements in plasma samples. In addition, we compared c-Fos expression as a marker of neural activation after central PACAP administration. We found that ICV administration of PACAP-38 increased c-Fos expression in PVN and LS. Moreover, ICV as well local administration of PACAP-38 in these brain areas increased the immobility time and reduced active coping behavior during the forced swim stress exposure as PACAP-38 treated animals showed enhanced floating and reduced struggling behavior compared to controls. Furthermore, administration of PACAP-38 into the LS and PVN significantly increased ACTH stress response without changing basal ACTH levels. Thus, our data show that the PACAP/PAC1 receptor system mediates the modulatory effects on neuroendocrine and behavioral stress function within distinct forebrain areas such as PVN and LS.

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Gating defects induced by a CACNA1D gain-of-function mutation linked to autism spectrum disorder

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Background: Low voltage-gated $Ca_v1.3$ L-type Ca^{2+}-channels are key regulators of neuronal excitability controlling neuronal development and different types of learning and memory. Recently, large-scale genetic analysis revealed human *de-novo* missense mutations in their pore-forming α_1-subunit (*CACNA1D* gene) in 6 patients associated with a broad spectrum of neurodevelopmental disorders including the A749G mutation identified in a patient suffering from autism spectrum disorder (ASD) and intellectual disability. A typical hallmark of all these mutations are severe gating changes compatible with a gain-of-channel-function. We have successfully generated a new ASD mouse model harboring the A749G mutation. As a first step to validate this model we investigated if the pathological gating changes observed in HEK-293 cells also occur in vivo in mouse chromaffin cells (MCCs), which are a suitable neuronal model due to their neuron-like firing pattern and the possibility to isolate L-type Ca^{2+}-channels.
Methods: Perforated voltage-clamp experiments were used to assess gating changes induced by the A749G mutation using 10 mM Ca^{2+} as a charge carrier followed by current-clamp experiments to investigate the physiological neuron-like firing patterns in heterozygous $Cav^{AG/WT}$ MCCs.
Results: The A749G mutation in MCCs recapitulates the phenotype previously observed in the recombinant system which is characterized by a negative shift in activation and steady-state inactivation (~6 mV) together with accelerated and more complete inactivation of L-type currents compared to WT. The window current of $Cav^{AG/WT}$ MCCs was negatively shifted allowing more Ca^{2+}-influx at more negative potentials which could lower the action potential (AP) firing threshold. However, current clamp experiments revealed a hyperpolarized resting membrane potential together with a 3 mV more negative AP threshold resulting in less spontaneous AP firing at rest whereas the onset of APs under stimulation is facilitated. The negative resting membrane potential could be explained by an enhanced SK channel activation due to the Ca^{2+}-accumulation at negative potentials. Moreover, the single AP shape was altered featured by broadening of single APs and less marked afterhyperpolarization suggesting a possible reduction of $Ca_v1.3$/BK channel coupling in $Cav^{AG/WT}$ MCCs. In addition, evoked APs show faster firing adaptation which can be explained by diminished $Ca_v1.3$ Ca^{2+}-currents upon sustained depolarizations.
Discussion: Here we provide evidence that this mutation induces severe gating defects in $Cav^{AG/WT}$ MCCs very similar to the previously observed effects in the recombinant system. Therefore, this mouse model is suitable to study aberrant signaling in neurons which might relate to the neurodevelopmental phenotype. Patients carrying such mutations may benefit from treatment with already available L-type Ca^{2+} channel blockers, such as nimodipine.

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Subtype-selective inhibitors of voltage-gated Cav2.3 Ca^{2+} channels to treat CNS disorders

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Background: Voltage-gated Ca^{2+} channels transform membrane depolarizations into intracellular Ca^{2+} signals and are strongly associated with CNS disorders. More recently there is emerging evidence that also Cav2.3 (R-type) Ca^{2+} channels are involved in CNS diseases such as Parkinson's disease (PD) and epilepsies. Cav2.3 channels are considered "pharmacoresistant" and no high affinity non-peptide inhibitors are known (Schneider, Pharmaceuticals, 2013). As there are no selective blockers available to date we aim to biophysically and pharmacologically characterize this channel subtype using a stably expressing Cav2.3 cell line that is currently established in our laboratory.

Methods: Cav2.3 stably expressing (splice variant Cav2.3e) cell lines were generated using the Flp-In™ T-REx™ system (Invitrogen) by Flp-recombinase dependent integration of Cav2.3 cDNA into the host cell line (HEK293 cells constitutively expressing human β3 and α2δ1). Channel properties were characterized using whole cell patch clamp recordings using 2mM Ca^{2+} or Ba^{2+} as charge carriers 12-48 hours after inducing channel expression with doxycycline. The clone with properties most closely resembling transiently transfected cells was chosen for further pharmacological experiments.

Results: All four newly established cell line clones showed electrophysiological properties comparable to transiently transfected cells. Current-voltage (I-V) relationships (20ms depolarizing steps to different voltages, holding potential: -80mV) revealed current amplitudes ranging from 200-5000pA (current densities 67.9±15.7pA/pF, n=12, mean±S.E.), a half-maximal activation voltage ($V_{0.5act}$) of 9.6±1.4mV (slope 5.1±0.2mV), and a maximum of the I-V-curve (V_{max}) of 20.4±1.6mV using 2mM Ca^{2+} (I_{Ca}) as a charge carrier (n=12). The inactivation time course of I_{Ca} was fast during a 5 sec pulse to V_{max} with residual currents of 37.9±2.2%, after 50ms and 1.54±0.5% (n=11) at the end of the pulse. Inactivation was slightly slower in 2 mM Ba^{2+}.

Discussion: We were able to successfully establish a Cav2.3 stably expressing cell line containing all auxiliary subunits with the expected biophysical properties. It also gives rise to high current amplitudes which will allow current recordings using different simulated neuronal activity patterns as well as a detailed pharmacological characterization including identification of highly selective blockers.

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Voltage-gated Calcium channels in retinal rod bipolar cells

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The retina is a dense network made up from a large number of distinct cell types (>100 in mouse) implementing synaptic release of almost all neurotransmitter classes. Yet, glutamatergic excitatory transmission within the retina almost exclusively relies on ribbon-type synapses. These specialized synapses provide sustained transmitter release regulated by graded potentials in photoreceptors and their fundamental connection to the inner retinal neurons, the bipolar cells. The presynaptic voltage-gated calcium channel (VGCC) responsible for sustained transmitter release from retinal photoreceptors is the L-type calcium channel (LTCC) Cav1.4. The identity of the VGCC that triggers release from bipolar cells is still controversial. Our aim was to identify and characterize the VGCC that is responsible for synaptic transmission from rod bipolar cells (RBC), which is the crucial excitatory interneuron in the primary rod photoreceptor pathway.
All four LTCC isoforms have been reported in RBCs, so our first goal was to clarify which LTCC gene is expressed predominantly. We collected fluorescently labelled RBCs from two different mouse lines (Pcp2-ChR2-YFP and Pcp2-cre X Ai9) by FACS-sorting as well as harvested single RBCs of wild type and Cav1.4 knockout mice from dissociated retinal cell suspensions using glass capillaries. We performed qPCR for VGCC genes on both sample types with a focus on the Cav1 genes. To estimate contributions of contaminating rod, cone and cone bipolar cell mRNA to the gene expression profiles we quantified cell type-specific marker genes (Prkca, Pde6a/c, Scgn) in our samples and compared them to whole retina mRNA extractions. Our preliminary results from FACS-sorted Pcp2-ChR2-YFP mouse RBC suggested the predominant expression of Cav1.4 in RBC. In fact, we found almost exclusive expression of Cav1.4, β2 and α2δ-4. We revisited the VGCC expression pattern with the second RBC reporter mouse line and could further corroborate the presence of Cav1.4 in RBC. When we investigated the synaptic terminal morphology of RBCs in Cav1.4 mutant mice we found alterations in RBC synaptic terminal morphology that depended on the status of Cav1.4: RBC in Cav1.4 I745T retina had enlarged terminals whereas RBC terminals in Cav1.4 knockout retina were smaller than in wild type. Taken together our experiments suggest the relevance of Cav1.4 in RBC synaptic transmission. Indeed the expression profile of VGCC genes in RBC was similar to what has been described for photoreceptors and suggested for pineal gland confirming the specificity of auxiliary subunit pairing of Cav1.4 with β2 and α2δ-4. Further investigation of RBC calcium channel characteristics will reveal important insights into scotopic signal processing and could open new possibilities to study synaptic adaptation mechanisms in this crucial visual pathway.

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posters

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Determination of the absolute configuration of sesquiterpenes from roots of *Ferula hezarlalehezarica* using electronic circular dichroism

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The genus *Ferula* comprises 30 species of which 15 are endemic to Iran [1]. *Ferula hezarlalehzarica* was reported for the first time in 2008 from Hezar Mountain at Kerman province, Iran [2]. Since there was no phytochemical investigation on this plant we conducted this study to isolate, structurally elucidate and to determine the absolute configuration of purified compounds. Extraction of the root was done using dichloromethane and purification of its constituents achieved using different chromatographic techniques. In order to establish the absolute configuration of isolated compounds, 3D structures were created based on NOESY data and conformational analysis was done by using MacroModel package (Schrödinger LLC) with OPLS-3 force field. The conformers occurring in an energy window of 5kcal were subjected to geometrical optimization and UV/ECD simulation by Gaussian 09 software. Absolute configurations of the compounds were finally determined by the comparison of measured and calculated ECD spectra. Nine known and one new sesquiterpene were isolated. Due to the lack of information regarding their absolute configuration, ECD spectra were calculated using quantum chemical calculation methods. All the calculated spectra were fitting with the experimental spectra, emphasizing that the used approach is well suited for the determination of absolute configuration.

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Phytochemical and analytical study on *Urceola rosea* leaves

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Purpose of this study was to investigate and quantify major chemical constituents of *Urceola rosea* leaves, which are widely used as vegetable and anti-inflammatory herbal medicine in Vietnam and China. The isolation of compounds was possible by various chromatographic techniques and their chemical structures were established by a combination of NMR and MS methods. Furthermore, the quantification of five major compounds could be achieved by HPLC, using a Synergi MAX-RP column with acetonitrile and 0.1% formic acid in water as mobile phase. Our efforts resulted in the isolation of thirteen compounds, mainly flavonoid and chlorogenic acid derivatives. Validation of the HPLC method confirmed its good linearity ($R^2 \geq 0.9997$), precision (intra-day R.S.D ≤ 4.31%, inter-day R.S.D ≤ 3.52%), and accuracy (recovery rates were from 96.8 to 102.6%). Limits of detection (LOD) were always lower than 0.07 µg/mL. The quantification of three plant samples collected in Vietnam revealed a variable flavonoid content from 0.30 to 1.07% and a chlorogenic acid concentration ranging from 0.14 to 0.66%. Accordingly, phenolic compounds were determined as major constituents in *U. rosea* leaves and with a relatively high content of up to 1.7% their contribution to the plant's bioactivity is very likely.

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