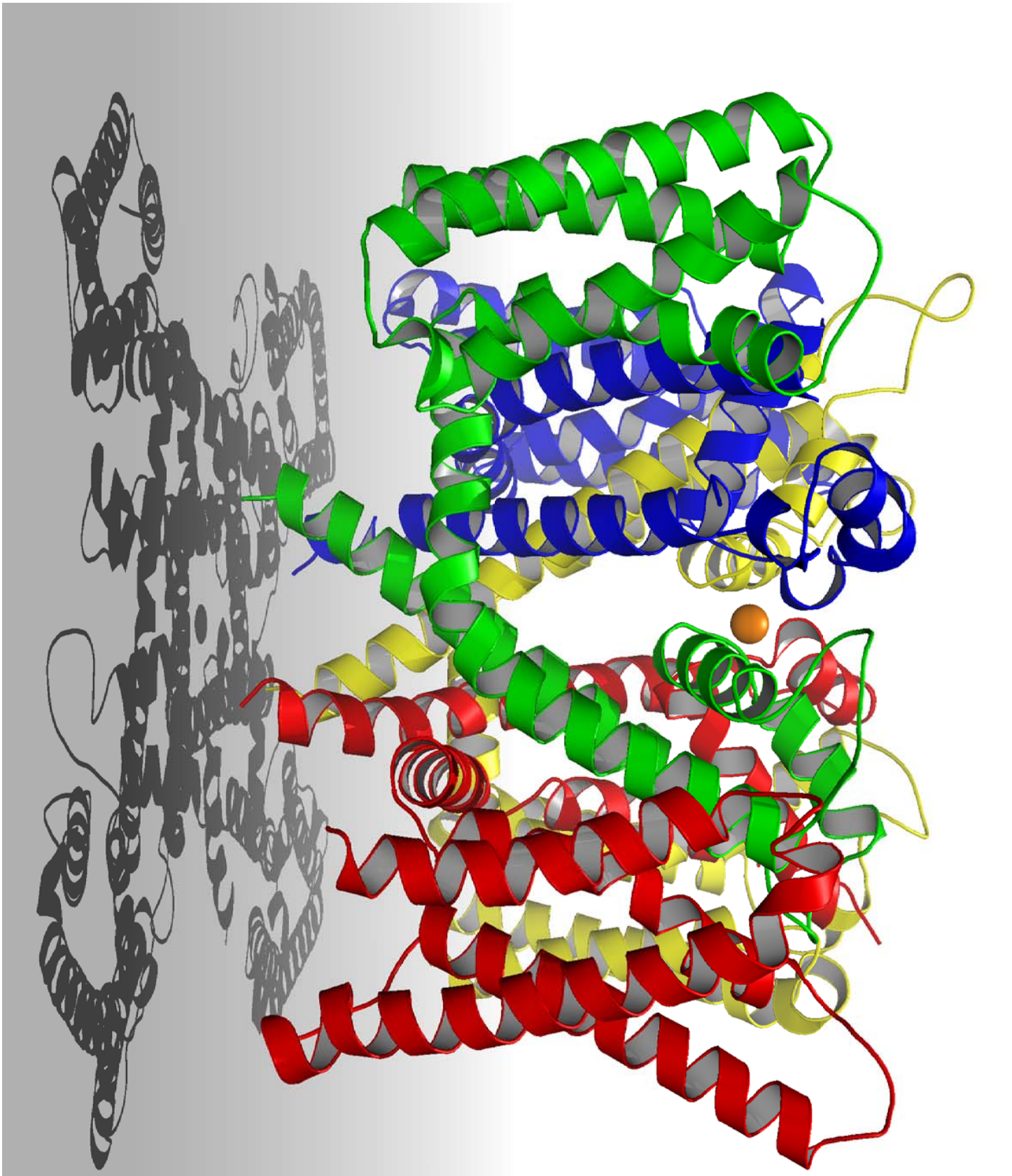
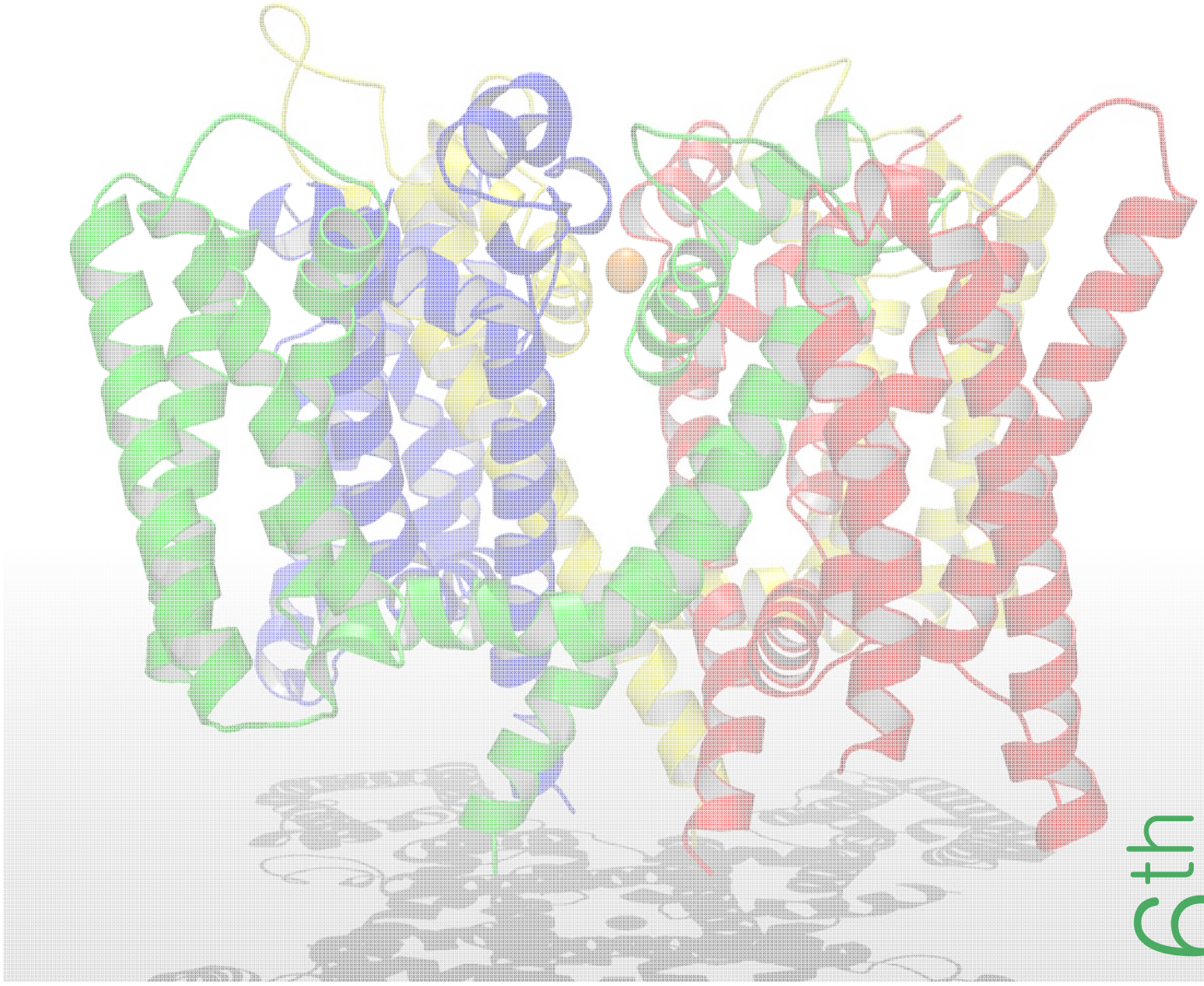


6th CMBI Meeting

University of Innsbruck

Gnadenwald, March 3 – 4, 2016



6th CMBI Meeting

Gnadenwald, March 3 – 4, 2016

Program

Organizers: *Bert Hobmayer, Ronald Micura, Jörg Striessnig*

Meeting office: Gabi Reiter, CCB, University of Innsbruck
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CMBI Center for Molecular Biosciences Innsbruck

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Thursday, March 3, 2016

8:30 – 8:45	Opening remarks
8:45 – 9:45	Plenary lecture — Chair: Hermann Stuppner **Christian Griesinger** NMR based structural Biology, Max Planck Institute for Biophysical Chemistry Göttingen, Germany ***Small molecule interference with neuro and beta-cell degeneration***
9:45 – 10:00	Break
	Session 1: Drugs/biotechnology — Chair: Alexandra Pinggera Short talks
10:00 – 10:15	*Synthesis, HPLC stability studies and biological investigations of novel NHC gold(I) complexes as potential anticancer agents* **Caroline Gallati**, Department of Pharmaceutical Chemistry
10:15 – 10:30	*In vitro L-type Ca^{2+} channel current properties and isradipine sensitivity during substantia nigra neuron-like activity patterns* **Nadine Ortner,** Department of Pharmacology and Toxicology
10:30 – 10:45	*Pharmacophore modeling and virtual screening for the discovery of potent soluble epoxide hydrolase (sEH) inhibitors* **Birgit Waltenberger,** Department of Pharmacognosy
10:45 – 11:00	*Towards novel biomimetic glues for medicine and industry* **Julia Wunderer**, Institute of Zoology
11:00 – 11:30	Break
	Session 2: Proteomics/metabolomics/modelling — Chair: Birgit Waltenberger Short talks
11:30 – 11:45	*Pharmacophore-based virtual screening for identification of novel inhibitors of CYP17* **Muhammad Akram,** Department of Pharmaceutical Chemistry
11:45 – 12:00	*Seed germination and seedling establishment are associated with distinct metabolic transitions in Brassica oleracea* **Erwann Arc,** Institute of Botany

Thursday, March 3, 2016

12:00 – 12:15	*Metal Complexes of Phyllochromobilins* **Chengjie Li,** Institute of Organic Chemistry
12:15 – 14:00	Lunch
14:00 – 14:20	Introduction lecture Chair: Dirk Meyer *Exploiting cellular reprogramming to study development and organismal regeneration* **Frank Edenhofer,** Institute of Molecular Biology
14:20 – 14:35	Session 3: Protein structure Chair: Christoph Kreutz Short talks *Advanced vibrational spectroscopic technologies for natural products quality control and cancer tissue screening* **Christian Huck,** Institute of Analytical Chemistry and Radiochemistry
14:35 – 14:50	*Explaining the recognition process of ice binding proteins* **Michael Schauperl,** Institute of General, Inorganic and Theoretical Chemistry
14:50 – 15:05	*Characterization of RNA-protein binding interfaces by mass spectrometry* **Eva-Maria Schneeberger,** Institute of Organic Chemistry
15:05 – 15:20	*Using BioGPS and molecular dynamics simulations to identify molecular determinants of serine protease substrate specificity* **Birgit Waldner,** Institute of General, Inorganic and Theoretical Chemistry
15:20 – 16:50	Poster session (odd numbers – 1, 3, 5, ...)
16:50 – 17:50	Plenary lecture Chair: Martin Tollinger **Remco Sprangers** Max Planck Institute for Developmental Biology, Max Planck Campus Tübingen, Germany ***Novel insights into dynamic processes involved in mRNA degradation***
17:50	Get-together & dinner for registered participants

Friday, March 4, 2016

	Session 4: Signaling mechanisms — Chair: Robin Kimmel Short talks
8:30 – 8:45	*tRNA-derived fragments target the small ribosomal subunit to fine-tune translation* **Jennifer Gebetsberger,** Institute of Organic Chemistry
8:45 – 9:00	*Atypical transcriptional switching via TCF/ß-catenin for germ layer segregation* **Willi Kari,** Department of Evolutionary and Developmental Biology, Institute of Zoology
9:00 – 9:15	*Cancer driver MYC and calcium signaling* **Philipp Raffeiner,** Institute of Biochemistry
9:15 – 9:30	*Identification of fumarylacetoacetate hydrolase domain containing protein (FAHD1) as oxaloacetate decarboxylase* **Alexander Weiss,** Research Institute for Biomedical Aging Research
9:30 – 11:00	Poster session (even numbers – 2, 4, 6, ...)
	Session 5: Disease and stress mechanisms — Chair: Philipp Raffeiner Short talks
11:00 – 11:15	*Human voltage gated Ca^{2+} channel mutations in autism spectrum disorder* **Alexandra Pinggera,** Department of Pharmacology and Toxicology
11:15 – 11:30	*The stress response to excess light: A signalling role for singlet oxygen-mediated acrolein production in the chloroplast* **Thomas Roach,** Institute of Botany
11:30 – 11:45	*Impact of kinase activating and inactivating patient mutations on binary PKA interactions* **Ruth Röck,** Institute of Biochemistry
11:45 – 12:00	*Role of the medial prefrontal cortex in mediating the extinction-promoting effects of L-DOPA in extinction-resistant subjects* **Simone Sartori,** Department of Pharmacology and Toxicology
12:00 – 12:15	Break

Friday, March 4, 2016

12:15 – 13:15	Plenary lecture — Chair: Pidder Jansen-Dürr **Almut Schulze** Department of Biochemistry and Molecular Biology, University of Wuerzburg, Germany ***Targeting Cancer Metabolism***
13:15 – 13:30	Award session and poster prizes and closing of the symposium

Posters

P1*	*NMR spectroscopy of PR-10 allergens* Ahammer L, Grutsch S and Tollinger M
P2*	*The first GPCR with A-kinase anchoring function* Bachmann VA, Mayrhofer JE, Ilouz R, Röck R, Tschaikner PM, Raffeiner P, Aanstad P, Taylor SS, Stefan E
P3*	*Comparison of HPLC-UV and NMR methodologies for the quantification of Silymarin complex in Silybum marianum fruit extracts* Antigoni Cheilari, Sonja Sturm, Christoph Seger, Hermann Stuppner
P4*	*Influence of particle size on the extraction performance of Ethanolic Extracts from Bupleuri radix based on UHPLC-ToF-MS* Delueg Stefanie, Jakschitz Thomas, Intelmann Daniel, Bonn Günther K.
P5*	*Systematic Conformational Studies with Density Functional Theory* Dennis Dinu, Klaus R. Liedl, Maren Podewitz, Michael Schauperl
P6*	*Nodal-dependent endoderm formation requires inhibition of Fgf-signalling via Mixer* Patrick Fischer, Dominik Regele, Frederic Pacho, Anming Huang, Dietmar Rieder, Dirk Meyer
P7*	*Structure activity relationship of novel GPR30 ligands* Valeria Follia, Sandra Alscher, Brigitte Kircher and Ronald Gust
P8*	*Remarkable rate differentiation for local conformational changes in response to ligand binding of a preQ$_1$ riboswitch* Frener M, Micura R
P9*	*The Role of PI(3)K in Endocrine Cell Migration* Julia Freudenblum, Tanja Walsen, Martin Hermann, Dirk Meyer, Robin Kimmel
P10*	*Facile synthesis of 6-thioguanosine building blocks for RNA synthesis and subsequent labeling applications* Catherina Gasser and Ronald Micura
P11*	*Characterization of RNA base methylations by top-down mass spectrometry* Glasner H, Riml C, Falschlunger C, Micura R, Breuker K
P12	*Wnt/β-Catenin Target Genes in Hydra Patterning and Regeneration* Gufler S., Eder M-K., Falschlunger J., Artes B., Bollmann A., Kuen S., Zitzelsberger L., Ostermann T., Valovka T., Hartl M. and Hobmayer B.

Posters

P13	*Wnt/β-catenin signaling in Hydra leads to specific repression of the myc1 gene* Hartl M, Glasauer S, Gufler S, Breuker K, Hobmayer B, Bister K
P14*	*A novel HILIC-Method for the analysis of Mycosporine-like Amino Acids and their biological relevance on anti-inflammatory and collagenase inhibitory targets* Anja Hartmann, Johanna Gostner, Kathrin Becker, Markus Ganzera
P15*	*Radiation damage of DNA unraveled by molecular cluster studies* Bea Haslwanter, Julia Reitshammer, Michael Neustetter, Julia Aysina, Stephan Denifl
P16*	*Multidimensional Approach in Diagnosis of the Oral Squamous Cell Carcinoma* Raphael Henn, Verena Huck-Pezzei, Christian Huck
P17*	*The role of β-catenin during regeneration in Macrostomum lignano* Hilchenbach J, Egger B
P18	*Protein quality control in UVB-induced senescence of human dermal fibroblasts* Maria Cavinato, Rafal Koziel, Pidder Jansen-Dürr
P19*	*Expanding the scope of 2'-SCF_3 modified RNA* Lukas Jud, Marija Košutić, Veronika Schwarz, Markus Hartl, Christoph Kreutz, Klaus Bister and Ronald Micura
P20*	*Mapping Conformational States of Thrombin by Comparison of X-ray Structures* Ursula Kahler, Julian E. Fuchs, Klaus R. Liedl
P21*	*Efficient characterization of local millisecond dynamics: Dihedral entropy from accelerated MD* Anna S. Kamenik, Julian E. Fuchs, Klaus R. Liedl
P22*	*Towards a new type of antivitamins B_{12}* Kieninger Christoph, Markus Ruetz, Bernhard Kräutler
P23*	*Design and synthesis of new bivalent stilbene analogues as anti-breast cancer agents* Alexandra Knox, Ronald Gust
P24*	*Combining Knowledge Based Approaches and Free Energy Calculations to Predict Binding Affinities* Johannes R. Loeffler, Julian E. Fuchs, Klaus R. Liedl

Posters

P25	*Regulation of the longevity-promoting transcription factor DAF-16/FOXO by germline signals in the nematode Caenorhabditis elegans* Hildegard Mack, T. Richard Parenteau, Cynthia Kenyon
P26*	*Elucidating the "LIM-code" in rat pancreatic endocrine cells* Fabian Martin, Armin Wilfinger, Herbert Lindner and Dirk Meyer
P27*	*Water Wires in Calcium Channels and Prediction of Disease-associated Mutations* Monteleone S, Fuchs JE, Pinggera A, Tuluc P, Striessnig J, Liedl KR
P28*	*A novel monolithic stationary phase for the separation of phenolic acids by capillary electrochromatography* Murauer A, Bakry R, Schottenberger H, Bonn G, Ganzera M
P29*	*Functional properties of newly identified somatic CACNA1D mutations in aldosterone-producing adenomas* Negro Giulia, Pinggera A, Tuluc P, Monteleone S, Liedl K, Striessnig J
P30*	*A Mini-Twister Variant and Impact of Residues/Cations on the Phosphodiester Cleavage of this Ribozyme Class* Sandro Neuner, Marija Košutić, Aiming Ren, Sara Flür, Christoph Wunderlich, Elisabeth Mairhofer, Nikola Vušurović, Jan Seikowski, Kathrin Breuker, Claudia Höbartner, Dinshaw J. Patel, Christoph Kreutz and Ronald Micura
P31*	*Characterization of post-translationally modified histones in peripheral blood mononuclear cells (PBMCs) of panic disorder patients* Verena Maurer, Michael Oberhauser, Christiane Ziegler, Bettina Sarg, Klaus Faserl, Herbert Lindner, Katharina Domschke and Nicolas Singewald
P32*	*Antitumour active cobalt alkyne complexes derived from acetylic salicylic acid: studies on impact of fluorination and chlorination of Co-ASS* Obermoser V, Schuster C, Schuster P, Kircher E, Braun V, Lettenbichler SM, Gust R
P33*	*The role of inflammaging in the age-related impairments in the maintenance of immunological memory* Pangrazzi L, Meryk A, Trieb K, Grubeck-Loebenstein B
P34*	*A protein fragment complementation assay for measuring iron-sulfur-cluster formation* Pfurtscheller S, Eigentler A, Bösch S, Schneider R
P35*	*The Proseriate flatworm Minona ileanae: a new model for Bioadhesion* Robert Pjeta, Willi Salvenmoser, Peter Ladurner
P36*	*Understanding and Improving B_{12} Antivitamins by Molecular Dynamics Simulations* Maren Podewitz, Julian E. Fuchs, Bernhard Kräutler, Klaus R. Liedl

Posters

P37*	*Alpine plants in cosmetics* Revoltella S, Waltenberger B, Baraldo G, Kohl C, Kohl R, Jansen-Dürr P, Stuppner H
P38*	*Secondary electron interaction with radiosensitizers* Anita Ribar, Katrin Tanzer, Katharina Fink, Stefan Matejcik and Stephan Denifl
P39*	*Syntheses of 5-hydroxymethylcytidine and -uridine phosphoramidites and their incorporation into RNA by solid–phase synthesis* Christian Riml, Ronald Micura
P40	*'Clickable' Chlorophyll Catabolites – III* Matthias H. Roiser, Bernhard Kräutler
P41*	*Acute vortioxetine, but not fluoxetine, promotes synaptic plasticity in the hippocampal CA1 region: possible mechanism for pro-cognitive effects* Sah A, Waller JA, Chen F, Dale E, Kharitonova M,Wegener G, Sanchez C, Singewald N
P42*	*Silvering and parasitism affect ROS defense capability in swimbladder tissue of the European eel (Anguilla anguilla)* Schneebauer G, Pelster B
P43	*Characterization of the effects of carbon monoxide on the cardiovascular system under hypoxia in zebrafish (Danio rerio)* Schult J, Schwerte T
P44*	*Risk assessment on toxic relevant destruxin analytes – do they enter the food chain?* Judith Taibon, Sonja Sturm, Christoph Seger, Hermann Strasser, Hermann Stuppner
P45*	*Impact of kinase activities on RNA:protein interactions* Torres-Quesada O, Stefan E
P46*	*Competition for protons in the dissociation of RNA-small molecule complexes* Jovana Vusurovic and Kathrin Breuker
P47*	*Facile synthesis of a 2'-O-aminoalkylated guanosine building block for RNA synthesis and subsequent labeling applications* Nikola Vušurović and Ronald Micura
P48*	*Effect of Amphiphilic Block Copolymer on Stratum Corneum Lipid Bilayer by Coarse-Grained Molecular Simulations* Yin Wang, Klaus R. Liedl

Posters

P49*	*Boron Carbide As a New SPE Sorbent for the Isolation of Curcuminoids* Arko Jatmiko Wicaksono, Shah Hussain, Dieter Schemeth, Matthias Rainer, Christian W Huck and Günther K Bonn
P50	*Role of mnx1 in zebrafish endocrine cell fate determination* Armin Wilfinger, Elisabeth Ott, Frederic Pacho, Valeriya Arkhipova, and Dirk Meyer
P51*	*Mechanism of Ets factor repression during neural fate acquisition* Johannes Paul Will, Ute Rothbächer
P52*	*Bioadhesion in ascidian* Fan Zeng, Julia Wunderer, Peter Ladurner, Ute Rothbächer
P53*	*Development and characterization of thiolated α-cyclodextrin as an ocular drug delivery system* Muhammad Ijaz, and Andreas Bernkop-Schünrch

*) Participation in poster prize competition

Synthesis, HPLC stability studies and biological investigations of novel NHC gold(I) complexes as potential anticancer agents

Caroline M. Gallati, André Betz, Ronald Gust
Department of Pharmaceutical Chemistry, CCB - University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria

Platinum complexes are widely used in cancer treatment since their discovery in the 1960s. However, chemotherapy with platinum drugs is accompanied by severe side effects and development of resistance. Therefore, new metals have been investigated to replace platinum. Several complexes of gold(I) and gold(III) have been synthesized and tested against tumor cells and showed promising results [1]. Their mechanism of action is fundamentally different to the one of platinum drugs, which, together with the fact that gold complexes are already in clinical use for the treatment of rheumatoid arthritis [2], makes gold such an interesting alternative. Of all known ligands for gold complexes, N-heterocyclic carbenes (NHCs) are especially interesting because of their strong bonding to gold(I), leading to complexes with high stability. Stability, however, is an issue that needs to be addressed when evaluating the properties of metal complexes for pharmacological use. A series of NHC gold halide complexes derived from 4,5-diarylimidazoles has been synthesized by Liu et al., showing interesting activity [3]. Based on this work, a new series of NHC ligands with an imidazole core bearing a methoxy-pyridine ring and a substituted phenyl ring in positions 4 and 5, respectively, was designed. A versatile synthetic pathway for the preparation of the imidazolium ligand precursors has been developed, enabling the synthesis of a series of derivatives with various substituents. The corresponding gold(I) halide complexes were prepared and characterized. An HPLC method for analysis of the complexes was developed and the purity of the complexes was determined. Detailed stability studies were performed on one of the complexes, revealing a very peculiar chemistry of the complex in solution. Cytotoxicity of the gold(I) complexes was evaluated on the MCF-7 cell line and the IC_{50} values were found to be in the micromolar range.

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In vitro L-type Ca^{2+} channel current properties and isradipine sensitivity during substantia nigra neuron-like activity patterns

Nadine J. Ortner[1], Petronel Tuluc[1], Thomas Pomberger[1], Antonios Dougalis[3], Birgit Liss[3] Thomas Ciossek[2], Henning J. Draheim[2] and Jörg Striessnig[1]
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Background: Voltage-gated L-type Ca^{2+} channels (LTCCs) are characterized by their high sensitivity towards organic Ca^{2+} channel blockers (CCBs), such as isradipine. Cav1.3, one of the two brain LTCC isoforms, has been recently implicated in the pathophysiology of Parkinson's disease. There are controversial data on neuroprotection by blocking LTCC-mediated Ca^{2+} transients in substantia nigra pars compacta (SNc) neurons in animal models. Since isradipine acts in a highly state-dependent manner, the shape and frequency of the depolarizing stimuli can strongly influence drug responsiveness. IC_{50} values for CCB-mediated inhibition during neuronal activity patterns are unknown. For this study, we generated stable cell lines expressing different Cav1.3 splice variants and Cav1.2 to quantify the pharmacological activity of isradipine under physiological recording conditions (2 mM Ca^{2+}) and neuronal activity stimuli.

Methods: Inducible cell lines stably expressing human Cav1.2 and Cav1.3 long and short C-terminal splice variants (hCav1.2, $hCav1.3_L$, $hCav1.3_S$, together with β3 and $\alpha2\delta1$) were generated using the Flp-In™ T-REx™ system. Channel properties were measured using the whole-cell patch-clamp technique (2 mM Ca^{2+} or Ba^{2+} as charge carrier). Cells were stimulated with action potential (AP) command voltages recorded from identified mouse SNc neurons or obtained by computational modelling. IC_{50} values for inhibition of the steady-state LTCC Ca^{2+} currents (I_{Ca}) by isradipine during simulated SNc regular pacemaking (2.5 Hz) were determined.

Results: Stable cell lines expressing hCav1.2, $hCav1.3_L$ and $hCav1.3_S$ reproducibly exhibited large current amplitudes (500 – 3000 pA) and similar biophysical and pharmacological properties compared to transiently transfected constructs. When starting from a holding potential of -80 mV, tonic pacemaking (2.5 Hz) caused a pronounced peak I_{Ca} decay with steady-state availabilities of 16.1 %, 15.4 % and 19.6 % for $hCav1.3_S$, $hCav1.3_L$ and hCav1.2, respectively. The time for peak I_{Ca} equilibrium being reached differed significantly ($hCav1.3_S$ < $hCav1.3_L$ < hCav1.2) and was much slower for barium currents. Isradipine IC_{50} values for steady-state Ca^{2+} current inhibition were ~15 nM for $hCav1.3_S$ and ~5 nM for $hCav1.3_L$ and hCav1.2. At present we are also investigating the influence of different pacemaker frequencies as well as burst activity on LTCC I_{Ca}.

Conclusion: Our data show that Cav1.3 C-terminal splicing and Ca^{2+}-dependent inactivation affect the availability of LTCCs during SNc-like electrical activity. The measured IC_{50} values for channel block by isradipine are in the range of human therapeutic plasma levels. Whether this translates to a neuroprotection in Parkinson's disease is currently investigated in a large clinical trial (~5 - 10 nM plasma concentration for 10 mg/d; STEADY-PD III trial: NCT02168842).

This work was supported by the Austrian Science Fund (FWF F44020, W11).

Pharmacophore modeling and virtual screening for the discovery of potent soluble epoxide hydrolase (sEH) inhibitors

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As a key enzyme within the arachidonic acid cascade, the soluble epoxide hydrolase (sEH) plays an important role in the regulation of inflammation. While the cyclooxygenase (COX) and lipoxygenase (LO) enzymes produce largely pro-inflammatory metabolites, the cytochrome P450 epoxygenases metabolize arachidonic acid into anti-inflammatory epoxyeicosatrienoic acids (EETs). These endogenous compounds are rapidly oxidized to the corresponding dihydroxyeicosatrienoic acids (DHETs) by sEH. Inhibitors of sEH block this degradation and therefore stabilize EET levels, which leads to an enhancement or extension of the anti-inflammatory effect. Hence, there is an increasing interest in this potential therapeutic strategy for treating inflammatory disorders [1-3].
This study aimed at the identification of novel potent sEH inhibitors. Therefore, several structure- as well as ligand-based pharmacophore models for sEH inhibitors were developed and theoretically validated using data from literature. The best eight models were used as a search query to virtually screen the chemical database supplied from the Specs. For each model, six virtual hits showing high fit values were selected for biological investigation in a fluorescence-based enzyme activity assay. At least one of the six virtual hits, respectively, displayed a sEH remaining activity of less than 35% of control at a concentration of 10 µM. In total, out of 48 compounds, eight compounds of different chemical scaffolds showed a sEH remaining activity of less than 60% of control at a concentration of 0.1 µM and IC_{50} values in the low nanomolar range. The most active compound exhibited an IC_{50} of 5.0 nM.
Within this study, pharmacophore modeling and virtual screening led to the identification of novel potent inhibitors of sEH, a promising anti-inflammatory target.

Acknowledgement: Supported by the Austrian Research Fund (S10703, S10711) and the Standortagentur Tirol.

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Towards novel biomimetic glues for medicine and industry

Julia Wunderer[1], Birgit Lengerer[1], Robert Pjeta[1], Marcelo Rodrigues[1], Herbert Lindner[2], Willi Salvenmoser[1], Peter Ladurner[1]
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Biological adhesion is widely spread among all kinds of organisms and can be found in both dry and wet environments. The increasing need for new, non-toxic and strong adhesives in industry and medicine makes these biological attachment systems subjects of numerous investigations [1].
The free-living, marine flatworm *Macrostomum lignano* (Rhabditophora, Macrostomorpha) possesses an elaborate adhesive system, which allows the animals to repeatedly adhere and release. The adhesive system comprises about 130 adhesive organs at the tip of the tail, each consisting of three cells: one adhesive gland cell, one releasing gland cell and one anchor cell [2].
To reveal the adhesion mechanisms and the adhesive and releasing substances of *M. lignano* we screened 298 mainly tail specific transcripts with whole mount *in situ* hybridization. A newly established double fluorescent *in situ* protocol allowed us to assign the transcripts with expression in the adhesive organs to a distinct cell type. Additionally the recently published *Macrostomum lignano* genome [3] helped to identify two big adhesion genes *Mlig-ap1* and *Mlig-ap2*.
Functional analyses of both genes was done by RNA interference. A knock-down of both genes led to a non-adhesive phenotype respectively. Ultrastructural analysis of RNAi worms revealed an alteration in the adhesive vesicles in both cases whereas the structure of the cells was not affected. These findings lead to the suggestion that we have found two essential parts of the *Macrostomum* glue. Furthermore we could confirm the secretion of both glue components by analyzing footprints with Mass spectrometry.
Understanding the mechanisms of adhesion and release in *M. lignano* can pave the way for new bioinspired adhesives.

Supported by FWF grant P25404-B25

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Pharmacophore-based virtual screening for identification of novel inhibitors of CYP17

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Endocrine receptor modulation by environmental chemicals is widely studied. However, the inhibition of cytochrome P450c17 (CYP17) by environmental chemicals is not very well scrutinized [1]. CYP17 catalyzes the key reaction for the biosynthesis of dehydroepiandrosterone (DHEA) and androstenedione, which are precursors of testosterone and estradiol [2]. Because of its importance in the biosynthesis of androgens, CYP17 inhibition may be a promising target for the treatment of castration resistant prostate cancer (CRPC) [3,4,5,6,7]. One of the reasons of prostate cancer progression is the androgen receptor signaling despite of the androgen deprivation therapy. Even after castration, some prostate cancers have the ability to convert adrenal steroids into androgens at sufficient level to activate the adrenal receptors. That's why CYP17 inhibition can be a cure for CRPC [8].

In this study, we have created structure-based and ligand-based pharmacophore models for CYP17 inhibition. These pharmacophore models were employed to perform virtual screening of environmental chemical databases to identify the potential CYP17 inhibitors. The used environmental chemical databases include approved drugs, cosmetic ingredients, endocrine disruptors, food contact materials, food flavoring agents and pesticides. The most relevant hits will be tested for CYP17 inhibition *via* enzymatic assay. Molecular docking studies will be performed to propose protein-ligand interactions of the experimentally tested hits.

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Seed germination and seedling establishment are associated with distinct metabolic transitions in Brassica *oleracea*

Julian WIMMER[1], Erwann ARC[1], William FINCH-SAVAGE[2], Ilse KRANNER[1], Wolfgang STÖGGL[1]
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Plants are the basis of life on earth. Most human and animal food is derived directly or indirectly from plants, mostly from seeds. Seed germination, the transition from seed to seedling, is the most critical phase in the plant life cycle, where the emerging new plant is highly vulnerable to environmental stress factors, with significant downstream effects on agricultural yields. Seed germination comprises a sequence of molecular and physiological events initiated upon seed imbibition, and is completed when the radicle protrudes through the seed envelopes and cell division starts. This transition is associated with drastic changes in gene expression and a sequential remodelling of the proteome.
Here, GC-MS-based metabolite profiling was used to unravel the metabolic changes occurring during the different phases of seed germination up to the early stages of seedling establishment in Brassica oleracea, a relative of the model plant Arabidopsis thaliana. Moreover, several plant hormones were simultaneously quantified using LC-MS/MS. Comparing two seed lots with almost identical genetic background that only differ in germination vigour allowed us to gain new insights into molecular events that define seed quality.

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Metal Complexes of Phyllochromobilins

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Degradation of chlorophyll is the visible sign in senescent leaves and vegetables, as well as in ripening of fruit.[1] As we now know, this process decomposes chlorophyll into a variety of linear tetrapyrroles, named as phyllobilins.[1] In senescent leaves, colourless, 'nonfluorescent' bilane-type catabolites (NCCs) and the related dioxobilin-type NCCs (DNCCs) typically accumulate as "final product". These linear tetrapyrroles show low affinity for biological relevant transition metal ions.
Recently, some unsaturated coloured phyllobilins, phyllochromobilins, were discovered, called yellow chlorophyll catabolites (YCCs) and pink chlorophyll catabolites (PiCCs).[2] Coordination of biologically relevant transition metal ions to the PiCC or YCCs is rapid. Coordination of the Zn- or Cd-ions to the PiCC leads to the intensive red fluorescence of the complexes Zn- or Cd-PiCC.[3] Coordination of the Zn-ions to YCCs affords the complexes Zn-$(YCCs)_2$ which exhibit bright green fluorescence.[4] Formation of complexes M-PiCC or M-YCCs by coordination of transition metal-ions to the phyllochromobilin could be a biologically significant capacity of chlorophyll catabolites. This may give such coloured phyllobilins unsuspected physiological functions in plants, e.g. as sensitizer for singlet oxygen, as toxins against pathogens or in 'heavy metal transport and detoxification'.

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Advanced Vibrational Spectroscopic Technologies for Natural Products Quality Control and Cancer Tissue Screening

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Natural product´s properties are related to defined classes of potent ingredients including e.g. flavonoids, alkaloids, essential oils and others. Conventional analytical techniques routinely applied are mainly based on separation technologies (TLC, GC, LC, LC-MS, CE, CE-MS) offering the advantages of high selectivity/sensitivity but the drawbacks of being time consuming, expensive and hardly offering an efficient fingerprint analysis. Therefore, infrared spectroscopic technologies combined with multivariate analysis (MVA) enjoy an excellent reputation, because of the fast and non-invasive analysis enabling the measurement of several parameters simultaneously.
Near infrared (NIR; 4.000-10.000 cm^{-1}) and attenuated total reflection (ATR; 400-4.000 cm^{-1}) spectroscopy have permanently increased their efficiencies for the quality control of different natural products including foodstuff and medicinal plants since the early sixties. These techniques enable both a quantitative analysis of ingredients and in many cases also a determination of the geographical provenience. Miniaturized and customized handheld spectrometers allow on one side avoiding deception of consumers, on the other side low-quality products from third countries can be easily identified. In the field of medicinal plant analysis they are powerful for in-field measurement to determine the optimum harvest time based on a maximum of potent ingredients and properties (e.g. anti-oxidative potential). Fourier transform infrared (FTIR) spectroscopic imaging/mapping is suitable not only for the differentiation of plant species, but also to localize individual ingredients within a sample (resolution 4 µm). This technique shows promising attempts being used as an early screening method for different types of cancer. As an alternative Raman imaging spectroscopy might offer additional benefits due to the higher resolution and the lack of water based spectral influences
In the present contribution, the principle and technique of the different vibrational spectroscopic methodologies are described in details followed by several selected applications for both potent quality / quantity control and cancer tissue screening [1], [2].

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Explaining the recognition process of ice binding proteins

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Pure water freezes at -37 °C, because the formation of ice is kinetically hindered at higher temperatures.[1] The common known property of water, freezing at 0 °C, results only from impurities in the water acting as ice nuclei. Ice nuclei are substances catalyzing the formation of ice. This phenomenon is especially significant, when water is found in small droplets, as it is in the artificial snow production and the formation of clouds in the atmosphere, where it has a huge impact on the earth's climate.[3]
Experimental studies showed that the most efficient class of known ice nuclei are proteins. These proteins are termed ice nucleation proteins [2] and catalyze freezing of water already at higher temperatures (0 to -5 °C).
The mechanism of ice nucleation itself is poorly understood, when seen in relation to its relevance. In our studies we investigated the ice nucleation process by molecular dynamics simulations to gain insight in the underlying principle. The focus in this study was to describe the hydration properties of water molecules in the surrounding of the protein. Our study revealed that ice nucleation is facilitated, if the enthalpic interaction between water and the protein is low, and in contrast, the entropy of the surrounding water molecules is medium or high. Based on these results, we proposed a new improved mechanism for the ice nucleation process of ice nucleation proteins.

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Characterization of RNA-protein binding interfaces by mass spectrometry

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The HIV1 TAR-TAT complex is an important model system in studies of RNA-protein binding, in which the arigine-rich motif (ARM) of TAT interacts with the TAR ribonucleic acid (RNA) primarily through electrostatic interactions, although hydrogen bonds and hydrophobic interactions are essential to providing binding specificity. Here we demonstrate that native electrospray ionization (ESI) mass spectrometry (MS) can preserve the intra- and intermolecular interactions of HIV1 TAR-TAT, and that low-energy collisionally-activated dissociation (CAD) provides site-specific data that confirm the known binding pattern of the TAT motif (aa 48-57) with TAR RNA (31 nt). Moreover, studies of complexes with 1:1 and 1:2 TAR:TAT stoichiometries revealed a second, specific binding site for TAT. The known binding motif in the major groove of TAR RNA involves residues U23, C24, and U25, whereas the newly identified second interaction site could be located to the TAR RNA loop region. In future studies, we will explore the general potential of native ESI and CAD for the structural probing of protein-RNA interactions.

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Using BioGPS and Molecular Dynamics Simulations to Identify Molecular Determinants of Serine Protease Substrate Specificity

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Proteases are enzymes that catalyze the cleavage of peptide bonds and are important in numerous fundamental cellular processes, ranging from food digestion over blood coagulation to apoptosis. Proteases also account for 1-5% of the genome of infectious organisms such as bacteria, parasites and viruses [1].
While proteases involved in cellular signaling pathways such as the blood coagulation pathway or the apoptosis pathway show high specificity, proteases involved in processes such as the digestion of food proteins show rather low specificity. The specificity of a series of proteases has been quantified by Fuchs et al. through calculation of the so-called cleavage entropy as a sub-pocket-wise and overall specificity score [2] based on cleavage data from the MEROPS database [3].
To understand the mechanism of protease recognition on a molecular level, we applied the recently developed BioGPS method [4] to a set of apo X-ray structures of serine proteases with chymotrypsin-fold:
Individual serine protease sub-pockets were identified via a combination of the FLAP*site* algorithm implemented in FLAP [5] and manual sub-pocket definition. Pseudo molecular interaction fields (Pseudo MIFs) using probes to probe for shape (H), hydrophobicity (R), acceptor (A) and donor (D) characteristics of sub-pockets were determined. Sub-pockets were then compared via three-dimensional superposition of the Common Reference Framework [5] and sub-pocket similarity was calculated in an all-against-all approach. In addition to the similarity score for the individual probes, a global similarity score combining the information of all four probes was determined. Similarity matrices for individual probes and global similarity score were then subjected to non-metric multidimensional scaling (NMDS) to reduce data dimensionality. In addition to BioGPS analysis of X-ray structures, we also used an ensemble of representative conformations obtained from clustering of molecular dynamics trajectories to study the influence of sub-pocket flexibility on sub-pocket specificity. The results give detailed insight into the molecular determinants of serine protease specificity and provide the basis for the development of selective inhibitors by targeting properties that distinguish the desired target from similar targets. In addition the knowledge on key drivers of sub-pocket specificity can be used for the prediction of specificity of targets whose substrate specificity has not yet been characterized. The methods applied to this set of serine proteases with chymotrypsin-fold can readily be applied to serine proteases with different fold or proteases in general.

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tRNA-derived fragments target the small ribosomal subunit to fine-tune translation

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Post-transcriptional cleavage of RNA molecules to generate smaller fragments is a widespread mechanism that enlarges the structural and functional complexity of cellular RNomes. In particular, fragments deriving from both precursor and mature tRNAs represent one of the rapidly growing classes of post-transcriptional RNA pieces. Importantly, these tRNA-derived fragments (tRFs) possess distinct expression patterns, abundance, cellular localizations, or biological roles compared with their parental tRNA molecules.[1]

Here we present evidence that tRFs from the archaeon *Haloferax volcanii* directly bind to ribosomes. In a previous genomic screen for ribosome-associated small RNAs we have identified a 26 residue long fragment originating from the 5' part of valine tRNA (Val-tRF) to be by far the most abundant tRF in *H. volcanii*.[2] The Val-tRF is processed in a stress- dependent manner and was found to primarily target the small ribosomal subunit *in vitro* and *in vivo.* Translational activity was markedly reduced in the presence of Val-tRF, while control RNA fragments of similar length did not show inhibition of protein biosynthesis. Crosslinking experiments and subsequent primer extension analyses revealed the Val-tRF interaction site to surround the mRNA path in the 30S subunit. In support of this, binding experiments demonstrated that Val-tRF does compete with mRNAs for ribosome binding. Therefore, this tRF represents a ribosome-bound non-protein-coding RNA (ncRNA) capable of regulating gene expression in *H. volcanii* under environmental stress conditions probably by fine-tuning the rate of protein production.[1]

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Atypical transcriptional switching via TCF/ß-catenin for germ layer segregation

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In ascidians, it is well established that vegetal accumulation of ß-catenin suppresses the animal fate. In *Ciona*, we previously showed that β-catenin/TCF dependent repression of GATAa transcription factor activity mediates germ layer segregation[1]. Moreover, tight temporal correlation of ß-catenin accumulation with GATA activity repression is consistent with direct repression of GATAa target genes. A GATA site multimer is similarly repressed, indicating that GATA factor binding sites are responsive to this repression and that canonical TCF binding sites (normally mediating activation) are not involved. Indeed, atypical TCF sites were linked to ß-catenin/TCF mediated repression in *Drosophila*[2]. In addition, we very recently described a direct repressive TCF/ß-catenin mechanism in *C. elegans* on other transcription factor binding sites[3].
In ascidian germ layer segregation, GATAa target genes may be directly repressed by ß-catenin/TCF and we are currently testing the hypothesis that atypical repressive ß-catenin/TCF signaling works through GATA sites. To investigate the repressive activity of ß-catenin/TCF at GATA sites, we separated atypical TCF sites from GATA sites by generating activity reporter constructs (G12 constructs) with different GATA binding site composition. Interestingly, we found that their differing animal GATA responsiveness did not perfectly correlate with their decrease in repression by β-catenin/TCF in the vegetal region. This suggests that activation of GATA sites depends on a different signature than their repression. Presently, we are analysing the biochemical interaction of GATAa/TCF/ß-catenin on "repressive" GATA sites for atypical transcriptional switching in *Ciona*.

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Cancer driver MYC and calcium signaling

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The MYC protein is a transcriptional regulator that controls fundamental cellular functions. Deregulated MYC expression leads to neoplastic transformation and is a hallmark of most human cancers [1, 2]. MYC forms binary complexes with its binding partner Max [2] and this protein-protein interaction (PPI) is currently a target for drug development [3-5]. MYC activity and protein stability are regulated by a variety of molecular interactions, most notably PPIs [2]. Here we present evidence for a physical interaction between MYC and the calcium binding protein calmodulin (CaM).
The MYC-CaM interaction is calcium-dependent and PPI analyses using serial deletion mutants of MYC show that the binding site maps to the basic region helix-loop-helix leucine zipper (bHLH-LZ) domain. NMR spectroscopy using recombinant ^{15}N-labeled bHLH-LZ domain of MYC and CaM corroborates this finding. This carboxyl-terminally located protein motif of MYC is required for dimerization with the Max protein and for DNA-binding [2]. In electrophoretic mobility shift assays (EMSAs) we found that Ca^{2+}/CaM does not interfere with DNA-binding of MYC. Co-immunoprecipitation experiments carried out with cytoplasmic and nuclear cell extracts show that CaM is in complex with the cytoplasmic pool of MYC. Although MYC fulfills most of its cellular functions as a transcription factor within the cell nucleus, critical cytoplasmic MYC functions have recently been reported [6].
CaM is a highly conserved protein that serves as a molecular switch to regulate a network of calcium signaling pathways [7]. This is achieved by calcium-dependent and independent PPIs of CaM with effector molecules. MYC function and protein modification have already been linked to calcium signaling [6]. Investigations how MYC-CaM PPI influences the carcinogenic activities of Myc are currently underway.

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Identification of fumarylacetoacetate hydrolase domain containing protein (FAHD1) as oxaloacetate decarboxylase

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Fumarylacetoacetate hydrolase domain containing protein 1 (FAHD1) is known to act as acylpyruvase in mitochondria [1]. It has recently been described as oxaloactetate decarboxylase [2], rendering it as a key role enzyme in the TCA cycle. Mouse [2] and *c. elegans* [3] FAHD1 knockdown models provide evidence for the linkage of this enzyme's activity to relevant diseases, such as type II diabetes, and neurological disorders. Single-point mutations are found to alter the enzyme's activity with respect to the wild type. Current research investigates Michelis Menten kinetics profiles of prominent homologues and mutant protein structures, as well as possible inhibition of the wild type.

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Human voltage gated Ca^{2+} channel mutations in autism spectrum disorder

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Background: L-type voltage gated calcium channels ($Ca_v1.1$-1.4) are expressed in electrically excitable cells and control many physiological functions. Among these $Ca_v1.2$ and $Ca_v1.3$ are the only isoforms expressed on postsynaptic locations in neurons and are thus involved in controlling brain functions including mood behavior, long term fear memory and neuronal development.
Recently, a number of $Ca_v1.3$ mutations have been identified in whole exome sequencing studies of patient cohorts with autism spectrum disorders (ASD). We analyzed two *de novo* mutations (G407R and A749G) and one identified in a case-control study (A59V) which occurred in conserved domains and regions of the channel which are known to be crucial for proper channel function.
Methods: We introduced the mutations into the major $Ca_v1.3$ long and short splice variants and functionally analyzed them by whole-cell patch-clamp recordings, after co-expression with β_3 and $\alpha_2\delta$-1 subunits in tsA-201 cells. To gain insight in the structure-function relationships from mutation induced gating changes we generated a 3D model of $Ca_v1.3$ α1 subunit using homology (MOE) and Rosetta *ab initio* modeling.
Results: Mutation G407R is situated in the activation gate of repeat I and showed significantly reduced current amplitudes but dramatically slowed the inactivation time course of the channel during depolarizing pulses. Moreover, it significantly shifted the voltage dependence of activation to hyperpolarized voltages when introduced in the short splice variant. Mutation A749G is located in a similar position of the activation gate in repeat II. In comparison to wild-type it caused a strong gain-of-function phenotype evident from a hyperpolarizing shift of the voltage dependence of activation and inactivation. In addition, it showed a 3-fold higher ratio of peak tail current versus ON-gating charge, suggesting a higher estimated open probability. The biophysical changes induced by mutations G407R and A749G imply that they can induce enhanced Ca^{2+} currents through $Ca_v1.3$ channels in neurons (gain-of-function mutations). For mutation A59V we did not find a phenotype so far. However, this mutation is located in a conserved region (NSCaTE) in the N-terminus which is involved in the inactivation of the channel. Possible alterations in this mechanism are currently investigated.
Conclusion: Our data suggest that the $Ca_v1.3$ mutations G407R and A749G confer a major part of the risk for autism in the two probands and may even have a causal role in the development of the disease. Based on our studies probands may benefit from treatment with clinically available L-type calcium channel blockers. Furthermore, we have evidence for the implication of an additional *de novo* mutation in ASD which is currently under investigation.
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The stress response to excess light: A signalling role for singlet oxygen-mediated acrolein production in the chloroplast

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Due to their sessile nature, plants must often tolerate environment stresses to survive, including light intensity fluctuations of orders of magnitude that can occur over a few seconds. Absorption of excess light enhances the production of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), which damages photosynthetic machinery, but also induces signalling responses. For example, treating the green alga *Chlamydomonas reinhardtii* with excess light induces the expression of glutathione peroxidase (GPXh) [1], which subsequently increases the tolerance of cells to otherwise lethal levels of $^1O_2$ [2]. However, it is not clear how $^1O_2$ is involved in signal transduction. $^1O_2$ can oxidise thylakoid trienoic fatty acids (18:3), releasing short chain oxylipin carbonyls, such as acrolein [3]. Here, it is shown that treating *C. reinhardtii* with very low concentrations of acrolein stimulated similar responses to high light and improved tolerance to $^1O_2$. Photosynthetic organisms use a mechanism called non-photochemical quenching (NPQ), which reduces the production of $^1O_2$ under excess light [4]. It is also shown here using the *npq4* mutant, deficient in NPQ, that the transcription of GPXh is increased compared to wild-type cells under excess light and this also corresponds to enhanced tolerance to $^1O_2$. In summary, $^1O_2$-mediated production of acrolein by photosynthetic organisms is a very likely candidate for signal transduction to excess light.

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Impact of kinase activating and inactivating patient mutations on binary PKA interactions

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The second messenger molecule cAMP links extracellular signals to intracellular responses. The main cellular cAMP effector is the compartmentalized protein kinase A (PKA). Upon receptor initiated cAMP-mobilization, PKA regulatory subunits (R) bind cAMP thereby triggering dissociation and activation of bound PKA catalytic subunits (PKAc) [1]. Mutations in PKAc or RIa subunits manipulate PKA dynamics and activities which contribute to carcinogenesis, hormone excess or hormone deficiency and resistance [2-4]. Here we extended the application spectrum of a Protein-fragment Complementation Assay based on the *Renilla* Luciferase to determine binary protein:protein interactions (PPIs) of the PKA network [5]. We compared time- and dose-dependent influences of cAMP-elevation on mutually exclusive PPIs of PKAc with the phosphotransferase inhibiting RIIb and RIa subunits and the protein kinase inhibitor peptide (PKI). We analyzed PKA dynamics following integration of patient mutations into PKAc and RIa. We observed that oncogenic modifications of PKAc(L206R) and RIa(Δ184-236) as well as rare disease mutations in RIa(R368X) affect complex formation of PKA and its responsiveness to cAMP elevation. With the cell-based PKA PPI reporter platform we precisely quantified the mechanistic details how inhibitory PKA interactions and defined patient mutations contribute to PKA functions [6].

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Role of the medial prefrontal cortex in mediating the extinction-promoting effects of L-DOPA in extinction-resistant subjects

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Despite its success in treating specific anxiety disorders, the effect of exposure-based therapy is limited by problems with treatment resistance and fear relapse after initial response. Recent evidence suggests that activation of the dopaminergic system contributes to successful fear extinction, which is the main process underlying exposure based therapy in healthy subjects (1). Here, we exploited the extinction-facilitating potential of the dopamine precursor L-DOPA in extinction-resistant 129S1/SvImJ (S1) mice using a classical cued fear conditioning paradigm followed by extinction training and several extinction test sessions to study fear relapse. Pre-extinction application of L-DOPA promoted fear extinction in S1 mice in the short-term. In an attempt to identify the neuronal substrated mediating the observed behaviorual effects, we next performed microinjection studies targeting the medial prefrontal cortex (mPFC) where successful fear extinction has been shown to coincide with enhanced expression of dopamine-related genes in our preceding study. The intra-mPFC infusion of dopamine constantly reduced fear responses during the extinction training in S1 mice and caused lower freezing levels than vehicle-treatment in the extinction retrieval test, similar to the systemic application of L-DOPA. 10 days later this extinction-enhancing effect was still present suggesting the formation of a persistent extinction memory. Likewise, microinfusions of the dopamine D1 receptor agonist SKF81297 or the dopamine D2 receptor agonist sumanirole prior extinction training attenuated fear responses of S1 mice in the long-term. Overall, the present findings suggest that activation of both the D1R and D2R in the mPFC contributes to the promotion of a persistent extinction memory in the extinction-deficient S1 mouse.
Acknowledgement: Supported by the Austrian Science Fund FWF (SFB F4410 to NS).

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Small molecule interference with neuro and beta-cell degeneration

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NMR based structural Biology, Max Planck Institute for Biophysical Chemistry Göttingen, Germany

Novel insights into dynamic processes involved in mRNA degradation

Remco Sprangers

Max Planck Institute for Developmental Biology, Max Planck Campus Tübingen, Germany

Targeting Cancer Metabolism

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P1

NMR spectroscopy of PR-10 allergens

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Pathogenesis-related (PR) proteins are encoded by different genes that are induced to defend the plants against infections with fungi, bacteria or viruses. The exact function of PR proteins of class 10 is still unclear.[1] Most of the identified plant allergens can be grouped into the PR-10 protein family. A prominent member of this protein family is Bet v 1 (*Betula verrucosa*), the birch pollen allergen. Bet v 1 proteins represent one of the best-characterized group of model allergens in immunology. These proteins are one of the main causes of allergic reactions worldwide, with an estimated 100 million people affected.[2] More than 70 % of birch pollen allergic patients are also reacting allergic to apple.[3] Mal d 1 (*Malus domestica)* is the main allergen found in apples. Cross-reactivity between Bet v 1 and Mal d 1 may result from a high degree of amino acid sequence identity (55-68 %) and the structural similarity of these two proteins.

Among the factors that can contribute to the allergenic potential of proteins,[4] we hypothesize that intrinsic dynamic and structural processes are not only critical but even essential for their immunologic properties.[5]
The goal of our study is to characterize and compare the structures, the stabilities and the flexibilities of allergenic PR-10 proteins from various sources (*i.e.* birch pollen and apple) by different NMR spectroscopic techniques. We are attempting to rationalize the different allergenic and immunological properties of Bet v 1 and Mal d 1 and obtain structrual insight into the cross-reactivity of these two allergens. The data will provide a comprehensive picture of the interplay between structure, dynamics and function of different allergens from the PR-10 family.

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P2

The first GPCR with A-kinase anchoring function

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Signal transmission emanating from the G protein-coupled receptor (GPCR) family to intracellular effector cascades is organized by pleiotropic scaffolding proteins. Signaling scaffolds such as A-kinase anchoring proteins (AKAPs) compartmentalize kinase activities and ensure substrate specificity [1]. In previous studies we have identified dynamic protein:protein interactions (PPI) of compartmentalized protein kinase A (PKA) [2] with distinct molecular switches downstream of receptor cascades [3,4]. In order to gain a more comprehensive and mechanistic understanding of cAMP-controlled macromolecular PKA complexes we chose a phospho-proteomics approach to map dynamic PPIs. We affinity-purified endogenous PKA complexes from osteosarcoma cells and generated a dynamic PPI network. Besides well-known connections to AKAPs, we identified possible links of PKA to metabolic pathways, protein transport, RNA binding, GTP binding, calcium signaling, and nuclear signaling. We selected and investigated a novel PPI between the cAMP/PKA cascade and an orphan GPCR, the GPR161, which has been shown to be involved in Hedgehog signaling [5]. We show that the orphan GPCR GPR161 contains the structural features to function as selective high-affinity type I AKAP. Binary complex formation of GPR161 exclusively with type I PKA regulatory subunits affect plasma membrane targeting in cells and provokes GPR161-mediated PKA recruitment into the primary cilium of zebra-fish embryos. We illustrate that receptor-anchored PKA complexes enhance cAMP-mediated GPR161 phosphorylation, which is regarded as one general principle for regulating GPCR desensitization. In addition we revealed that distinct 'rare disease' mutations of PKA regulatory subunits differentially contribute to spatially restricted interactions of GPR161-anchored PKA type I holoenzyme complexes. We propose that the ciliary *GPR161-PKA signalosome* is a compartmentalized signaling hub that directly integrates receptor-sensed input signals with spatiotemporal cAMP dynamics.

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P3

Comparison of HPLC-UV and NMR methodologies for the quantification of Silymarin complex in *Silybum marianum* fruit extracts

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Silybum marianum (L.) Gaertn. (Asteraceae) is one of the most investigated plant extracts with known mechanism of action. Milk thistle preparations have been used to treat a variety of ailments, particularly against liver damage [1]. The beneficial properties of *S.marianum* are ascribed to Silymarin, a mixture of (at least) six flavonolignans and one flavonoid, used in clinical research as well as in dietary supplements. The separation of individual compounds from the complex mixture of regioisomers remains a challenging task. This study presents two validated HPLC-DAD and NMR methods, according to the ICH guidelines, for the simultaneous determination and quantification of six bioactive compounds in *S.marianum* extracts.

A HPLC method was developed for determination of flavonolignans incorporating rapid separation with highly sensitive UV detection. The method, beside the analysis, was used for their quantification in the fruit extracts. As *Silybum* flavonolignans are difficult to obtain as fully characterized pure compounds, the development of a non-targeted approach by quantitative 1H NMR represented an attractive alternative to conventional chromatographic analysis. Besides quantification, qNMR provides valuable structural information, requires simple sample preparation and reasonably short measuring times, especially with contemporary NMR instrumentation. Silybins and Isosilybins exhibit near-identical 1H NMR spectra, which led us to quantify them in pairs. Measurement of two different types of extracts showed comparable results in composition between the two different techniques. For more than five decades, Silybins and Isosilybins have presented a wealth of challenging interdisciplinary research problems. The successful implementation of the current work could comprise an established approach for future investigations, giving new insight into the quantification of bioactive compounds of other plant species containing complex mixtures of isomers.

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P4

Influence of particle size on the extraction performance of Ethanolic Extracts from Bupleuri radix based on UHPLC-ToF-MS

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Bupleurum falcatum L. was chosen to compare the influence of different extraction parameters. This plant was used because of its high chemical diversity and biological activity. Bupleuri radix is a commonly used herbal drug in Traditional Asian Medicine. [1] It is part of the genus of Atiaceae. The major constituents that are associated with the pharmacological activity of Bupleuri radix include flavonoids, saponins, fatty acids, polysaccharides and polyacetylenes. [2] This large profile induces anti- inflammatory, antibacterial and antiviral properties to bupleuri extracts. [3]

In this study specific extraction parameters were evaluated for the comparison of the secondary metabolite profile. The extracts of Bupleuri radix were assembled with maceration and Ultrasound Assisted Extraction (UAE). [4] In addition the particle size, the concentration of plant material in solvent (10 g/L – 100 g/L), the extraction time (< 24h) and the temperature (20 °C - 80 °C) were modified.

The analytical characterization was performed with an UHPLC-ToF-MS. The large data set was analysed with different statistical tools. The results show that particle size has greater impact on the measured profiles of Bupleuri radix extracts than the chosen extraction methods. The different physical parameters influence the extraction efficiency, too. This approach shows how strong the kinetics are influenced by different extraction techniques. Furthermore a different stability of specific substances, appeared by varying extraction temperature and extraction time.

In summary, the variation of parameters on the extraction process is very important to get more information and to allow optimization.

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P5

Systematic Conformational Studies with Density Functional Theory

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In any molecule the rotation around a bond yields an infinite number of conformers in theory. However, only certain conformers, namely rotamers, represent (local) energy minima, which are separated by energy barriers. Some of these rotamers are so stable that they can be isolated at standard conditions. Such conformers are termed atropisomers. If the molecule is a potential drug, the effect of atropisomerism has a crucial impact in the drug design process. Previous studies dealt with this effect and proposed a classification of atropisomers with respect to their rotational barrier height, and a potential way to estimate those rotational barriers using the Hartree Fock Theory (HF). As the shortcomings of HF are well documented, the intention of our study is to evaluate whether Density Functional Theory (DFT) yields more reliable results. Multiple datasets are analyzed and 18 density functionals plus empirical dispersion correction are tested. Resulting rotational barriers are
compared to experimental data and advanced quantum mechanical calculations to benchmark the performance of DFT. We find out that DFT results are similar to experimental data and advanced quantum mechanical calculation, and that the differences between the various density functionals
are marginal. Furthermore, we experience that relaxed PES scans are suitable for fairly accurate estimations of the rotational barrier.

P6

Nodal-dependent endoderm formation requires inhibition of Fgf-signalling via Mixer

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The formation of the three germ layers – exoderm, endoderm and mesoderm – is an important step from the single cell to a multicellular vertebrate organism. Different signalling pathways are involved and well regulated. Current models characterize Nodal as a morphogenic factor which is important for the patterning of endoderm and mesoderm, but new data focus more on a temporal window of action and interplay with other signalling pathways, e. g. FGF-signalling.

First own experiments on the interaction between Nodal- and FGF-signalling were done on the level of Mixer, a central transcriptional factor within the Nodal cascade. A loss of Mixer circumvents the formation of endoderm. *In-situ* hybridisations and qPCR analyses illustrate the downregulation of endodermal markers, but simultaneously the upregulation of some FGFs, e.g. *fgf3* and *fgf4*. Additional analyses pointed out Mixer binding sites at the respective loci. It is known from previous studies that a high-level of FGF induces the formation of mesoderm and represses endoderm. Many studies describe the role of FGF for mesoderm, but less is known about its function in endodermal patterning.
In this study the crosstalk between Nodal- and FGF-signalling is investigated - especially the role of Fgf3 and Fgf4. Due to the former results a negative and counteracting role of FGF over Nodal signalling must be propagated.
Some mutants of *fgf3* and *fgf4* loci were established with CRISPR/Cas and TALEN technique. The aim is to gain a more detailed view on the processes which define endoderm and mesoderm. Especially the reciprocal impact of Mixer and Fgf3/4 is central. The results of the examinations will be presented.

P7

Structure activity relationship of novel GPR30 ligands

Valeria Follia, Sandra Alscher, Brigitte Kircher and Ronald Gust

The GPR30 receptor is a seven transmembrane Gs protein-coupled receptor, involved in the development of breast and ovarian cancer, able to modulate the non-genomic estrogenic signaling in different cell types. Overexpression of the GPR30 is linked to tumor size (> 2 cm) and strongly correlated to proliferation, invasion, metastasis and drug resistance in many cancer cell lines.
The gene for GPR30 was cloned by different working groups in the late 1990s [1-4] and only in 2006, the first GPR30 agonist, 1-(4-(6-brombenzo[d][1,3]dioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]chinolin-8-yl)ethanone, known as G1, has been discovered [5]. Therefore, as an alternative treatment for breast and ovarian cancer, new derivatives of the GPR30 agonist, G1, were synthesized and tested for biological activity. The compounds elicited strong concentration-dependent anti-proliferative effects which were highly dependent on their structure. Induction of apoptosis was also dependent on the substitution pattern, but also on the tumor cell line.
Our compounds may represent novel drugs targeting aggressive breast and ovarian cancers which overexpress GPR30 and establish a basis for the development of new G1-derivatives.

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P8

Remarkable rate differentiation for local conformational changes in response to ligand binding of a $preQ_1$ riboswitch

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The assessment of kinetics for ligand-induced structure rearrangements that are encountered in RNA riboswitches is crucial to comprehend the intricate mechanism of these RNA domains that regulate gene expression.[1,2] Thereby ligand sensing by the aptamer domain is one of the most critical steps. This process, in particular the induced conformational changes at the secondary and tertiary structure level can be thoroughly analyzed in vitro by the 'ApFold approach'.[1,3]
Here we present the surprisingly large rate differentiation for conformational rearrangement of individual nucleobases that occur during ligand recognition of the $preQ_1$ class-I riboswitch aptamer from *Thermoanaerobacter tengcongensis*. The rate discrimination is 22-fold and significantly larger compared to other aptamers, such as TPP (7-fold), adenine (3-fold) or SAM-II (2-fold) sensing RNAs.[4-6] Our findings provide novel insights into how compact RNA folds that follow the induced-fit recognition mechanism adapt local structural elements in response to ligand binding on a rather broad time scale and how this process culminates in a structural signal that is responsible for efficient down-regulation of ribosomal translation.

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P9

The Role of PI(3)K in Endocrine Cell Migration

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Cell migration is a crucial process during vertebrate development, as in many cases progenitor cells are far away from their final location. In the vertebrate pancreas, the migration of endocrine cells is essential for the formation of functional islets, which produce critical endocrine hormones such as insulin and glucagon. However, the mechanisms which are involved in this migration are almost unknown [Serafimidis, et al., 2011]. To determine the molecular mechanisms behind this migration we use the zebrafish as a vertebrate model organism. With the help of live-cell fluorescence microscopy in combination with quantitative image analysis approaches, it is possible to observe cell migration dynamics *in vivo* and investigate the signaling pathways involved. From other systems it is known that cell migration is regulated by a complex network of signal transduction pathways, which are regulated and act through lipid second messengers, small GTPases, membrane receptors, kinases, cytoskeleton-modifying proteins and motor proteins [Welf and Haugh; 2011]. We first examined PI(3)K as a candidate regulator, as zebrafish neutrophils lose directional migration following PI(3)K inhibition [Yoo, et al.; 2010]. To test the function of PI(3)K in pancreatic cell migration, we are using pharmacologic and genetic approaches. Examination of PI(3)K-inhibited cells revealed changes in cell morphology, and further studies are investigating the effects on directed migration and islet assembly.

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P10

Facile synthesis of 6-thioguanosine building blocks for RNA synthesis and subsequent labeling applications

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6-Thioguanine has been used to treat acute myeloid leukemia since the early eighties,[1] however, its natural occurence is still uncertain. Here, the main goal was to synthesize the corresponding nucleoside and to functionalize it as building block for RNA solid-phase synthesis. After incorporation into RNA oligonucleotides, the influence of the 6-thio modification onto RNA base pairing properties is planned to be investigated. Moreover, 6-thioguanine appears to carry the potential for postsynthetic functionalization. We aim to test various reagents, such as hypervalent iodine compounds[2] or their reactivity against the thio moiety. Ideally, covalent bond formation will be encountered in order to attach a tether carrying an azido unit to allow further functionalization of the RNA with a broad spectrum of labels. Additionally, reagents will be tested that potentially transform the sulphur unit into an amino group; such a thioguanine–into–diaminopurine transformation could impact on RNA sequencing approaches.
The synthesis of 6-*S*-(cyanoethyl)-6-thioguanosine has been described previously,[3] however, because of low overall yields we pursued an alternative strategy. First, a di-*tert*-butylsilylene group was introduced to protect the 5' and 3' hydroxyl groups of guanosine simultaneously, followed by *tert*-butyldimethylsilyl protection of the 2' hydroxyl group and acetylation of the nucleobase 2-amino group[4]. Subsequently, the lactam 6-oxygen atom was converted into a good leaving group using mesitylenesulfonyl chloride. The 6-*S*-cyanoethyl residue was introduced by reaction of the sulfonyl precursor with 2-mercapto ethannitril that was obtained by reduction of 3-(2-cyanoethyldisulfanyl)propanenitrile. Thereafter, the ribose 3' and 5' hydroxyl groups were selectively liberated while the 2'-*O*-tBDMS group was retained, followed by functionalization with 5'-*O*-DMT and 3'-*O*-phosphoramidite moieties to achieve the desired building block for RNA synthesis in high overall yields.

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P11

Characterization of RNA base methylations by top-down mass spectrometry

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Large-scale analysis of ribonucleic acids (RNA) by deep sequencing methods has recently revealed extensive transcription of mammalian genomes without any evidence for translation into proteins. These noncoding RNAs can be highly modified. Methylations are among the most widespread modifications found in posttranscriptionally modified RNA. They can affect all bases as well as the ribose moiety, and they can occur in a highly dynamic fashion. By increasing the structural diversity of RNA, modified nucleosides play important roles in gene expression, and in regulating many aspects of RNA functions. Nevertheless, deep sequencing does not provide information on posttranscriptional modifications (PTMs). A promising alternative approach for the characterization of PTMs, i.e., their identification, localization, and relative quantitation, is top-down mass spectrometry (MS) using collisionally activated dissociation (CAD) and electron detachment dissociation (EDD).
Here we show how top-down MS can serve as a powerful tool for the characterization of RNA base methylations. For this purpose, we systematically studied a variety of methylated RNA forms and isomers, among them 15 nt and 23 nt RNA forms, and 27 nt RNA forms and isomers whose sequence corresponds to the spanning repeat 8 of XIST RNA, with different types, sites, and extent of methylations.

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P12

Wnt/β-Catenin Target Genes in Hydra Patterning and Regeneration

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As in higher animals, also in cnidarians the Wnt/β-Catenin pathway plays a crucial role in developmental and regenerative processes and shows high conservation of structure and function of its signaling components. Wnt/β-Catenin signaling acts in the formation of a head/blastoporal organizer, establishing positional information along the body axis of cnidarian polyps. How this positional information affects cell behavior and to what extent it is evolutionary conserved is unknown. To approach this, a systematic expression profiling of β-catenin target genes was performed. Pharmacologically treated *Hydra* polyps showed increased or decreased β-catenin levels along the body column. RNA was isolated for quantitative sequencing and compared with wildtype transcripts. Genes with the strongest change in mRNA expression were independently tested via semi-quantitative RT-PCR. Whole mount *in situ* hybridizations were performed with 10 critical candidates, seven of the genes are so far not described as β-catenin targets. Overall, these genes show activation in the hypostome, in the tentacles, or in an apical gradient in the body column - areas of Wnt/beta-Catenin action. To uncover direct binding of Tcf/β-catenin to the enhancer regions, CHIP analyses are performed using an α-*Hydra*Tcf antibody.
Surprisingly, all target gene candidates show an upregulation in early head and foot regeneration, which provides evidence for direct Wnt/β-Catenin regulation and may suggest a Wnt independent regulation by β-Catenin in foot regeneration.

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P13

Wnt/β-catenin signaling in *Hydra* leads to specific repression of the *myc1* gene

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The c-Myc protein represents a transcription factor with oncogenic potential controlling fundamental cellular processes. Genes homologous to the c-*myc* protooncogene have been recently identified and characterized in the early diploblastic cnidarian *Hydra* (*myc1*, *myc2*)[1-3]. 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 which is regulated by multiple upstream signaling pathways implicated in oncogenesis and development. Human T-cell factor 4 (Tcf4), the effector of Wnt/β-catenin signaling, has been identified as an oncogenic regulator of the c-*myc* gene in colon cancer[4], but a tumor suppressive function has been also ascribed to this transcription factor[5].
Here we show by *in situ* and Northern hybridization that pharmacological or genetic stimulation of canonical Wnt/β-catenin signaling in *Hydra* leads to specific downregulation of *myc1*, whereas expression of the *myc2* paralog is not affected. Mapping and analysis of the *myc1* and *myc2* promoter regions revealed the presence of consensus Tcf motifs in the regulatory regions immediately upstream of the transcription start sites. *In vivo* binding of Tcf to both promoter regions was demonstrated by chromatin immunoprecipitation, however, luciferase reporter gene assays showed that only the *myc1* promoter is repressed in presence of ectopically expressed β-catenin/Tcf proteins. The identification of the canonical Wnt/β-catenin/Tcf axis as a possible regulatory pathway of the *Hydra myc1* gene indicates that principal interactions in the Wnt signaling network may have been conserved throughout evolution.

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P14

A novel HILIC-Method for the analysis of Mycosporine-like Amino Acids and their biological relevance on anti-inflammatory and collagenase inhibitory targets

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Depletion in stratospheric ozone and subsequent increases in solar radiation promoted research on UV absorbing compounds from different organisms. Mycosporine-like amino acids (MAAs), a group of small secondary metabolites predominantly found in algae, cyanobacteria, lichen and fungi came in the focus because of their pronounced photo protective and anti-oxidative activities [1,2]. In literature the HPLC analysis of MAAs has always been described using reversed phase material [3]. In our study, a fully validated HILIC method is presented, which offers many advantages like a precise quantification of the analytes, adequate retention times and a direct coupling to MS. Excellent linear correlation coefficients (R^2> 0.9998) were obtained. LOD (0.1-0.16µg/ml) and LOQ (0.31-0.48µg/ml) of the pure compounds achieved good results. Additionally three major MAAs, namely shinorine, palythine and porphyra were investigated for collagenase inhibition and anti-inflammatory properties. Collagenase activity was measured in a previously validated fluorogenic assay indicating a dose dependent inhibition of the enzyme by all three derivatives, with IC_{50} values in the range of 37.73 µg/mL (porphyra) to 34.42 µg/mL (shinorine). Their effect on the inflammatory pathway was investigated in a cellular context (NF_kB activity on THP-1 blue cells). Interestingly, shinorine and porphyra were able to induce and not inhibit NF-$_k$B activity, which might suggest pro-inflammatory or immune stimulating properties of these compounds. A significant activation was measured especially in unstimulated cells for porphyra and shinorine but also to a minimum in LPS-stimulated cells for shinorine.

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P15

Radiation damage of DNA unraveled by molecular cluster studies

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In recent years there has been a significant interest in the understanding of damage processes of DNA, also induced by low energy electrons (LEEs). Ionizing radiation releases a large amount of secondary electrons in human cells. These electrons possess kinetic energies up to a few of tens of eV [1]. In this energy range electron ionization as well as electron attachment could cause chemical transformation in biological tissue. Investigations by Sanche and co-workers [1] indicated that these LEEs can induce single and double strand breaks in a film of plasmid DNA upon dissociative electron attachment (DEA). In the process of DEA an electron attaches resonantly to a molecule leading to the formation of a transient negative ion which then may decay into a stable anion and neutral(s) upon the fragmentation of the molecule. This dissociation can be fast and often has only spontaneous electron emission as competitive channel. A large number of studies on electron ionization and electron attachment to various simple biomolecules (e.g. DNA bases, sugars, amino acids) in the gas phase have been carried out [2]. Thereby, it turned out that single biomolecules in the gas phase often substantially decompose upon electron collisions. In addition, DEA is a site selective process, i.e. only certain bonds are cleaved in a molecule after capture of an electron with specific kinetic energy [3].
However, in order to understand radiation damage of complex matter it is highly important to understand how the fragmentation process of biomolecules is modified by a surrounding environment.
In the present study we used biomolecular clusters to observe electron induced reactions in DNA [4]. We chose biomolecules as pyrimidine ($C_4H_4N_2$), tetrahydrofuran (C_4H_8O) and triethylphosphate ($C_6H_{15}O_4P$) as model compounds of DNA building blocks. To meme the surrounding tissue in the human body, the biomolecules were solvated by several water molecules. The clusters were produced via supersonic expansion of the solvated biomolecules into vacuum and crossed with a low energy electron beam. Anions formed were analysed by mass spectrometry.
Our cluster studies gave evidence to the answer how DNA strand breaks are formed by LEE and confirmed a recent postulate [1] how a single LEE can induce a double strand break in DNA.

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P16

Multidimensional Approach in Diagnosis of the Oral Squamous Cell Carcinoma

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Wrong decisions in diagnostic of carcinomas can lead to drastic consequences for the patient, such as surgery or radiotherapy [1]. Due to the fact that decision making process from pathologists is somehow subjective in diagnosing OSCC there is a need for objective analysis of tissue samples. This kind of tumor shows a 5 year survival rate of 53% and 62% for men and women respectively [2]. Due to the high malignancy of this disease early diagnosis is vitally important. IR-imaging is a label free method for molecular imaging in the µm scale and one of the most promising tools to accomplish the task of objective classification [3]. 20 samples of different patients were analyzed and different cluster techniques (hierarchical cluster analysis = HCA, k-means cluster analysis = KMC, fuccy-c-means cluster analysis = FCM) applied to the huge amount of data (100.000 - 200.000 spectra per sample) to compare the results with the usually used (H&E) staining procedure. Good correlations could be observed between the spectroscopic dataset and the reference staining method. By evaluating each pixel of the gained results a rough but objective percentage of cancerous tissue can be estimated. Furthermore a classification model (PCA) among multiple sample areas and patients was established to show excellent differentiation possibility between cancerous and noncancerous areas highlighting the potential usage of IR-imaging in clinical diagnostics.

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P17

The role of β-catenin during regeneration in *Macrostomum lignano*

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Macrostomum lignano is a basally branching flatworm, which is unable to regenerate its head. Different to this flatworm, most some other flatworms are able to regenerate all body parts including the head [1]. Even in some regeneration-deficient species, in which head regeneration is not possible from posterior pieces, head regeneration can be rescued by silencing of [2-4]. β-catenin is an important bifunctional protein in cell adhesion and the canonical Wnt-signalling pathway [5], the latter of which is necessary for establishing anterior-posterior regeneration polarity [6].
Here *M. lignano* is used to study the role of *β-catenin* during regeneration of a species which is unable to regenerate a head from any level of amputation. *β-catenin* orthologues were identified and knocked-down through RNAi treatment. Additionally chemical inhibitors of known β-catenin partners were used to down- and upregulate β-catenin protein activity. Preliminary results suggest that *β-catenin* has partially conserved functions in anterior-posterior regeneration in flatworms.

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P18

Protein quality control in UVB-induced senescence of human dermal fibroblasts

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Cellular senescence is now recognized as a physiological process with defined functions in tumor suppression, mammalian development, tissue reorganization, fibrotic diseases and last but not least, organismic aging of metazoans. UVB-induced senescence of diploid human dermal fibroblasts is used as an experimental model to study the process of photoaging in the skin. We found that the inhibition of proteasomal degradation of damaged proteins and the activation of autophagosome formation are early events in UVB-induced senescence of human dermal fibroblasts (HDF), dependent on UVB-induced accumulation of reactive oxygen species (ROS). Our data suggest that autophagy is required for the establishment of the senescent phenotype in UVB-treated HDF, and that inhibition of autophagy is sufficient to change the cell fate from senescence to cell death by apoptosis. Studies in reconstructed skin equivalents revealed that UVB irradiation triggers hallmarks of autophagy induction in the dermal layer. These findings have potential implications for fundamental as well as translational research into skin aging, in particular photoaging.

P19

Expanding the scope of 2'-SCF_3 modified RNA

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The 2'-trifluoromethylthio (2'-SCF_3) modification endows ribonucleic acids with exceptional properties and has attracted considerable interest as a reporter group for NMR spectroscopic applications.[1,2] However, only modified pyrimidine nucleosides have been generated so far. Here, the syntheses of 2'-SCF_3 adenosine and guanosine phosphoramidites of which the latter was obtained in highly efficient manner by an unconventional Boc-protecting group strategy, are reported.[3] RNA solid-phase synthesis provided site-specifically 2'-SCF_3-modified oligoribonucleotides that were investigated intensively. Their excellent behavior in ^{19}F NMR spectroscopic probing of RNA ligand binding was exemplified for a noncovalent small molecule-RNA interaction. Moreover, comparably to the 2'-SCF_3 pyrimidine nucleosides, the purine counterparts were also found to cause a significant thermodynamic destabilization when located in double helical regions. This property was considered beneficial for siRNA design under the aspect to minimize off-target effects[4] and their performance in silencing of the *BASP1* gene was demonstrated.[3]

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P20

Mapping Conformational States of Thrombin by Comparison of X-ray Structures

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The serine protease thrombin plays a vital role in the blood coagulation cascade, where it activates a variety of substrates to promote blood clotting, supports platelet aggregation and shows an anticoagulant effect by cleaving protein C. [1] Different conformational states of thrombin were described in literature. Prothrombin is the inactive precursor of thrombin and shows large structural differences to the activated form. [2] The transition from the less active slow form to the more active fast form is promoted through the allosteric binding of a Na+-ion. [3]
We align all available native X-ray structures of thrombin (> 300) of the Protein Data Bank [4] to obtain a better understanding of its conformational states. We use the RMSD matrix as the basis for the construction of a structure tree [5]. We describe those amino acid residues that occupy characteristic conformations for the identified states.
This approach systematically compares the available thrombin structures and categorizes them on a state map. It facilitates an automated structure classification that is clear-cut and non-ambiguous and allows the identification of protein sites with high conformational diversity, which is a key property in the substrate readout of thrombin. [6]

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P21

Efficient characterization of local millisecond dynamics: Dihedral entropy from accelerated MD

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We demonstrate a method to capture local dynamics on a time scale three orders of magnitude beyond state-of-the-art simulation approaches. We apply accelerated molecular dynamics [1] for conformational sampling and extract reweighted backbone dihedral distributions. [2] We characterize local dynamics by integration of torsional probabilities, resulting in residue-wise dihedral entropies. We successfully validate our approach for three different protein systems of increasing size: alanine dipeptide, bovine pancreatic trypsin inhibitor (BPTI) and major birch pollen allergen bet v 1. We demonstrate excellent agreement of flexibility profiles with vast scale computer simulations [3] and experimental dynamics data from NMR. [4] Thus, our method provides efficient access to biologically relevant time scales of local protein dynamics.

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P22

Towards a new type of antivitamins B_{12}

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Antivitamins B_{12} are novel organometallic derivatives of vitamin B_{12}.[1] They are presumed to be toxins for any B_{12} dependent organisms. Indeed the antivitamin B_{12}, ethylphenylcobalamin (EtPhCbl), caused "functional B_{12} deficiency" in mice.[2] An unsuspected general impairment of B_{12} uptake and an unequal distribution of EtPhCbl was found in this *in vivo* study.[2]
We are interested in developing new types of antivitamins B_{12} as molecular tools to generate B_{12} deficiency in animals. In addition, we search for effective new antivitamins B_{12} that can be tuned to other organisms than animals and humans, then potentially creating specific antibiotics.[3]

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posters

P23

Design and synthesis of new bivalent stilbene analogues as anti-breast cancer agents

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The estrogen receptor (ER) is a member of the group of nuclear receptors and target of estrogenic hormones. It is found in multiple areas in the human body such as in many reproductive tissues (mammary gland, uterus) the skeletal and cardiovascular system and also specific parts of the brain[1]. Regulation of the estrogen receptor activity can be achieved via different ways. The target predominantly chosen for ER-antagonists is the ligand binding domain (LBD)[2]. One of the main therapy strategies in mammary carcinoma treatment is the resulting ER antagonistic effect on breast cancer cells of the stilbene derivative tamoxifen[3]. However, in the last few years, inhibitors of the coactivator binding have been developed to achieve a new targeting of ER-alpha. Previously, ER-beta was crystallized with two 4-hydroxytamoxifen (4-OHT) molecules. 4-OHT is bound in the LBD, as well as at the hydrophobic surface of the coactivator binding groove[4].
Docking studies using the program GOLD demonstrated that also other stilbene derivatives interact in a similar way at these sites. With this information, compounds with a stilbene substructure were synthesized and linked with an aliphatic spacer through diamide formation in order to target both sites.
The main goal of developing novel ER inhibitors is the design of anti-breast cancer agents against ER-positive tumors that do not respond to currently available antihormonal therapy. A combined LBD and coactivator inhibitor may overcome this drug resistance. An additional aim of a dual mechanism of action is to achieve a more specific targeting of the ER-alpha leading to higher potency and less adverse effects.

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P24

Combining Knowledge Based Approaches and Free Energy Calculations to Predict Binding Affinities

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Structure based optimization is an established approach to improve the affinity of a lead compound in terms of drug discovery. We investigate the comparability of knowledge-based approaches such as Matched Molecular Pairs [1] to Thermodynamic Integration Calculations. [2]
The results are obtained for four different protein-ligand systems: Cycloxygenase II [3], Carbonic Anhydrase I [4], Thrombin [5] and the Vascular-Endothelial Growth Factor-2 Tyrosine Kinase Receptor [6]. We show that Matched Molecular Pairs and Thermodynamic Integration Calculations show a high conformity for most of the investigated transformations.
We conclude that combining knowledge-based approaches and physics based molecular dynamics simulations can provide new insights on ligand-protein interactions and therefore guide lead optimization.

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P25

Regulation of the longevity-promoting transcription factor DAF-16/FOXO by germline signals in the nematode *Caenorhabditis elegans*

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The FOXO transcription factor is required for longevity in response to reduced insulin-like signaling in many organisms (Kenyon, 2010). In the nematode *Caenorhabditis elegans*, the FOXO ortholog, DAF-16, is also required for the lifespan increase that occurs when germline stem cells are ablated while the somatic gonad remains intact (Hsin and Kenyon, 1999). Interestingly, previous work from our group has found that removing the germline precursor cells from long-lived insulin-receptor (*daf-2*) mutant worms extends their lifespan even further (Hsin and Kenyon, 1999), in a manner dependent on different upstream regulators (Berman and Kenyon, 2006). These and other findings are consistent with the notion that DAF-16 regulation and function differ between the Insulin signaling- and the germline longevity pathway. Here, we present our biochemical screening approach to identify and our ongoing work to validate novel regulators of *C. elegans* germline longevity.

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P26

Elucidating the "LIM-code" in rat pancreatic endocrine cells

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The so-called "LIM-Code" describes a model for generating distinct neuronal cell types by combinatorial expression of distinct sets of LIM-domain transcription factors in an initially similar progenitor population. Support for this model comes from studies in C*. elegans, Drosophila* and various vertebrates, which demonstrated cell-type specific requirements for LIM-proteins and their interaction during differentiation, maturation and survival of various CNS and PNS neuron. As key components of this LIM-code are not only expressed in the nervous system, we hypothesized for a more general role of "LIM-Codes" in generating cellular diversity among related cell populations.
In our previous studies in zebrafish we showed that one of these key components, the LIM-homeobox factor Islet1/isl1, also has cell-type specific functions in the developing pancreatic islet. Consistent with a potential pancreatic "LIM-code", we further found distinct pancreatic expression pattern for several LIM-proteins encoding genes. In order to study the molecular basis for cell-type specific differences of Isl1 activities, we decided to study islet1 interactions in rat cell lines resembling characteristics of pancreatic β- (INS1) and δ-cells (MSL-G2 TU6), respectively. In a first set of experiments we confirmed the cell line specific physiological characteristics and revealed differences of Isl1 interacting partners by RT-qPCR. Further, we established immunoprecipitation of endogenous Isl1 bound co-factors to identify the cell-type specific Islet1-protein complexes by mass spectrometry.
In further experiments we will use loss- and gain-of-function studies in these cell lines to determine the role of the identified co-factors as part of a potential pancreatic Lim-code. Recent data will be presented.

P27

Water Wires in Calcium Channels and Prediction of Disease-associated Mutations

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The development of water wires in the voltage sensors of ion channels has severe consequences on the channel functions and cellular patterns [1][2][3]. We focused on their molecular mechanisms for voltage-gated calcium channels, where the measurement of omega currents is still challenging so far.
Since crystal structures of α_1 subunit are not yet available, we applied molecular modeling to predict potential functional changes upon amino acid mutations.
We generated homology models based on the crystal structure of a sodium channel [4] and performed molecular dynamics simulations in the membrane.
Our models show the formation of continuous water wires in the resting state α upon mutation of the top arginine residue (R1) in helix S4 to smaller uncharged amino acids.
The state dependence of water wires has a great impact not only on the activation mechanism, but also on the relationship between genotype and phenotype.
Indeed, public databases report thousands of single nucleotide polymorphisms (SNPs) at calcium channel α_1 subunits, but only few of them have been found in patients as cause of diseases. Still today most SNPs remain unexplored and may hold the answer to several unexplained questions on the development of diseases.
In absence of assay data, we applied computational tools to perform the screening of huge databases [5] and select the most relevant mutations to be afterwards tested experimentally.
In detail, we based the predictions on our complete 3D models for calcium channels and calculated descriptors to for each mutation. Our in silico model predicted the probability of single nucleotide polymorphisms to be either benign or disease-associated, facilitating the selection of mutants for the upcoming assays.

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P28

A novel monolithic stationary phase for the separation of phenolic acids by capillary electrochromatography

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CEC (Capillary Electrochromatography) is a hybrid technique combining features of HPLC (stationary phase) and capillary electrophoresis (electroosmotic flow) in a unique way [1]. CEC impresses with versatility, high separation efficiency and economy [2]. Yet, its practical relevance is still limited due to missing commercial sources for stationary phases and few convincing applications [3].
An innovative zwitterionic stationary phase for CEC was developed by UV-induced polymerisation of 1-allyl-3-(butyl-4-sulfonate)imidazoline. Using a porogen mixture of 1-propanol, 1-butandiol and H_2O, a homogeneous monolith was obtained. With an appropriate buffer system (12 mM ammonium acetate; pH 8.5; 88% acetonitrile) this stationary phase permitted the separation of six phenolic acids (salicylic, cinnamic, syringic, rosmarinic, caffeic and chlorogenic acid) in 10 min. The method was fully validated and successfully applied to the quantitative analysis of respective compounds in several food plants (green coffee beans, black tea leaves, thyme and dill leaves). The results were reproducible and in accordance to established procedures.

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P29

Functional properties of newly identified somatic *CACNA1D* mutations in aldosterone-producing adenomas

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Background: Almost 5 % of hypertension is caused by aldosterone-producing adenomas (APAs). We have previously identified seven new mutations occurring in the pore-forming α_1 - subunit (*CACNA1D* gene) of $Ca_v1.3$ voltage-gated Ca^{2+} channels. For four of them (G403R, I750M, P1336R and V259D) we identified a gain-of-function phenotype which explains excess of aldosterone production. Here we report the functional characterization of the three APA mutations not studied so far (F747L, R990H and M1354I) to determine if they also enhance calcium entry through these channels.
Methods: TsA-201 cells were transiently transfected with the cDNA of mutations F747L, M1354I and R990H in $Ca_v1.3$ α_1 – subunits (long splice variant, $Ca_v1.3_L$), together with the auxiliary β_3 and $\alpha_2\delta$ subunits. Biophysical properties of Ca^{2+} inward currents (15 mM Ca^{2+}; I_{Ca}), were measured in whole-cell patch-clamp recordings. Western blot analysis was used to quantitate α_1 protein expression levels.
Results: Mutation F747L severely affected $Ca_v1.3_L$ gating by significantly shifting $V_{05,act}$ (-13.9 mV, n > 15) and $V_{05,inact}$ (-4 mV, n > 7) to more negative voltages and by significantly slowing inactivation (remaining current [%] after 250 ms: 43.3 ± 2.25 %, n = 28 for WT, 70.6 ± 3.96 %, n = 14 for F747L, p-value < 0.001, unpaired Student's t-test). Moreover, ON-gating charge was not detectable in mutant channels suggesting also enhanced open probability. In contrast, mutation M1354I did not show significant differences in channel gating ($V_{05,act}$, $V_{05,inact}$), or inactivation time course. For the R990H mutant an increased I_{Tail}/Q_{ON} ratio was observed, suggesting an enhanced open probability indicative of a gain-of-function phenotype. In addition, molecular modelling studies strongly suggest that this mutation affects a critical arginine residue in the S4 helix of the voltage sensor in domain III, thus creating an additional pore in this voltage sensor domain (VSD) permitting a depolarizing flow of protons ("ω-current") in the resting state. Total protein expression levels were increased for F747L and R990H mutations compared to wild-type $Ca_v1.3_L$, but were unchanged for M1354I.
Conclusions: Mutation F747L results in strong gating changes compatible with a channel gain-of-function and therefore supports its disease-causing role in APAs. Molecular modelling of mutation R990H strongly points to a disease-relevant creation of a pore in the VSD, where ω-currents may induce depolarization of the cell. In contrast, the disease-causing potential role of M1354I could not be confirmed in our studies.

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P30

A Mini-Twister Variant and Impact of Residues/Cations on the Phosphodiester Cleavage of this Ribozyme Class

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Nucleolytic ribozymes catalyze site-specific cleavage of their phosphodiester backbones. A minimal version of the twister ribozyme[1-3] is reported that lacks the phylogenetically conserved stem P1 while retaining wild-type activity.[4] Atomic mutagenesis revealed that nitrogen atoms N(1) and N(3) of the adenine-6 at the cleavage site are indispensable for cleavage. By NMR spectroscopy, a pK_a value of 5.1 was determined for a ^{13}C2-labeled adenine at this position in the twister ribozyme, which is significantly shifted compared to the p*K*a of the same adenine in the substrate alone. This finding pinpoints at a potential role for adenine-6 in the catalytic mechanism besides the previously identified invariant guanine-48 and a Mg^{2+} ion, both of which are directly coordinated to the non-bridging oxygen atoms of the scissile phosphate; for the latter, additional evidence stems from the observation that Mn^{2+} or Cd^{2+} accelerated cleavage of phosphorothioate substrates. The relevance of this metal ion binding site is further emphasized by a new 2.6 Å X-ray structure of a 2′-OCH3-U5 modified twister ribozyme.[1]

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P31

Characterization of post-translationally modified histones in peripheral blood mononuclear cells (PBMCs) of panic disorder patients

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Epigenetic mechanisms, such as histone acetylation and methylation, have been shown to be involved in the etiology of anxiety disorders (El-Sayed et al., 2012). Peripheral blood mononuclear cells (PBMCs) provide an easy accessible biomarker source to measure epigenetic changes in patients with psychiatric disorders (Sharma, 2012). Here, we report on the application of capillary electrophoresis electrospray ionization mass spectrometry (CESI-MS) for fast and sensitive analysis of post-translational modifications at the N-terminal tail of histone H4 isolated (Sarg et al., 2013) from PBMCs of panic disorder patients and healthy controls. Histone H4 was chosen based on the previous observation that acetylation levels on this specific histone protein change during fear extinction learning in rodents (Bredy et al., 2007), which forms the basis of exposure-based therapy in humans. Core histones were pre-separated by reversed-phase high performance liquid chromatography (RP-HPLC) and histone H4 fraction was subsequently analyzed by CESI-MS. However, high levels of histone H4 citrullination were present in several samples, interfering with the quantification of acetylation levels using CESI-MS. To overcome this technical limitation, we tried to apply a highly sensitive capillary electrophoresis (CE) method coupled to ultraviolet (UV) detection (CE-UV). Indeed, CE-UV led to sufficient separation of acetylated and citrullinated histone H4 proteoforms, enabling reliable quantification of acetylation levels.
Preliminary results revealed no differences in baseline histone H4 mono-acetylation in PD patients compared to healthy controls. Moreover, we did not observe changes in histone H4 mono-acetylation in response to treatment. Interestingly, however, histone H4 mono-acetylation positively correlated with age in healthy subjects, but not in patients with panic disorder, potentially indicating a role for histone acetylation in the altered regulation of gene expression associated with aging and panic disorder.
Taken together, the current study suggests that CE-UV is a reliable technique to study posttranslational histone modifications in PMBCs in the course of epigenetic studies in humans. We further find that epigenetic mechanisms involving histone acetylation could be implicated in aging and anxiety disorders, both of which are associated with a wide range of gene expression changes.

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P32

Antitumour active cobalt alkyne complexes derived from acetylic salicylic acid: studies on impact of fluorination and chlorination of Co-ASS

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[(Prop-2-ynyl)-2-acetoxybenzoate]dicobalthexacarbonyl (Co-ASS), a derivative of the irreversible COX-1/-2 inhibitor acetylic salicylic acid (ASS) chelated to cobalt, demonstrated high growth-inhibitory potential against various tumour cell lines with inhibition of COX-1/-2 as a probable mode of action. [1] We modified the ASS moiety esterified by a propargyl group and obtained its chlorinated and fluorinated derivatives, respectively, and investigated them in biological studies to identify the impact of a fluorine or chlorine substitute in different positions of the acetylic salicylic acid moiety on cytotoxic activity, COX inhibition in general and COX isoenzyme selectivity in particular compared to the lead structure Co-ASS.
Compounds and ligands only were evaluated for cytotoxicity in breast [MCF-7 (hormone dependent), MDA-MB-231 (hormone independent)] and colon cancer [HT-29] cell lines and for COX-1/-2 inhibitory effects at human recombinant or ovine isoenzymes. For selected compounds with strong COX-1/-2 inhibition, the major COX metabolite prostaglandine E2 (PGE_2) was quantified in arachidonic acid-stimulated MDA-MB 231 breast tumor cells via enzyme immunoassay (EIA). Whereas the ligands only showed insignificant cytotoxic effects, cobalt complexes were able to inhibit tumor cell growth in all cell lines to a larger extent. Further, complexation of ligands to cobalt led to a tenfold increase in the potency to inhibit COX-1/-2 compared to ligands only. Fluorination and chlorination of the ASS propargylester moiety led to a shift in COX selectivity and a cell line specific cytotoxicity. The chlorinated derivatives, however, showed higher cyclooxygenase inhibition and cytotoxicity than the fluorinated derivatives. Compounds with a weak inhibition of COX also showed a low cytotoxic potential in the cell based assay and vice versa, indicating the interference with the arachidonic acid as a potential mode of action.

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P33

The role of inflammaging in the age-related impairments in the maintenance of immunological memory

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Aging is characterized by a decline of immune functions, a process known as immunosenescence. For this reason it is very important to find new strategies to counteract the problem, in particular through the maintenance of immunological memory. It has been demonstrated that memory T cells and long-lived plasma cells survive in bone marrow niches, well organized structures which promote the homeostatic proliferation of these cells[1]. $CD4^+$ and $CD8^+$ T cells maintenance is supported by IL-7 and IL-15 while plasma cells require APRIL and IL-6 producing cells [2, 3, 4].
Aging induces a basal level of inflammation throughout the body, a condition known as inflammaging which is believed to play an important role in the onset of most age-related diseases[5]. Reactive oxygen species (ROS), whose production is particularly high in old age, may contribute in this process.
We investigated bone marrow mononuclear cells (BMMC) expression of memory T cell and plasma cell survival factors by qPCR and FACS, finding that IL-7 and APRIL decrease while IL-15 and IL-6 increase with age. Stimulation of peripheral blood mononuclear cells (PBMC) with IFNγ and TNFα leads to an increased IL-15 and IL-6 expression. Incubation of PBMC and BMMC with ROS scavengers N-acetylcysteine (NAC) and vitamin C leads to a reduction of IL-15 and IL-6 levels, suggesting that oxidative stress may play an important role in the age-related impairments in the maintenance of immunological memory.

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P34

A protein fragment complementation assay for measuring iron-sulfur-cluster formation

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Friedreich's Ataxia (FA) is an autosomal recessive disease and the most common inherited ataxia [1]. It is caused by an intronic GAA-trinucleotide expansion in the frataxin gene. This leads to reduced levels of Frataxin [2]. Among other functions, frataxin a small mitochondrial protein, is also involved in assembly of iron-sulfur-cluster (FeS) proteins. As there are several isoforms of Frataxin and their individual exact physiological role is unknown [3] we wanted to develop a readout for frataxin´s ultimate function, namely the formation of FeS-clusters [4]. For this purpose, a dynamic protein interaction system based on protein fragment complementation was established. Human glutaredoxin 2 (GRX2), a protein that is only able to dimerize by binding 2Fe2S clusters [5], has been fused to two renilla luciferase fragments that can only complement each other when brought into close proximity [6]. Thus FeS-cluster dependent dimerization of GRX2 can be measured via the bioluminescence of complemented luciferase [5]. As a negative control the C37A-mutant of GRX2, that is no longer able to coordinate a metallocluster has been chosen. With this assay it will be possible to measure whether small molecules or hormons, like erythropoietin have an influence on the frataxin-dependent formation of FeS-clusters. It could thus be a useful tool to screen for novel drugs to combat Friedreich´s Ataxia.

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P35

The Proseriate flatworm *Minona ileanae*: a new model for Bioadhesion

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Adhesives are used in many different fields in industry, technology and medicine. Numerous synthetic materials are used in manufacturing processes and the trend for miniaturization in many fields require new innovations in adhesive technology. Man-made adhesives normally share disadvantages like poor biocompatibility or weak underwater performance. Biological systems have developed a great variety of different glues for their needs and therefore are of interest in the adhesion field.
Our investigations focus on the bioadhesive properties of flatworms. We will present our findings on the temporary adhesive system of the Proseriate flatworm *Minona ileanae*. Proseriates have developed highly sophisticated adhesives which allow them to live in between sand of beaches with high wave impact. By the use of transmission electron microscopy, we were able to morphologically characterize the *Minona ileanae* duo-gland adhesive system. In addition, we generated a differential transcriptome to identify tail-specific genes. This data is currently being used as basis for a *in situ* hybridization screen. First *in situ* hybridizations already identified one possible adhesion related transcript. In future investigations we aim to characterize and compare adhesion molecules on a molecular level of several flatworm species.

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P36

Understanding and Improving B_{12} Antivitamins by Molecular Dynamics Simulations

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B_{12} antivitamins are novel cobalamin derivatives that block the B_{12} metabolism by inhibition of the processing enzyme *homocystinuria type C* (CblC). They may be used in animal studies to induce a functional B_{12} deficiency in a selective and noninvasive way. Thus, they are ideally suited to study the consequences of cobalamin deficiency because they mimic the symptoms of reduced or impaired B_{12} uptake.
B_{12} Antivitamins represent a new class of cobalamin derivatives with novel Co_{β}-aryl and Co_{β}-alkynyl ligands [1,2]. The unique feature of these species is that their Co_{β}-aryl and Co_{β}-alkynyl bond cannot be reversibly cleaved by the enzyme. Thus, these species are not transformed into a physiologically active form, and further metabolism is impeded.
Until today, no crystal structures are reported, where antivitamins are bound to CblC. Therefore, we used molecular dynamics simulations to study the CblC-antivitamin interactions and understand their functioning. Results are compared to protein-ligand interactions found for physiologically active species such as methylcobalamin.
Although the reported antivitamins inhibit the CblC, they neither UV-Vis stable [1] nor proteolysis resistant [2]. Based on the size and the electrostatics of the protein cavity, we suggest modified antivitamins that increase the protein-substrate interactions by maximizing the number of favorable protein-substrate interactions.

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P37

Alpine plants in cosmetics

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Plant extracts represent a valuable source for the identification of novel bioactive natural products with anti-ageing effects. Of particular interest are plants from the Alps that, due to the different conditions present in higher altitudes compared to the valleys, often comprise unique ingredient patterns. They accumulate certain secondary metabolites in larger quantities [1], especially substances that protect plants against UV radiation [2], which is known to contribute to the ageing of human skin [3, 4]. Therefore, Alpine plants provide enormous potential for the development of new cosmeceuticals, i.e., cosmetics with proven pharmacological effects.
Within this project, 150 plant species, mainly from the Alpine region, were selected, collected, and extracted with ethyl acetate and methanol, respectively. The extracts were forwarded to pharmacological investigation of their ability to inhibit NADPH oxidase 4 (NOX4) and to activate proteasome in cell-based assays as well as to analysis of their phytochemical profile. Up to 10 extracts with potent anti-aging effects and suitable chemical profiles will be selected for further studies. Bioactive compounds will be isolated, identified, and subjected to analysis of their molecular mechanism of action. Between 1 and 3 extracts with cosmeceutical potential will be chosen for evaluation of their effectiveness *in vivo*. Moreover, active compounds present in these extracts will be quantified and analytical methods for quality control will be developed. The final aim of this project is the identification and generation of high-quality plant extracts with anti-ageing activity *in vitro* and *in vivo*, which can be used for the development of cosmeceuticals.

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P38

Secondary electron interaction with radiosensitizers

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Nitroimidazolic compounds are currently investigated as potential radiosensitizers, some of which, i.e. misonidazole and nimorazole, have been already tested in clinical trials [1, 2, 3]. The clinical trials with nimorazole have been especially successful and as a result it is currently used during radiotherapy of head and neck cancers as part of the standard treatment schedule in Denmark [1].
The effect of radiosensitizers, i.e. enhanced damage of tumor cells, is ascribed partially to the action of low-energy secondary electrons which are formed in abundant amounts during the irradiation of biological tissue. The kinetic energy distribution of secondary electrons formed finds its maximum below 10 eV, where the (dissociative) electron attachment (DEA) may significantly contribute to rate of damage of the tissue. In the present study we investigated low energy electron interaction with radiosenzitizers in means of DEA, thus we observed charged fragments of the molecule after the reaction with the electrons. We were mainly focused on production of OH radicals from the interaction between the electrons and radiosensitizers, since it is known that they are responsible for the strand breaks of the DNA. Further on, we are examining reactivity of radiosensitizers with similar structure, i.e. nitroimidazole and its methylated derivative. In one of our studies it was shown that the methylated form of nitroimidazole is less reactive than nitroimidazole itself when exposed to electrons in low energy range [4].
The search for alternatives for currently used radiosensitizers with low neurotoxicity but high sensitizing abilities is still ongoing and therefore other possible radiosensitizers are being investigated.

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P39

Syntheses of 5-hydroxymethylcytidine and -uridine phosphoramidites and their incorporation into RNA by solid–phase synthesis

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5-Methylcytosine is one of the most abundant epigenetic base modifications in DNA. Its function and metabolism have been extensively studied over the past decade.[1] Recently, light has also been shed on the molecular basis of reversibility of cytosine methylation patterns.[2] Thereby, the major demethylation pathway involves a conversion of 5-methylcytosine into 5-hydroxymethylcytosine. In contrast to DNA, "*Epigenetics of RNA*" is underexplored as becomes evident by the fact that 5-methylcytosine has been identified only very recently in other types of RNA than tRNA and rRNA.[3,4] Likewise, the mechanism of cytosine methylation in RNA, and in particular that of demethylation, is far from being intensively explored. In order to investigate the biological function of 5-hydroxymethylcytosine and its role in RNA demethylation, chemically synthesized oligoribonucleotides that contain this modification are an important tool. We report on the syntheses of 5-hydroxymethyl-uridine (5hm(rU)) and -cytidine (5hm(rC)) phosphoramidites and their incorporation into RNA by solid-phase synthesis. Deprotection was accomplished in straightforward manner using standard conditions. The approach provides robust access to 5hm(rC/U) modified RNAs which await applications in pull-down experiments to identify potential modification enzymes. They will also serve as synthetic probes for the development of high-throughput sequencing methods in native RNAs.

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P40

'Clickable' Chlorophyll Catabolites – III

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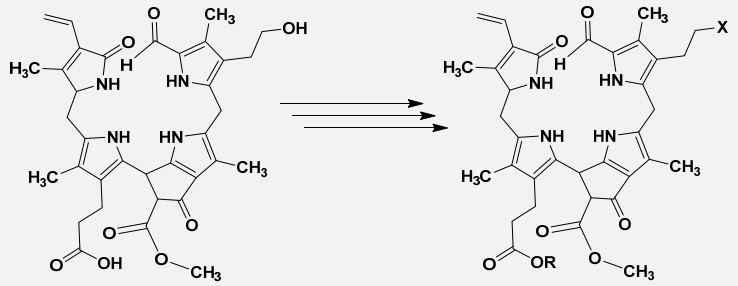
Breakdown of chlorophyll in plants is a focus of investigation in our laboratory [1]. An open issue is the biological interactions of chlorophyll catabolites. This refers, in particular to non-fluorescent chlorophyll catabolites (NCCs), which are also found in our nutrition [2]. To find an answer for this question, we thought to bind NCCs to a solid phase for affinity chromatography. This would generate the possibility to find still elusive biomolecular binding partners for NCCs. We used the Cj-NCC-1 (1) from leaves of the deciduous tree Cercidiphyllum japonicum [3] as our synthetic "workhorse".



Cj-NCC-1 (1) NCC Derivatives (2a, 2b)

We converted the Cj-NCC-1 (1) to new stabile azide derivatives (2a, R = methyl, X = N3; 2b, R = 2-trimethylsilylethyl, X = N3), which underwent the click reaction (azide-alkyne-cycloadditon reaction [4]) readily. Herein we present our approaches directed of finding biomolecular interactions with NCCs by affinity matrices.

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P41

Acute vortioxetine, but not fluoxetine, promotes synaptic plasticity in the hippocampal CA1 region: possible mechanism for pro-cognitive effects

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Preclinical and clinical studies have demonstrated the antidepressant properties of the multimodal acting antidepressant vortioxetine and a clinical study in elderly depressed patients showed superiority to placebo in cognitive tests. Depression-related impaired cognition is associated with aberrant synaptic plasticity in the hippocampus. Therefore, we compared the effect of vortioxetine against the established antidepressant fluoxetine on hippocampal synaptic plasticity in the rat hippocampal CA1 region. Male Sprague-Dawley rats were dosed acutely/subchronically with vortioxetine/fluoxetine. Subsequently, immediate-early-gene-mapping, patch-clamp recordings, golgi-staining and spine morphology analysis was performed in the CA1 region.
Immediate-early-gene-mapping revealed that vortioxetine, but not fluoxetine, reduced the total number of activated neurons (at the basal state) in the CA1 region. The number of activated excitatory neurons remained unchanged, indicating a possible reduction in the inhibitory neuronal activity by vortioxetine. In line with these findings, patch clamp recordings revealed that vortioxetine disinhibited pyramidal cells by blocking serotonin-induced excitation of GABAergic interneurons in CA1 and the golgi staining revealed that subchronic vortioxetine, but not fluoxetine, increased dendritic length in the pyramidal CA1 neurons. Finally, hippocampal neuronal culture studies showed that vortioxetine promoted dendritic spine maturation relative to vehicle, suggesting a transition to a more mature neuronal morphology. In contrast, fluoxetine treatment promoted an increase length of dendritic protrusions only, indicative of an immature phenotype. Given the central role of the hippocampus in cognition, and the key role played by pyramidal cells for hippocampal output, these findings indicate possible cellular correlate to the observed effects of vortioxetine on cognition.

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Silvering and parasitism affect ROS defense capability in swimbladder tissue of the European eel (Anguilla anguilla)

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In a process called silvering, European eels prepare for their long-distance migration from European fresh water systems to the Sargasso Sea for reproduction. During this journey, eels perform extended diel vertical migrations [1], and the concomitant changes in hydrostatic pressure significantly affect the swimbladder, functioning as a buoyancy organ [2]. As the swimbladder is primarily filled with oxygen, the tissue has to cope with extreme hyperoxic conditions, which typically are accompanied by the generation of reactive oxygen species (ROS) and oxidative stress [3]. In addition, since the introduction of the parasitic nematode *Anguillicola crassus* in the early 1980's, swimbladder function of most of the European eels is impaired by the infection with this parasite [4].
To answer the question how the swimbladder tissue can cope with hyperoxic conditions and whether silvering or the infection with *A. crassus* affect this capability, we analyzed muscle and swimbladder tissue from uninfected yellow, infected yellow, uninfected silver and infected silver eels for the enzymes superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (GPx) and glutathione reductase (GR), the metabolite glutathione (GSH+GSSG) and additionally the level of lipid peroxidation which increases with ROS stress.
In swimbladder tissue we found higher concentrations of GSH+GSSG as well as higher activities of SOD, GPx and GR, suggesting SOD and the glutathione cycle to be of high importance for ROS detoxification. Comparing uninfected yellow with uninfected silver eels, the concentration of GSH+GSSG and the activity of SOD were elevated after silvering, corresponding with lower levels of lipid peroxidation. While in yellow eels the infection with *A. crassus* had no effect, in silver eels the capacity to cope with ROS was significantly impaired and resulted in increased oxidative stress.

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P43

Characterization of the effects of carbon monoxide on the cardiovascular system under hypoxia in zebrafish (*Danio rerio*)

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Periods of environmental hypoxia are extremely common in aquatic systems due to manifold natural, for example biotic or abiotic diurnal oscillations. Fish provide a number of hypoxia tolerant species. Over the past decade the zebrafish *Danio rerio* was introduced as a popular model organism to study the effects of oxygen deprivation. Depending on the animal's developmental stage, duration and severity of deprivation, hypoxia or anoxia affect cell cycle, gene expression, development and physiology in many ways. In the most extreme case heart beat and other vital signs can suspend in case of extreme hypoxia[1].
This study tries to shed some light on the effects of hypoxia combined with carbon monoxide (CO) on the cardiovascular system of zebrafish. CO is a well-known toxic gas, which is colorless, odorless and tasteless. The toxicological effect results of its binding to haemoglobin. CO has a stronger affinity for haemoglobin then oxygen. Therefore, CO was mainly known as a killer and its negative aspects. But since a while the negative connotation of CO is changing and the possible positive effects of CO are investigated. Preclinical studies show positive effects of CO in cardiovascular disease, sepsis and shock, cancer, acute and chronic rejection of a transplanted organ and acute lunge, kidney and liver injury. CO has potent anti-inflammatory effects and can cause vasorelaxation and hypotension[2]. We hypothesize that zebrafish survive hypoxia better in the presence of high CO levels. We tested this hypothesis on 3 dpf old zebrafish larvae. A custom-made glass chamber containing the animals in a petridish was perfused with a gas mixture consisting of 0,94 vol. % oxygen, 5 vol. % CO in nitrogen. After six hour of incubation survival rate and vitality of the animals was compared to a control group without CO. Our hypothesis was not confirmed. Although heartbeat restarted after reoxygenation in some animals, 48h after incubation these animals showed heart edema which was not observed in animals incubated in hypoxia alone.
Further experiments are needed to understand the phenomenon of suspended animation and to find strategies to enhance survival after oxygen deprivation.

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P44

Risk assessment on toxic relevant destruxin analytes – do they enter the food chain?

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The entomopathogenic fungus *Metarhizium brunneum* plays an important role as a biological control agent (BCA) and is used successfully to control the western corn root worm (*Diabrotica virgifera virgifera*) and the vine weevil (*Otiorhynchus sulcatus*). Since there are still concerns that the use of fungal biocontrol agents or their bioactive secondary metabolites impart risks to humans or the environment , their use has to be flanked by a risk management approach, which includes the need to monitor the fate of these relevant toxic metabolites in crops.
Therefore, a novel QuEChERs-based extraction protocol in combination with a fast and selective UHPLC-QTOF-MS assay has been established, which allowed the quantitative analysis of the relevant destruxin congeners, dtx A, dtx B and dtx E, down to the parts per billion range.
The optimized assay was used to screen a total of 19 strawberry and maize samples, eight control and eleven treatment samples, obtained from INBIOSOIL field trials. All samples were prepared in duplicates and analysed three times with the result that no destruxin residues were found in any of the analysed samples.

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P45

Impact of kinase activities on RNA:protein interactions

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Post-transcriptional gene regulation is essential to maintain cellular metabolism, coordinate maturation, transport, and degradation of all classes of RNAs. These processes are tightly regulated by versatile RNA-binding proteins [1]. Especially RNA-binders of the FET family with the three members *protein-fused in sarcoma* (FUS), the *Erwing sarcoma protein* (ESWR1), and the *TATA-binding protein-associated factor 2N* (TAF15) are critical implicated in physiological and pathological RNA-related functions [2]. TAF15, for example, is able to bind a large and ubiquitous RNA population, controlling biogenesis and maturation of mRNAs and microRNAs [3]. Deregulation of this FET protein has been linked to the progression of certain types of cancer and it has been implicated in the etiology of Amyotrophic Lateral Sclerosis (ALS) [4]. However, the molecular mechanism how TAF15 contribute to these different disease patterns is poorly understood. In a phospho-proteomics screen for protein kinase A (PKA) [5] substrates and interaction partners we have identified possible links between kinase signaling and TAF15 phosphorylation. Interestingly, we have allocated a PKA consensus phosphorylation site in one of the RNA binding modules of TAF15. Recently, we confirmed the specific phosphorylation of TAF15 by PKA, which we assume could affect its RNA-binding properties. We plan to perform individual-nucleotide resolution cross-linking and immunoprecipitation (iCLIP) experiments [6] to identify RNA species bound to TAF15. The characterization of this phospho-protein bound RNA population could connect receptor/kinase signaling cascades with deregulated RNA metabolism. We assume that kinase-controlled dynamics of the molecular interaction of RNA with TAF15 could become a pharmaceutical target to tackle deregulated TAF15 functions in diseases such as ALS.

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P46

Competition for protons in the dissociation of RNA-small molecule complexes

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The characterization of ribonucleic acids (RNA) by mass spectrometry (MS) using electrospray ionization (ESI) often suffers from undesired alkali cation (Na^+, K^+) attachment resulting in heterogeneous signals from various ion species derived from a single RNA molecule of mass M, i.e., $(M - nH + mNa + oK)^{(n-m-o)-}$. However, solution additives such as piperidine can be used to efficiently suppress alkali cation attachment. We have previously shown that additives of differing proton affinity (PA) can be used to efficiently manipulate the net charge n of $(M - nH)^{n-}$ ions of RNA by a mechanism that is not yet understood [1], and that charge manipulation is highly important for RNA characterization by MS using collisionally activated dissociation (CAD) and electron detachment dissociation (EDD) [2]. Here we investigate the mechanism of RNA-additive dissociation and show that separation of oppositely charged species, $(M - nH)^{n-}$ ions of RNA and positively charged additive ions, can become favorable when additive cluster ions are involved. This new insight has important implications for gas phase proton transfer reactions of biomolecules in general, and for RNA-ligand interactions in particular.

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P47

Facile synthesis of a 2'-*O*-aminoalkylated guanosine building block for RNA synthesis and subsequent labeling applications

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Modern spectroscopic techniques – such as single-molecule-fluorescence-resonance energy transfer (smFRET) – require labeling of the biomolecule of interest[1,2]. For RNA, labeling usually involves a multiple-step approach that starts with RNA solid-phase synthesis using an appropriately prefunctionalized building block that is site-specifically incorporated. Consequently, the deprotected and purified RNA provides an anchor group that, in turn, is transformed with the actual label, e.g. a fluorophore. Most frequently, a pyrimidine nucleoside phosphoramidite with a protected aminoalkyl tether either at the 5 position of the nucleobase or the 2'-*O* position of the ribose unit represents the "appropriately prefunctionalized building block". While the pyrimidine building blocks are commercially available, access to the corresponding aminoalkyl tether purine nucleosides represents a severe bottleneck, in particular concerning guanosines. Here, we present the synthesis of a 2'-*O*-aminopropyl guanosine building block that has been carefully optimized based on an existing report in the literature[3]. The key steps are direct alkylation of 2,6-diaminopurine nucleoside followed by enzymatic transformation into guanosine through adenosine deaminase. Only five steps were needed to obtain the fully functionalized nucleoside phosphoramidite which makes this synthesis highly efficient and superior to other routes towards tethered guanosines building blocks.

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P48

Effect of Amphiphilic Block Copolymer on Stratum Corneum Lipid Bilayer by Coarse-Grained Molecular Simulations

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Amphiphilic block copolymers are widely investigated as drug delivery systems. The ABA-type triblock copolymers Poloxamers are known to be no cytotoxic at low concentrations and commercially available in a wide range of block ratios and molecular weights.

We performed molecular dynamics simulations with the MARTINI coarse-grained force field to investigate the phase behavior of Poloxamers in water. The building blocks were shown to be able to assemble themselves. Different structures such as micellar, hexagonal and lamellar structures were observed in different concentrations. Phase diagram was compared with experimental results. In order to evaluate the ability of Poloxamers acting as transdermal delivery system, we performed simulations with polymer micellar on the *stratum corneum* lipid bilayer which locates at the upmost later of human skin. The results showed the speed of copolymer molecules penetrating into lipid bilayer was highly dependent on the hydrophilic–lipophilic balance. The local bilayer structure was seriously affected once the copolymer micellar submerged in the lipid bilayer.

The understanding of this interaction mechanism can help to explain the *stratum corneum* barrier function against toxic particles and to develop novel drug delivery systems.

P49

Boron Carbide As a New SPE Sorbent for the Isolation of Curcuminoids

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Curcuminoids are natural products present in Curcuma with promising health promoting effects[1,2]. According to the literature a high affinity between boron and curcumin is reported[3,4,5]. In this study we applied for the first time boron carbide (-200 mesh and <10 µm), as selective sorbent for the specific isolation of curcuminoids from *Curcuma longa* and *Curcuma xanthorriza*.
The selectivity of boron-carbide for the enrichment of curcuminoids was compared to polystyrene-co-divinylbenzene particles, OASIS® HLB (Waters, Milford, USA) and silica C18 sorbents to investigate the enrichment efficiency. A recovery study was carried out using Reversed-Phase Liquid Chromatography (RP-LC). Several eluents were applied to get the highest recovery.
It was observed that boron carbide provides the best selectivity among all of the tested sorbents. Therefore, boron carbide represents a promising sorbent for the selective extraction of curcuminoids from natural sources.

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P50

Role of *mnx1* in zebrafish endocrine cell fate determination

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Mnx1 is a mainly repressive homeobox transcription factor with conserved roles in motoneuron formation, pancreas morphogenesis and β-cell differentiation. How Mnx1 conducts its fate-determining functions is currently not known.
Recent work in fish and mouse showed that loss of β-cells in *mnx1* deficient animals is caused by fate conversion of β-cell progenitors either into α- or δ-cells, respectively. Based on these data it was proposed that Mnx1 supports β-fate implementation by repressing α- or δ-fate determinants. This model predicts that an ectopic activation or repression of Mnx1-targtes in the developing pancreas should result in the formation of more or less α-/δ-cells. By using transgenic animals for conditional activation of Mnx1, and a transcriptional activating form of Mnx1 (Mnx1-VP16) we tested this model. In contrast to the expected roles in defining a specific endocrine lineage, our findings suggest a role of *mnx1* in the general progression of early endocrine differentiation. To enable more detailed analyses of the role of *mnx1* we now generated loss-of-function mutants by using TALEN genome editing technology. These fish serve as a model for addressing molecular and cellular requirements of Mnx1 in β-cell differentiation and maintenance. Recent data including cell-tracking analyses and molecular approaches for the identification of direct Mnx1-target genes will be presented.

P51

Mechanism of Ets factor repression during neural fate acquisition

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Previous results have shown that neural induction in *Ciona intestinalis*, an invertebrate chordate and sister group to vertebrates, is mediated by Ci-*Ets1/2* and Ci-*GATA*, upon FGF signal from vegetal cells that activate the early neural marker *Otx* (Betrand et al. 2003). A minimal enhancer that contains *Ets* and *GATA* binding sites, called a-element was shown to activate *Otx,* specifically in neural cells. It was observed that repression through Ets-factor binding sites in the a-element is important for keeping *Otx* robustly shut off in non-induced ectodermal cells (fated to become epidermis) (Rothbächer 2007). Candidate repressors *Erfa* and *Elk* were isolated in the host lab that may be responsible for repression of *Ci-Otx* (Rothbächer unpublished). Ets factors are generally effectors of the FGF-RAS/MAPK/ERK-pathway that is well studied (Yordy and Helmericks 2000). During the development of the neural lineage in *Ciona* FGF signals are expressed at different time points and in different regions, which could have different influence on corresponding Ets-factors (activators and repressors). In mouse cell culture *Erf* and *Ets* seem to be shuttled out or in the nucleus, respectively upon *Erk*-phosphorylation when FGF is active (Gallic et al. 2004).
Nuclear shuttling of *Erfa* could therefore play a role in neural fate acquisition *in vivo* in *Ciona*. Competition for *Ci-Ets* transcription factor binding site that may be bound alternatively by *Ci-Erf* and *Ci-Ets* could be regulated by active FGF-Ras/MAPK/ERK signaling. We testing this hypothesis using the *Otx* a-element. We expect that the neural fate is regulated by diverse Ets-activators and repressors in *Ciona*. Preliminary results show nuclear Ets-factors shuttling upon different levels of FGF visualized with fluorescently tagged Ets-factors. Finally we hope to link the mechanism of Ets-factor shuttling to the timing of neural fate development by analyzing Ets-factor activity localization at different time point in development.

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Bioadhesion in ascidian

P52

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Marine bio-adhesion research has increasingly inspired the design of bio-mimetics 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[1]. In recent years it therefore became of great interest to understand how bio-adhesion is functioning in ascidians. In the host lab we use the ascidian *Ciona intestinalis* as model organism to study the adhesive properties of their larvae. Recent research demonstrated that larval adhesion organs (papillae) of *Ciona intestinalis* mainly consist of glue secreting collocytes, papillary neurons and supporting cells. However, detailed structure of cellular components of papillae and neurons, the exact secretion mechanism and the involved molecules are not fully described and need further investigation. Consequently, we aim at visualizing papillary cellular components by fluorescent labelling of neurons accompanied by immune-histochemical staining. Furthermore, we analyse candidate genes that might be involved in papillae organogenesis. Finally, we plan to biochemically analyse (Protein pulldown, Mass-spectrometry) candidate adhesion proteins and required post-translational modification such as glycosylation.

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P53

Development and characterization of thiolated α-cyclodextrin as an ocular drug delivery system

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Aim: Eye drops are rapidly drained from ocular surface and time for drug local effects is only a few minutes. Therefore, the aim was to develop thiolated α-CD conjugates making them mucoadhesive via disulfide bond formation with cysteine-rich substructures of the ocular mucus layer in order to provide a prolonged residence time at the site of action.
Methods: For this, cysteamine was covalently attached to oxidized α-CD via reductive amination. Thiolated α-CD was characterized regarding the amount of free thiol groups attached to the polymer backbone via Ellman`s reagent, resazurin assay was conducted for cytotoxicity and mucoadhesive properties were evaluated on porcine ocular mucosal tissues. Furthermore, albino rabbits were used for assessing the irritation masking effects of thiolated α-CD.
Results: showed that free thiol groups attached to the backbone were in the range of 558 ± 24 to 1143 ± 92 µmol/g. All the α-CD-cys conjugates were not significantly affecting the viability of Caco-2 cells in concentrations of 0.5 - 1%. Mucoadhesive properties were 32.11-fold improved compared to unmodified α-CD. Encapsulation of cetirizine into thiolated resulted into significantly reduced irritating effects of cetirizine. According to these results thiolated α-CD conjugate might prove a new promising tool for safe and prolonged drug action on the ocular mucosal surface.

guest poster