Molecular
Life Sciences

Molecular Life Sciences

2013

International Symposium of the
German Society for Biochemistry
and Molecular Biology (GBM)

GBM

3.–6. Oct. 2013

Campus Westend
Goethe University
Frankfurt a. M. • Germany

www.molecular-life-sciences.de

ABSTRACTS

List of Content

Transport and Folding

ORAL PRESENTATIONS

POSTER PRESENTATIONS

AWARDEES

AWV01
Biogenesis and function of the autophagosome

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The pathway of autophagy has assumed an important position in the analysis of mammalian cellular response to stress, hypoxia and pathogen infection. Autophagosomes mature by growth and envelopment of cytosolic proteins and organelles that are trapped within the inner membrane of a two-membrane organelle. Trapped proteins are delivered by autophagosome fusion to the lysosome where protein and polysaccharide degradation permit amino acids and sugars to be recycled. A pre-autophagsosomal membrane matures by the addition of membrane material from various intracellular sources and the attachment of peripheral proteins that remain bound through a covalent lipidation reaction. The origin and the mechanism of generation of the pre-autophagic membrane are poorly understood. Published evidence suggests an origin of this membrane on the ER, on the mitochondrial surface or possibly the Golgi or plasma membrane. We have addressed these issues with the development and analysis of a cell-free reaction that reproduces the lipidation of a major peripheral autophagosomal protein, LC3. Mouse embryonic fibroblasts (MEFs) taken from a mouse strain deficient in Atg5 are unable to lipidate LC3 and other targets of lipidation involved in the autophagic process. A crude membrane fraction isolated from these MEFs was mixed with cytosol harvested from normal cells that were untreated or subjected to a stress regimen known to induce autophagy. On addition of ATP, incubation of the mixture resulted in the formation of lipidated endogenous LC3 or exogenous recombinant LC3. The reaction required both membranes and cytosol and was stimulated 2-5 fold when the cytosol was taken from stress-induced cells, but was inactive when cytosol was used from stressed atg5 mutant MEFs. Autophagosome maturation requires a class III PI-3 kinase (VPS34 homolog); LC3 lipidation in our cell-free reaction was inhibited by wortmannin and 3-methyladenine, known inhibitors of this kinase, and by the addition of a peptide containing a PI3P-bnding sequence, the FYVE domain. Using cell fractionation techniques including differential centrifugation and buoyant density sedimentation, we have identified an ERGIC fraction enriched in Sec22 and ERGIC-53 that has the highest specific activity for LC3 lipidation in comparison to other cellular membranes. Membranes isolated from cells depleted of the ERGIC by use of selective inhibitors of traffic did not retain lipidation activity, but the activity reappeared quickly when traffic and the ERGIC was restored by removal of the inhibitor. Other early markers of the autophagosome membrane, Atg14L and DFCP1, are recruited to the ERGIC in cultured cells and in our cell-free reaction. This approach may now be exploited to understand the molecular mechanism of growth, closure and cargo capture by the maturing autophagosomal membrane.

References

Ge, L,. Melville, D., Zhang, M., and Schekman, R. (2013) The ER-Golgi intermediate compartment is a key membrane source for the LC3 lipidation step of autophagosome biogenesis. *eLife* 2:e00947. DOI: 10.7554

AWV02
60 Jahre DNA-Doppelhelix – Translation eines Naturgesetzes

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Sixty years ago Watson and Crick discovered the universal genetic principle, how biological information is encoded. And eight years later, scientists discovered with the deciphering of the genetic code a second universal biological principle.
The fact that encoding and decoding of genetic information is universal among all living organisms on this earth is the basis for biotechnology. This allows for example to integrate a genetic building instruction from a plant protein into a bacterium, which in turn will transform this instruction into a protein, basically identical to the original plant protein.
During the years biotechnology has developed in three main directions, which can be assigned the colors red, white and green.
Red biotechnology designates the medical/pharmaceutical branch of biotechnology. Bacteria, fungi, or cell cultures are used to produce drugs such as insulin. Manipulating an animal's genome to establish a disease model for the development of new drugs is another very important use of red biotech.
In white or industrial biotechnology, industrial products are produced with the aid of living microorganisms or cellular components. One exciting use of white biotech is a way of cleaning a contaminated environment by releasing bacteria that help break down or degrade the unwanted chemical.
In green biotechnology plants are genetically modified to establish resistance to a certain chemical or pest or to increased crop yield. Proponents of this technology believe that such modified organisms will help to solve food crises throughout the world. Opponents argue that transgenic plants pose a threat to biodiversity, and may pose health risks as well.

AWV04
Mechanism of microRNA-mediated mRNA Decay

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During my PhD I studied the mRNA decay pathway, which contributes to post-transcriptional regulation of gene expression. mRNA degradation is initiated by shortening of the mRNA poly(A) tail. Deadenylated mRNAs can be decapped, which ultimately targets them for complete degradation. I aimed to improve our understanding of the molecular mechanism of general and target-specific mRNA decay in metazoa and chose therefore human and Drosophila cell lines as model systems.
To obtain insights into the mechanism of general mRNA decay I dissected with coimmunoprecipitation experiments the proteins involved in mRNA decapping and the protein complexes they form. I studied the roles of the characterized domains and functional sequences in mRNA decay with reporter assays in cells. My studies provided an explanation at the molecular level for the functions of mRNA decay factors and revealed an unexpected complexity and connectivity of the mRNA decay machinery.
Furthermore, I studied target-specific mRNA decay taking the microRNA (miRNA) pathway as an example. It was well-established that miRNAs are incorporated into a miRNA-induced silencing complex (miRISC) and promote mRNA decay of miRNA targets, however it was unclear how this was achieved by the miRISC. I systematically screened for interactions between the miRISC and mRNA decay proteins both in the cellular context and with purified proteins. I could identify two deadenylation factors as new interaction partners of miRISC core components. This work showed how the miRISC can directly promote target degradation and established a mechanistic connection between miRNA guided target recognition and target degradation.

AWV05
Structure, Function and Regulation of the Spliceosomal Helicase Brr2

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Splicing entails the removal of non-coding intervening sequences from eukaryotic pre-mRNA and the ligation of the neighboring coding regions and is carried out by the spliceosome. The spliceosome requires elaborate assembly mechanisms to be put to work at the right place and time. Major conformational and compositional RNP remodeling driven by helicases of the superfamily 2 is required to convert an initially inactive complex into an active spliceosome. The Brr2 protein is the key player in this catalytic activation process. It stands out both architecturally and functionally among all other spliceosomal helicases. Brr2 belongs to a unique group of nucleic acid helicases, whose members are exceptionally large and which contain two expanded helicase units fused in tandem. Furthermore, unlike other spliceosomal helicases, Brr2 is preassembled with a known substrate, the U4/U6 di-snRNP, before incorporation into the spliceosome, and remains stably associated with the spliceosome throughout the splicing cycle. Thus, Brr2 must be tightly regulated to ensure correct timing of spliceosome activation. Since no detailed structure and very little mechanistic information were available on this class of enzymes, we set out to understand how the unusual architecture of Brr2 meets these specific functional and regulatory requirements. We have determined the crystal structure of a 200 kDa portion of Brr2, showing that its two ring-like helicase cassettes intimately interact with each other suggesting that they functionally cooperate. We showed that one of the helicase cassettes is inactive in ATP hydrolysis, RNA binding and RNA duplex unwinding, although it still binds ATP and strongly stimulates the active helicase unit. Using structure-guided mutagenesis, we delineated communication lines between the cassettes required for this modulation. While our results suggest that the inactive cassette acts as an intra-molecular cofactor of Brr2, recent findings have also pointed out the Prp8 protein as an important regulator of the Brr2 helicase. By solving the structure of Brr2 in complex with a C-terminal fragment of Prp8, we demonstrated that Prp8 can insert its last 15 flexible residues (C-terminal tail) into the RNA-binding tunnel of the active cassette, thereby blocking RNA binding by Brr2. Biochemical analyses have shown that Brr2's ATPase and U4/U6 unwinding activities can be reversibly inhibited by this C-terminal tail of Prp8 through a direct competition for RNA substrate binding. Our results highlight the potential for versatile regulation of Brr2 on various levels and further suggest that spliceosomal proteins may exploit the inactive cassette to modulate Brr2 activity.

AWV06
Biogenesis and Functional Architecture of Mitochondrial Membranes

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The inner mitochondrial membrane consists of a boundary region closely apposed to the outer membrane and large tubular invaginations termed *cristae* that protrude into the mitochondrial matrix. Whereas the inner boundary membrane is enriched in protein complexes devoted to the transport of metabolites and mitochondrial precursor proteins synthesized in the cytosol, the cristae are the main sites of oxidative phosphorylation. The border regions between inner boundary and cristae membranes are morphologically well defined and have been named *crista junctions*. Little is known about how the characteristic morphology of the inner mitochondrial membrane is generated and how the specific membrane architecture controls vital mitochondrial functions.
We have recently identified an evolutionary conserved inner membrane protein complex that we termed *Mitochondrial Inner Membrane Organizing System (MINOS)*. MINOS consists of the core subunits *Mio10/MINOS1)* and *mitofilin/Fcj1* together with at least four other proteins and forms large oligomeric structures of several megadaltons in size. Mutants with defective assembly of MINOS show a dramatically altered inner membrane morphology with extended stacks of sheet-like cristae that are virtually devoid of crista junctions and thus detached from the inner boundary membrane. Our preliminary data indicate that Mio10 plays a crucial role in the structural and functional organization of MINOS. Moreover, MINOS components are engaged in multiple interactions between inner and outer mitochondrial membranes (*contact sites*) and contribute to mitochondrial protein biogenesis. Mitofilin-deficient mitochondria are impaired in both, import of proteins into the intermembrane space and biogenesis of outer membrane b-barrel proteins. We propose that mitofilin acts as a scaffold that brings together different protein import systems in the intermembrane space for efficient preprotein transfer.
We are studying the molecular mechanisms of MINOS function and dynamics. We aim to understand the mutual relationships between MINOS and other protein machineries of both mitochondrial membranes that are involved in membrane organization, transport and protein biogenesis.

AWV07
Arabidopsis thaliana and its relatives as model system for the study of evolutionary questions

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My group is addressing fundamental questions in evolutionary biology: (i) How, and how frequently, do new genetic variants arise? (ii) Why do some variants increase in frequency? (iii) And why are some combinations of new variants incompatible with each other?
In collaboration with several other labs, we are describing whole-genome variation in natural accessions of Arabidopsis thaliana, under the auspices of the 1001 Genomes project (http://1001genomes.org). To better understand the patterns we observe in A. thaliana, we are comparing within-species variation with differences to the closest relatives, A. lyrata, and to variation in a closely related genus, Capsella. On the other end of the spectrum, we are analyzing new mutations that have arisen spontaneously under laboratory conditions or in a natural mutation accumulation experiment. The latter studies take advantage of an A. thaliana lineage that was apparently introduced to North America in historic times and accounts for about half of the population there. We have been able to support what we see in the extant North American population by whole-genome sequencing of herbarium samples from the 19th century. Since there has been much recent excitement about the potential contribution of heritable epigenetic variation, we are complementing our studies of genetic variation with analyses of DNA methylation differences, again over different time scales: in isogenic laboratory lines, in isogenic natural lines and across species boundaries.
The motivation for our top-down studies is to understand how new genetic and epigenetic variation interacts with reassortment of variants after crosses and natural selection to shape geographic patterns of genetic and epigenetic diversity. To this end, our population genomic work is complemented by forward genetic analyses, especially of detrimental combinations of sequence variants found in separate lineages. The ultimate goal is to understand at the molecular and biochemical level how changes in DNA sequence increase of decrease the fitness of organisms through their effects on protein levels and activity.
Additional information about our work can be found on our website, http://weigelworld.org.

AWV08
Gene transcription and genomic regulation

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Our laboratory has recently provided a molecular movie of RNA polymerase II transcription that reveals many aspects about how mRNA molecules are made and provides a useful teaching tool (Cheung and Cramer, Cell 2012). In a collaboration with Dirk Eick's lab we also described that Pol II is phosphorylated on the tyrosine-1 residue of its C-terminal repeat domain (CTD) and that this phosphorylation impairs binding of transcription termination factors (Mayer et al., Science 2012).
This led to an 'extended CTD code' that explains transcription cycle coordination including the elongation-termination transition. We also recently developed protocols to monitor the dynamics of mRNA synthesis and degradation system-wide and used these to demonstrate that cells have mechanisms to buffer mRNA levels by globally compensating between mRNA synthesis and degradation (Miller et al., Mol Sys Biol 2011; Sun et al., Genome Res 2012). In my talk I will concentrate on two unpublished functional studies.
In the first study, we found that the RNA exonuclease Xrn1 is required for global buffering of mRNA levels in a eukaryote and robustness of genome expression (Sun, Schwalb et al., unpublished). In the second study, we show how aberrant non-coding RNAs are globally removed by early transcription termination, resulting in a mechanism for transcriptome surveilance (Schulz, Schwalb et al., unpublished). The latter studies are collaborations with computational biologists Achim Tresch, Julien Gagneur, and Johannes Soeding, and use new algorithms to analyze a combination of functional genomics data.

PLENARY TALKS

PLV01
Designing Biological Systems

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Biology presents us with an array of design principles. From studies of both simple and more complex systems, we understand some of the fundamentals of how Nature works. We are interested in using the foundations of biology to engineer cells in a logical and predictable way to perform certain functions. By necessity, the predictable engineering of biology requires knowledge of quantitative behavior of individual cells and communities and the ability to construct reliable models. By building and analyzing synthetic systems, we learn more about the fundamentals of biological design as well as engineer useful living devices with myriad applications. For example, we are interested in building cells that can perform specific tasks, such as remembering past events and thus acting as a biological computer. Moreover, we design cells with predictable biological properties that serve as cell---based sensors, factories for generating useful commodities and improved centers for carbon fixation. Recently, we have engineered naturally occurring gut bacteria to report on the environment within the mammalian gut. This is one of the first practical applications for synthetic biology.

PLV02
Exploring the Human Protein Atlas to study biology and disease

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The human genome has approximately 20,000 protein-encoded genes. An import quest for the future is to characterize the expression, localization, modification of all the human proteins. The current version 11.0 of the Human Protein Atlas (www.proteinatlas.org) contains more than 18,000 validated antibodies targeting 14,200 genes corresponding to more than 75% of the protein-encoded genes in humans. The Protein Atlas contains more than 14 million high-resolution images generated by immunohistochemistry and confocal microscopy. The antibodies have been generated to regions of low homolog and the long-term objective is to generate paired antibodies towards the protein targets with separate and non-overlapping epitopes (6). Pilot projects have been initiated to also generate recombinant affinity reagents, a pilot version of a Rodent Brain Protein Atlas (8) and to study human biology. In addition, we have developed a targeted MS-proteomics strategy based on the recombinant protein fragments (PrESTs) generated within the frame-work of the Protein Atlas project. We have used the human protein atlas to study the global protein expression patterns in human cells, tissues and organs as well as a discovery tool to find potential biomarkers for disease, such as cancer.

PLV03
DNA demethylation and repair by Gadd45

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Gadd45a is member of a small gene family of stress response genes encoding 18 kDa acidic histone fold proteins mediating active DNA demethylation. Gadd45a mediated demethylation involves recruitment of the nucleotide excision repair and/or base excision repair machineries to excise 5mC. Gadd45 proteins function in gene-specific demethylation of numerous target genes such as *rDNA*, *Bdnf IX, Fgf-1B, S100*, *CD11a*, *CD70*, *RARβ2*, *osterix*, *Dlx5, Runx2* and *BGP,* in mammals, *Xenopus,* and zebrafish (Niehrs and Schäfer, 2012). A common theme of these studies is that demethylation by Gadd45 is a highly selective process: Not only is it gene-specific, but within a given gene it typically affects distinct mCpGs, often in the promoter region. This specificity highlights a set of general, unresolved key questions in DNA demethylation: What determines the target site specificity of DNA demethylation? Is there a relationship between site specific DNA demethylation and the epigenetic landscape? What may be the cofactors involved in regulating targeting? We found a histone mark reader which interacts with GADD45a to promote gene specific DNA demethylation. The reader binds GADD45a and recruits it to sites marked by histone H3 lysine 4 trimethylation (H3K4me3) via its PHD domain. We show that reduced H3K4 methylation impairs recruitment of GADD45a and demethylation of target genes. Our results indicate that histone methylation determines DNA demethylation.

References

Niehrs C and Schäfer A. **Active DNA demethylation by Gadd45 and DNA repair**.
Trends Cell Biol. 2012 22:220-7.

PLV04
Roles of Pink1 kinase and Parkin E3 ligase in mitophagy and Parkinson's disease

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The products of two genes mutated in autosomal recessive forms of Parkinson's disease, Pink1 and Parkin, have been identified in Drosophila to work in the same pathway to maintain healthy flight muscles and dopaminergic neurons. PINK1 is a kinase located on mitochondria whereas Parkin is an E3 ubiquitin ligase normally located in the cytosol. Upon mitochondrial damage Pink1 recruits cytosolic Parkin to mitochondria to mediate mitophagy revealing a cell biology pathway in mammalian cells where Pink1 works upstream of Parkin. Although PINK1 has a predicted mitochondrial import sequence, its cellular and submitochondrial localization remains unclear in part because it is rapidly degraded. Pink1 appears to be constitutively targeted to mitochondria and cleaved by PARL. Upon membrane damage it becomes stabilized on the outer mitochondrial membrane to recruit Parkin. Thus Pink1 serves to flag damaged mitochondria for removal. Whole genome RNAi screens reveal new gene products involved in regulating PINK1 targeting to mitochondria and Parkin translocation to mitochondria. How defects in mitochondrial quality control may contribute to parkinsonism will be discussed.

PLV05
Transport Machineries in Adaptive Immunity and Viral Immune Evasion

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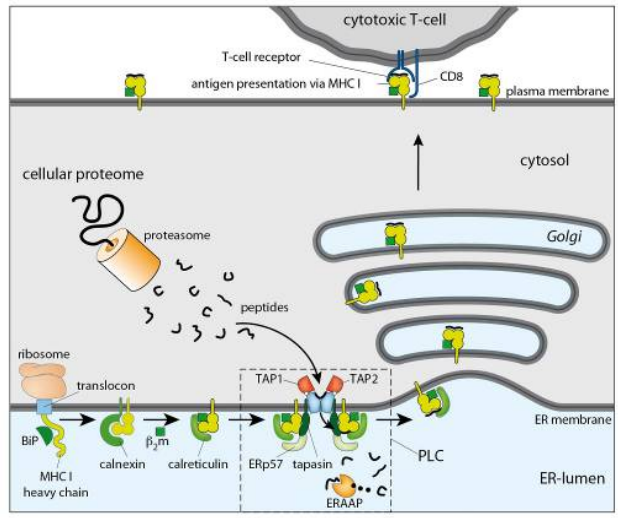
The recognition and elimination of virally or malignantly transformed cells is the pivotal task for the adaptive immune system. For immune surveillance, a snapshot of the cellular proteome is displayed on major histocompatibility (MHC) class I molecules as immunodominant epitopes for recognition by cytotoxic T-cells. The knowledge about the track from the equivocal protein to the presentation of peptides has greatly expanded, leading to an astonishingly elaborated understanding of the MHC I peptide loading pathway. This seminar will report on this complex process, which rests on ABC transporters, chaperones, and ER quality control. The contribution of the individual proteins and subcomplexes as well as the architecture and dynamics of the peptide-loading complex will be discussed, including mechanisms of viral immune evasion.



PLV06
parallel Plenary 2a - Chaperones for the folding, assembly and activation maintenance of RuBisCO

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Life on earth is dependent on the ability of photosynthetic organisms to sequester inorganic carbon dioxide of the atmosphere into organic carbon via the Calvin-Benson-Bassham (CBB) cycle. The key enzyme responsible for this process is ribulose-1,5-bisphosphate carboxylase/ oxygenase (RuBisCO), the most abundant protein in nature. It is also arguably the most important protein, since all biomass, and thus all food source, results either directly or indirectly from the action of RuBisCO. Despite its central role in biomass production, RuBisCO is an inefficient enzyme and also catalyses the wasteful reaction of photorespiration (oxygen as the substrate instead of carbon dioxide). Recent forecasts suggest that global food production will need to rise more than 30 % by 2050 to meet the ever increasing demand of the growing human population. Hence, engineering a more efficient RuBisCO enzyme will be important to increase agricultural output and may also be useful in controlling the greenhouse gas induced climate change. However, efforts to evolve RuBisCO must take into account the complex cellular pathways and machineries for its folding, assembly and activation. I will discuss our recent structural and functional analysis of the molecular chaperones that mediate RuBisCO biogenesis and maintenance, specifically the assembly chaperone RbcX and the AAA+ chaperone RuBisCO activase.

PLV07
Dielectric Heterogeneity in the Hetero-Oligomeric Photosynthetic Cytochrome b_6f Complex

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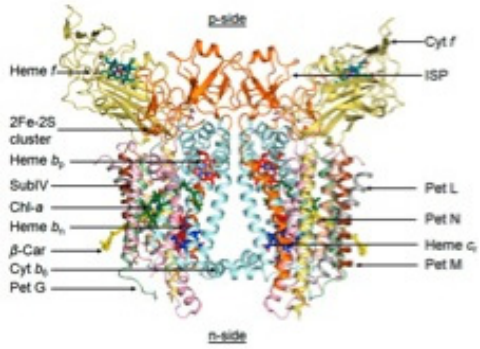
The pathway of trans-membrane electron transfer in the dimeric cytochrome b_6f complex (Fig. 1), derived from crystal structures from cyanobacteria [1,3-6] and a green alga [2], includes 4 *b*-type hemes organized as 2 heme pairs, b_p and b_n, on the electrochemically positive and negative side of the complex in 2 adjacent symmetric monomers. The pathway was studied by simultaneous measurement of the kinetics of dithionite reduction of the hemes and an associated heme-heme exciton interaction assayed through Soret band split circular dichroism (CD) spectra. Simulations show that the CD signal is dominated by the 2 intra-monomer hemes. Reduction of the 2 trans-membrane intra-monomer hemes and the increase in amplitude of the 2 lobes of the split Soret band CD spectra have similar kinetics, with pH-dependent half-times occurring on a time scale of minutes. Simulation of the kinetics is fit best by a model in which the pathway of trans-membrane heme reduction preferentially occurs pair-wise through the 2 intra-monomer *b*-hemes having indistinguishable kinetics of reduction over the time scale of the experiment. These observations can be explained energetically only by spatial heterogeneity of the intra-protein dielectric constant (ε) between the heme pairs. The largest ε exists between the intra-monomer b_n-b_p hemes, which decreases the energy of this reduced pair below that of the pair of reduced hemes b_n in adjacent monomers. A Stark Effect of the Q_y-band absorbance of the integral chlorophyll *a* molecule, generated with kinetics similar to heme reduction, is attributed to uncompensated electronic charge introduced by charged dithionite into the low dielectric protein interior.

References

(1) Hasan, S. S. *et al*. (2013), PNAS, 110: 4297-4302.



PLV08
Molecular networks for enhancing crop productivity

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Growth of plants and plant organs is orchestrated by complex molecular networks that integrates both intrinsic development signals encoded by the genome as well as a wide variety of environmental cues such as light, availability of water and minerals, temperature, e.a. Understanding the molecular composition and topology of these networks ultimately will accelerate advanced breeding and gene engineering for higher yielding crops. We have chosen leaves as a model organ to understand growth and size control mechanisms. Detailed cellular and molecular analysis of numerous *Arabidopsis* mutants revealed the existence of at least five mechanisms that contribute to final leaf size: i) the initial size of the leaf primordium; ii) cell cycle duration; iii) the developmental timing of the transition from cell division to cell expansion; iv) the timing of meristemoid division; and v) cell expansion. For each mechanism, multiple genes have been identified that when overexpressed or mutated enhance leaf organ size. The predominant mechanism acts at the transition from cell division to cell expansion during leaf development and is mediated by the gibberellic acid (GA) dependent activity of a chromatin remodeling complex. GA levels in the growth zones are regulated by the activity of two transcription factors ERF5 and ERF6 that also have a pivotal role in regulation growth in response to the environment. Furthermore, GA also was shown to have an important function in mediating leaf growth in maize and by engineering GA metabolism maize leaves that have a 40% increase in length were obtained. We will discussed our current understanding of growth regulatory networks and how we can use this information to improve crop yield.

PLV09
Molecular mechanisms of mitochondrial behavior

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Mitochondria are double membrane-bounded organelles that perform a myriad of diverse and essential functions in cells, which are dependent on the integrated behavior of the organelle. We have characterized key features of mitochondrial behavior. Mitochondrial behavior is determined by mitochondrial division and fusion, which govern overall mitochondrial shape, connectedness and distribution and by contact sites that intimately link mitochondria with the ER, which play roles in mitochondrial positioning and dynamics. We have also addressed the fundamental question of how mitochondrial membranes are sub-compartmentalized to reveal how the complex internal architecture of the organelle is generated. Our current challenge is to gain a complete picture of how pathways that regulate mitochondrial behavior are integrated with one another and to translate our mechanistic cell biological models into an understanding of how mitochondrial dysfunction affects the physiology of cells and organisms.

PLV10
Leveraging evolutionary information for protein structure and function production

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More than 10 years after the human genome project, we still know only a fraction of the structures and functions of human proteins. My lab develops methods for very sensitive sequence search and alignment methods that are based on the pairwise comparison of sequence profiles. Such methods have led to remarkable improvements in protein structure and function prediction over the last 10 years. I will explain how the evolutionary information contained in sequence profiles can enable us to detect homologous relationships across billions of years. I will then give an overview of the state-of-the-art as well as our contributions to this field and also sketch ideas that offer great potential to further enhance the scope of sequence-based protein structure and function prediction methods.

PLV11
Modern Concepts in Protein Engineering: from Directed Evolution to in silico Approaches

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Protein engineering has developed in the past decade to a highly important technology [1] as it is a useful tool to create enzymes with desired properties (with respect to e.g., substrate specificity, stereoselectivity or thermostability), but also helps to understand how proteins evolved and how they function. In this lecture, the principle strategies and current challenges in protein engineering will be highlighted. Examples will include the creation of an epoxide hydrolase from an esterase scaffold [2] the inversion of enantioselectivity of an esterase [3] and a method for *in vivo* selection and cell sorting [4]. The vast number of protein sequences available from databases substantially facilitates protein engineering. We used this plethora of information to analyze >2.800 sequences of enzymes from the a/b-hydrolase fold family using the 3DM database [5] from which enzyme variants with substantially enhanced thermostability, enantioselectivity and altered substrate range could be identified [6]. More recently, a detailed *in silico* analysis enabled the identification of a toolbox of novel (*R*)-selective transaminases [7] as well as (*S*)-selective enzymes from a structure-guided search [8].

References

[1] *Nature*, **485**, 185-194 (2012), *Nature Chem. Biol.*, **5**, 526-529 (2009)
[2] *Angew. Chem. Int. Ed.*, **48**, 3532-3535 (2009)
[3] *Angew. Chem. Int. Ed.*, **47**, 1508-1511 (2008)
[4] *Angew. Chem. Int. Ed.*, **49**, 8584-8587 (2011)
[5] *ChemBioChem*, **11**, 1635-1643 (2010)
[6] *ChemBioChem*, **11**, 1861-1866 (2010) *Protein Eng. Des. Sel.*, **23**, 903-909 (2010)
[7] *Nature Chem. Biol*, **6**, 807-813 (2010), *Adv. Synth. Catal.*, **353**, 2439-2445 (2011)
[8] *ChemCatChem*, **5**, 150-153 (2013), *ChemCatChem*, **5**, 154-157 (2013)

PLV12
Development and Manufacturing of Next Generation Biopharmaceuticals

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The development of recombinant antibodies and vaccines has allowed us to treat and prevent a number of life-threatening diseases. However, for highly variable viruses such as HIV and influenza, or even parasites such as plasmodium falciparum no vaccines exist and vaccine researchers want to elicit antibodies that protect against most or all viral strains - not just a few
such as seasonal flu vaccines currently on the market. One of the latest strategies is the development of broadly neutralizing antibodies from long-term pathogen-positive survivors, enriching antibody-producing B cells from blood samples and then to identify those that produce antibodies capable of neutralizing multiple strains of the relevant pathogen. Such broadly neutralizing antibodies typically work by blocking crucial functional sites on a pathogen that are conserved among different strains despite high mutation elsewhere. Technologies for identifying such B-cell clones to clone the relevant immunoglobulin genes and then to apply ecombinant antibody technologies to improve the performance of such antibodies will be discussed here. Another key issue for biopharmaceuticals is the capacity and scalability of current production systems which is beginning to place limitations on this crucial technology. The large-scale production of antibodies, vaccines and other pharmaceutical recombinant proteins is restricted

by the industry's current reliance on fermenter technology, particularly the culture of mammalian cells. This expensive and time-consuming production platform is preventing the distribution of recombinant protein drugs to those most in need. One way in which the above limitations can be addressed is through the use of plants and plant-based expression systems for recombinant pharmaceutical protein production.

PLV13
Lipid transport and membrane remodeling to build and protect chloroplasts

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The galactoglycerolipids mono- and digalactosyldiacylglycerol, MGDG and DGDG respectively, are the main polar lipid constituents of the photosynthetic membrane. Establishment and maintenance of the photosynthetic membrane is a dynamic process throughout the life cycle of plants and algae and involves finely tuned mechanisms to adjust synthesis, breakdown and transport of polar lipids. The TRIGALACTOSYLDIACYLGLYCEROL (TGD1, 2, 3 and 4) proteins of Arabidopsis constitute a transport system in the chloroplast envelopes required for the transfer of lipid droplets from the ER to the plastid. Disruption of this transport system in mutants causes indirectly the activation of SENSITIVE TO FREEZING2 (SFR2), which processively catalyzes the transfer of a galactosyl residue from MGDG to a galactoglycerolipid acceptor generating DGDG and oligogalactolipids as well as diacylglycerol, which is further converted by a separate enzyme to triacylglycerol (TAG). In Arabidopsis SFR2 is naturally activated by freeze stress and sfr2 mutants lose their ability to withstand moderate freezing. The proposed function of SFR2 is to stabilize the chloroplast envelope membranes against adverse affects of severe dehydration accompanying the formation of ice crystals, by removal of the non-bilayer forming lipid MGDG and sequestering its diacylglycerol moiety through formation of triacylglycerol into lipid droplets, and by increasing the hydration of the envelope membrane through formation oligogalactolipids. In the unicellular green algae Chlamydomonas, ER-to-plastid trafficking is not thought to occur. However, the Chlamydomonas genome contains at least TGD1, 2 and likely TGD3 proteins, but not TGD4. A Chlamydomonas mutant lacking one of the TGD protein orthologs shows reduced viability, increased accumulation of TAG, and an increased conversion of mature MGDG into TAGs. As Chlamydomonas lacks an ortholog of SFR2, the tgd mutant does not accumulate oligogalactolipids as observed for Arabidopsis. Comparing the two TGD lipid transport systems in Arabidopsis and Chlamydomonas, should provide a better mechanistic understanding of the biogenesis and maintenance of chloroplast membranes in photosynthetic organisms.

PLV14
Redox Regulation and Oxidative Stress - A Biochemical Perspective -

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Living an aerobic lifestyle is hazardous since organisms are constantly exposed to highly reactive oxygen species (ROS). Though necessary at low concentrations to control physiological signaling processes, high ROS levels damage DNA, lipids, and proteins and induce oxidative stress. Oxidative stress appears to be involved in numerous pathological conditions, and is considered to be one of the underlying causes of aging.
An increasing number of proteins have been identified that use reversible ROS-mediated thiol modifications to regulate their protein function. One of these proteins is Hsp33, a highly conserved molecular chaperone, which protects cells against oxidative stress conditions that lead to protein unfolding. Inactive when reduced, Hsp33 is rapidly activated by oxidative-stress mediated disulfide bond formation. Intriguingly, activation of Hsp33 involves the oxidative unfolding of its own C-terminal redox sensor domain, making Hsp33 a member of a new class of stress-specific chaperones that need to lose their structure to gain function. Hsp33 uses its intrinsically disordered regions to selectively bind to structured folding intermediates during stress conditions, addressing the long-standing question of how chaperones recognize their substrate proteins. Reduction of Hsp33's disulfide bonds upon return to non-stress conditions triggers destabilization of the bound substrate proteins, which is necessary for their return onto a productive folding pathway. These results beautifully illustrate how "simple" changes in a protein's redox status are utilized to rapidly adjust the protein's activity to the prevailing redox conditions in the cell, and help organisms in their defense against oxidative stress.

INVITED TALKS

INV01

C-type lectin receptors as targets for immune modulation: from glycan arrays to murine studies

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C-type lectin receptors (CLRs) are pattern recognition receptors mainly expressed by antigen presenting cells. CLRs recognize glycan structures present on pathogens, but also self-antigens. The primary interaction of a dendritic cell and a pathogen shapes the following immune response. Thus, searching for immune modulators that can either enhance or modulate an initiated immune response is of importance.(1)
To identify immune modulatory CLR ligands, we have developed a glycan array-based platform. The respective CLR carbohydrate-recognition domains (CRDs) were eukaryotically expressed as fusion proteins with the F_C fragment of human IgG_1 molecules. CLR-F_C fusion proteins represent valuable tools for high-throughput screening as they display the CRD in a dimeric form, are properly glycosylated and can be detected by a secondary fluorophore-conjugated antibody. By using these CLR-F_C fusion proteins in the glycan microarray, several glycan structures were identified as novel binders of CLRs. The CLR-binding carbohydrates were covalently coupled to the model antigen ovalbumin (OVA). These OVA-carbohydrate conjugates were used in DC/T cell co-cultivation assays to stimulate transgenic T cells *in vitro*. In addition, mice were immunized with these conjugates to identify immune modulatory CLR ligands *in vivo*. In conclusion, this platform brings together CLR ligand identification and their immunologic evaluation.(2)
Recently, we have shown the relevance of lectin receptors in the context of cerebral malaria(3), inflammatory bowel disease(4), and influenza(5). Thus, CLR targeting during infection or inflammation might be a means to dampen immune-mediated pathology.

We thank the Max Planck Society for the very generous support. This work is also funded by the BMBF (Fkz. 0315446) and the SFB 765.

References

(1) Lepenies, B.*; Lee, J.; Sonkaria, S.*; *Adv. Drug Deliv. Rev.* **2013**, in press.
(2) Eriksson, M.; Maglinao, M.; Schlegel, M.K.; Seeberger, P.H.; Lepenies, B.*; in preparation.
(3) Maglinao, M.; Klopfleisch, R.; Seeberger, P.H.; Lepenies, B.*; *J. Immunol.* **2013**, 191, 2551-59.
(4)Eriksson, M.; Johannssen, T.; von Smolinski, D.; Gruber, A.D.; Seeberger, P.H.; Lepenies, B.*: *Front. Immunol.* **2013**, 4, 196.
(5) Hütter, J.; Rödig, J.V.; Höper, D.; Seeberger, P.H.; Reichl, U.; Rapp, E.*; Lepenies, B.*; *J. Immunol.* **2013**, 190, 220-30.

INV02
Use of Zinc Finger Nuclease gene targeting for Functional Glycomics

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Glycosylation is the most abundant and diverse posttranslational modification of proteins. While several types of glycosylation can be predicted by the protein sequence context, and substantial knowledge of these glycoproteomes is available, our knowledge of the GalNAc-type O-glycosylation is highly limited. This type of glycosylation is unique in being regulated by 20 polypeptide GalNAc-transferases attaching the initiating GalNAc monosaccharides to Ser and Thr (and likely some Tyr) residues. We have developed a genetic engineering approach using human cell lines to simplify O-glycosylation (SimpleCells) that enables proteome-wide discovery of O-glycan sites using "bottom-up" mass spectrometric analysis. We implemented this on 12 human cell lines from different organs, and present a first map of the human O-glycoproteome with almost 3,000 glycosites in over 600 O-glycoproteins as well as an improved NetOGlyc4.0 model for prediction of O-glycosylation. The finding of unique subsets of O-glycoproteins in each cell line provides evidence that the O-glycoproteome is differentially regulated and dynamic. The greatly expanded view of the O-glycoproteome should facilitate the exploration of how site-specific O-glycosylation regulates protein function.

INV03

The influence of the intracellular concentration of sialic acid on the sialylation of glycoconjugates

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The sialic acids are acidic monosaccharides typically found at the outermost ends of the sugar chains of animal glycoconjugates. Due to their exposed position at the cell surface they participate in a variety of intermolecular and intercellular interactions. The key enzyme for the biosynthesis of sialic acid is the UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosaminekinase (GNE). Mutations in the binding site of the feedback inhibitor CMP-sialic acid of the GNE leads to sialuria, a disease in which patients produce sialic acid in gram scale. Patients with this abnormality of allosterism show variable degrees of developmental delay and hepatomegaly, along with vastly increased urinary excretion of free sialic acid and an elevated concentration of free sialic acid in the serum [1]. The reason for the variable degrees of developmental delay in sialuria patients still remains elusive. To further analyse this dysregulation of the sialic acid biosynthesis we generated a sialuria transgenic mouse model. This transgenic mouse over-expresses a GNE with an amino acid exchange in the feedback inhibition domain at amino acid position 263 from arginine to leucine (GNE-R263L). We measured the concentration of free sialic acid in the urine of the GNE-R263L mice and found more than five-fold increase compared to the wild-type mice. The analysis of overall glycan-bound sialic acid in different tissues like brain, lung or muscle showed no significant difference in the sialuria mice. However, as we looked in detail at the sialylation of the neural cell adhesion molecule (NCAM) in the brain we found an increased polysialylation of NCAM in sialuria mice. An altered polysialylation after birth in sialuria could play a role in the manifestation of developmental delay in sialuria patients.

1. Enns (2001) Clinical course and biochemistry of sialuria. J Inherit Metab Dis. Jun;24(3):328-36

INV04

Bacterial Life on CO and CO_2

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Anoxic niches are providing habitats for microorganisms adapted to seemingly hostile environments. Carbon monoxide and carbon dioxide are used by diverse anaerobic microorganisms as carbon and energy source. Different pathways of growth with CO/CO_2 have been described under which the reductive acetyl-CoA pathway is likely primordial [1]. Productive utilization of carbon dioxide is relying on the a of oxygen sensitive metalloenzymes exploiting the metal organic chemistry of nickel and cobalt to synthesize acetyl-CoA from two molecules of CO_2. In addition to the central enzymes, CO dehydrogenase and acetyl-CoA synthase, we recently started to investigate accessory proteins of the pathway, which are exploiting ATP hydrolysis to drive electron transfer and metal incorporation to (re)activate the central players of the pathway. The talk will give an overview on the current status of our work and will focus on a novel type of metallo-ATPase in the reductive acetyl-CoA pathway, which uses the energy released by hydrolysis of ATP to transfer an electron against a redox potential gradient [2]. Possible strategies to achieve electron transfer between two metalloproteins will be discussed.

References

[1] Fuchs, G. (2011) Annu. Rev. Microbiol. 65, 631-658.
[2] Hennig, S.H., Jeoung, J.H., Götzl, S., Dobbek, H. (2012) Proc. Natl. Acad. Sci, US 109, 5235-5240.

INV05

High-resolution 3D Structure Determination of Dynamic Macromolecular Complexes by Single Particle cryo-EM

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Using the latest developments in electron microscopic hardware combined with advanced computational image processing it is now possible to determine structures of large and dynamic macromolecular complexes at near atomic resolution. We have determined the structure of a 70S ribosome-SelB complex at 3.8 Å resolution which is sufficient to determine a *de novo* structure of SelB bound to the ribosome. SelB is the elongation factor specific for the delivery of the selenocysteine-tRNA to the ribosome. This also requires a stop codon in the mRNA being recoded into a signal for selenocysteine incorporation by a SECIS element in the pre-mRNA. Selenocystein incorporation is a rather inefficient process *in vivo* making the structure determination of SelB bound to the ribosome an evasive target in structural biology for a long time. Successful structure determination in fact requires extensive optimization of the ribosome-SelB complex preparation and image sorting of a rather heterogeneous population of ribosome complexes. This strategy allows not only the structure determination at very high resolution but also the simultaneous structure determination of numerous functionally distinct states of the ribosome-SelB complex.
As a control we also have determined the structure of the ribosome-EF-Tu complex at 3.2 Å resolution. EF-Tu is the elongation factor responsible for transport of all canonical aminoacyl-tRNAs to the ribosome. Having both structures available at high resolution we obtained a detailed view of how the ribosome can be hijacked by SelB to allow the recoding of a stop signal into a signal for selenocysteine incorporation.

INV06

Structure Determination from sparse NMR Data using CS-Rosetta: Hidden States, Large Proteins, and Automation.

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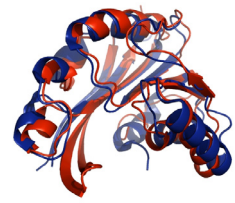
We develop algorithms based on the structure prediction methodology Rosetta for structure determination in cases where NMR data is sparse or of low-quality. Recently, we have introduced a genetic algorithm approach that recombines topological and structural features of low-energy conformations with the goal to enrich low-energy and near-native features until accurate structures in atomic-resolution are generated. The algorithm has been applied successfully to determine structures of 11 proteins ranging from 15 to 40 kDa, seven of which were previously unsolved. Moreover, the algorithm has been coupled with iterative NOE cross-peak assignment and thus allows automatic and unsupervised determination of NMR structures from unassigned NOESY peak-lists. The method, autoNOE-Rosetta, was tested in a benchmark of 50 protein data sets and showed high robustness against unrefined or errorneous input data and an enhanced radius of convergence when compared to established programs. In 20 cases, it was possible to compare the accuracies of autoNOE-Rosetta structures with respect to X-ray structures with those of PDB-deposited NMR solution structures. This comparison demonstrated that the here presented black-box approach significantly outperforms the expert-work reflected in the PDB-deposited NMR solution structures.



INV07

Structure and function of the Amyloid Precursor Protein APP in Alzheimer's Disease

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The Amyloid Precursor Protein (APP) and its neurotoxic cleavage product Ab are key players in the development of Alzheimer's disease (AD) and appear to be essential for neuronal development and cell homeostasis in mammals. The proteolytic processing of APP by the α-, β- and γ-secretases as well as the regulation of proteolysis seems to be hereby central to its physiologic and pathologic effect(s). We have investigated the 3D-architecture of APP and solved the structures of its rigidly folded E1 and E2-domains by protein crystallography. Within E1, the two constituting subdomains GFLD and CuBD interact tightly and in a pH-dependent manner *via* an evolutionary conserved interface. Two such E1-entities, and in consequence two APP-molecules, dimerize upon binding of heparin to a positively charged surface area that is created upon dimer formation. Within E2, we identified a novel, tight metal binding site, which results in large metal-dependent conformational changes of this protein at physiologic concentration of Zn^{2+} and Cu^{2+} - basically representing a metal-dependent conformational switch, that is likely to interfere with the intracellular trafficking and the proteolysis of APP.
We thus see the molecular basis of several widely discussed effects of the multi-domain protein APP, such as APP-dimerization, cell-cell- and/or cell-ECM-interactions, metal-binding, the metal-influenced processing of APP, etc., which will be discussed. Due to its general architecture, APP potentially fulfills multiple functions and adapts to different sub-cellular environments based on their different intrinsic pH.

References

Dahms, S. O., et al., (2010) *Proc. Natl. Acad. Sci.* **107**, 5381-5386.
Dahms, S. O., et al., (2012) *J. Mol. Biol.* **416**, 438-452.

INV08
Mechanism of membrane protein transport by a chaperone

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The biosynthesis of integral outer membrane proteins (Omps) in Gram-negative bacteria relies on molecular chaperones that convey the aggregation-prone Omp polypeptides in an unfolded form to the outer membrane. The trimeric 51 kDa protein Skp is one such transport chaperone [1,2], with a broad substrate range of more than 15 different Omps [3]. Importantly, Skp binds its substrates with nanomolar affinity, but being periplasmic, it can not rely on cellular energy to release them. It is so far not understood in which conformation the substrates bind to Skp and how the periplasmic Skp fulfills its biological function of substrate holding and release.
Here, we address these questions by extensive solution NMR spectroscopy experiments of Skp-Omp complexes. Our measurements on these 70 kDa assemblies provide a complete description of their conformation and dynamics at the atomic level. The Skp trimer provides a dynamic architectural scaffold that rigidifies upon binding of the Omp substrate. The chaperone-bound Omps populate an equilibrium of rapidly interconverting backbone conformations, with exchange rate constants larger than 1 ms^{-1}. Skp binds these Omp polypeptide ensembles with a global lifetime that is seven orders of magnitude longer than any of the local interaction lifetimes involved, showing that the high global affinity is added up by avidity from multiple local weak interactions. The unfolded Omp polypeptide is thus maintained stably in a dynamic, high-entropy state, from which it can rapidly release and subsequently fold without the need for external energy. This “fluid globule” state is a functional polypeptide conformation in bacterial outer membrane protein biogenesis and possibly a general paradigm for energy-independent polypeptide transport in all kingdoms of life.

References

1. Korndörfer IP, Dommel MK & Skerra A. *Nat. Struct. Mol. Biol.* **11**, 1015-1020 (2004).
2. Walton TA & Sousa MC. *Mol. Cell* **15**, 367-374 (2004).
3. Jarchow S, Luck C, Gorg A & Skerra A. *Proteomics* **8**, 4987-4994 (2008).

INV09
Design and application of cytokine receptor-based interaction traps

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MAPPIT (Mammalian Protein-Protein Interaction Trap) is a cytokine receptor-based two-hybrid method that operates in intact mammalian cells (www.mappit.be). The bait is linked to a signaling-deficient receptor, allowing complementation assays upon interaction with a prey. Protein-protein interactions (PPIs) can thus be studied in their close-to-normal physiological habitat. MAPPIT can be used for detailed analysis of selected PPIs, but also for large-scale interactomics, e.g. for mapping the human interactome. Interactor hunts for novel human protein-protein interactions can be performed using either a FACS-based format for cDNA library screening or using a semi-automated protocol to interrogate proteome-wide ORF arrays.
Over the past years, we have observed two types of technical “false positives” intrinsic to the MAPPIT system.
Type 1 artifacts represent bait-independent PPIs with the cytokine receptor complex. These are useful as they provide novel insights into cytokine receptor functioning. As an example, we will discuss the role of the ring finger protein RNF41 in the regulation of receptor turn-over and shedding.
Type 2 artifacts are autonomously signaling preys that operate completely independent of the cytokine receptor. One such case led to the development of a novel mammalian two-hybrid method that we termed KISS (KInase/Substrate Sensor). This alternative strategy accommodates PPI analysis of integral membrane and nuclear proteins and considerably expands the PPI repertoire seen by MAPPIT. As KISS uses the same read-out as MAPPIT, we expect that a combination of both methods will yield high coverage combined with the high sensitivity of the MAPPIT system. We will demonstrate KISS applications in the context of ER stress.

INV10
Chemical Ubiquitination

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Although 8 ubiquitin chain topoisomers are found in nature, we can enzymatically generate and study only few of them. The same holds true for ubiquitinated or ubiquitin-like modified polypeptides. Only for few polypeptide sequences and proteins the ligase combinations are known and can be isolated, needed for ubiquitin or ubiquitin-like modification in vitro. We have developed chemical strategies that allow the construction of virtually any ubiquitin or ubiquitin-like conjugate. This technology has made custom ubiquitination services reality and the basics of this technology will be explained in detail.
In addition, a series of novel ubiquitin-based probes will be discussed that have been designed to study the activity of deubiquitinating enzymes (DUBs). These probes are based on the ubiquitin structure and are equipped with a reactive moiety that allows covalent inhibition of DUBs through reaction with their active site cysteine nucleophiles. This strategy allows for example the identification of novel DUBs and the simultaneous activity measurement of multiple cysteine-dependent DUBs present in a given example.
We recently discovered by serendipity DUB probes based on alkynes as a reactive moiety. Interestingly, these alkynes have so far been considered to be unreactive but it appears that DUBs can trigger a chemical reaction not yet known. The discovery and implications of this unexpected alkyne reactivity will be discussed. Alkyne-based DUB probes react with a wider range of DUBs compared to other probes previously developed while they seem inert to reactions other than reactions with active site cysteine residues in deubiquitinating enzymes.

INV11
Reverse engineering a hierarchical regulatory network downstream of oncogenic KRAS

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RAS mutations are highly relevant for progression and therapy response of human tumours, but the genetic network that ultimately executes the oncogenic effects is poorly understood. We used a reverse-engineering approach in an ovarian cancer model to reconstruct KRAS oncogene-dependent cytoplasmic and transcriptional networks from perturbation experiments based on gene silencing and pathway inhibitor treatments. We measured mRNA and protein levels in manipulated cells by microarray, RT-PCR and western blot analysis, respectively. The reconstructed model revealed complex interactions among the transcriptional and cytoplasmic components, some of which were confirmed by double pertubation experiments. Interestingly, the transcription factors decomposed into two hierarchically arranged groups. To validate the model predictions, we analysed growth parameters and transcriptional deregulation in the KRAS-transformed epithelial cells. As predicted by the model, we found two functional groups among the selected transcription factors. The experiments thus confirmed the predicted hierarchical transcription factor regulation and showed that the hierarchy manifests itself in downstream gene expression patterns and phenotype.

References

(Molecular Systems Biology 8: 601 (2012); supported by Collaborative Research Center (SFB 618) and BMBF grants (NGFN2, ColoNet, FORSYS and Virtual Liver)

INV12
Allosteric Modulation of Protein Function by Supramolecular Ligands

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The selective shut-down of protein function by external agents, e. g., in signal transduction pathways, has hitherto been largely restricted to the presence of well-defined binding sites. Recent progress in supramolecular chemistry has now opened a new avenue for the construction of synthetic protein ligands which bind to specific regions on the protein surface or polypeptide backbone. The lecture presents molecular tweezers for exposed lysine and arginine residues, trimeric aminopyrazoles for the ß-sheet peptide backbone and designed polymers for larger epitopes. These chemical tools introduce new mechanisms of enzyme inhibition, prevent pathological protein aggregation and disrupt protein protein interactions. They are designed *in silico*, prepared in multistep organic synthesis, and mechanistically studied with isolated proteins, in cell culture and finally in animals. Although still in the stage of fundamental research, this new supramolecular approach to protein manipulation holds promise for entirely new mechanistic, diagnostic and therapeutic strategies

INV13
Berberine: New aspects of an ancient remedy

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Berberine, an isoquinoline alkaloid found in extracts from bark, roots and rhizomes of the genus *Berberis* and *Coptis* has a long history in traditional medicine. Extracts containing berberine are used as anti-microbial agents to treat intestinal infections. Besides its anti-inflamatory activities, berberine has been reported to exhibit cardiovascular and metabolic effects, anti-neoplastic and growth-suppressive activities. In our recent experiments we tested berberine and 13-arylalkyl derivatives thereof for their effects on Wnt/β-catenin signaling.
HEK-293 and colon cancer cells exhibiting upregulated Wnt-signaling were treated with berberine and its derivatives. Cytotoxicity, viability and anchorage-independent growth were monitored. The effects on Wnt-signaling were studied by reporter gene, qRT-PCR and Western blot analysis.
At concentrations below 20µM viability of HEK-293 and HCT116 cells was not influenced and no cytotoxicity was observed. Berberine significantly inhibited β-catenin transcriptional activity as determined by luciferase-reporter gene assays and qRT-PCR. Furthermore anchorage independent growth was decreased and a concomitant increase in E-cadherin expression was observed. Simultaneously an increased expression of E-cadherin was observed. Screening of 13-arylalkyl derivatives of berberine showed that several compounds had a much stronger effect compared to the naturally occurring parent compound, some exhibiting 100-fold lower EC50 values for Wnt-repression.
These observations indicate that berberine and its derivatives are potential drug candidates to inhibit β-catenin-driven carcinogenesis.

INV14
Small-molecule stabilization of 14-3-3 Protein-Protein Interactions: new way of pharmacological intervention

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Targeted pharmacological modulation of Protein-Protein Interactions (PPIs) is a promising strategy in Chemical Biology and Drug Development. However, in the vast majority of cases this concept has been realized only for inhibition of PPIs despite the fact that in many biomedical contexts stabilization of PPIs would be desirable. The natural product fusicoccin A is stabilizing the binding of 14-3-3 adapter proteins to the plant H^+-ATPase PMA serving as proof-of-principle molecule for the possibility to address the widespread interactome of 14-3-3 proteins. In humans, these proteins interact with partner proteins implicated for example in cancer (p53, Raf, YAP/TAZ, β-catenin) or neurodegenerative diseases (Tau, α-Synuclein, LRRK2). We have devised a fusicoccin-derivative (FC-THF) that stabilizes the interaction of 14-3-3 with the K^+ channel TASK-3. Protein crystallography shows how this molecule binds to the rim of the interface of 14-3-3 proteins and a TASK-3-derived phosphopeptide contacting both protein partners simultaneously. Since binding of 14-3-3 proteins mediates trafficking of TASK-3 to the plasma membrane administration of FC-THF enhances surface expression of the channel in cells and increases K^+ currents. Together with the demonstration that 14-3-3 PPI stabilizers can be identified by screening conventional compound libraries these studies support the concept of small-molecule PPI stabilization for biomedical research. In addition we have also shown that inhibition of 14-3-3 PPIs is a feasible approach in certain physiological settings.

INV15
Atypical Rho GTPases: Roles in cancer cell contraction and migration

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The Rho GTPases have mainly been studied in association with their roles in the regulation of actin filament organization. These studies have shown that the Rho GTPases are essential for basic cellular processes, such as cell migration, contraction and division. We have previously shown that the Rho family consists of 20 members and that their effects on cytoskeletal dynamics are more intricate and complex than recognized before. Lately, we have focused on the less studied Rho GTPases, in particular on the atypical member RhoD, and have identified several novel signaling pathways downstream of this protein. For example, we found that RhoD binds the actin nucleation-promoting factor WHAMM as well as the related Filamin-A-binding protein FILIP1. Of these two RhoD-binding proteins, WHAMM was found to bind to the Arp2/3 complex, while FILIP1 bound Filamin A. WHAMM was found to act downstream of RhoD in regulating cytoskeletal dynamics. In addition, cells treated with siRNAs for RhoD and WHAMM showed increased cell attachment and decreased cell migration. We have put forward a model stating that RhoD functions as a central coordinator of actin dynamics, Golgi homeostasis and endosome trafficking. We are currently studying how signaling pathways controlled by additional atypical Rho GTPases can regulate cytoskeletal organization and membrane dynamics, and how these signals coordinate adhesion, contraction and migration of cancer cells.

INV16
Genetic analysis of signaling pathways that direct the formation of axons

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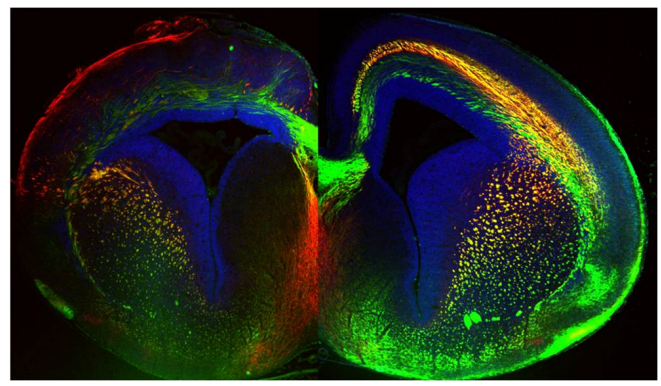
The establishment of polarity is an essential step in the differentiation of a neuron that results in the formation of a single axon and multiple dendrites. Cultures of dissociated neurons from rat embryos have been widely used as a model to identify several signaling pathways directing axon formation. Our previous results showed that Rap1 GTPases are essential for neuronal polarity to initiate axon formation in cultured neurons. We have now analyzed the in vivo function of Rap1 GTPases in neuronal development using conditional knockout mice. Rap1 GTPases are encoded by two genes in mice (Rap1a and Rap1b). Knockout of either Rap1a or Rap1b alone does not affect neuronal development indicating that they act redundantly in neuronal development. The knockout of both Rap1a and Rap1b in neuronal precursors of the cortex using Emx1-Cre results in a severe disorganization of the brain. A detailed analysis reveals defects in cadherin-dependent adhesion between radial glia and in integrin-mediated attachment of radial glia endfeet to the extracellular pial surface. The disorganization of radial glia results in defects of neuronal migration and disruption of cortical layers. In addition, a loss of axons was observed in the cortex and the hippocampus, showing that Rap1 GTPases are required for axon formation also in vivo. Using cultured neurons, we identified Rap1gef1 as an activator of Rap1 GTPases required for axon formation. Conditional knockout of Rap1gef1 results in a less severe disruption of radial glia while the loss of axons is comparable to that in the Rap1a/b double knockout. Taken together, our genetic analysis shows that Rap1 GTPases are required for the polarized morphology of radial glia and the formation of axons in vivo.



INV17
Mechanisms of neurotransmitter receptor clustering at GABAergic Synapses

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In many brain regions, gephyrin and $GABA_A$ receptor clustering at developing inhibitory synapses depends on the guanine nucleotide exchange factor collybistin (Cb). The vast majority of Cb splice variants contain an autoinhibitory SH3 domain, and several synaptic proteins are known to bind to this SH3 domain and to thereby activate gephyrin and $GABA_A$ receptor clustering. However, many functional GABAergic synapses form independently of the known Cb activating proteins, Neuroligins 2 and 4, indicating that additional Cb activators must exist. Two relevant Cb-interacting proteins in this regard are the closely related small GTPases TC10 and Cdc42, which we identified recently as potential activators of Cb in the formation of GABAergic postsynapses. Our data indicate that the small GTPase TC10 stimulates Cb-dependent gephyrin clustering by binding in its active, GTP-bound state to the pleckstrin homology domain of Cb. Overexpression of a constitutively active TC10 variant in neurons causes an increase in the density of synaptic gephyrin clusters and mean mIPSC amplitudes, while a dominant negative TC10 variant has opposite effects. The enhancement of Cb-induced gephyrin clustering by

GTP-TC10 does not depend on the guanine nucleotide exchange activity of Cb but involves an interaction that resembles reported interactions of other small GTPases with their effectors. Our data indicate that GTP-TC10 activates the major SH3 domain-containing Cb variants by relieving autoinhibition and thus define a novel GTPase-driven signalling pathway in the genesis of inhibitory synapses.

INV18
Autoimmune and paraneoplastic syndroms, antigens and mechanisms of disease

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Abstract not submitted.

INV19
Reactivation of codogenic human endogenous retroviruses in endometrial carcinoma and prestages: Emergence of new molecular targets

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Abstract not submitted.

INV20
The role of the ubiquitin proteasome system in the pathogenesis and therapy of B-cell malignancies

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B-cell neoplasms are characterized by high levels of genomic instability that correlate with disease progression. The ubiquitin proteasome system (UPS) plays a central role in the DNA damage response, therefore representing a potential target for directed therapies. As such, proteasomal inhibition has been successfully introduced into the therapy of B-Cell neoplasms, indicating the presence of specific deregulated
ubiquitylation events with potential therapeutic implications. However, specific deregulated ubiquitylation events have remained largely unknown. Starting from systematic analyses of genome-wide aCGH and expression studies, we identified two previously orphan E3 ubiquitin ligases of the SCF family (Fbxo9 and Fbxo25) as promising candidates which are overexpressed or deleted in multiple myeloma (MM)
and Non-Hodgkin lymphoma (B-NHL), respectively. Our unbiased quantitative mass spectrometry based screens have led to the identification of substrates that suggest important functions in the response to growth factor withdrawal (Fbxo9), and the regulation of apoptosis (Fbxo25). Specifically, we found the proteins that control the cellular abundance of mammalian PIKKs: Tel2 and Tti1, to be targeted for
degradation by Fbxo9, and the potent pro-survival protein Hax-1 (HS-1 associated protein X-1) to be ubiquitylated by Fbxo25. Subsequent studies in different in vivo models and patient samples suggest that Fbxo9 and Fbxo25 may be both promising new therapeutic target structures and potential markers to predict clinical response to proteasomal inhibition.

INV21
Targeting DNA repair pathways for personalized cancer therapy

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In response to genotoxic stress, cells activate a complex, kinase-based signaling network to arrest the cell cycle, initiate DNA repair, or, if the extend of damage is beyond repair capacity, induce apoptotic cell death. ATM lies at the heart of this signaling network, which is collectively referred to as the DNA damage response (DDR). ATM is involved in all three of these DDR-regulated cellular responses - cell cycle arrest, DNA repair and apoptosis. Disabling ATM mutations occur frequently in various human tumor entities, including lung cancer and hematological malignancies. Here we show that ATM-deficiency protects human and murine cancer cells from apoptosis induced by genotoxic chemoptherapy. Using genetic and pharmacological approaches we then demonstrate *in vitro* and *in vivo* that ATM-defective murine and human cells display a strong non-oncogene addiction to DNA-PKcs signaling. We further show that this dependence of ATM-defective cells on DNA-PKcs offers a window for therapeutic intervention. We show that pharmacological or genetic abrogation of DNA-PKcs in ATM-defective settings leads to the accumulation of DNA double-strand breaks and the subsequent CtIP-dependent generation of large single-stranded DNA (ssDNA) repair intermediates. These ssDNA structures trigger the activation of pro-apoptotic signaling through the RPA/ATRIP/ATR/Chk1/p53/Puma axis, ultimately leading to the apoptotic demise of ATM-defective cells exposed to DNA-PKcs inhibitors. Lastly, we demonstrate that DNA-PKcs inhibitors show remarkable preclinical activity as single agents against ATM-defective lymphomas *in vivo*. Together, our data implicate DNA-PKcs as a novel drug target for the treatment of ATM-defective malignancies.

INV22
Study Group Session 6 - Soluble Lipid Bilayer Systems for Structural and Functional Studies of Membrane Proteins

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Membrane proteins are involved in numerous vital biological processes, including transport, signal transduction and the enzymes in a variety of metabolic pathways. Integral membrane proteins account for up to 30% of the human proteome and make up more than half of all currently marketed therapeutic targets. Unfortunately, membrane proteins are inherently recalcitrant to study using the normal toolkit available to scientists, and one is most often left with the challenge of finding inhibitors, activators and specific antibodies using a denatured or detergent solubilized aggregate. Importantly, since membrane proteins are inherently insoluble and prone to aggregation and oligomerization in solution, the active state of interest is obscured. The Nanodisc platform circumvents these challenges by providing a self-assembled system that renders typically insoluble yet biologically and pharmacologically significant targets such as receptors, transporters, enzymes, and viral antigens soluble in aqueous media. Because Nanodisc constructs provide a native-like bilayer environment that maintain a target's functional activity, they are a versatile tool in the study of membrane proteins such as ion channels, GPCRs, cytochrome P450s, blood coagulation factors, various toxins and viral entities and a plethora of pharmaceutical targets. In addition to the opportunities in drug discovery, the Nanodisc provides a nanometer scale vehicle for the *in vivo* delivery of amphipathic drugs, therapeutic lipids, tethered nucleic acids, imaging agents and active protein complexes. Since an incorporated membrane protein is in its native structural state, this platform can also be used to raise specific therapeutic antibodies.

INV23
SNARE-mediated Vesicle fusion at the synapse

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Abstract not submitted.

INV24
Building a channel into the nuclear envelope - how nuclear pore complexes assemble

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Nuclear pore complexes are the gatekeepers of the nucleus. They exclude most macromolecules but mediate the selective and regulated transport of nuclear proteins and nucleic acids across the barrier of the nuclear envelope. Having a mass of approximately 125 MDa, nuclear pore complexes are the largest protein complexes in most vertebrate cells. Despite their enormous size they are only composed of about 30 different proteins called nucleoporins. Because of the eight-fold symmetry of the pore, 8, 16 or even more copies of each nucleoporin are present per nuclear pore complex. The stepwise co-ordinated assembly of these huge complexes from more than five hundred individual components and their integration into the nuclear envelope is a fascinating example of molecular self-organisation in cells.
Using a combination of biochemical and cell biological experiments we dissect the molecular mechanisms of nuclear pore complex assembly. I will present recent progress in our understanding of how nuclear pore complexes embed into the two membranes of the nuclear envelope and form a transport competent pore.

INV25
Vesicle Formation by the COPII Complex
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The coat protein complex II (COPII) is responsible for cargo sorting and vesicle formation at the endoplasmic reticulum. The small GTPase Sar1 is activated by GTP in a Sec12-catalyzed reaction, resulting in membrane binding. Membrane-bound Sar1 recruits the heterodimer Sec23/Sec24, forming an inner coat layer. Furthermore, the rod-shaped heterotetramer Sec13/Sec31 is recruited, leading to the assembly of an outer protein coat.
Reconstitution of COPII vesicle formation from liposomes using this minimal set of proteins and density gradient ultracentrifugation produces separated COPII-coated vesicles under non-GTP-hydrolyzing conditions. However, unfissioned tubular membranes with unconstricted and constricted morphologies are primarily observed when the same set of COPII proteins is employed with less perturbative microscopy techniques.
We try to further elucidate the mechanism of coated bud formation and vesicle fission in *in vitro* reconstituted samples by means of biophysical characterization and visualization of morphologies and structures. *In vitro* reconstituted COPII-coated tubular membranes have permitted the reconstruction of the inner and outer coat structures of membrane-bound COPII. Langmuir film balance measurements assess the ability of the Sar1 N-terminal amphipathic helix to embed into the proximal leaflet of the membrane. Factors influencing the membrane binding and remodelling activity of the COPII complex are investigated along with the Sar1 enzymatic activity with the goal of a mechanistic understanding of the process of transport vesicle formation, including the still elusive fission step.

INV26
Tba
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Abstract not submitted.

INV27
A fast clathrin-independent endocytic mechanism
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Cell shape is adapted to function. Organelle shape and local membrane architectures are likewise optimised for the processes that take place on and within these microenvironments. We focus on the dynamic regulation of membrane shape, which can occur by the interplay between the transient and regulated insertion of membrane bending motifs and the detection and stabilisation of membrane shape. This approach has allowed us not only to describe the biophysics of membrane shape changes but also to take a fresh look at membrane dynamics in physiological processes like exocytosis and endocytosis. In doing so we have noted that proteins with amphipathic helices or hydrophobic membrane-inserting loops are likely to effect or respond to curvature and that the membrane interaction surfaces of proteins can sense shape (like proteins of the BAR Superfamily). This molecular view has allowed us to ascribe novel cell-biological functions to proteins (e.g. the mechanistic affect of synaptotagmin in membrane fusion) and to give a more insightful view of how these processes work. Thus we can now go from the biophysics of a molecule, to better understanding of known pathways and to the molecular characterisation of novel cellular trafficking pathways both of endocytosis and exocytosis. I will present one such novel pathway that we are in the midst of characterising. It is a ubiquitous pathway operating especially in synapses but also in all cell types we have tested. It is clathrin-independent and dynamin dependent and operates at a much faster timescale that clathrin vesicle formation. We believe that a molecular understanding of this pathway will lead to fresh insights into fast membrane trafficking responses, like synaptic vesicle retrieval. For further details of our approaches see:http://www.endocytosis.org/

References

Emmanuel Boucrot, Adi Pick, Gamze Çamdere, … Harvey T. McMahon, Michael M. Kozlov (2012) Membrane fission is promoted by insertion of amphipathic helices and is restricted by crescent BAR domains. **Cell** Mar 30;149(1):124-36. PMID: 22464325
William Mike Henne, Emmanuel Boucrot, … Harvey T. McMahon (2010) FCHo Proteins are Nucleators of Clathrin-Mediated Endocytosis. **Science** 328, 1281-1284. PMID: 20448150
Alexander J. Groffen, Sascha Martens, … (Harvey T. McMahon and Matthijs Verhage) (2010) Doc2b Is a High-Affinity Ca^{2+} Sensor for Spontaneous Neurotransmitter Release. **Science** (article), 327(5973), 1614-1618. PMID: 20150444
Harvey T. McMahon, Misha Kozlov and Sascha Martens (2010) Membrane Curvature in Synaptic Vesicle Fusion and Beyond. **Cell**, 140, 601-605. PMID: 20211126
Richard Lundmark, Gary J. Doherty, … Harvey T. McMahon (2008) The GTPase-activating protein GRAF1 regulates the CLIC/GEEC endocytic pathway. **Current Biology,** 18, 1802-1808. PMID: 19036340
Sascha Martens, Michael M. Kozlov and Harvey T. McMahon (2007) How Synaptotagmin Promotes Membrane Fusion. **Science**, 316, 1205-1208. PMID: 17478680
Eva M. Schmid and Harvey T. McMahon (2007) Integrating molecular and network biology to decode endocytosis. **Nature**, 448, 883-888. PMID: 17713526
William Mike Henne, Helen M. Kent, … Philip R. Evans, and Harvey T. McMahon (2007) Structure and Analysis of FCHo2 F-BAR Domain: a Dimerising and Membrane Recruitment Module that Effects Membrane Curvature. **Structure,** 15, 839-852. PMID: 17540576
Oli Daumke, Richard Lundmark, Yvonne Vallis, Sascha Martens, Jo Butler and Harvey T. McMahon (2007) Architectural and mechanistic insights into an EHD ATPase involved in membrane remodelling. **Nature,** 449, 923-927. PMID: 17914359

INV28
Single Molecule Mechanics of Protein Folding and Interaction
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The development of nano-mechanical tools like Atomic Force Microscopy and optical traps has made it possible to address individual biomolecules and study their response to mechanical forces. In my talk, I will show how single molecule mechanical methods can be used to study the folding and interaction of proteins. Examples include the folding of calmodulin as well as the interaction of the cytoskeletal protein filamin with transmembrane proteins.

INV29
The ribosome-bound RAC/Ssb chaperone system of yeast prevents premature release of nascent chains from pausing ribosomes
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Protein synthesis is a highly sophisticated process. Errors in any step of protein synthesis may result in the translation of defective polypeptides, which can be detrimental for a cell. One major problem encountered during translation is stalling of ribosomes on defective mRNA molecules. Stalling may occur for different reasons; in any case, cellular maintenance machineries have to attend to the defective mRNA, the stalled ribosome, and the incomplete translation product.
The yeast ribosome-associated complex (RAC), consisting of the Hsp70 homologue Ssz1 and the Hsp40 homologue Zuo1, enables its Hsp70 partner Ssb to interact with nascent polypeptides as they emerge from the ribosome. RAC/Ssb is involved in the early steps of protein biogenesis, however, in addition performs functions distinct from those of a classical, cotranslational chaperone. Here we report that RAC/Ssb is required to repress translation of nonstop-proteins and proteins containing C-terminal polylysine tails. The release from translational repression in the absence of RAC/Ssb is due to premature translation termination on pausing ribosomes, which carry a sense codon in the A-site.

INV30
Prolyl isomerization in protein folding and its catalysis
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Prolyl cis/trans isomerizations determine the rates of protein folding reactions and can serve as molecular switches and timers. The gene-3-protein (G3P) of filamentous phage contains a *cis* proline (Pro213) in an extended hinge region between the domains N1 and N2 that mediate phage infection. The *trans*-to-*cis* isomerization of Pro213 is the rate-limiting step in the folding of G3P. *Cis*-Pro213 allows the two domains N1 and N2 to assemble into a compact structure. The reverse reaction, Pro213 *cis*-to-*trans* is required for activating the phage for infection. This reaction is elicited by binding of the phage to a bacterial pilus and is coupled with a local unfolding reaction, followed by domain disassembly. The kinetics, the structural determinants, and the energetic basis of this proline-limited activation reaction were elucidated by a kinetic analysis and, at the level of individual residues, by a mutational analysis and by real-time 2D NMR. The results reveal how the local *cis*/*trans* switching at a proline residue is embedded into intramolecular conformational signalling that is propagated specifically and over a long distance to elicit a function, which, in this case, was the initiation of phage infection.

References
Hoffmann-Thoms, S., Weininger, U., Eckert, B., Jakob, R. P., Koch, J. R., Balbach, J., & Schmid, F. X. Initiation of phage infection by partial unfolding and prolyl isomerization. (2013) J. Biol. Chem. 288, 12979-12991 [JBC Paper of the Week, May 3, 2013].

INV31
Processive movement and pausing of ribosomes during mRNA translation

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Translation of mRNA on the ribosome is a vectorial process that is modulated by codon usage, mRNA structure, and auxiliary factors. The local translation rates along the mRNA are not uniform: rapidly translated regions are separated by transient pauses. The duration of individual pauses upon synthesis of a model protein PrmC is 8-20 s, whereas the rate of protein synthesis during periods of rapid translation about 5 amino acids per s. For the full length PrmC, we find 17 pausing sites, many of which coincide with the boundaries of the protein secondary structure. Our data indicate a yet unappreciated frequency and mechanistic complexity of translation pauses, which are transient intermediates in movement of the ribosome on the mRNA. In a special case when the ribosome encounters sequences of consecutive prolines such as PPP, PPG, PPPP, PPPG, and longer proline runs, the ribosome stalls. Our recent data suggest that irreversible stalling of ribosomes on proline clusters in prevented by EF-P, which is an evolutionary conserved translation factor that binds at the interface between the ribosomal subunits and mimics a tRNA bound between the P and E site. In the cell, EF-P is post-translationally modified by a hydroxylated beta-lysine residue attached to Lys34; we show that the modification increases the catalytic proficiency of the factor by 100-fold. EF-P appears to be an elongation factor which works only on subset of cellular proteins, which explains the highly pleiotropic effects of deletions of the genes coding for EF-P or its modification enzymes.

INV32
The function of the universally conserved translation elongation factor EF-P

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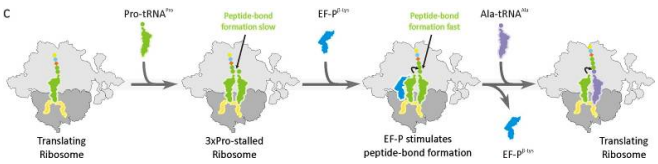
Translation elongation factor P (EF-P) is critical for virulence in bacteria. EF-P is present in all bacteria and orthologous to archaeal and eukaryotic initiation factor 5A, yet the biological function has so far remained enigmatic. We can demonstrate that EF-P is an elongation factor that enhances translation of polyproline-containing proteins: In the absence of EF-P, ribosomes stall at polyproline stretches, whereas the presence of EF-P alleviates the translational stalling. Moreover, we can demonstrate the physiological relevance of EF-P to fine-tune the expression of the polyproline-containing pH receptor CadC to levels necessary for an appropriate stress response. Bacterial, archaeal, and eukaryotic cells have hundreds to thousands of polyproline-containing proteins of diverse function, suggesting that EF-P and a/eIF-5A are critical for copy-number adjustment of multiple pathways across all kingdoms of life.

References
Ude et al. Science. 2013 Jan 4;339(6115):82-5.



INV33
Compartmentation of thiol-redox control in the eukaryotic cell

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The glutathione (GSH) and thioredoxin pathways are the two independent arms of the system that reduce cellular disulfide bonds. This system is needed to reduce catalytic disulfides in enzymes such as ribonucleotide reductase (RNR), PAPS reductase, thiol-based peroxidases, regulatory disulfides in redox-controlled proteins that participate to ROS signaling, and for providing a thiol-reducing power for the periplasm of prokaryotic cells. Due to its high abundance and low redox potential, GSH is also given the attribute of a redox buffer that prevent disulfide bonds accumulation. *E. coli* has provided the model of the thiol redox system that indicates a total redundancy of its two arms. Eukaryotic systems, although similar in architecture, diverges profoundly: in yeast **(i)** they are not redundant, thioredoxin having an exclusive role in H_2O_2 metabolism and sulfate assimilation, and a dominant one in RNR reduction; **(ii)** GSH is required for aerobic and anaerobic viability, due to an essential function in iron metabolism, but has a negligible impact on cytosolic thiol-redox control when the thioredoxin pathway is present. More importantly, the compartmentalized nature of the eukaryotic cells adds a layer of complexity that has been hitherto unsuspected. Use of redox probes that equilibrate with the GSSG/GSH redox couple in yeast have provided strikingly different values for the redox potential of GSH in these different compartments. However, only one enzyme dedicated to the reduction of the oxidized form of GSH exists, glutathione reductase, and this enzymes is only present in the cytosol and mitochondrial matrix. Accordingly how is the redox state of GSH regulated in the other compartments? We have addressed this question by combining ectopically expressed GSH redox probes and a cellular model allowing instantaneous change of GSH intracellular concentration from undetectable levels to levels > 10 fold those of wild type cells. Such a system has allowed us to decipher routes of GSH and GSSG traffic between compartments. We will present the data obtained using this method.

INV34
Mitochondrial Complex I and Redox Signaling

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Complex I of the mitochondrial respiratory chain is not only a major energy converter that operates as a redox driven proton pump, but it is as an important source of reactive oxygen species. To what extent this secondary function contributes to cellular oxidative stress or redox signaling is still unclear. The regulation of the active/deactive transition of complex I by modification of a specific cysteine suggests that complex I itself may be a target for redox signaling. The X-ray structure of mitochondrial complex I now gives insight into the structural basis of the function and regulation of this giant membrane bound multiprotein complex at the atomic level. Connecting structural data to functional analysis and identifying generator specific targets of reactive oxygen species by redox proteomics provides clues on the role of complex I within the cellular redox signaling network.

INV35
Reversible protein S-thiolation in *Mycobacterium tuberculosis*: structural and mechanistic insights into mycoredoxins

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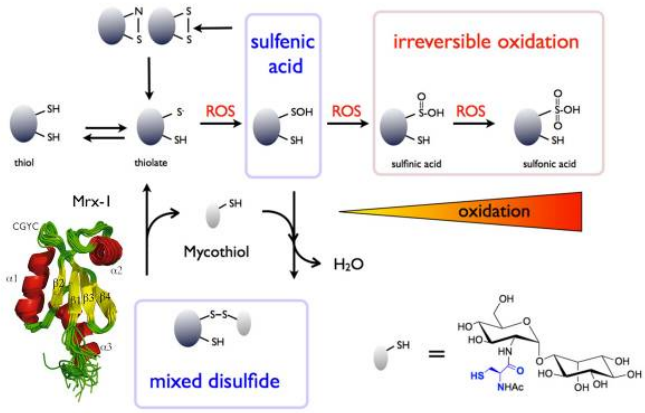
Mycobacteria produce millimolar concentrations of mycothiol (MSH) as their major low molecular weight thiol redox buffer. MSH-deficient mutants display increased sensitivity towards reactive oxygen, nitrogen and electrophilic species as well as alkylating agents and antibiotics. MSH is maintained in its reduced thiol state by the NADPH-dependent mycothiol disulfide reductase (Mtr). However, the redoxin that uses the MSH/Mtr/NADPH pathway for reduction of MSH-mixed protein disulfides, formed during oxidative stress, has long remained unknown. Here, we report that MSH provides the reducing power for mycoredoxin-1 (Mrx1) to reduce MSH-mixed disulfides. We solved the reduced (dithiol) and oxidized (disulfide) solution structures of Mrx1 by nuclear magnetic resonance (NMR) spectroscopy. NMR time course experiments also demonstrate the transient S-mycothiolation of the active site Cys14 of oxidized Mrx1 during reduction by the MSH/Mtr/NADPH electron pathway. Mrx1 has a redox potential of -218 mV and hydrogen bonding with neighboring residues lowers the p*Ka* of its N-terminal nucleophilic cysteine (1).
Furthermore, we report a newly identified S-mycothiolated Mrx1 substrate. Our findings open a new era of research to identify more S-mycothiolated Mrx1 substrates, and the function of MSH in redox regulation and the survival of *Mycobacterium tuberculosis* under oxidative stress like experienced in human macrophages.

References
1. Van Laer, K., Buts, L., Foloppe, N., Vertommen, D., Van Belle, K., Wahni, K., Roos, G., Nilsson, L., Mateos, L. M., Rawat, M., van Nuland, N. A., and Messens, J. (2012) Mycoredoxin-1 is one of the missing links in the oxidative stress defence mechanism of Mycobacteria. *Mol Microbiol* **86**, 787-804



INV36

Molecular mechanism of superoxide production by cytochrome bc_1

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Cytochrome bc_1 (mitochondrial complex III) is a key component of respiratory and photosynthetic energy conversion. It transfers electron between quinone and the mobile electron carriers (cytochrome *c*) and couples this process to vectorial transfer of protons across the membrane. The most crucial chemistry of the enzyme involves the reversible oxidation of quinol at the Q_o site upon which electrons are delivered to two separate chains of cofactors, initially reducing the Rieske cluster (FeS) in one chain and heme b_L in the other. Unwanted side reactions at the Q_o site can lead to superoxide production. Semiquinone intermediate (SQ_o) is believed to be a direct source of electrons that react with oxygen. Recent experiments performed with bacterial and mitochondrial enzyme suggest that incomplete reduction of quinone by heme b_L is a dominant reaction responsible for formation of SQ_o that reacts with oxygen. They also suggest that leaks of electrons on oxygen can compete kinetically with energy-wasting short-circuits reactions that retain electrons within the enzyme. A factor that appears to influence the effectiveness of short-circuits vs leaks is a position of the FeS head domain (this domain naturally undergoes constant large-scale movement between the Q_o site and heme c_1 to support catalysis) during the time SQ_o is formed and resides within the site: probability that SQ_o will react with oxygen increases as the FeS head domain moves away from the Q_o site and loses ability to directly exchange electrons with quinol/quinone/semiquinone. This concept offers new insights to better understand mechanism of quinol oxidation at the Q_o site and conditions under which complex III might contribute to generation of reactive oxygen species in vivo.

INV37

Imaging RNA and RNA biology using RNA mimics of green fluorescent protein

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Green fluorescent protein (GFP) and its derivatives have transformed the use and analysis of proteins for diverse applications. Like proteins, RNA has complex roles in cellular function and is increasingly used for various in vitro and in vivo applications, but a comparably robust and simple approach for fluorescently tagging RNA is lacking. We will describe the generation of RNA aptamers that bind fluorophores resembling the fluorophore in GFP. These RNAs activate the fluorescence of these fluorophores, resulting in a palette of RNA-fluorophore complexes that span the visible spectrum. An RNA-fluorophore complex resembling enhanced GFP (EGFP), termed Spinach, emits a green fluorescence comparable in brightness to fluorescent proteins. Spinach is markedly resistant to photobleaching, and Spinach fusion RNAs can be used to image RNA localization and other RNA regulatory processes in cells. We will also discuss the use of Spinach to image other biological molecules in cells. These RNA mimics of GFP provide novel approaches to image RNA biology and other processes in cells.

INV38

Combining chemo-enzymatic capture and deep sequencing in the search for new functional RNAs

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A multitude of chemical modifications decorate RNA molecules from all domains of life to expand their nucleotide repertoire and to support their diverse coding, structural, and catalytic functions[1,2]. Recent additions include enzymatic cofactors, such as coenzyme A and nicotinamide adenine dinucleotide (NAD)[3,4]. Given the central role of NAD in cellular processes such as redox chemistry, post-transcriptional protein modification, and signaling, its attachment to RNA points to unknown roles of RNA in these processes and to undiscovered pathways in RNA biogenesis and regulation. However, the unknown identities of the involved RNAs so far precluded a functional analysis. Here, we present a first set of RNAs bearing an NAD modification, identified by a new chemo-enzymatic protocol for the capture of NAD-RNA conjugates from bacterial total RNA preparations, combined with small-RNA deep sequencing. For the most abundant captured RNA, the covalent linkage to NAD was mass-spectrometrically established. Our results suggest a new link between RNA modification and regulation and the existence of a specific biochemical mechanism for the attachment of NAD to RNA.

References

1 Dominissini, D. *et al. Nature* **485**, 201-206 (2012).
2 He, C. *Nat. Chem. Biol.* **6**, 863-865 (2010).
3 Chen, Y. G., Kowtoniuk, W. E., Agarwal, I., Shen, Y. & Liu, D. R. *Nat. Chem. Biol.* **5**, 879-881 (2009).
4 Kowtoniuk, W. E., Shen, Y., Heemstra, J. M., Agarwal, I. & Liu, D. R. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 7768-7773 (2009).

INV39

Study Group Session 10 - RNA Chemical Biology: Functional nucleic acids for RNA ligation and labeling

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Monitoring RNA folding pathways, RNA-ligand, and RNA-protein interactions by spectroscopic methods requires site-specifically labeled RNA. Fluorescent or paramagnetic reporter groups are usually installed by chemical synthesis in combination with enzymatic ligation strategies. Challenges that need to be addressed include the synthesis of new labels and the preparation of long RNA beyond the length limit that is routinely achievable by solid-phase synthesis.
We capitalize on the catalytic ability of DNA and develop deoxyribozymes into general tools for site-specific labeling of RNA. Expanding the scope of DNA enzymes for RNA research is facilitated by a more detailed understanding of their molecular functions. With our recently developed combinatorial DNA probing methods,[1] we have identified the most critical nucleotides and optimized DNA enzymes for practical utility. Recent results on the synthesis of paramagnetic RNAs will be discussed, based on deoxyribozymes as alternatives to proteins for ligation of spin-labeled RNA.[2] In an alternative strategy, DNA enzymes are used to site-specifically address internal 2'-OH groups for covalent ligation of chemically modified nucleotides. This approach is exemplified for the synthesis of spin-labeled and fluorescently labeled *S*-adenosylmethionine (SAM)-binding riboswitch RNAs.

References

[1] a) F. Wachowius, F. Javadi-Zarnaghi, C. Höbartner. *Angew. Chem. Int. Ed.* **2010**, 49, 8504. b) F. Wachowius, C. Höbartner. *J. Am. Chem. Soc.* **2011**, 133, 14888. c) B. Samanta, C. Höbartner. *Angew. Chem. Int. Ed.* **2013**, *52*, 2995. [2] L. Büttner, J. Seikowski, K. Wawrzyniak, A. Ochmann, C. Höbartner. *Bioorg Med Chem.* **2013** doi: 10.1016/j.bmc.2013.04.007.

INV40

Structure-guided Discovery of a Novel Aminoglycoside Conjugate Targeting HIV-1 RNA Viral Genome

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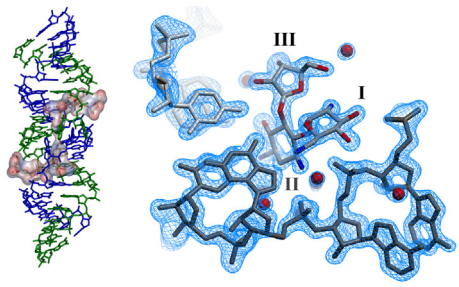
HIV-1 Dimerization Initiation Site (DIS) is a conserved hairpin in the 5' UTR of the genomic RNA. The DIS loop initiates genome dimerization by forming a loop-loop complex and is further stabilized into an extended duplex form by the viral the NCp7 nucleocapsid protein. X-ray structures of the DIS loop-loop complex and duplex forms revealed similarities with the bacterial 16S ribosomal RNA A-site, which is the target of aminoglycoside antibiotics.
As a result, we have shown that aminoglycosides bind the HIV-1 DIS and solved X-ray structures of DIS kissing-loop complex and extended duplex bound to

several aminoglycosides. However, identification of molecular driving forces important for the DIS/aminoglycoside binding could not be achieved by consideration of structural data alone, stressing the importance of collecting both thermodynamic and structural data for a complete understanding of the molecular recognition process.
Using ITC microcalorimetry, thermodynamics of aminoglycosides binding to the HIV-1 DIS duplex and kissing-loop forms was investigated. Together with structural data provided by high-resolution crystal structures, we could establish the basis for the specificity of drug/RNA recognition and allow discriminating between specific binding sites and potential competing secondary sites. We could also show that drug binding to the DIS kissing-loop complex inhibits the NCp7-assisted conversion into the extended duplex form. By using all these data, we could rationally design an aminoglycoside conjugate that specifically binds the HIV-1 DIS RNA. Our results show the feasibility of targeting the HIV-1 DIS dimer before and after the NCp7-assisted RNA maturation with the same molecule.



INV41

Systems Biology modeling techniques for metabolic networks

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Theoretical and experimental studies of the systemic interactions in metabolism are an important part of Systems Biology. The motivation comes mainly from medical and biotechnological applications. Cellular metabolism has a complex structure due to the large number of reactions involved and the fact that many of these are bi- or trimolecular. To understand this complex interplay, theoretical methods are needed. These methods include, among others, dynamic simulation, stability and bifurcation analyses, Metabolic Control Analysis (MCA), Metabolic Pathway Analysis (MPA), Metabolic Flux Analysis (MFA), optimization and game-theoretical approaches.
In this talk, I focus on Metabolic Pathway Analysis (MPA). In particular, the concept of elementary flux modes analysis has become a well-established theoretical tool. It allows one to decompose complex metabolic networks into the smallest functional entities, which can be interpreted as biochemical pathways or, as a special case, substrate cycles. Moreover, it allows the maximization of molar yields, which has important applications in biotechnology. Several illustrative examples of application of that analysis are presented. Current trends and future prospects are discussed.

References

S. Schuster, D.A. Fell, T. Dandekar: A general definition of metabolic pathways useful for systematic organization and analysis of complex metabolic networks. Nature Biotechnol. 18 (2000) 326-332.
E. Ruppin, J.A. Papin, L.F. de Figueiredo, S. Schuster: Metabolic reconstruction, constraint-based analysis and game theory to probe genome-scale metabolic networks. Curr. Opin. Biotechnol. 21 (2010) 502-510.
M. Bartl, M. Kötzing, S. Schuster, P. Li, C. Kaleta: Dynamic optimization identifies optimal programmes for pathway regulation in prokaryotes. Nature Commun. 4 (2013) 2243

INV42

Systematic characterization of human platelets in arterial vascular disorders by quantitative proteomics

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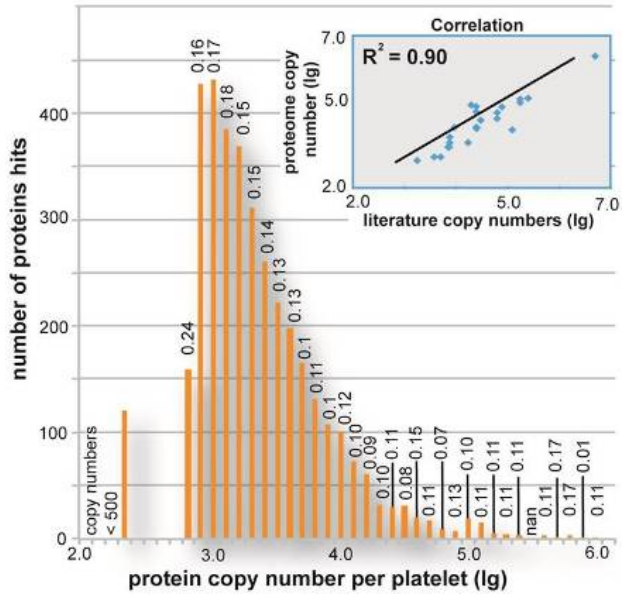
[4]*VIB, Department of Medical Protein Research, Gent, Belgium*

Anti-platelet treatment is of fundamental importance in combatting functions/dysfunction of platelets in the pathogenesis of cardiovascular and inflammatory diseases. Dysfunction of anucleate platelets is likely to be completely attributable to alterations in protein expression patterns and post-translational modifications. Combining elaborate protocols for platelet isolation from fresh blood donations in conjunction with quantitative mass spectrometry, we created the first comprehensive and quantitative proteome of human platelets, comprising almost 4,000 unique proteins with copy number estimates for ~3,700 of those and relatively quantified ~1,900 proteins between four different healthy donors - with negligible contamination by leukocytes, erythrocytes and plasma, respectively. For the first time, our data allow for a systematic and weighted appraisal of protein networks and pathways in human platelets, and indicate the feasibility of differential and comprehensive proteome analysis from small blood donations. Since 85% of the platelet proteome show no variation between healthy donors, this study represents the starting point for disease-driven platelet proteomics. These findings allow for correlation to genome-wide association studies which identified in a retrospective manner a set of chromosomal regions affecting the risk of cardiovascular diseases. In order to improve cardiovascular risk management, genomic and proteomic analyses of respective corresponding gene loci and proteins using next generation sequencing and targeted MS strategies are applied with the final goal to characterize valuable biomarkers for biomedical screenings.



INV43

Study Group Session 11 - Mathematical modeling of plant metabolic processes

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Plants are of vital significance as a source of food, feed, energy and feedstocks for the chemical industry. Given the close connection between plant metabolism and usability of plant products, there is a growing interest to understand and predict the behavior and regulation of plant metabolic processes. Mathematical modeling of plant metabolism offers new approaches to understand, predict and modify complex plant metabolic processes.
In my talk I will present a multiscale metabolic modeling approach which integrates static organ-specific metabolic models with a whole plant dynamic model. Allowing for a dynamic flux balance analysis (dFBA) on a whole plant scale, the approach provides a framework for the in silico analysis of the metabolic dynamics of the barley plant. The application of the approach is shown by a case study of source-sink interactions during barley seed development.

INV44

Metabolomics-based functional genomics in plants

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Metabolomics plays a major role in systems biology, functional genomics and biotechnology in plants (1). At the hypothesis generation phase, systems biology plays an important role for prediction of gene-to-metabolite relations in a model plant *Arabidopsis thaliana*. A metabolomic database of *A. thaliana*, AtMetExpress, was developed as a systems biology tool. AtMetExpress Development was designed to be compatible with AtGenExpress to allow the efficient elucidation of metabolite-transcript networks during tissue development. AtMetExpress 20 Ecotypes is representing metabolic diversity of 20 natural variation of *A. thaliana*. Detailed analysis of co-regulation frameworks of genes and metabolites in the pathways of sulfur-containing metabolites, flavonoid and lipid revealed the functions of novel genes and metabolites. Metabolomics developed in Arabidopsis is further applicable to crops and medicinal plants to decipher their genes' functions and evaluate the traits of biotech crops. An excellent coverage of chemical diversity of our

metabolomics platform was suitably applied to the study metabolite quantitative loci (QTL) analysis in rice. The technology was further applied to identify genes involved in the biosynthesis of specialized (secondary) metabolites, e.g., saponins and alkaloids, in medicinal plants (2). The crucial roles of metabolomics in phytochemical genomics and application to crop biotechnology will be discussed.

References

1. Saito, K., Matsuda, F. (2010) Metabolomics for functional genomics, systems biology, and biotechnology. *Annu. Rev. Plant Biol*. **61**: 463-489
2. Saito, K. (2013) Phytochemical genomics - a new trend. *Curr. Opin. Plant Biol*., **16**: 373-380

INV45

Plant transport engineering - Exploitation of transport processes to remove anti nutritional compounds

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In plants, transport processes are important for the reallocation of defense compounds to protect tissues of high value, as exemplified in the model plant Arabidopsis where the major defense compounds, glucosinolates, are translocated to seeds upon maturation. Knowledge about the molecular basis for long distance transport of glucosinolates as well as other defense compounds remains limited. We have identified and characterized two members of the NRT/PTR family, AtGTR1 and AtGTR2, as high-affinity, proton-dependent glucosinolate-specific transporters. Remarkably, the Atgtr1 Atgtr2 double mutant did not accumulate glucosinolates in the seeds and had over-accumulation in leaves and silique walls, which shows that both AtGTR1 and AtGTR2 are essential for long-distance transport of glucosinolates to the seeds (1). This represents the first example of a successful transport engineering strategy to eliminate glucosinolates from the seeds of Arabidopsis has been reported (2). The close synteny between Arabidopsis and B. napus (rapeseed) enables rapid translational biology strategies to improve the nutritional value of a globally important crop which will function as case study for development of novel generic approaches for specifically eliminating anti-nutritional specialized metabolites from edible parts of crops.

References

1.Nour-Eldin HH, Andersen TG, Burow M, Madsen SR, Jørgensen ME, Olsen CE, Dreyer I, Hedrich R, Geiger D, and Halkier BA (2012) NRT/PTR transporters essential for allocation of glucosinolate defense compounds to seeds. Nature. 488, 531-534.
2.Nour-Eldin HH and Halkier BA (2012) The emerging field of transport engineering of plant specialized metabolites Current Opinion in Biotechnology, 24, 263-270

INV46

Gene clustering of plant secondary metabolite pathways

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Opium poppy (*Papaver somniferum* L.) is best known as the only commercially viable source of pharmaceutically important morphinan alkaloids, such as the potent analgesics, morphine and codeine. Besides these, the opium poppy also produces the phthalideisoquinoline alkaloid noscapine, which has been used for decades as a safe, non-addictive cough-suppressant and also shows some promise as an anti-cancer compound.
We recently discovered a cluster of ten genes encoding five enzyme classes for the synthesis of noscapine in opium poppy. Our analysis of the arrangement and homology of the clustered genes in opium poppy suggests that genome reorganisation involved in cluster formation is an ongoing process that can occur either before or after gene duplication. However, similar to other plant secondary metabolites, whose biosynthetic genes are clustered, the precise ecological function of noscapine and the evolutionary forces that led to the clustering of its biosynthetic genes as well as the sequence of clustering events are unknown. Since production of noscapine and other structurally related alkaloids is not restricted to opium poppy but occurs in other species of the genus *Papaver*, a comparison of the genomic organisation and arrangement of homologues in these species will shed light on noscapine gene cluster formation in particular and formation of plant metabolic gene clusters in general.

INV47

Multiscale metabolic modeling: dynamic flux balance analysis on a whole plant scale

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Plant metabolism is characterized by unique complexity on the cellular, tissue and organ level. On a whole plant scale changing source- and sink-relations accompanying plant development add another level of complexity to metabolism. With the aim of getting a spatio-temporal resolution of source-sink interactions in crop plant metabolism, a multiscale metabolic modeling (MMM) approach was applied which integrates static organ-specific models with a whole plant dynamic model. Allowing for a dynamic flux balance analysis (dFBA) on a whole plant scale, the MMM approach was used to decipher the metabolic behavior of source and sink organs during the regenerative phase of the barley plant. It reveals a sink-to-source shift of the barley stem caused by the senescence-related decrease in leaf source-capacity which is not sufficient to meet the nutrient requirements of sink organs such as the growing seed. The MMM platform represents a novel approach for the *in silico* analysis of metabolism on a whole plant level, allowing for a systemic, spatio-temporally resolved understanding of metabolic processes involved in carbon partitioning, thus providing a novel tool for studying yield stability and crop improvement.

BIOANALYTICS AND BIOINFORMATICS – JOINT FORCES TO UNDERSTAND CELLULAR NETWORKS

PKV13

NOVA: Evaluation of complexome profiling data

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Motivation: The isolation of large native macromolecular complexes, identification of components and their dynamics, is a difficult task, requiring advanced proteomic strategies like complexome profiling (Heide *et al.*, 2012). Complexome profiling uses blue-native electrophoresis (BNE) to separate protein mixtures. Proteins that are subunits of the same complex are expected to have similar migration profiles which are measured by label-free quantitative mass spectrometry. Because manual comparison of all migration profiles is not feasible, cluster analysis is required to process the data sets. To the best of our knowledge no tool is available that offers statistical methods to evaluate complexome profiling data.
Results: We developed NOVA - a new tool for the analysis of complexome profiling data. A graphical user interface (GUI) provides various visualization modes, such as heat maps and 2D plots. Several hierarchical clustering algorithms (e.g., average linkage, Wards linkage), different distance measures (e.g., Euclidean distance, Pearson distance), and various normalization techniques are implemented. Migration profiles of several complexome profiling experiments can be easily compared (e.g. knockdown *vs.* wild typ). Many additional functions like zooming, searching for proteins, image export, and automatic file format recognition support intuitive handling. We demonstrate the functionality of the program by its application to recent experimental data obtained by complexome profiling.

References

H. Heide, L. Bleier, M. Steger, J. Ackermann, S. Dröse, B. Schwamb, M. Zörnig, A. S. Reichert, I. Koch, I. Wittig, and U. Brandt. Complexome Profiling Identifies TMEM126B as a Component of the Mitochondrial Complex I Assembly Complex. Cell Metabolism, 16(4):538-549, 2012.

AIP01

Lats2 phosphorylates p21 and induces apoptosis in response to UV irradiation

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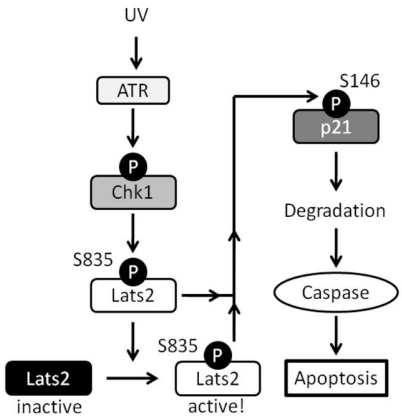
Lats2 is a tumor suppressor and one of the kinases in the Hippo pathway. Lats2 has been demonstrated to interact with the ATR-Chk1 pathway after UV radiation. However, the detailed functions of the Lats2 in DNA damage response remain elusive. We searched the relation between Lats2 and p21 under DNA damage. It was reported that p21 inhibits apoptosis by binding to caspase. Here we show that Lats2 was phosphorylated by Chk1 at Ser835 in response to UV radiation. Since Ser835 located at the kinase domain of Lats2, we assume this phosphorylation enhances kinase activity of Lats2. On the other hand, after UV irradiation, p21 protein levels decreased rapidly; however, p21 remained markedly higher in cells transfected with Lats2 siRNA. *In vitro* kinase assay revealed that Lats2 phosphorylated p21 at Ser146; this phosphorylation is known to destabilize p21.We found that overexpression of activated state Lats2(Lats2-S835D) induced apoptosis. Interestingly, p21 degradation and caspase activation was detected in Lats2-S835D expressing cells. Moreover, overexpression of p21 inhibited Lats2-S835D-induced cell death. In summary, we propose that Ser835-phosphorylated Lats2 by Chk1 after UV radiation phosphorylates and activates another Lats2. Next, Active Lats2 phosphorylates at Ser146 of p21, thereby inducing apoptosis by activation of caspase-3/9. p53 is stabilized by DNA damage and induces transcription of p21, which is also an apoptosis inhibitor, simultaneously with apoptosis-inducing factors. On the other hand, Lats2 is activated by Chk1 and inhibits p21. We believe the function of p53 is to unify to apoptosis induced by Lats2.



AIP02

The *Streptomyces* papain inhibitor from *Streptomyces mobaraensis* is unusually composed of a proteinaceous transglutaminase substrate and a small inhibitor molecule

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The heat-resistant *Streptomyces* papain inhibitor (SPI) from *S. mobaraensis* inhibits cysteine proteases like papain, bromelain, gingipain K and R1, and staphopain B, but also serine proteases like trypsin, and an unknown secreted hydrolase from *Bacillus anthracis*. It also inhibits bacterial growth of both Gram-positive and Gram-negative bacteria in a bacteriostatic or bactericide manner depending on the micro-organism (Zindel *et al*., 2013). Additionally, SPI acts as a substrate of the intrinsic transglutaminase (TGase) from *S. mobaraensis*. SPI shows no sequence homology to any known inhibitory family but only to a putative protein from *S. lavendulae* (Q9X5U4) (Sarafeddinov *et al.*, 2011).
In this study we clearly proved that SPI is unusually built out of two components with high affinity to each other: a protein that acts as a substrate of the intrinsic TGase of *S. mobaraensis,* thus being a potential vehicle/platform for the second component, a small organic molecule of unknown size and structure that is responsible for the protease inhibitory effect. Recombinant production with *E. coli* resulted in inactive SPI that may also be obtained by ethanol precipitation of *wt*-SPI from *S. mobaraensis* or dialysis against water. The active compound discovered in the watery dialysate was purified and analysed using culture supernatants from *S. mobaraensis*.

AIP03

Identifying genes relevant to specific biological conditions in time course microarray experiments

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The analysis of microarray data is a challenging task. One of the key problems in microarray analysis is the classification of unknown expression profiles. Specifically, the often large number of non-informative genes on the microarray adversely affects the performance and efficiency of classification algorithms. Furthermore, the skewed ratio of sample to variable poses a risk of overfitting. Thus, in this context, feature selection methods become crucial to select relevant genes and, hence, improve classification accuracy.
In this study, we investigated feature selection methods based on gene expression profiles and protein-protein interactions. As a result, we developed a novel feature selection method, which we call "relative Signal-to-Noise ratio" (rSNR), which ranks genes based on their specificity to an experimental condition. The rSNR compares intrinsic variations, i.e. variations in gene expression within an experimental condition, with extrinsic variations, i.e. variations in gene expression across experimental conditions. Genes with low variation within an experimental condition of interest and high variation across experimental conditions are ranked higher, and help in improving classification accuracy. We compared different feature selection methods on two time-series microarray datasets and one static microarray dataset. We found that the rSNR performed generally better than the other methods.

AIP04

Basic topological features for metabolic pathway models

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The development and analysis of metabolic networks an / or signal transductionnetworks is an important topic in systems biology. Graph theory, which networks are based on, is a subject of intensive research since the 18th century, and concerns, besides other topics, with the meaning of topological properties.
In the raising importance of the analysis of biological networks in the last decades, researchers are aiming to find correlations between topological properties and biological interpretation. A first investigation of biological networks, concerning their node degree distribution, was done by Joeng *et al.*, suggesting scale free network structure in biological networks. Ravasz *et al.* analyzed properties like the cluster coefficient of metabolic networks. Both studies are based on the same dataset of 43 metabolic networks. The common hypergraph representation of the networks limits the investigation to the properties of the metabolites, without considering the impact of the reactions on these properties. In contrast, bipartite graphs allow to explore the effect of the reactions on the topological properties. We choose the Petri net models that characterize the topological properties of both, reactions and metabolites. We focus on three topological properties: node degree, cluster coefficient, and the shortest path length. Our study is based on 1846 different whole-genome metabolic networks from the path2models database. We found that in this graph representation the node degree of metabolites follow, in contrast to reactions, a scale free distribution. Also, we show the big influence of the secondary metabolites on these distributions. The small world property applies for both, but only because of the secondary metabolites.

AIP05

Modelling NF-κB signal transduction using Petri nets

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Tumor necrosis factor receptor 1 (TNFR1) mediates an important pathway in immune response regulation. Binding of tumor necrosis factor-α can either trigger death or survival of the cell. If transcription factor nuclear factor-κB (NF-κB) is activated, the expression of survival genes is enhanced. A dysregulation of signal transduction may result in inflammatory diseases or cancer. Accordingly, this system requires a strictly controlled regulatory network to conduct the cell response following TNFR1 stimulation. To elucidate the dynamics and regulations, we developed a Petri net (PN) in a systems biology approach. Our focus is to model the interactions and regulatory processes of signalling to NF-κB, which was not described in a PN model so far. Recently gained insights like linear ubiquitylation events are considered in the model. We applied P/T-Petri nets to qualitatively model NF-κB signalling according to the literature, since no

quantitative data are available. The MonaLisa tool was used for the construction, analysis and simulation of the model. The mathematical analysis of the PN confirmed the consistency of the model by fulfilling the CTI property. Analysis of invariants points out regulatory effects, and PN simulation reveals dynamics. The established PN reflects the current understanding of signalling to NF-κB, while delivering a valuable basis for further quantitative mathematical analysis.

AIP06

Gene Encoding of β-Galactosidase from Sunn pest *Eurygaster integriceps*

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Sunn pest, *Eurygaster integriceps* Putton (Hemiptera: Scutelleridae) is one of the major pests of wheat and other cereals in a wide area of the world. They are dependent on their Beta galactosidase (EC 3.2.1.23). β-galactosidase catalyzes the hydrolysis of terminal, non-reducing beta-D-galactose residues in beta-D-galactosides. In this study, insects (*E. integriceps*) were collected from Pakdasht wheat farms of Tehran Province, Iran. They maintained on wheat kernels in the laboratory at 27±2℃ under a 14h light: 10h dark (LD 14:10) photoperiod. Total DNA was extracted from adults using the modified CTAB method. Two degenerate oligonucleotide primers were designed and synthesized based on the conserved amino acid sequence regions of several insect β-gal by AllellID 6.0 as follows:
β-gal Forward- 5́-AATTCCARGTNGARAAYGARTAY-3́
β-gal Reverse- 5́-AAGCTTNCCNCCRTARAACATRTA-3́
PCR was performed to amplify the fragment of β-gal gene with the two degenerate primers according to the protocol of Master Mix PCR Kit (Cinnagene). The products were separated on 1.2% agarose gel stained with ethidium bromide. The result has been deposit to NCBI after sequencing. Finally a part of β-galactosidase (β-gal) gene was isolated from *E. integriceps* (designated as *Ei*-βgal-JQ889818), which contained 328 bp encoding 109 amino acids. BLAST analysis revealed that the deduced *Ei*-βgal-JQ889818 had extensive homology with other insect β-gals. Phylogenetic tree analysis showed that it has a close relationship with *Arabidopsis thaliana* and *Synechococcus sp.*. Accordingly, β-gals should be functional proteins involved in the biosynthesis of lactose and are derived from a common ancestor.

Keywords: β-Galactosidase, Carbohydrate enzyme, BLAST analysis, Phylogenetic tree, *Eurygaster integriceps*

AIP07

Tumor Treatment Analysis by kinetic Cell Models with the EPN-test of Exponential growth detects Proliferation Inhibition and Necrosis after Radiotherapy

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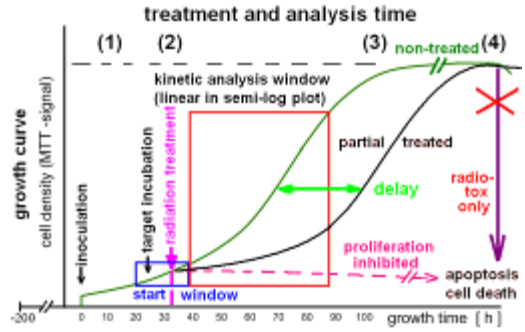
Cell cultures as tumor models offer a fast and precise estimation of a cancer treatment success, e.g. after administration of radio- or chemotherapy. In therapy development they have to distinguish between unwanted direct cell killing (necrosis: embolic and metastasis risks), and the clinically wanted proliferation inhibition with delayed apoptosis, corresponding to tumor growth stop with later remission (months).
We have developed a kinetic cell culture tumor treatment test, which is based on the analysis of theexponential cell growth, yielding proliferation inhibition andnecrosis contributions (EPN-test). After initial work with cell suspensions, the EPN-test was developed to a high throughput test with adherent cancer cells in multiwell plates (typ. 384 well). After seeding at 2% confluence the cell cultures pre-grow to 10-20% confluence (preparation, 2 d). In the treatment, the drug is administered, e.g. radiotherapy enhancer-nanoparticles. After incubation, which depends on cell line and nano-drug type, e.g. 1h, the therapeutic irradiation is done (treatment window). After medium exchange the cultures are grown until full confluence (analysis window, 7 d). For a high throughput analysis an analysis is done daily for one row of the plate with the photometric MTT test , which was shown to be equivalent to the colony test [Buch, Peters, Nawroth, Langguth, Schmidberger (2012) Rad. Oncol. 7, 1]. The proliferation inhibition is observed as a delay of the growth curve, the radio-necrosis as a negative jump. After development at the ESRF synchrotron (60 keV PAT), the EPN-test was used for PT at the radiotherapy clinics Mainz (accelerator 8 MeV); cold neurons (ILL Grenoble) and thermal neutrons (TRIGA reactor Mainz) for B-NCT, Gd-NCT.



AIP08

The interactome of *Salmonella* Typhimurium and human host cells

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Pathogenicity is inevitably linked to specific changes in the cellular proteome, and hence in gene expression, both for the pathogen as well as the host cells. We aimed at establishing a protocol that allows for exact and reliable characterization of differentially expressed genes during the host-pathogen interaction of pro- and eukaryotic cells without prior disruption of the interaction. Therefore, we applied the tag-based, next-generation sequencing-coupled transcriptome profiling techniques deepSuperSAGE and MACE (Massive Analysis of cDNA ends) combined with TrueQuant (PCR-bias free quantification) to specifically assess the transcriptomes of cultivated and interacting *Salmonella* Typhimurium and human (HeLa S3) host cells. This reduction in complexity significantly decreases the necessary sequencing depth for a good coverage of both the pro- and eukaryotic transcriptomes, which is especially important in respect of the relatively low abundance of pathogen-derived transcripts. The combination of the published *S.* Typhimurium transcriptome from Kröger and colleagues (2012) with the operon structure identified by Ramachandran and co-workers via dRNA-Seq (2012) enabled us to accurately quantify polycistronically transcribed genes of the prokaryote despite the fact that we employed tag-based methods for transcription profiling. Furthermore, different annotation routines were tested for their efficiency to identify the pro- and eukaryotic-derived transcripts as accurately as possible, and the best performing routine was finally employed to analyze the interaction of the human-virulent model pathogen with epithelial cells at several points of time post infection.

BIOENERGETICS

PKV19

Supramolecular organization of the respiratory chain of hyperthermophilic eubacterium *Aquifex aeolicus*

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The respiratory chain in eukaryotic mitochondria mainly consists of four transmembrane protein complexes. In contrast, the protein components of bacterial respiratory chains are much more diverse and complex, which enables microorganisms to cope with various living environment. *Aquifex aeolicus* is Gram-nagative, hyperthermophilic, chemolithotrophic and microaerophilic eubacteria. Previously, a supercomplex composed of complex III and IV was isolated in *A. aeolicus*. In this project, *A.aeolicus* native membranes were solubilized using mild detergents. The protein sample was fractionated by sucrose gradient ultracentrifugation, resolved by Blue Native PAGE, and the respiratory chain complexes were indicated by in-gel activity assay and identified by mass spectrometry. The preliminary results indicated that a stable subcomplex of complex I might functionally associate with an unknown ATPase in the membrane, as well as the F_1F_o ATP synthase. In addition, several hypothetical membrane proteins of *A. aeolicus* were also shown to potentially interact with some subunits of complex I, yet their functional relationships are still be to investigated.

BEV01

The structure of the Na^+-translocating NADH:ubiquinone oxidoreductase from *Vibrio cholerae at 3.7* Å *resolution*

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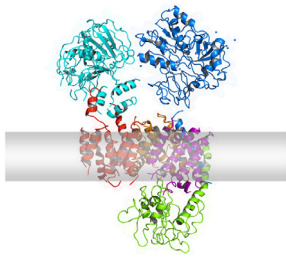
Vibrio cholerae maintains a Na^+ gradient across the cytoplasmic membrane [1,2]. The generated sodium motive force is essential for substrate uptake, motility, pathogenicity, or efflux of antibiotics. This gradient is generated by a NADH:ubiquinone oxidoreductase (NQR) that is related to the RNF complex of archaea and bacteria. NQR is an integral membrane protein complex consisting of six different subunits, NqrA-NqrF [3]. In order to get insights into the redox driven Na^+-transport mechanism we have isolated and crystallized the NQR of *Vibrio cholerae* [4]. The crystals of the entire membrane complex diffract to 3.7 Å. Phases were obtained using different metal-clusters and Se-Met as derivatives. Since some parts could not be resolved at this resolution in the experimental electron density, we determined independently the structure of two large soluble domains at 1.5 Å and 1.9 Å, respectively. Both domains could be fitted into the electron density improving phasing and refinement.

References

[1] Türk et al. (2004) *J. Biol Chem* **279**:21349-55
[2] Juárez et al (2010) *PNAS* **107**:12505-10.
(3) Casutt et al (2010) *J .Biol. Chem.* **285**:27088-99.
[4] Casutt et al. (2010) *Acta Cryst F* **66**:1677-9.



BEP01

Structural and Thermodynamic Characterization of the Cohesin and Dockerin Proteins Complex from Bacterial Celullosome

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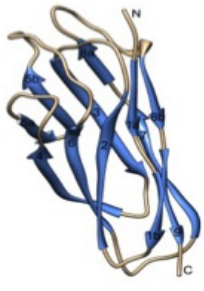
Anaerobic microorganisms have extracellular multi-enzyme complexes (cellulosomes) responsible for cellulose degradation. The structural organization of the cellulosome depends on the interaction pair's cohesin (Coh): dockerin (Doc). The cellulosome of *Ruminococcus flavefaciens* is described as the most elaborate system identified so far. In the present study two structure of cohesin from scaffolding B (*Rf*-CohB) were determined at 1.46 Å and 2.30 Å resolution both in the orthorhombic $P2_12_12_1$ space group by single-wavelength anomalous diffraction, and it is features were compared with known type-I, type-II and type-III cohesin structures. Based on structures analysis it was obtained an insight into the binding mechanism of *Rf*-CohB and its dockerin pair from SCaA using a combination of molecular dynamics (MD) simulations, Isothermal Titration Calorimetry (ITC) and Differential Scanning Calorimetry methods (DSC). It was observed the importance of a loop located between the strands 5b and 6 for binding using a series of structure-based site-directed alanine scan mutants (G94A, Q95A, G96A, G98A and R99A). These results leads to a induced-fitting mechanism of *Rf*-CohB upon Doh binding involved in the complex high affinity.



Supported by: FAPESP and CNPq

BEP02

The c-ring ion-binding site of the ATP synthase from *Bacillus pseudofirmus* OF4 is adapted to alkaliphilic cell physiology

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F_1F_o-ATP synthases contain c-rings, which shuttle ions across membranes as a part of a unique rotary mechanism. The concerted and reversible binding of ions to and from the c-ring is tightly coupled to ATP synthesis. We investigated whether a water molecule found within each of the c_{13} ring ion-binding sites of the alkaliphile *Bacillus pseudofirmus* OF4 and a high rotamer flexibility found for the conserved glutamate E^{54} enhances the ability to capture protons and support enzyme operation at high environmental pH. By X-ray crystallography, we solved the structure of a 'neutralophile-like' mutant (P51A) at 2.8Å showing reduced presence of water. Using the ATP synthase inhibitor dicyclohexyl-carbodiimide (DCCD) we kinetically traced (de-)protonation events at the conserved ion-binding site's glutamate at various pH using the mutant and WT c-rings. Another 'neutralophile-like' mutant (V21N) was investigated on a bioinformatic level revealing a reduced rotamer flexibility of E^{54}. Cell-growth studies illustrate the reduced growth rate at high pH in the neutral-like mutants4. Our results directly connect delicate structural changes of the c-ring ion-binding site and its altered proton binding affinity with in vivo growth effects on the cell physiology level of an alkaliphile.

BEP03

Biogenesis factors of respiratory chain complex I

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The NADH:ubiquinone oxidoreductase is the first and largest complex of the mitochondrial respiratory chain. It has gained much attention since many human neurodegenerative diseases are caused by a dysfunction of complex I. This dysfunction often results from an impaired assembly, which is poorly understood so far. For identification of biogenesis factors the Pentatricopeptide repeat (PPR) proteins were examined due to their involvement in various steps of mitochondrial gene expression [1]. We determined the relevance of two PPR proteins for the assembly of complex I by characterizing their corresponding knockout mutants obtained by a high-throughput program [2]. Two knockout mutants were specifically affected in the assembly of complex I. The presence of a peripheral arm and the absence of a detectable membrane arm were demonstrated. A specific influence of the PPR proteins on processing respectively on amounts of mitochondrial RNA was observed by means of Northern Blots. In order to characterize their specific impact on biogenesis of complex I the two PPR proteins were expressed heterologously in *Escherichia coli* (*E. coli*) and purified in a two-step approach. Interaction studies with the proteins and the specific mRNA and/or DNA via electrophoretic mobility shift analysis are ongoing.

References

[1] Schmitz-Linneweber, C. and I. Small (2008). "Pentatricopeptide repeat proteins: a socket set for organelle gene expression." Trends Plant Sci 13(12): 663-70
[2] Colot, H. V., G. Park, G. E. Turner, C. Ringelberg, C. M. Crew, L. Litvinkova, R. L. Weiss, K. A. Borkovich and J. C. Dunlap (2006). "A high-throughput gene knockout procedure for Neurospora reveals functions for multiple transcription factors." Proc Natl Acad Sci U S A **103**(27): 10352-7.

BEP04
Isolation and proteomic analysis of a complex I assembly intermediate

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Mitochondrial complex I consists of fourteen central subunits harbouring the bioenergetic core functions and a number of accessory subunits acquired during evolution [1,2]. Assembly of the largest and most complicated multiprotein complex of respiratory chain proceeds via intermediates and is supported by assembly factors [3].
Accessory subunit NUMM of aerobic yeast *Yarrowia lipolytica* complex I (bovine 13kDa, human NDUFS6) harbours a conserved zinc binding motive comprising 3 Cys and 1 His. Exchange of one of the conserved cysteine residues into tyrosine in human NDUFS6 subunit (corresponding to C128 in NUMM of *Y. lipolytica*) was reported to affect complex I assembly and result in fatal neonatal lactic acidemia [4].
We found that assembly of complex I of *Y. lipolytica* is stalled in a late stage after deleting the accessory subunit NUMM or mutating residues forming the zinc binding motive. Complex I isolated from the strains lacked the EPR signature for iron - sulphur cluster N4. Subunit N7BM (bovine B17.2, human NDUFA12) was missing as well, while assembly factor N7BM-L (B17.2-L; NDUFAF2) remained associated with the assembly intermediate. Those findings suggest a role of the chaperone in assembly of subunits NUMM and N7BM as well as in the iron - sulphur cluster insertion. We have no indication that N7BML is involved in attachment of the flavoprotein domain (51 kDa and 24 kDa central subunits) as previously reported [5].

References

1. Angerer H. et al. Biochem. J., 437 (2) (2011), pp. 279-288
2.Carroll J. et al. J Biol Chem., 281(43) (2006), pp. 32724-7
3. Hoefs S.J.G. et al., Mitochondrion, 12 (5) (2012), pp. 520 - 532
4. Spiegel R. et al., Eur. J. Hum. Genet., 17 (2009), pp. 1200-1203
5. Lazarou M. et al., Mol. Cel. Biol., 27 (12) (2007), pp. 4228 - 4237

BEP05
Accessory subunit NB4M is essential for the activity of complex I from *Yarrowia lipolytica*

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Mitochondrial NADH:ubiquinone oxidoreductase (complex I, CI) is a large 1 MDa membrane protein complex with a central function in energy metabolism. Complex I from the yeast *Yarrowia lipolytica* comprises 14 central subunits and at least 28 accessory subunits. The association with two acyl carrier proteins (ACPM1 and ACPM2) suggested that complex I functions as a platform for a biosynthetic pathway not directly linked with energy conversion. ACPM1 is essential for *Yarrowia lipolytica* and the deletion of ACPM2 results in a complex I assembly defect. The soluble accessory subunit NB4M contains the conserved *Complex1_LYR* motif (pfam05347). The orthologous human subunit (NDUFA6/LYRM6) has been shown to be down-regulated in response to HIV-1 infection. We identified NB4M and ACPM1 as components of the Fe/S clusters containing hydrophilic peripheral arm of complex I. The deletion of subunit NB4M did not affect the overall assembly of complex I; however subunit ACPM1 was not present in complex I-ΔNB4M. In intact mitochondria of deletion strain *nb4mΔ* ACPM1 was exclusively detected as free soluble matrix protein suggesting functional integrity of ACPM1 in its free form. No physiological ubiquinone-1 and decylubiquinone reduction was observed in mitochondrial membranes of *nb4mΔ*. All EPR-detectable Fe/S clusters of complex I-ΔNB4M were reduced by NADH, however the signal of Fe/S cluster N2 was decreased to 60%. The results from EPR spectroscopy suggest an impact of subunit NB4M on Fe/S cluster N2. Electron microscopy of complex I-ΔNB4M revealed the lack of domain 6 of the peripheral arm. Mitochondrial complex I undergoes the so-called active/de-active (A/D) transition that has been monitored by differential accessibility of a cysteine residue in the long inside loop of subunit ND3. Complex I missing subunits NB4M and ACPM1 (domain 6) might be conformationally blocked in the D-form rendering the enzyme inactive.

BEP06
The role of conserved active site residues in pentaheme cytochrome *c* nitrite reductase

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Cytochrome *c* nitrite reductase (NrfA) catalyzes the six-electron reduction of nitrite to ammonium as the final enzymatic step in the metabolic pathway of respiratory nitrite ammonification within the biogeochemical nitrogen cycle. Although no intermediate products are released, NrfA is able to reduce various other compounds such as nitric oxide and hydroxylamine, but notably also sulfite, providing the only known direct link between the nitrogen and sulfur cycles. NrfA proteins are widespread and well-studied pentaheme cytochromes *c* (for example from the Epsilonproteobacterium *Wolinella succinogenes* or the Gammaproteobacterium *Escherichia coli*) that characteristically contain an unconventional CXXCK heme binding motif in addition to four common CXXCH motifs. The lysine residue of the active site heme acts as an axial heme ligand and it has been shown that the replacement of this lysine by histidine substantially impaired NrfA activity [Pisa *et al*. (2002) *Mol Microbiol* 43, 763-770]. Nonetheless, genome sequence databases reveal an increasing number of NrfA proteins with five regular CXXCH motifs (for example from *Campylobacter* species) questioning the need of the lysine-ligated heme.
To study the role of conserved active site residues, various site-directed variants of the NrfA proteins from *W. succinogenes* and *C. jejuni* were purified and characterized with respect to their catalytic activity and substrate range.

BEP07
Molecular evolution of the quinol oxidation motif of cytochrome *b*

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The oxidation of quinol is the key step in the catalytic mechanism of the cytochrome bc_1 complex. The latter is a central component of biological energy conversion in cellular respiration. Bifurcated electron transfer upon substrate oxidation at the Qo site ensures the operation of the proton motive Q cycle, as it links electron transfer towards cytochrome *c* and release of protons to the positive membrane side with reduction of quinone and proton uptake at the opposite membrane side [1]. The PEWY loop of cytochrome *b* is so far considered as signature motif of quinol oxidation, and the enclosed glutamate E272 is regarded as catalytic residue which acts as substrate ligand and primary proton acceptor [1,2]. Yet, the role of E272 is debated as the structure of the enzyme-substrate complex is still not known, and substitutions of E272 by site-directed mutagenesis in different organisms did not fully abolish quinol oxidation activity [3,4]. Furthermore, E272 is not fully conserved across all species. We noted replacements with valine and leucine in β- and γ-proteobacteria [4]. Thus, a comprehensive phylogenetic analysis of all available cytochrome *b* genes was carried out. A surprising natural variance of the quinol oxidation motif was found. The approach led to the identification of clade-specific quinol oxidation motifs and sheds light on the evolution of cytochrome bc_1 complexes and their active site.

References

[1] Hunte C. *et al*. Results Probl. Cell Differ. 2008, **45**, 253-78.
[2] Palsdottir H. *et al*. J. Biol. Chem. 2003, **278**, 31303-31311.
[3] Osyczka A. *et al*. Biochemistry 2006, **45**,10492-10503.
[4] Wenz T. *et al*. Biochemistry 2006, **45**, 9042-9052.

BEP08
Structural characterization of mitochondrial complex I

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Proton-pumping respiratory complex I (NADH:ubiquinone oxidoreductase) is among the largest and most complicated membrane protein complexes. Its function is critical for efficient energy supply in aerobic cells and malfunctions are implicated in neurodegenerative disorders. It is the entry point for electrons from NADH into the respiratory chain and it couples electron transfer to ubiquinone with vectorial proton pumping across the inner mitochondrial membrane, thus contributing about 40 % of the proton motive force that drives ATP synthesis by ATP synthase. The X-ray crystallographic analysis of the complete mitochondrial complex I from *Yarrowia lipolytica* provided insights in its modular architecture, revealed positions of all iron-sulfur clusters relevant for electron transfer, indicated the position of the ubiquinone reduction site ~30 Å

above the membrane, and identified a long lateral helix in the membrane arm. This analysis provided clues on the mechanism of redox-linked proton translocation [1], yet, a complete mechanistic understanding of complex I is lacking. The key open question is the coupling between redox chemistry and proton pumping, processes which are spatially separated in peripheral and membrane arm, but which are connected *via* the ubiquinone chemistry. Here, we present the structure determination and X-ray structure of *Y. lipolytica* and discuss it in mechanistic context.

References
[1]Hunte, Zickermann, Brandt (2010), Science 329, 448-451

BEP09

will be presented as an oral presentation in the Study-Group-Session 9 "ROS: Generating complexes and intracellular signaling". See page XX.

BEP10

Structural basis for redox-linked proton translocation by mitochondrial complex I

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Mitochondrial complex I (proton pumping NADH:ubiquinone oxidoreductase, EC 1.6.5.3) is a 1 MDa membrane protein complex with a central function for efficient energy production of the cell. Coupling electron transfer from NADH to ubiquinone with transmembrane proton translocation complex I contributes substantially to the proton motive force that drives ATP synthesis by ATP synthase. Complex I is known to release toxic oxygen radicals and complex I dysfunction caused by mutations or toxins is associated with a number of neuromuscular and neurodegenerative human diseases, e.g. Parkinsons disease. In our laboratory the aerobic yeast *Yarrowia lipolytica* was established as a model system to study mitochondrial complex I in health and disease. We have determined the arrangement of functional modules within the 42 subunit enzyme complex by X-ray crystallography [1]. At improved resolution and employing anomalous diffraction of brominated tool compounds we characterized the ubiquinone and inhibitor binding site of complex I. New structural information combined with extensive mutagenesis data provides clues on the yet poorly understood mechanism of redox-linked proton translocation.

References
[1]Hunte, Zickermann, Brandt (2010),Science 329, 448-451

BIOPHYSICAL AND EVOLUTIONARY ASPECTS OF PROTEIN ENGINEERING

PEV01

A toolbox of dihydroxyacetone dependent aldolases for enzymatic C-C bond formation

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The formation of C-C bonds is at the heart of organic synthetic chemistry. The enzyme catalyzed C-C bond formation by aldolases and transaldolases is advantageous with respect to its high regio- and stereoselectivity which is especially important for the synthesis of bioactive compounds like pharmaceuticals. Recently, dihydroxyacetone (DHA) dependent aldolases, such as fructose-6-phosphate aldolase (FSA) and variants of transaldolase B (TalB), gained particular interest.[1]
FSA can use an unprecedented large number of donor substrates (dihydroxyacetone, hydroxyacetone (HA), 1-hydroxy-2-butanone (HB) and glycolaldehyde).[1] FSA was successfully applied in the synthesis of ketoses, aldoses, deoxysugars and iminocyclitols. By protein engineering, we generated by just one amino acid replacement a TalB variant (TalB F178Y) with a similar catalytic efficiency as wild-type FSA for the aldol addition of DHA and D-glyceraldehyde-3-phosphate.[2] This identified a tyrosine and its H-bond to a catalytic water molecule in the active site as crucial for aldolase activity. A comparative analysis of FSA and TalB F178Y revealed that both aldolases exhibit a complementary donor specificity.[3] FSA prefers HA and HB whereas TalB F178Y uses predominantly DHA. Currently, we explore the differences in substrate specificity as well as the molecular determinants for the different reaction type in aldolases and transaldolases by mutagenesis studies. Our latest results of the mutagenesis studies and their implications for the aldolase mechanism will be presented.

References
[1] Samland, A. K., et al. (2011) *Chembiochem.* 12, 1454-74.
[2] Schneider, S., et al. (2008) *J Biol Chem.* 283, 30064-72.
[3] Rale, M., et al. (2011) *Chem Eur J.* 17, 2623-32.

PEV02

Characterization and application of novel naturally split inteins from metagenomic sources with unusual properties

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Inteins are *in*ternal pro*teins,* which can excise themselves out of a precursor polypeptide chain and connect the external protein chains (exteins) by a native peptide bond. There are only few examples known where inteins are naturally split and therefore translated as two independent protein chains. In these cases, the N- and C-terminal fragments (Int^N and Int^C) have to associate first before *trans*-splicing can occur.[1, 2] Split inteins have recently received much attention due to their great potential for many biotechnological applications, e.g. protein semi-synthesis, and production of cyclic polypeptides. Therefore, new split inteins with superior properties are urgently needed. Several sequencing projects of metagenomic DNA have accumulated large amounts of data, in which also new split inteins were identified.[3] We now have identified a novel split intein with interesting and unusual sequence characteristics. This intein exhibits a unique combination of the key catalytic residues that may point to a protein splicing mechanism differing from the canonical pathway. Furthermore, the novel split intein displays an unusual split site, which will be advantageous for several applications in protein labelling. Here, we report the biochemical characterization and application of the protein *trans*-splicing reaction of this unusual split intein. Furthermore, we have studied the applicability of another unusual split intein which also holds great potential.[4]

References
1) Mootz HD. *ChemBioChem*, 2009, **10**, 2579-2589
2) Volkmann G., Iwaï H., *Mol. BioSyst.*, 2010, **6**, 2110-2121
3) Dassa B. et al., *NAR*, 2009, **8**, 2560-2573
4) Carvajal-Vallejos P. et al., *J Bio Chem*, 2012, **34**, 28686-28696

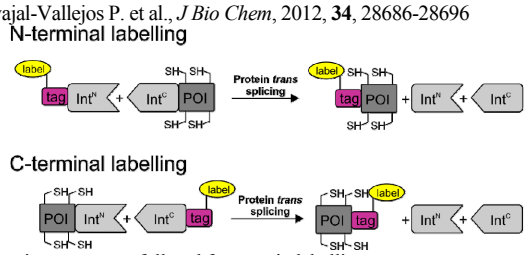

Fig 1: Inteins as a powerfull tool for protein labelling.

PEV03

Interfacial activation of *Candida antarctica* lipase B: combined evidence from experiment and simulation

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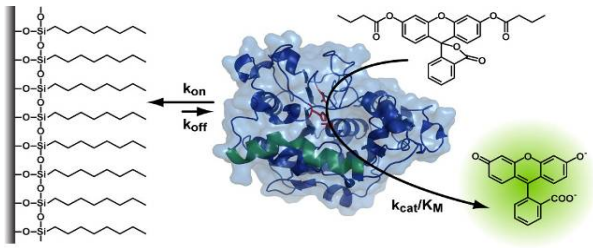
Candida antarctica lipase B (CalB) is an important biocatalyst used in many industrial applications. Lipases are frequently activated after binding to hydrophobic interfaces. For CalB, clear experimental evidence proving this interfacial activation is currently lacking.
Having performed a systematic study with CalB non-covalently immobilized on surfaces of different hydrophobicity and using different fluorogenic substrates, we show that the surface properties and the size of the substrate have a clear effect on CalB activity. Enhanced activity is only observed when CalB hydrolyzes the large, bulky substrate fluorescein dibutyrate AND is immobilized on a highly hydrophobic octyl-silane surface.
This enhancement, currently observed in ensemble measurements and supported by molecular docking simulations, is most likely caused by a conformational change. We hypothesize that the hydrophobic surface stabilizes a more open active site conformation that shows increased activity for bulky substrates. Ongoing single molecule experiments give additional insight into the mechanism of this activation indicating that the activity of all enzymes in the population increases as a response to the hydrophobic environment. Taken together, our combined approach shows that CalB explores a large conformational space, influenced by a number of external parameters. These findings will improve our

understanding of the parameters regulating CalB activity and lay the foundation for its further optimization.



PEV04

Change in protein-ligand specificity through binding pocket grafting

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Recognition and discrimination of small molecules is crucial for many biological processes. Understanding the rules that underlie binding specificity is of particular interest to synthetic biology, e.g. the engineering of biosensors with novel ligand affinities. Promising scaffolds for such biosensors are the periplasmic binding proteins (PBPs) due to their ligand-mediated structural change that can be translated into a physically measurable signal.
In our study we focused on the two homologous polyamine binding proteins PotF and PotD. Despite their structural similarity, PotF and PotD have very different binding specificities for the polyamines putrescine and spermidine. To elucidate how specificity is determined, we grafted the binding site of PotD onto PotF. The introduction of seven mutations in the first shell of the binding pocket leads to a swap in the binding profile as confirmed by isothermal titration calorimetry. Furthermore, the 1.7 Å crystal structure of the new variant complexed with spermidine reveals the interactions in the binding pocket including a defined water network. Additionally, the individual contributions of the specificity determining residues were investigated by biophysical and structural analysis of reduced sets of mutants. Altogether our study shows that specificity is encoded in the first shell of the PotF binding pocket and that it is possible to swap the binding specificity through transplantation. It also indicates that second shell mutations should be considered to increase affinity and to fine-tune ligand-mediated structural changes.

PEP01

Functional analysis of single amino acids in the active site of a dandelion (*Taraxacum officinale*) polyphenol oxidase via site-directed mutagenesis

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Polyphenol oxidases (PPOs; EC 1.10.3.1) are type-3 copper proteins whose active site incorporates the two copper-binding motifs CuA and CuB, each coordinating a copper ion by three histidine residues. In contrast to tyrosinases (EC 1.14.18.1), PPOs exclusively catalyse the oxidation of *ortho*-diphenols to the corresponding *ortho*-quinones.
Sequence analyses of plant PPOs, fungal as well as bacterial tyrosinases showed conserved amino acids to be crucial for protein folding, copper coordination and catalytic activity. Yet the molecular basis for the diverse enzymatic properties of PPOs such as substrate specificity is unknown. Due to the lack of an efficient expression system, mutation studies to reveal specific amino acids causing these differences have been missing so far.
Using a recently-established procedure we succeeded in heterologously expressing soluble and active dandelion (*Taraxacum officinale*) PPO muteins in *Escherichia coli*. By analyzing PPO and tyrosinase sequences from different organisms we selected several potentially important amino acids. Mutants of a dandelion PPO were generated by replacing these amino acids located in the immediate vicinity to the CuB motif.
Activity studies with a panel of diphenolic model substrates offering different functional groups did not indicate an influence of the mutations on SDS-dependent activation or the optimum pH. In contrast, enzyme kinetics revealed a significant impact of certain amino acid substitutions on maximum velocity as well as the substrate specificity.
Our data therefore provide new insights into the molecular basis of substrate selectivity of plant PPOs and constitute great potential for further studies on the catalytic mechanism of type-3 copper proteins.

PEP02

Improvement of antimicrobial activity of lysozyme by the modification of polyarginine at the C-terminus

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Introductions: Human lysozyme (HLY) is an anti-bacillus protein taking the place of antibiotic, because HLY is effective against antibiotic-resistant bacteria. To improve the antibacterial activity, arginine residues were connected to the C-terminus of HLY in order to raise the affinity between HLY and bacteria.
Materials and methods : The genes of hybrid HLYs connecting arginine residues at the C-terminus, were expressed in insect cells using baculovirus expression system. Three kinds of hybrid HLYs were produced as follows: HLY-Ser-Arg (HLY-SR), HLY-Ser-Arg-Arg-Arg (HLY-SR3), HLY-Ser-Arg-Arg-Arg-Arg-Arg-Arg (HLY-SR6). The lytic activity to *Micrococcus.lysodeikticus* was measured in various pH or in various NaCl concentrations.
Results: From pH 6 to 9, the rising of pH decreased the activities of native HLY and HLY-SR, but not HLY-SR3 and HLY-SR6. In high NaCl concentrations (200mM), native HLY and HLY-SR completely lost their activities, though HLY-SR3 and HLY-SR6 showed still 8-12% of the original activity. Even in 500mM NaCl which is almost equal to sea water, HLY-SR6 was still effective to bacteria.
Conclusions: With increasing the number of arginine at the C-terminus, lytic activity of HLY was strengthened. Especially, HLY-SR6 exhibited significantly strong and wide optimal pH range and resistance to higher ionic strength conditions than other HLYs. HLY may have more strong activity, if basic residues are added further at the C-terminus.

PEP03

The evolution of the tryptophan synthase complex is accompanied by a change in quaternary structure

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Early phases of the evolution of enzyme complexes have remained obscure to date. Here, we have used crystal structure and mutational analysis to retrace the evolution of the ubiquitous tryptophan synthase complex (TS), which consists of the α- and β-subunits TrpA and TrpB. Previous experimental findings allowed us to postulate an evolutionary pathway starting from the putatively most archaic TrpB2a protein [ββ dimer, no complex formation with TrpA] *via* TrpB2i [transient αββ complex, uni-directional activation of TrpA, no substrate channelling] to TrpB1 [permanent αββα complex, bi-directional subunit activation, substrate channelling]. Now, we have reproduced the structural details of this pathway by solving the crystal structures of ssTrpB2a and the ssTrpA-ssTrpB2i complex from *Sulfolobus solfataricus*, which we compared with each other and with the canonical stTrpA-stTrpB1 complex from *Salmonella typhimurium*. The N-terminal sequences of ssTrpB2a and ssTrpB2i differ in length and composition, which prompted us to create ssTrpB2i: ssTrpB2a chimeras. Their characterization showed that few amino acid exchanges in the N-terminal interface of ssTrpB2i are sufficient to confer transient binding of ssTrpA by ssTrpB2a. Strikingly, the quaternary structure of ssTrpA-ssTrpB2i differs significantly from the quaternary structure of stTrpA-stTrpB1, which suggests the need for a larger rearrangement of the interfaces to enable substrate channelling and bidirectional subunit activation. In summary, these protein complexes most plausibly represent frozen states of TS evolution and confirm the hypothesis that protein assembly pathways mimic evolutionary pathways.

PEP04
New Bacterial Members in the Geranylgeranylglyceryl Phosphate Synthase Family

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Geranylgeranylglyceryl phosphate synthase-like enzymes (GGGPS) catalyze the formation of an ether bond between glycerol-1-phosphate and polyprenyl pyrophosphates. Since ether lipids are a unique feature of archaeal cell membranes, the emergence of GGGPS enzymes is discussed as a key event in the evolution of archaea. We could show that there exist bacterial homologues of GGGPS enzymes which differ considerably in their specificity to the hydrophobic substrate. While GGGPS enzymes from archaea prefer a geranylgeranyl (C20) pyrophosphate (GGPP) as substrate, the representatives from Bacillales prefer the longer heptaprenyl (C35) pyrophosphate. The physiological role of ether lipids in the domain of the bacteria is unknown, to date.
We now report an additional group of bacterial GGGPS-like enzymes from Bacteroidetes. We studied their phylogenetic context and found that the GGGPS-like enzymes split into two distinct subgroups (group I and group II). While some archaeal representatives and the Bacillales enzymes belong to group I, most archaea and the Bacteroidetes possess a group II enzyme. Only group I enzymes have been studied extensively during the last years, their substrate specificity is determined by certain amino acids acting as a "ruler" and supported by their dimeric structure. We solved the crystal structures of a bacterial and an archaeal group II enzyme with bound substrates and characterized additional group II representatives. We found that they all prefer GGPP as substrate, use the same "ruler" mechanism to determine the substrate specificity, and some of them have a hexameric structure. These results provide new insights into the evolution of substrate specificity determination in the family of GGGPS-like enzymes.

PEP05
Tailoring acetaldehyde dependent aldolases for organic synthesis

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Acetaldehyde dependent aldolases were demonstrated to have a high potential as biocatalyst: They form chiral building blocks for organic synthesis via a highly selective aldol reaction. Several complex natural products have been synthesized in good yields and high enantioselectivity without harsh reaction conditions.[1] A prominent example is the key building block for the pharmaceutical block buster atorvastatin synthesized by Jennewein et al.[2]
For our synthetic purposes, we use the 2-deoxy-D-ribose-5-phosphate aldolase (DERA) of *R. erythropolis* which catalyzes the aldol reaction between two aldehydes.[3] Although the reaction conditions are quite mild, acetaldehyde tends to give side reactions. Therefore, we are searching for new, yet uncharacterized DERAs that are able to work under even milder conditions. Thus, we are exploring DERAs from psychrophilic as well as thermophilic organisms for their potential in organic synthesis. For optimizations the structural information of DERAs is used to design new DERAs with increased stability towards temperature and organic solvents.

References

[1] a) Clapés, P.; Fessner, W.-D.; Sprenger, G. A.; Samland, A. K.; *Curr. Opin. Chem. Biol*. **2010**, 14, 154-167. b) Domínguez de María, P.; Pohl, M.; Gocke, D.; Gröger, H.; Trauthwein, H.; Stillger, T.; Walter, L.; Müller, M.; *Eur. J. Org. Chem.* **2007**, 2940-2944.
[2] a) Jennewein, S.; Schürmann, M.; Wolberg, M.; Hilker, I.; Luiten, R.; Wubbolts, M.; Mink, D.; *Biotechnol. J.* **2006**, 1, 537-548. b) Wolberg, M.; Dassen, B. H. N.; Schürmann, M.; Jennewein, S.; Wubbolts, M.; Schoemaker, H. E.; Mink, D.; *Adv. Synth. Catal.* **2008**, 350, 1751-1759.
[3] Kullartz, I.; Pietruszka, J.; *J. Biotechnol.* **2012**, 161, 174-180.

PEP06
Biophysical Characterization of the Nore1-MST1 SARAH interaction

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Ras regulates apoptosis signaling by binding its effector protein Nore1. Therefore, Nore1 binds Ras via its RBD and thus mediates growth inhibition. Further, the Ras-Nore1 complex activates the pro-apoptotic kinase MST1, which finally leads to apoptosis. The MST1 activation occurs through the interaction of Nore1 and MST1 by association of their homologous coiled-coil SARAH domains. Additionally, besides the heterodimeric Nore1-MST1 interaction, both also form homodimers with other Nore1 or MST1 proteins, respectively. These dimers coexist in a complex thermodynamic equilibrium. Thereby, the K_D values indicate the Nore1 homodimer as the weakest complex, whereas the MST1 homodimer shows the highest affinity. Thus, the heterodimeric interaction is favored by Nore1 while MST1 prefers homodimer formation.
The kinetics of the Nore1 and MST1 dimers have been investigated by T-jump perturbation (TgK-Scientific). These measurements gave an insight into the relaxation rates as well as the concentration dependency of the binding and folding of the dimers. Due to the complex association/dissociation reaction of Nore1 and MST1, a covalently linked Nore1-MST1-SARAH heterodimer was designed. This heterodimer enabled further investigations of the inter- and intramolecular Nore1 and MST1 interactions by fluorescence titrations.
In the future, further fluorescence studies and the heterodimeric crystal structure shall deepen the understanding of the Nore1 and MST1 interactions.

PEP07
Engineering of weak helper interactions for high-efficiency FRET probes

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FRET between genetically encoded fluorescent proteins allows for the direct detection and visualization of transient protein-protein interactions. However, interaction FRET sensors usually require custom design and protein truncations in order to optimize the geometric arrangement of donor and acceptor probe. We introduce two different strategies for the rational design of weak helper interactions (hi) that co-recruit donor and acceptor fluorophores for the robust detection of bimolecular FRET: (i) in silico design of electrostatically driven encounter complexes, (ii) the fusion of tunable domain - peptide interaction modules based on WW or SH3 domains. Each strategy was tested for optimization of FRET between mCitrine and non-Aequorea mCherry. Both approaches yield comparable and large increases in FRET efficiencies with little or no background. Helper interaction modules can be fused to any pair of fluorescent proteins (such as mTFP1 / mCherry as well as mTurquoise2 / mCitrine). We apply hiFRET probes to study the binding between H-Ras and full length Raf1 and develop apoptosis sensors with increased dynamic range. In addition, hiFRET, but not conventional FRET probes, can visualize a weak transient interaction between Raf1 and B-Raf which is implicated in the frequent clinical failure of anti-proliferative Raf kinase inhibitors. We expect the design of deliberately weak protein interactions to find many additional applications.

PEP08
(4*R*)- and (4*S*)-Fluoroproline in the Conserved *cis*-Prolyl Peptide Bond of the Thioredoxin Fold: Tertiary Structure Context Dictates Ring Puckering

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Among the natural amino acids, proline (Pro) plays a crucial role for the integrity of protein
structure. Moreover, *cis* Pro peptide bonds are essentially the only *cis* peptide bonds occurring in protein structures, and their formation is often the rate-limiting step in protein folding.
Here, we "edited" the conserved *cis*-proline residue 76 of the thiol/disulfide oxidoreductase thioredoxin (Trx) by substitution with 4-fluoroproline (Flp), i.e., insertion of an electron-withdrawing substituent on the pyrrolidine ring.[1] First, we constructed the thioredoxin variant Trx1P, in which all prolines except for *cis* Pro76 were replaced by alanines. High-resolution X-ray structures of the oxidized forms of Trx1P and its 4R-Flp and 4S-Flp variants showed that the molecular architecture of wt thioredoxin was preserved, including the *cis* 75-76 peptide bond. The structures of the variants Trx1P-4R-Flp and Trx1P-4S-Flp only showed the γ-endo puckering although 4R-Flp disfavors this conformation, showing that tertiary structure context dominates the *cis* proline ring puckering.
This study represents the first comprehensive, high-resolution structural analysis of a globular protein in which either (4*R*)-Flp or (4*S*)-Flp was incorporated at a single position, combined with quantitative data on thermodynamic stability and biological activity. Trx1P is also the first case where both Flp isomers have identical effects on protein stability (i.e., both stereoisomers stabilize the reduced form of the protein, and both destabilize the oxidized form). Our results underline the potential of Pro-to-Flp substitutions for increasing or fine-tuning protein stability without affecting biological activity.

References

(1) Rubini M, Schärer M, Capitani G, Glockshuber R. (2013) *Chembiochem.* 14:1053-

PEP09

CysTag: An easy, traceless and general method to label proteins C-terminally

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A still challenging problem in protein modification is the regio-selective labelling of proteins. A multitude of methods is available, but they each have certain advantages and disadvantages. Often they require the incorporation of a big tag into the POI, which may perturb its activity. Some modification reagents are hardly accessible or the approach as such is difficult.
Here we present an improved method to selectively modify the C-terminus of proteins using split inteins. The CysTag-strategy, established by Kurpiers *et al.* allows the selective modification of target proteins using a 119 aa tag sequence, fused to the C-terminus of the target protein on DNA level. This sequence corresponds the N-terminal part of an intein (Int^N) and is recognized by its complementary fragment (Int^C). The fragments form the active intein which rapidly ligates the flanking sequences by protein *trans* splicing with concomitant excession of the intein parts. By fusing the Int^C with a short aminoacid sequence, including an modified cysteine, a system to modify the C-terminus of proteins is obtained. An important aspect of this approach is that well established thiol-directed reagents can be used and that the POI may contain additional cysteines.
Although this strategy is a powerful tool for protein modification, there were some drawbacks remaining, i.e. the poor folding and catalytic properties of the reported intein system. Also, the tag had a size of 119 aa. By redesigning the strategy based on the M86 mutant of the *Ssp*-DnaB intein the tag could be reduced to 11 aa and the reaction time was shortened to one hour.

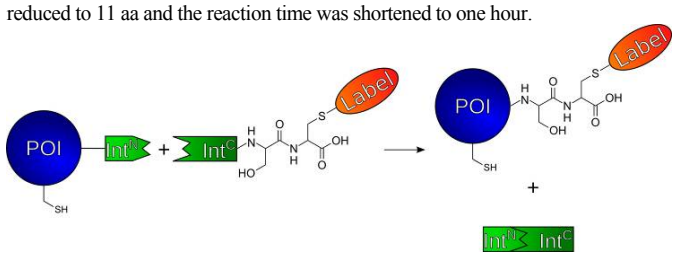

Figure 1: The CysTag strategy. The POI is modified C-terminally using a prelabeled tag. The intein used to mediate the ligation is excised in the process.

PEP10

Comparative studies on recombinant polyphenol oxidases from dandelion correlate sequence differences to substrate specificities

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Plant polyphenol oxidases (PPOs, EC 1.10.3.1) are ubiquitous type-3 copper proteins which occur in gene-families coding for different isoenzymes. They are closely related to tyrosinases and hemocyanins and enzymatically convert *ortho*-diphenols to the respective quinones. Though PPOs traditionally are of economical interest due to their contribution to fruit and vegetable browning, their physiological functions in plant tissues are mostly not clarified nor are structure-function relationships. Our newly established bacterial expression system, allowed us to separately characterize different isoenzymes giving a direct connection between sequence and measured enzymatic activity and therefore reaching new insights into PPO-function.
Dandelion (*Taraxacum officinale*) possesses a remarkably large PPO-family with eleven genes which clearly separate into two phylogenetic groups. To elucidate a potential functional basis for this grouping we performed detailed sequence comparisons, molecular modeling and *in silico* substrate docking. Furthermore we heterologously expressed two PPOs from each group and characterized the purified enzymes regarding their substrate specificities.
Our results reveal significant differences in enzymatic parameters for members of the two groups. *In silico* docking studies suggest this to result from differences in the amino acid composition at the entrance of the active site influencing substrate binding. These results strongly imply special functions for different PPO-isoenzymes *in planta* connected to different natural substrates.
Ongoing studies aim to identify those natural substrates and further elucidate substrate binding and processing in plant PPOs.

PEP11

Effects of naringin and dimethyl sulfoxide (DMSO) on the thermal aggregation of *Bacillus amyloliquefaciens* alpha-amylase

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Introduction
Proteins tend to form inactive aggregates by the assembly of unfolded protein molecules. Protein aggregation is a concern in cell biology and biotechnological industry, and should be prevented.
Alpha-amylases (EC 3.2.1.1) are enzymes that are used in starch liquefying industry. In the present study, *Bacillus amyloliquefaciens* alpha-amylase (BAA) was exposed to thermal denaturation, and the effect of Naringin and its solvent DMSO was also checked on the aggregation process of the enzyme.

Material and methods
Solution containing 0.05 mg/ml of BAA in phosphate buffer (pH=5) and different concentrations of Naringin and DMSO were prepared and incubated at 60°C for a period of 600 or 800 seconds. Protein aggregation was determined by UV-Visible spectroscopy using absorbance at 400 nm.

Results
Upon enzyme incubation in high temperature, amorphous aggregates are formed. Both Naringin and DMSO were found to be effective on the process. Next, DMSO concentration was decreased by dilution with phosphate buffer to assess the individual effect of Naringin. A final range of 0.6-18% DMSO and 0.2-6 mM naringin were tested. Interestingly, Naringin was found to actually increase the aggregation of BAA.

Discussion
Naringin is a glycosylated flavonoid, and flavonoids have been shown to have either inhibitor or activator effect on alpha-amylase enzyme activity, while various carbohydrates are known to be aggregation inhibitors. However, Naringin was found to increase aggregation, and further studies are needed to clarify its mechanism of action. Interestingly, DMSO was found to be able to inhibit the enzyme aggregation, which may be related either to the fact that it is a solvent, or to a more specific interaction with the enzyme.

PEP12

Effect of DMSO, propanol, isopropanol, acetone and TFE on porcine pancreatic lipase aggregation

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Introduction
Protein aggregation is observed as a consequence of harsh environmental conditions. Porcine pancreatic lipase (PPL) (E.C. 3.1.1.3) is a cheap commercially produced enzyme, able to catalyze various transformations used in chemicals synthesis, where organic solvents may be used. Here, the effect of these solvents was checked on PPL aggregation.

Material and methods
Aggregation of porcine pancreatic lipase was determined by UV-Visible spectroscopy at 400 nm for 600 seconds and at 50°C. Samples containing lipase in phosphate buffer at (pH=5), were tested in presence or absence of DMSO (5-25%), propanol (1-20%), isopropanol (1-25%), acetone (1-25%) and TFE (1-10% due to safety limitations) .

Results
Native enzyme forms visible amorphous aggregates. Adding 5-25% (v/v) of DMSO showed increasing reductive effect on PPL aggregation. On the other hand, an increase occurred in PPL aggregation by adding any of acetone, isopropanol, propanol or TFE, with the last two solvents being more effective. The aggregation inductive effect was stronger above a particular concentration of these solvents, being 10% for propanol , 15% for isopropanol, 10% for acetone and 5% for TFE. Speed of protein aggregation was higher and aggregated protein particles were larger in these conditions.

Conclusion
Propanol and TFE increase protein aggregation speed, although TFE presence causes PPL to reach maximal aggregation in a shorter time period. DMSO, which has a different structure, and may affect the activity of PPL, could prevent aggregation of the enzyme. Further studies are needed to characterize the exact mechanism of action of these solvents, which have been reported to affect other lipases activities as either inhibitors or activators.

PEP13
Light driven proton pumps: What are the limitations of directed transport?

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All known light-driven proton pumps are microbial type rhodopsins that transport protons from the cytoplasm outward to the extracellular medium. These rhodopsins were found in bacteria, archaea and eukaryotes and contain all-*trans* retinal as chromophore. Bacteriorhodopsin from *Halobacterium salinarum* is the most notable proton pump. Over the last decades bacteriorhodopsin has been intensively studied mainly by spectroscopic methods. More recently proton pumps have been employed optogenetics for silencing of neuronal activity by hyperpolarization. Surprisingly, the knowledge about the electrophysiological behavior in living cells is poor and the limitation of the pumping activity at negative voltage is for most light-driven transporters unknown. We reanalyzed a variety of proton pumps with a new focus using two-microelectrode voltage-clamp measurements (TEVC) of *Xenopus leavis* oocytes and whole-cell patch-clamp recordings in HEK293-cells. A special attention was laid on a rhodopsin from *Gloeobacter violaceus* (GR). Under standard conditions the GR photocurrents are outward directed but inward directed photocurrents can be observed at high electrochemical load, preferentially at low extracellular pHo. We demonstrated that these inward directed photocurrents are purely passive and based of a well defined ion leakage. We identified E132 as key position because passive ion leakage is completely blocked in the mutant GR-E132D. Mutations in other proton pumps at similar positions may induce leak currents and help to understand how proton pumps maintain their directed transport even at high electrochemical load.

PEP14
Therapeutic enzymes for the treatment of leukemia: Autoproteolytic activation of human asparaginase induced by free glycine

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Asparaginases (ASNase) catalyze the deamidation of the amino acid asparagine (Asn) to aspartate (Asp) and ammonia. Bacterial asparaginases [Elspar*] used in cancer chemotherapy deplete serum Asn, thus inducing apoptosis in several types of blood cancer cells which, in contrast to normal cells, depend for growth on the extracellular supply of the non-essential Asn. To avoid the immune response and other adverse effects caused by the bacterial enzymes, it would be beneficial to replace them with human ASNase. The human genome codes for at least three enzymes with ASNase activity. One of them, which we refer to as hASNase3, belongs to the N-terminal nucleophile (Ntn) hydrolase superfamily where the protein is made as a single polypeptide chain that is devoid of catalytic activity. Intramolecular processing releases the amino group of Thr168, a moiety required for catalyzing Asn hydrolysis. Increased expression of hASNase3 was observed in several tumors, but the functional relevance of this was unknown. Recombinant hASNase3 purifies as the uncleaved, ASNase-inactive form, and undergoes self-cleavage to the active form at a very slow rate [1]. We observed that the free amino acid glycine very selectively acts to accelerate autoproteolysis of hASNase3 both *in vitro* and in human cells. Crystal structures of hASNase3 in complex with glycine reveal the mechanism of glycine-accelerated cleavage [2].

References
[1] Nomme, J. et al. (2012) *Biochemistry* **51**,6816-6826.
[2] Su, Y. et al. (2013) *Chemistry & Biology* **20**,533-540.

PEP15
Engineering of chitin deacetylase activity from *Bacillus licheniformis* DSM13 by using different carbohydrate-binding modules

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Chitin, a β-(1-4)-linked homopolymer of *N*-acetyl-D-glucosamine, is commonly found in cell walls of fungi as well as in exoskeletons of arthropods and insects. It can be partially or fully de-*N*-acetylated to chitosan. Chitosan is positively charged, water-soluble at slightly acidic pH and therefore it offers a high potential of applications in industries. Currently, chitosan is produced chemically, but this process is environmentally harmful and unwanted side reactions such as degradation of the polymer chain may occur. An alternative approach is the use of chitin deacetylase (CDA, EC 3.5.1.41), when well defined products are required. However CDAs have only a low activity on crystalline substrates, the fusion of different carbohydrate-binding modules (CBM) to the catalytic domain of a CDA might increase the enzymatic activity on insoluble substrates.
In this study the CDA5 from *Bacillus licheniformis* DSM13 was fused to three different type A CBMs that bind to crystalline chitin: *Bl*CBM5_12 from *B. licheniformis*, *Pf*CBM2 from *Pyrococcus furiosus* and *Bc*CBM1 from *Botrytis cinerea*. The CDA5 and the fusion proteins were successfully heterologous expressed in *Escherichia coli* and purified. The temperature and pH optimum for wt CDA5 are 37°C and 8-10, respectively. As metalloenzyme Zn^{2+} is required for enzyme activity and the addition of EDTA abolished the activity. In comparison to the wt enzyme the CDA5-*Pf*CBM2 improved enzymatic activity on colloidal chitin particularly. All fusions showed a moderate activity on crystalline β-chitin, crystalline α-chitin was not deacetylated and the activity on soluble chitosan DA61% was decreased.
The enzyme characterizations confirm that the CDA5 could be optimized by enzyme engineering.

PEP16
Identification and characterization of *Alternaria alternata* chitinosanase, a novel chitosan-hydrolyzing enzyme with a specific pattern of activity

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Chitosans present numbers of biological properties which depend on their physicochemical characteristics and more precisely the degree of polymerization (DP), the degree of acetylation (DA) and the pattern of acetylation (PA). The degree of acetylation can be modified by chitin deacetylases and the degree of polymerization by chitin and chitosan hydrolysing enzymes. Until now, this group of enzymes was composed of chitinases, which cleave between GlcNAc and GlcNAc residues, and chitosanases, which cleave between GlcN and GlcN. Both chitinases and chitosanases can also cleave between GlcNAc and GlcN and/or GlcN and GlcNAc depending on their specificity.
A novel chitosan-hydrolyzing enzyme has been identified in the fungus *Alternaria alternata*. This enzyme is active on chitosans with DA from 10 to 70% and possesses the so far unique specificity not to be active on chitin, composed of GlcNAc-GlcNAc linkages, or on pure chitosan, composed of GlcN-GlcN linkages. With these properties, this enzyme cannot be defined as a chitinase or a chitosanase, and has been defined as the first member of the chitinosanase family.
In *Alternaria alternata*, the chitinosanase is secreted in the culture media in low amount. The purification has been optimized and allow us to study the specificity of its cleavage site. Experiments performed on chitosans with low DA have shown that this enzyme produces oligomers with GlcN-GlcNAc units at the reducing end. The activity on polymeric substrates with different DA as well as on oligomeric substrates will also be investigated. This novel and highly-specific enzyme is a promising tool for the production of chitosans with defined sizes, compositions and patterns of acetylation, interesting for downstream applications.

PEP17
Mutations in a hydrophobic cluster in human Guanylate Binding Protein 1 (hGBP1) induces striking differences in enzymatic activity and intramolecular arrangement

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HGBP1 is a member of the dynamin superfamily of large GTPases. Its expression is induced by interferons that mediate anti-proliferative and anti-pathogenic effects. The 68 kDa protein consists of an N-terminal globular enlarged G (LG)-domain and an elongated α-helical domain subdivided into a middle domain (α7-11) and a C-terminal domain (α12-13) which is connected to the LG-domain via saltbridges. Dimerization mediated by LG contacts stimulates GTP hydrolysis. Further, rearrangements of the LG upon GTP turnover lead to a release of the recently identified second interface located in α12-13 which in turn enables the protein to form higher oligomers or to interact with cellular compartments. Since the structural rearrangement is directly linked to GTP conversion the accessibility of the second interface is temporarily limited. Although this might be an important regulation mechanism for the biological function of hGBP1 studies on interaction mechanisms become complicated. In addition, hGBP1 has the unique feature to hydrolyze GTP not only to GDP but also to GMP, whereby product ratio is highly correlating with the degree of α12-13 flexibility.
Based on X-ray structures we identified a hydrophobic cluster crucially involved in nucleotide dependent structural rearrangements of hGBP1. With substituted residues in that region we created a mutant that shows altering enzymatic activity indicating a higher flexibility and therefore a longer accessibility of α12-13. Hence, this mutant might be useful to get insight into the unique mechanism of GTP hydrolysis and to investigate the contribution of the second interface α12-13. Both features are necessary to understand the antibacterial and antiviral activity of hGBP1.

CELLULAR BIOLOGY OF METABOLISM

PKV20
Lysine acetylation - a novel regulatory mechanism for OXPHOS assembly and stability?

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Mitochondria provide the majority of cellular ATP supply by the process of oxidative phosphorylation (OXPHOS) and are hence crucial for cellular function. OXPHOS can be regulated on different levels and post-translational modifications are increasingly recognized as an important regulator factor. Several OXPHOS proteins have been shown to be modified by lysine acetylation and that this pattern is dynamically changing in response to metabolic challenges. Here, we assessed the effect of changes in mitochondrial lysine acetylation state on OXPHOS function, assembly and stability and how lysine acetylation affects the adaptation of these features in response to metabolic challenges. We modulated the mitochondrial acetylation status by knockdown and over-expression of the mitochondrial deacetylase Sirtuin 3 (Sirt3) as well as of the component of the mitochondrial acetylase program GCN5L1. We could show that loss of Sirt3 decreases mitochondrial respiratory capacity associated with a specific defect in OXPHOS complex IV. Interestingly, loss of GCN5L1 showed the opposite phenotype. We could further demonstrate that Sirt3 is involved in the regulation of turnover of OXPHOS complexes and in supramolecular assembly of OXPHOS proteins in unchallenged as well as in metabolically challenged cells. Intriguingly, over-expression of GCN5L1 recapitulated the phenotype of Sirt3 knockdown and vice versa clearly indicating a causative role of mitochondrial lysine acetylation in the observed phenomenon. Altogether our data show that Sirt3 and GCN5L1 are important regulators of mitochondrial ATP synthesis and that part of this regulatory pathway involves augmenting OXPHOS protein assembly and stability. These findings add lysine acetylation as an important regulatory factor in assembly process within mitochondria and highlight Sirt3/GCN5L1 are important key players in this process.

MBP01
Arsenate stimulates Mrp1-mediated GSH export from neurons

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Introduction
Arsenate is an environmental toxin that contaminates the drinking water of millions of people. Chronic exposure of humans to inorganic arsenic species has been connected with various diseases including neuropathies and impaired cognitive function.

Materials & Methods
Cerebellar granule neuron cultures (CGNCs) were used as a model to investigate the effects of arsenate on the viability and glutathione (GSH) metabolism of neurons. Loss of cellular viability was measured by the extracellular appearance of the cellular enzyme lactate dehydrogenase (LDH). Extracellular and cellular contents of total glutathione (GSx) and oxidized glutathione (GSSG) were quantified by the enzymatic cycling assay using Ellman´s reagent.

Results
Concentrations of up to 10 mM arsenate did not compromise cell viability of cultured neurons during a 6 h incubation. Arsenate increased in a concentration-dependent manner the extracellular total glutathione (GSx) content of CGNCs almost linearly with a concomitant decrease in the cellular GSx contents. Maximal effects on the neuronal GSx content were observed for arsenate in a concentration of 0.8 mM. Cellular and extracellular contents of GSSG were not increased during arsenate exposure. Application of MK571, an inhibitor of multidrug resistance protein (Mrp) 1, completely prevented the arsenate-stimulated GSH export from viable neurons.

Conclusion
Cultured neurons appear to be remarkably resistant against arsenate-induced toxicity and appear not to suffer from severe oxidative stress during arsenate exposure. However, the arsenate-stimulated Mrp1-mediated GSH export suggests that arsenate can disturb the neuronal GSH metabolism.

MBP02
1-O-Acylceramides are natural components of human and mouse epidermis

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The lipid-rich stratum corneum functions as a barrier against pathogens and desiccation inter alia by an unbroken meshwork of extracellular lipid lamellae. These lamellae are composed of cholesterol, fatty acids and ceramides in an equimolar ratio. The huge class of skin ceramides consists of 3 groups, I) "classical" long and very-long chain ceramides, II) ultra-long chain ceramides, and III) ω-esterified ultra-long chain ceramides. The latter are esterified either with linoleic acid or with cornified envelope proteins and are essential for the water permeability barrier. In human and mouse epidermis we identified a class of 1-O-acyl ceramides with long to very-long chain acyl chains in both, N-and 1-O-position. They derive from the group I of classical ceramides (parents). Up to one out of 4 parent ceramides is 1-O-acylated. 1-O-acylceramides are the most water-repellent ceramides and may contribute substantially to an intact water permeability barrier. Loss of lysosomal phospholipase A2 or neutral glucosylceramidase activity did not influence 1-O-acylceramides levels. However, 1-O-acylceramides increased 7-fold upon deletion of the glucosylceramide synthase and then contributed 30% to all esterified ceramides. As glucosylation competes with 1-O-acylation of ceramides, we propose 1-O-acylceramides to be synthesized at ER-related sites.

MBP03
Optimization of cultural conditions for biosynthesis, partial purification and characterization of Invertase from *Aspergillus species* using potato and Carrot Peels as agro waste

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The present study was planned to produce invertase from indigenous wastes, carrot and potato peels, as substrates for five different *Aspergillus* species through solid state fermentation (SSF). Higher activities of invertase were observed by *Aspergillus niger* on carrot peels and *A.terreus* on potato peels at 30°C over 72h of incubation.
Invertase (β-D (fructofuranosidase, EC 3.2.1.26) is one of the important commercial enzymes used in food industry. The process parameters influencing the production of invertase by *A.niger* and *A.terreus* in SSF were optimized at 90% moisture content, 72 and 60 h of incubation period for *A.niger* and *A .terreus* respectively and 2.5% Inoculum's size.

Enzyme purification was carried out, and 512 IU of crude Invertase from *A.niger* was partially purified to maximum specific activity (11.5 IU/mg protein) after 40-80% ammonium sulphate precipitation. Similarly, in *A.terreus* enzyme purification was carried out, and 354.8 IU of crude Invertase was partially purified to maximum specific activity (9.46 IU/mg protein) after 40-80% ammonium sulphate precipitation.
During characterization, it was observed that invertase from *A.niger* was stable from pH 5.5 to 6.5 with maximum activity at pH 5.5. The temperature range for enzyme stability was between 20 to 50°C. When the effect of different substrates on invertase activity was assessed, it was found that sucrose showed highest activity both at higher and lower concentrations. As metal ions cobalt and sodium showed highest activity while mercury significantly inhibited invertase activity. While Invertase from *A.terreus* was stable from pH 4.0 to 6.0 with maximum activity at pH 6.0. The temperature range for enzyme stability was from 30 to 60°C. Similar results were observed in case of substrate specificity, metal ions inhibition.

MBP04
TLR4 plays a central role in Mac-1-mediated monocyte adhesion to endothelial cells via 5-lipoxygenase expression

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Toll-like receptors (TLR), in particular, TLR4 is shown to participate in the pathogenesis of atherosclerosis by the increased formation of macrophage foam cells. However, the role of this pathway in monocyte adhesion to vascular endothelial cells, an early event in macrophage foam cell formation, is still unclear. An *en face* immunohistochemistry of endothelial surfaces revealed a marked increase in monocyte adhesion to aortic endothelium in wild type (WT) mice treated with Kdo2-Lipid A (KLA), which was significantly attenuated in $TLR4^{(-/-)}$ mice. Likewise, the adhesion capacity of primary monocytes isolated from KLA-treated WT mice was higher than those of monocytes from $TLR4^{(-/-)}$ mice. In *in vitro* study, KLA increased monocyte adhesion to endothelial cells with an enhanced Mac-1 expression. These were attenuated by pretreatment with anti-human TLR4 antibody, suggesting a pivotal role of TLR4 in KLA-induced monocyte adhesion to aortic endothelium via Mac-1 wxpression. In addition, LTB_4 production was markedly increased in wild type (WT) mice treated with KLA, which was significantly attenuated in $TLR4^{(-/-)}$ mice. KLA increased monocyte adhesion to endothelial cells via enhanced Mac-1 expression, which were attenuated by pretreatment with a 5-LO inhibitor, MK886. Furthermore, KLA-induced 5-LO expression on monocytes was significantly inhibited by pretreatment with anti-human TLR4 antibody. Collectively, these data suggest that TLR4 plays a central role in Mac-1-mediated monocyte adhesion to endothelial cells through an increased 5-LO expression on monocytes, thus contributing to the initial process of atherosclerosis.

MBP05
5-Lipoxygenase plays a pivotal role in endothelial adhesion of monocytes via an increased expression of Mac-1

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5-Lipoxygenase (5-LO) is known to participate in the pathogenesis of atherosclerosis, however, the underlying mechanisms are unclear. Thus, this study investigated the molecular mechanisms responsible for 5-LO expression in monocytes as well as the role of 5-LO in monocyte adhesion to vascular endothelium, which is a key early event in macrophage foam cell formation. An *en face* immunohistochemistry of endothelial surfaces revealed a marked increase in monocyte adhesion to aortic endothelium in wild type (WT) mice treated with LPS, which was significantly attenuated in $5\text{-}LO^{(-/-)}$ mice. Likewise, the adhesion capacity of primary monocytes isolated from LPS-treated WT mice was higher than those of monocytes from $5\text{-}LO^{(-/-)}$ mice. In *in vitro* study, LPS increased monocyte adhesion to endothelial cells with an enhanced Mac-1 expression. These were attenuated by a 5-LO inhibitor, MK886, as well as by molecular depletion of 5-LO in monocytes. Furthermore, LPS-induced Mac-1 expression on monocytes was significantly inhibited by pretreatment with U-75302, a BLT_1-receptor antagonist, suggesting a pivotal role of 5-LO-derived leukotrienes. In promoter activity analysis and ChIP assays to identify transcription factors involved in 5-LO expression, both NF-kB and Sp1 played central roles to increase 5-LO expression in LPS-treated monocytes. Collectively, these data suggest that 5-LO expression in monocytes is modulated via NF-kB and Sp1 signaling pathways, and 5-LO plays a pivotal role in LPS-mediated monocyte adhesion to vascular endothelium through an increased expression of Mac-1 on monocytes.

MBP06
Role of AtETHE1 in amino acid catabolism during leaf senescence and seed development in *Arabidopsis thaliana*

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AtETHE1 (At1g53580) is a sulfur dioxygenase located in the mitochondrial matrix. Mutations in the human homolog lead to the fatal metabolic disease ethylmalonic encephalopathy due to disruption of the mitochondrial sulfide detoxification pathway. However, the function of ETHE1 in plant metabolism is still largely unknown. Complete knockout of the sulfur dioxygenase in *A. thaliana* is embryo lethal proving that this enzyme is essential for seed development. We extensively characterized three knockdown lines using physiological, biochemical, and proteomic approaches in order to elucidate the role of ETHE1 in plant metabolism. Our datasets indicate a function in nutrient remobilization during leaf senescence and seed development. Amino acids are an important alternative energy source in situations of carbon starvation, where the sulfur dioxygenase is probably involved in the degradation and detoxification of sulfur containing intermediates.

MBP07
Nuclear sequestration of PI4KIIα by oncogenic transcription factors leads to ERK1/2 inactivation.

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Acute Myeloid Leukemia (AML) is characterized by a block in the maturation of hematopoietic cells, leading to accumulation of immature blast cells in the bone marrow and blood. Acute promyelocytic leukemia (APL) is associated with t(15;17) and t(11;17) chromosomal aberrations, which causes the expression of the fusion proteins PML/RARα or PLZF/RARα (X-RARα). The expression of the fusion proteins leads to a differentiation block at the promyelocytic stage in human and murine cells and to the induction of leukemia in mouse models. Both X-RARα proteins build high molecular weight (HMW) complexes, which are critical for their leukemic potential. A proteomic screen was performed to identify novel interaction partners for PLZF/RARα and under other phosphatidylinositol 4-kinase IIα (PI4KIIα). We validated PI4KIIα as a binding partner of both X-RARα. PI4KIIα mediates the synthesis of PI(4)P and regulates several cellular processes. PI(4)P, the precursor of $PI(4,5)P_2$, is able to activate the PI3K/Akt and MAPK signaling pathways. Here, we show that the X-RARα trigger translocation of PI4KIIα from the cytoplasm to the nucleus. This is accompanied by an inhibition in PI4KIIα activity and downstream inactivation of ERK1/2. Moreover, ATRA treatment induced Erk1/2 activation in control and PML/RARα-expressing cells. Contrarily, PLZF/RARα further inhibited Erk1/2 phosphorylation in ATRA-treated cells and could thereby be interesting in regard to the PLZF/RARα ATRA resistance. The effect of PI4KIIα on differentiation was examined in m-HPCs. The knockdown of PI4KIIα decreased the amount of mature cells and engrafted the HSC pool. Thus, the PI4KIIα provides an interesting target for further investigations and AML treatment purposes.

MBP08
Sulfation pathways in health and disease

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The sulfation of biomolecules is a central process in biology. It has major implications in bone and cartilage development, biotransformation of drugs, within the blood clotting cascade or in the process of HIV infection and for the regulation of steroid hormones. The diverse human sulfation processes are catalysed by many sulfotransferases that all depend on provision of active sulfate in the form of 3'-phosphoadenosine-5'-phosphosulfate (PAPS), which is exclusively provided by two PAPS synthases, PAPSS1 and-PAPSS2. Human PAPSS2 mutations lead to a strikingly disturbed androgen metabolism where the sulfation of the androgen precursor DHEA is severely impaired resulting in increased conversion of DHEA to active androgen and androgen excess. Intriguingly, both PAPSS isoforms are expressed in the adrenal cortex; hence it is an open question why intact PAPSS1 could not functionally compensate for PAPSS2-loss in this patient. I could show that PAPSS2 is an unstable protein being partially unfolded at physiological temperature. Moreover, the PAPS biosynthesis intermediate APS stabilised PAPSS2. Functional divergence may also relate to differential subcellular localisation of PAPSS isoforms. Therefore, I have developed protein variants that had either nuclear localisation or export signals disrupted by point mutation resulting in cytoplasmic and nuclear protein variants, respectively. I am currently studying functional divergence by testing these variants in a DHEA/DHEAS conversion assay for their ability to support DHEA sulfation by cytoplasmic sulfotransferase SULT2A1. Finally, knockdown-studies in the adrenal NCI-295 cell line hold the promise to reveal the true functional differences between human PAPS synthases.

MBP09

Biocontrol of phytopathogenic bacteria by the use of plant extracts of Solanum nigrum L.

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The medicinal plants are a vast reservoir of potential phytochemical compounds that can be used as an alternative to synthetic microbicides and are being used to develop drugs. The present study deals with the anti-bacterial activity of aqueous extract of leaves, roots and stem of *S. nigrum, in-vitro*. The antibacterial activity was detected against phytopathogenic bacteria including *Xanthomonas axonopodis, Pseudomonas syringae, Burkholderia pseudomallei* and *Acidovorax temperans* by two methods i.e., agar-well diffusion and disc-diffusion method. Different concentrations i.e., 2%, 4%, 6%, 8%, 10% of each part of aqueous extract were prepared and used for antibacterial potential on Louri Bertani agar (LBA) medium. After incubation for 24 hrs, the zones of inhibitions obtained were recorded and analyzed against standard positive control chloromycetine and negative control (water). All the plant parts showed significant antibacterial activity against the tested organisms. Leaf extract exhibited higher inhibitory effects on bacterial growth as compared to root and stem. We also analyzed the phytochemicals of the plant i.e., test for terpenoids, saponins, tannis, carbonyle, flavonoids, phlobatanin. It is inferred from the present study that the inhibitory ability of aqueous extracts of *S. nigrum* is an indication of their broad spectrum antimicrobial potential which may be implicated in the management of microbial infections. Furthermore, the whole plant extracts of *S. nigrum* may recommend using in preparation of herbal chemicals against pathogens for sustainable crop production.

CURRENT TRENDS AND DEVELOPMENTS IN STRUCTURAL ANALYSIS OF BIOLOGICAL MACROMOLECULES

PKV26

How to kill a mocking bug

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Photorhabdus luminescens is an insect pathogenic bacterium that is symbiotic with entomopathogenic nematodes. Upon invasion of insect larvae, ***P. luminescens*** is released from the nematodes and kills the insect through the action of large tripartite ABC-type toxin complexes (Tcs). Tcs are typically composed of TcA-, TcB- and TcC proteins. Functioning as ADP-ribosyltransferases, TcC proteins were identified as the actual functional components that induce actin-clustering and cell death. However, little is known about the translocation of TcC into the cell by the TcA and TcB components. Here, we show that TcA (TcdA1) forms a transmembrane pore and report its structure in the prepore and pore state determined by cryo-electron microscopy and x-ray crystallography. We found that the TcdA1 prepore assembles as a pentamer forming a α-helical vuvuzela-shaped channel less than 1.5 nm in diameter surrounded by a large outer shell. The protomers are composed of eight domains. Comparisons with structures of the TcdA1 pore inserted into a membrane and in complex with TcdB2 and TccC3 reveal large conformational changes during membrane insertion suggesting a novel syringe-like mechanism of protein translocation. Our results demonstrate how ABC-type toxin complexes bridge a membrane to insert their deadly components into the cytoplasm of the host cell. Our proposed mechanism is paradigmatic for the whole ABC-type toxin family. It is an important step towards the understanding of the host-pathogen interaction and the complex life cycle of ***Photorhabdus luminescens*** and other pathogens**,** including human pathogenic bacteria.

Gatsogiannis, C. *et al. Nature* **495**(7442): 520-23 (2013).

PKV31

Analysis of Hsp90-cofactor complexes using analytical ultracentrifugation with fluorescence detection

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The ATP-hydrolysing dimeric molecular chaperone Hsp90 and its cofactors are important for the activation and stabilization of hundreds of client proteins, like protein kinases, steroid hormone receptors and myosin. In case of the steroid hormone receptor it has been shown that the client progresses through several distinct Hsp90-cofactor complexes to finally achieve a active state. These complexes are comprised of three, four or even more proteins, which regulate each others' binding. We employed a novel analytical ultracentrifugation (aUC) technology, which detects fluorescein-labelled proteins to describe the formation of these complexes and the influence that is exerted by the binding and hydrolysis of ATP to Hsp90-complexes. As critical intermediate we describe the formation of an asymmetric complex, which contains two distinct cofactors on each side of the Hsp90-dimer. Using FRET we also analyze the kinetics of complex formation to complement the binding studies in aUC. We further find that this asymmetric complex appears to be not restricted to the steroid hormone receptor cycle. Instead asymmetric Hsp90-complexes may also be relevant during chaperone cycles in muscle development and protein phosphorylation control.

SAP01

DNA-Lipid Recognitions: from Protocell Evolution to Bionanotherapy

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Spontaneously formed self-organized systems comprise the multidisciplinary research interest. Beside the biologically inspired interest in fundamental property of the living matter to form self-associating systems, they are approached by physical chemists, as well, in terms of pure supramolecular reasoning. Researchers from the origin of life field considered them as a primitive cellular forms in a RNA-dominated primordial soup. Subsequently, such self-assemblies have been looked for in crucial cellular events. In prokaryotes, example of such aggregate is mesosome, which itself has been considered as an accidental artifact formed during specimen preparation for microscopy, or another macromolecular biopolimers occurring during bacterial nucleoid packaging and segregation. mRNA maturation and transport through nuclear pores, cell division and phage infection are the examples of nucleic acid-lipid interactions among eukaryotes. The difficulty of describing these complexes at the cellular level are their instability, in relation to their kinetics of formation. Recently, the research interest on interactions between lipids and both DNA and RNA of different sizes and conformations has arisen after realization of their potential for employment as gene carriers in human gene therapy. Various spectroscopic, thermodynamic and microscopic measurements on their complex formation are presented and discussed in terms of their cell evolutionary and therapeutic viewpoints.

SAP02

High molecular weight macromolecule from Hyuganatsu extracts differentiates 3T3 fibroblast cells to osteoblast through BMP dependent MAP Kinase pathway

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In the present investigation, we studied the effect of high molecular weight peel extract of edible fruit orange, *citrus tamurana* (HNE) for differentiation of 3T3 fibroblast like cells to osteoblast. 3T3 cells were used for *in vitro* bone differentiation study. Cells are cultured in with (0.1, 0.25, 0.05, 0.75 and 1.0 mg/ml) or without HNE conditioned alpha medium in 10% FBS and anti-bacterial cocktail at 5% CO_2 and 37^{O}C. Calcification of cells are monitored by calcium nodule cell staining kit. RT-PCR and western blot techniques are used to study the fluctuations of osteogenic marker gens and WB for protein respectively. Initially we confirmed the bone formation parameter like calcification activity in HNE treated cells and result showed 0.50mg/ml showed significant deposition of Ca^{+} in the cells. Cells are treated with different concentrations of HNE (0, 0.1, 0.2, 0.5, 0.75 and 1.0 mg/ml) on fluctuations of BMP-2, Run X2, Collagen type 2, Osteopontin, Osterix and Osteoprotegerin. Results revealed the dose dependent fluctuations of genes upon treatment with different concentrations of HNE. We also studied the signaling pathways like MAPK, JNK, ERK, AkT through Western blot technique and accumulated resulted showed that highest significant activity of key proteins like MAPK, JNK and ERK but not AkT were observed at 0.5mg/ml of HNE. Furthermore, Noggin, antagonist to Run-X2 and BMP-2, treatment along with HNE (0.50mg/ml) confirmed that efficacy of HNE is dependent on BMP pathway since activity of RunX-2 and BMP-2 were diminished even after treatment with HNE. Here in this study we proved nutraceutical as osteogenic induction factor and further clinical evaluation may HNE serves as alternate medicine for osteoporosis.

SAP03

A new FRET-sensor to unravel conformational changes in nonribosomal peptide synthetases (NRPS)

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Nonribosomal peptide synthetases (NRPS) are complex protein machineries that synthesize a myriad of pharmaceutically highly interesting peptides (e.g. vancomycin). The structural and functional organization of NRPS consists of modules, which can be subdivided into domains, each of which is responsible for incorporation and modification of a single amino acid. Over the last years several crystal structures of multi-domain NRPS-constructs have been published, but the complex pattern of protein-protein interactions as well as the dynamics of their functional interplay remains poorly understood. The adenylation (A)-domain catalyses two distinct reactions, the activation and covalent binding of a cognate amino acid to the 4'-phosphopantetheine-cofactor (Ppant) of the peptidyl carrier protein (PCP)-domain. It is believed that this is achieved by remodelling of the active site for the respective catalytical step. The assumption of this complex mechanism is mainly based on crystal structure analysis and functional assays. We here report a study of catalytically competent enzymes in solution using a novel FRET-sensor. Our results support the presence of different conformations and show how these can be controlled by addition of small molecule ligands.

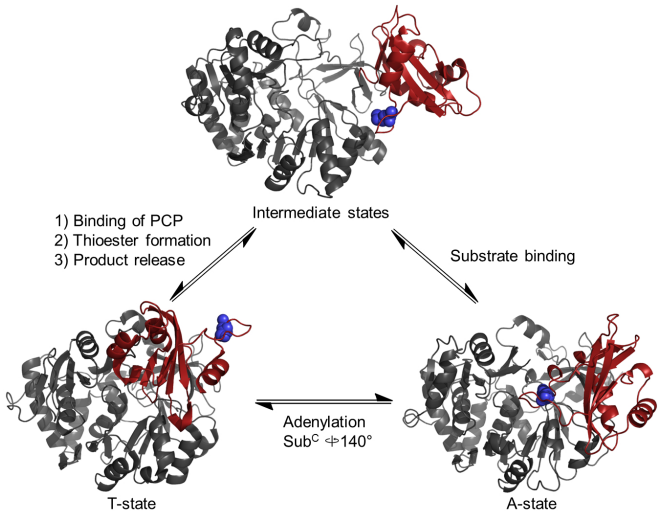

Figure 1: Catalytic cycle of NRPS A-domains; the active site is remodelled for catalysis of the respective reactions, activation of the cognate amino acid and transfer to the Ppant-arm of the PCP (PDB: 1ULT, 1AMU, 4DG9).

SAP04

Enhanced bactericidal effect of enterocin A in combination with thyme essential oils against *L. monocytogenes* and *E. coli* O157:H7

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The combined effects of enterocin A with *Thymus vulgaris* essential oils (EOs) against *Listeria monocytogenes* and *Escherichia coli* O157:H7 were investigated *in vitro* by enumeration of surviving populations of tested pathogens and minimal inhibitory concentration (MIC) determination. Enterocin A was purified to homogeneity by RP-HPLC from culture fluid of *Enterococcus* strain and thyme EOs were extracted from local *Thymus vulgaris* plants. The major constituent of thyme EOs oils determined by GC-MS was thymol (78.4%). Combination of enterocin A with thyme EOs enhances the antimicrobial effect against *Listeria monocytogenes* (Figure 1). Checkerboard assay and isobologram construction showed a synergetic interaction between these compounds (FIC index <0.5). Moreover, MIC value of enterocin A felled fivefold (from 4.57 to 0.9 µg/ml), while the MIC of thyme EOs decreased threefold (from 3.6 to 0.9 µg/ml). Treatments with enterocin A alone did not affect the growth of the enteric pathogen E. coli O157:H7. However, Combination of thyme EOs and enterocin A yielded a synergistic antimicrobial effect against *E. coli* (MIC thyme EOs decrease from 2.2 to 0.71 µg/ml). This is the first report on the combined effect of enterocin A and thyme EOs against food pathogens bacteria. This combination could be useful in food bio-preservation.

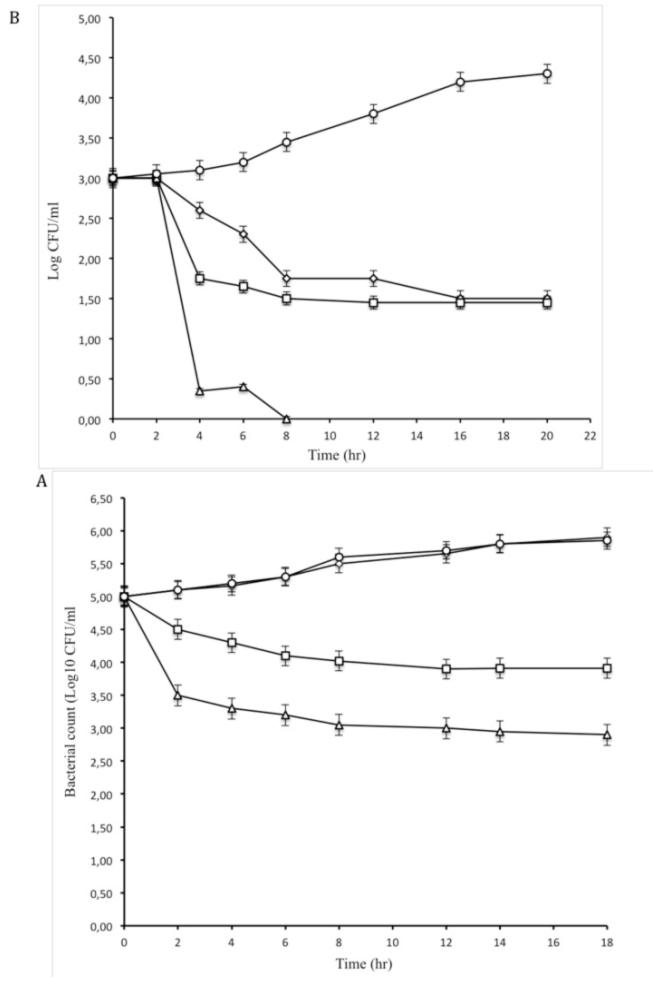

Figure 1: Growth of *L. monocytogenes* EGDe (A) and *E. coli* O157:H7 (B) in BHI broth in the presence of enterocin A, thyme EOs and both. The concentration of each agent is 1/2 MIC except for *E. coli* test. Results were obtained from duplicate. Enterocin A (◊), thyme EOs (Π), EntA+thyme EOs (Δ), blank test (O).

SAP05

Selective activators release the autoinhibitory mechanism of protein phosphatase 5

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Protein phosphatase 5 (PP5) is a ubiquitously expressed serine/threonine phosphatase that is strictly autoregulated by its tetratricopeptide repeat (TPR) domain and the C-terminal α-helix. Fine-tuning of its dephosphorylation activity modulates a diverse set of cellular key players including pathogenic proteins involved in neurodegenerative disorders. Here, we report on five allosteric PP5 activators that enhance the phosphatase activity up to 20-fold without affecting the PP5-Hsp90 interaction. Functional and crystallographic analyses reveal a relaxation of the autoinhibited state of PP5 upon ligand binding, thus facilitating substrate entry into the phosphatase domain. Application of the small molecule activators of PP5 in a tau-P301L mouse model decreased the pathology-relevant phosphorylation of tau and the degeneration of hippocampal neurons. Hence, the identified and characterized PP5-specific activators represent a promising alternative in the treatment of neurodegenerative disorders.

SAP06
D1054.3 a SGT1 homologue of *C. elegnas* lacking the TPR domain

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The eukaryotic suppressor of G2 allele of SKP1 (SGT1) protein is known to play a major role in cell cycle processes and the immune response of plants and animals. The protein itself is highly conserved and consists mainly of three domains - a tetraticopeptide repeat (TPR), a CHORD/SGT1 (CS) and an SGT1 specific (SGS) domain. So far the CS domain seems to be necessary for the interaction with the molecular heat shock protein 90 (Hsp90) while the TPR domain is assumed to play a major role in client interaction. Surprisingly, the TPR domain is missing in some organism like *Drosophila melanogaster* and *Caenorhabditis elegans*. Therefore we looked at the *C. elegans* SGT1 homologue D1054.3, only containing CS and SGS domain, in a more detailed way. As the CS domain of D1054.3 is very similar to the one of the Hsp90 inhibiting cochaperone p23 we compared the two proteins with each other using crosslinking, fluorescence anisotropy, ATPase assays and analytical ultracentrifugation. We could show that the two cochaperones have an overlapping binding site on Hsp90 but differ regarding the nucleotide dependency and conformational state of Hsp90. Using different Hsp90 mutants we analyzed this state more precisely, discovering mutants which were trapped in a certain conformation and disable to bind either D1054.3 or p23. Thereby we show how D1054.3 is acting during the Hsp90 cycle and propose how a putative client might be regulated.

SAP07
Structural and functional characterization of PTHR2

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The parathyroid hormone receptor 2 (PTHR2) is a recently found member of the mammalian parathyroid hormone (PTH) receptor family, binding three related ligands tuberoinfundibular peptide of 39 residues (TIP 39), PTH and PTH-related protein (PTHrP) as agonists or antagonist. PTHR2 belongs to class B G-protein coupled receptors (GPCR) and exhibited biological activity for TIP 39 and PTH. Diverse expression and secretion of PTHR2/TIP39 suggested the regulatory role in neurological, cardiac and bone related disease [1]. Despite, detailed knowledge of PTHR1 and its ligands (PTH and PTHrP) [2], less structural and functional information is available for PTHR2 and how this receptor may bind and discriminate different peptides such as TIP 39 and PTH. Extracellular N-terminal domain (ECD_{27-145}) of the PTHR2 plays a pivotal role in ligand recognition. For this purpose, SUMO fused ECD was expressed in *E coli* and purified from inclusion bodies. Circular-dichroism and fluorescence spectroscopic studies revealed that ECD was refolded and contains mixture of alpha helix and random coil. Disulfide bond pattern analyzed by mass spectrometry suggested the conserved pattern among different class B GPCR. TIP 39 and PTH 84 binding was explored by intrinsic tryptophan fluorescence quenching and resulted in lower micro molar affinity. Further NMR based studies will provide structural and topological information about the hormone while bound to the ECD of PTHR2. In conclusion, present studies provided an important prerequisite for subsequent structural and functional studies. Gaining the receptor-ligand complex (PTHR2-TIP 39/PTH) information on atomic level will lead to therapeutic application for human benefits.

References

[1] Panda D, Goltzman D, Jüppner H, Karaplis AC. (2009) Am J Physiol Endocrinol Metab 297: 1125-1136
[2] Pioszak AA, Xu HE. (2008) PNAS 105(13): 5034-5039

SAP08
De novo Protein Structure Prediction of the transmembrane domain TMD_0 of TAP

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The biochemical pathway of the major histocompatibility complex (MHC) class I peptide loading complex (PLC) has an essential role in the adaptive immune system by protecting vertebrates from virus, rejection of organ transplants, and cancer. Main investigations of this research project are focused on the structural clarification of the N-terminal TMD_0 domains of the transporter associated with antigen processing (TAP1/2), 4-TM bundles that are key interaction hubs of the MHC class I. Therefore we apply and compare the output of *de novo* methods to predict secondary and 3D structure, based on the raw amino acid sequence as an input. By using established transmembrane protein topology prediction tools (MEMSAT3, MEMSAT-SVM, OCTOPUS, TMHMM), the membrane spanning regions of helices can be determined and serve as an recommendation for further analysis in the *de novo* design approaches. These are mainly conducted by the structure prediction module of the ROSETTA software, a promising method based on fragment assembly. After generation of a certain number of models, clustering methods considering low scoring values and structural information like compactness, surface accessibility, and helix orientation will be performed to determine reliable and native-like structures. The detailed understanding of the structural organization of the TMD_0 domains of TAP1/2 will form the basis for further studies of structure-function relationships of the MHC class I supercomplex.

SAP09
FRET-based Determination of the Degree of Acetylation of Chitosan

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Chitin is a β-(1→4)-linked homopolymer of N-acetyl-D-glucosamine, which is one of the most abundant biopolymers in the world. The length of a polymer is given by the degree of polymerization (DP). While chitin is insoluble in water its partially or fully deacetylated cationic derivative called chitosan can be solved in slightly acidic solutions. The percentage of acetylated units is given by the degree of acetylation (DA). Chitosan is non-toxic, highly biocompatible, naturally degradable and has low immunogenic activity making it highly interesting for a wide range of applications. One characteristic influencing the specific activity of chitosan is the DA, which can be determined, e.g., via ^{1}H-NMR. For ^{1}H-NMR amounts in the range of milligrams of substrate are needed. A novel approach is the determination of a chitosans DA via FRET. Therefore one fluorophore of a FRET pair (eGFP) is fused to a chitin-binding domain of a chitinase, the other (eBFP) to an inactivated chitosanase generating fluorescent affinity proteins which specifically bind to acetylated or deacetylated regions. The DA-dependent binding specificity of these engineered proteins leads to a specific DA-dependent arrangement of the fluorophores on the polymer. The resulting FRET intensity is linearly correlated to the DA of the substrate in the range of soluble chitosan polymers (up to DA 60%). This method of DA determination is fast and needs substrate amounts in the range of few micrograms and thus is 1000 times more sensitive than the established ^{1}H-NMR method. The standard deviation of this method of DA determination is in the range of the ^{1}H-NMR method.

SAP10
Structural studies elucidate specificity determinants of Furin beyond the S4 pocket

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Furin, a member of the pro-hormone/pro-protein convertase (PCs) family of serine endoproteinases, is required for activation and maturation of many secreted proteins. Target proteins include peptide hormones, growth factors, matrix metalloproteases, blood clotting factors, bacterial toxins and viral capsid proteins. Therefore Furin is intensively investigated as pharmacological targets especially in cancer and infectious disease research (1, 2).
The aim of this study is the structure based development of competitive Furin inhibitors and their optimization for pharmaceutical and biochemical applications. A key step for structural investigation proved to be the developed of a large scale expression protocol for production of the homogenously glycosylated enzyme in HEK293S-cells. This approach was combined with a novel purification scheme, including an inhibitor-based affinity chromatography step (3). The structure shows a so far unknown specific interaction of the P5 group of the inhibitor with the substrate binding site of Furin. Our data elucidates a so far enigmatic specificity determinant, resulting in Ki-changes of more than two orders of magnitude as well as strong changes of the specificity for different PC family members upon variation of the P5 group. This work represents the basis for a comprehensive investigation of enzyme-substrate interactions in the substrate binding cleft of furin beyond the S4 pocket (I) to develop inhibitors and molecular probes of increased specificity and (II) to identify so far unknown Furin recognition sites and hence unknown Furin substrates.

References

1: Henrich, S. et al. Nat Struct Biol (2003) 11, 520-526
2: Seidah, Nabil G. et al. Nat Drug Dis (2011) 11, 367-383
3: Kuester, M et al. Biol Chem (2012), 392, 973-981

SAP11
The three-domain structure of plant polyphenol oxidases: functional insights using a domain swapping approach
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Plant polyphenol oxidases (PPOs, EC 1.10.3.1) belong to the type 3-copper proteins like tyrosinases and hemocyanins. They enzymatically convert *o*-diphenols to *o*-quinones using molecular oxygen as co-substrate. All plant PPOs show a conserved three-domain structure: (i) N-terminal domain including the active site, (ii) linker and (iii) C-terminal domain. So far only the crystal structure for the N-terminal domain is solved. The linker is believed to fulfill regulative functions and the C-terminal domain is postulated to cover the active site preventing the substrate to enter and thus leading to latency. Though anionic detergents (like SDS), proteases and fatty acids are well known activators for plant PPOs, neither the underlying activation mechanism nor *in vivo* activators are yet elucidated.
Recently, we established a bacterial expression system for plant PPOs which enables to yield high amounts of purified plant PPOs. For the first time it became possible to analyze the function of the different PPO-domains by means of protein engineering. We designed domain swap constructs of dandelion PPO-1 and PPO-2 without changing the natural order of the domains. The chimeric proteins were analyzed regarding activation and stability parameters and compared to the wildtype enzymes to gain new insights into the function of single domains.
Our results show that beside the N-terminal domain, both the C-terminal domain and even the linker do indeed have significant influences on the tested enzymatic parameters. Thus we propose both domains to have more specific functions for plant PPOs than thought so far. The linker and the C-terminal domain are most likely responsible for the fine tuning of pH optimum, activation by SDS and enzymatic stability.

SAP12
Spectroscopic Analysis and Identification of unlabeled ALPHASYN from CSF as a Proteomic Application in the early Diagnosis of Parkinson´s Disease
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Background
Parkinson's disease (PD) is the second most common neurodegenerative disease in the world. Aggregation of ALPHASYN (α-synuclein) are linked to PD and some other neurodegenerative diseases by substantial evidence. Lewy bodies and Lewy neurites, which are hallmarks of PD, comprise mostly the fibril of ALPHASYN. However, currently there is no routine neurochemical method for early diagnosis of PD. Effective marker or test model relating to ALPHASYN is needed, especially for CSF (Cerebrospinal fluid) analysis.

Aim
Presented study aims to demonstrate a potential method based on ATR-IR spectrum analysis for detection of lowly concentrated unlabeled proteins.

Methods
We have developed an in vitro artificial CSF test model aiming detection and specifying the secondary structure signal of ALPHASYN in low concentration from the artificial CSF test model by using infrared spectroscopy combined with attenuated total reflection system (ATR-IR). Here, CSF proteins, such as Albumin, were measured in a concentration gradient and a mixed spectra background calibration was established for ALPHASYN calculation. Furthermore, oligomerization induces secondary structural changes of ALPHASYN, which were also investigated in a model providing *in vitro* information of ALPHASYN undergoing pathological variation. Experimental data was also controlled by already established biochemical and biophysical methods.

Results
We have demonstrated that our analysed ATR-IR results strongly match with concentration of ALPHASYN in our artificial model CSF. We also have investigated possible interaction partners of ALPHASYN.

Conclusions
ALPHASYN undergoes structural reorganization depending on pH and ionic composition.

SAP13
Structural studies on eukaryotic 6-phosphofructokinases
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Phosphofructokinase (Pfk) catalyses the formation of fructose 1,6-bisphosphate from fructose 6-phosphate and MgATP in prokaryotic and eukaryotic cells. The catalytic activity is tightly regulated in a wide variety of organisms by diverse positive (e.g. AMP) and negative (e.g. ATP) effectors. Mutations in the gene of human muscle phosphofructokinase (*hm*Pfk) lead to Type VII glycogen storage disease (e.g. Tauri disease). In patients with Tauri diseases a complex systemic disorder is induced by intertwine of marked alterations in muscle bioenergetics and the erythrocyte metabolism. Eukaryotic Pfk evolved by a process of tandem gene duplication and fusion to yield a protein that has a multiple size and a much more complex structural organization and allosteric regulation compared to their bacterial counterparts. The *Mammalian* Pfks smallest active form is a tetramer of ~330 kDa. All human enzymes show a tendency to form larger aggregates. Additionally they can form isoforms in a tissue-dependent manner. The aim of this study is to characterize the molecular basis of allosteric regulation of the human Pfk-isoforms (M, L and P) by Small Angle X-ray Scattering (SAXS) and X-ray crystallography. Therefore we engineered the protein surface with the aim of obtaining mutants that are more suitable to crystallization than the wild type protein.

DISSECTION AND TARGETING OF SIGNAL TRANSDUCTION PATHWAYS

PKV01
Optogenetic control of cGMP mediated sensory transduction pathways in *Caenorhabditis elegans*
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Organisms respond to sensory cues by activation of a primary receptor followed by relay of information downstream to effector targets by secondary signalling molecules. Chemo-, thermo-, and oxygen-sensation in *C. elegans* involve sensory neurons that use cGMP as the main second messenger. For example, ASJ is the pheromone sensing neuron regulating larval development, AWC is the chemosensory neuron responding to volatile odours and BAG senses carbon dioxide in the environment. In these neurons, cGMP acts downstream of the GPCRs and functions by activating cGMP gated cation channels thereby depolarising the sensory neuron. Manipulating cGMP levels is required to access signalling between sensation and sensory neuron depolarization. We achieve this by implementing a photo-activatable guanylyl cyclase - bPGC - obtained from the bacteria *Beggiatoa* in the sensory neurons of *C. elegans*. bPGC is a BLUF (blue light sensing using flavin) domain containing cyclase which uses FAD as the co-factor and catalyses the synthesis of cGMP from GTP upon activation by blue light. Using cell specific promoters, we could drive the expression of bPGC in a variety of *C. elegans* sensory neurons (ASJ, AWC, BAG) that use cGMP as the second messenger. By optogenetically activating cGMP mediated signal transduction in these sensory neurons using bPGC, we intend to recapitulate the behavioural response to this variety of sensory stimuli and further on study signal transduction and integration in sensory neurons.

PKV03
Active Wnt proteins are secreted on exosomes
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Wnt signalling has important roles during development and in many diseases. As morphogens, hydrophobic Wnt proteins exert their function over a distance to induce patterning and cell differentiation decisions. Recent studies have identified several factors that are required for the secretion of Wnt proteins; however, how Wnts travel in the extracellular space remains a largely unresolved question. Here we show that Wnts are secreted on exosomes both during Drosophila development and in human cells. We demonstrate that exosomes carry Wnts on their surface to induce Wnt signalling activity in target cells. Together with the cargo receptor Evi/WIs, Wnts are transported through endosomal compartments onto exosomes, a process that requires the R-SNARE Ykt6. Our study demonstrates an evolutionarily conserved functional role of extracellular vesicular transport of Wnt proteins.

PKV04
Substrate recognition of SUMO-targeted ubiquitin ligases

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SUMO-targeted ubiquitin ligases (STUbLs) are a special class of RING-type E3 ubiquitin ligases that contain SUMO-interaction motifs (SIMs) and target sumoylated proteins. STUbLs are conserved in all eukaryotes. In humans, the RING-finger protein 4 (RNF4), which contains four putative SIMs, mediates the degradation of the promyelocytic leukemia (PML) protein in response to arsenic treatment. Furthermore, RNF4 regulates the repair of DNA double strand breaks (DSB) by targeting MDC1 and other DSB repair protein. Recently, RNF111/Arkadia has been shown to be a STUbL protein involved in the control of TGFb-signaling and of the PML protein.
In order to characterize the substrate recognition of RNF4 and Arkadia we have studied the interaction with polymeric SUMO chains in vitro and the STUbL activity in yeast and mammalian cells. For RNF4, we show that both, SIM2 and 3, are necessary for a high affinity interaction with SUMO-chains, while SIM4 is only contributing to the binding of long SUMO chains of three or more members. These in vitro data correlate with the activity of RNF4 in vivo to degrade poly-SUMO reporter constructs in yeast and to disrupt PML-nuclear bodies in HeLa cells. In contrast, Arkadia, which contains three putative SIMS, does interact stronger with SUMO chains than RNF4 and displays a preferred binding and degradation of SUMO-1 capped SUMO-2 chains. In summary, we present models of how the sequence and arrangement of SIMs in RNF4 and Arkadia determine their substrate specificities.

PKV10
Analysis in phytochrome hybrids of PhyB from *A. thaliana*

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Phytochromes are red/far-red (r/fr) light absorbing photoreceptors. Upon red light irradiaton a reversible isomerisation of the chromophore is triggered leading to the formation of the signalling active far-red absorbing state (Pfr). The N-terminal photosensory module of canonical phytochromes consists of a PAS-GAF-PHY tripartite domain [1]. Plant phytochromes possess an additional N-terminal extension. Whereas the PAS-GAF bidomain is essential for chromophore binding and exhibit some photochemistry, the PHY domain is required for Pfr stability. Deletion of the PHY domain leads to increasing rates of dark reversion of lit states and causes blue shifts in absorption maxima [2-3].
To investigate the structural aspects of plant phytochrome photoconversion, we created hybrids of *Synechocystis* Cph1 and *Arabidopsis thaliana* PhyB. The hybrid with the PAS-GAF bidomain from Cph1 and the PHY domain of PhyB was highly soluble. Contrary to Cph1, the hybrid showed only a monomeric behaviour in its size exclusion chromatography profile [4] and failed to achieve full photoconversion. Since all the PHY motifs ($W^A/_GG$, $^W/_FxE$ and PRxSF), expected to be essential for Pfr stability, are present in the hybrid, we propose that either additional motifs are required for full photochemistry or that certain structural elements of the PHY domain are displaced, thus failing to ensure an environment for full photoconversion.

Referemces

[1] Essen L O, Mailliet J, Hughes J, *PNAS*, **2008**, 38, 14709-14714.
[2] Oka Y, Matsushita T, Mochizuki N, Suzuki T,Tokutomi S, Nagatani A., *Plant Cell*, **2004**, *16*, 2104-16.
[3] Laan W W J, Phd thesis, University of Amsterdam, **2005**.
[4] Strauss H M, Schmieder P, Hughes J, *FEBS Lett.*, **2005**, *579*, 3970-3974.

PKV34
How does the mitochondrial IMS handle and communicate misfolding stress?

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To cope with misfolded and aggregated proteins cells developed organelle-specific control systems. In mitochondria, the unfolded protein response (UPR) and other quality surveillance mechanisms are poorly characterized. To identify new targets in the signaling pathway activated by misfolding stress in the intermembrane space of mitochondria (IMS) we will use model proteins that easily misfold and have to be recognized, and either refolded or removed from the IMS. In initial experiments we verified that overexpressed IMS proteins have a reduced half life in comparison to their endogenous counterparts, indicating a perturbation in IMS proteostasis. Moreover, we observed that known target genes of the IMS-UPR become activated under these conditions. Next, we will identify factors that signal or attenuate misfolding stress in the IMS by RNA-Seq and high-throughput siRNA screens. We will complement our studies on misfolded substrates by applying chemical inhibitors of the respiratory chain that will lead to physiological relevant oxidative stress, and by addressing possible crosstalks with UPRs of other compartments (cytosol, mitochondrial matrix and endoplasmic reticulum).

STP01
The other way round: how to use high throughput human monoclonal antibody generation to identify drug targets in signal transduction pathways

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Up to date, high content ex vivo screening methods struggle to deliver the expected abundance of new drug targets. Rapid in vitro generation of panels consisting of hundreds to thousands of standardized monoclonal human antibodies (yumabs) for research allows to overcome these limitations. We show that these designer-antibodies can be tuned in vitro to be capable to differentiate whether a single phosphorylation is present on a particular antigen. By using proximity ligation, this phosphorylation can be monitored in very high resolution upon activation of the signal transduction pathway. Further, real interactions with other proteins can be detected in vivo, i.e. directly on clinical material. Up to 100 different proteins per individual healthy vs. disease sample were mapped with 40nm resolution in situ. This for example will allow to individually analyze all of the different phosphorylations of an entire signal transduction pathway with unmatched resolution.
As the antibody genes are available right away, they can be used for the functional analysis of the biology of the targets in vivo. After simple subcloning into a mammalian intrabody expression vector, they provide a functional knock down of the target antigen, as demonstrated for membrane receptors on immune and nerve cells. Transgenic knock out mice were generated on the heterozygote level which allowed to rapidly analyze phenotypes of a protein knock out, providing a completely new level of in vivo gene function analysis.

STP02
Dissecting nucleo-cytoplasmic transport by exploiting natural products

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In eukaryotes, the compartmentalization into nucleus and cytoplasm allows the independent regulation of cellular programs. Hence, not only cellular homeostasis but also diseases critically depend on regulated nucleocytoplasmic transport of effector molecules. Despite intense investigation, the molecular details of transport are not yet fully resolved. Besides synthetic small molecules, natural products are considered a promising source for novel chemical tools to dissect transport pathways. Natural product-derived compounds have undergone an evolutionary selection process and hence, offer a source of increased structural diversity.
We engineered autofluorescent cellular translocation biosensors, and employed them on the Cellomics®ArrayScanVTI platform to screen 288 fungal extracts for cell-active export inhibitors. One 'hit-extract' was further fractionized resulting in the subsequent identification of the bioactive compound, FV-22MSC. As assayed in several tumor cell lines, FV-22MSC reversibly blocked nuclear export of nuclear export signal (NES)-containing proteins at µM concentrations. Inhibition occurred fast, but could be reversed upon compound removal. Similar to known export inhibitors, FV-22MSC targets the export receptor CRM1. Treatment with FV-22MSC triggered apoptosis of several solid tumor cell lines.
We here present the identification and characterization of a novel export inhibitor isolated from *Cyphellopsis anomala*. We provide first details of FV-22MSC's molecular mode of action and its effect on malignant cells. These results underline the power of our strategy to systematically exploit the chemical space of the fungal metabolome to identify novel drugs.

STP03
Cell-free expression of GPCRs as new platform for ligand screening

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The human endothelin receptor type A (ETA) and B (ETB) are prototypic GPCRs expressed in the human body on the surface of endothelial cells and smooth muscle cells present in the blood vessels tissue. These receptors are particularly important for the control of vasodilatation and vasoconstriction and

numerous diseases are occurring from the dysregulation of the endothelin system.
In our laboratory we have established protocols for high-level production of ETA, ETB and other GPCRs in an individual Continuous Exchange Cell-Free System (CECF). This expression system, based on *Escherichia coli* cell extracts, is a highly promising tool for the production of functionally active membrane proteins (Junge et al. 2010). We have optimized cell-free production protocols for the human ETA and ETB receptors by implementing a variety of co-translational expression approaches including supplied detergents, liposomes or nanodiscs (Junge et al. 2010, Roos et al. 2012, Proverbio et al. 2013). The functionality of the two receptors has been analysed in by a number of complementary assays including radioassays, surface plasmon resonance and fluorescence measurements. We demonstrate (i) the efficient co-translational production of GPCR/nanodisc complexes, (ii) characteristic differential binding pattern with specific agonists and antagonist, (iii) the formation of highly stable and SDS resistant complexes of the ETB receptor and the primary peptide ligand ET-1 and (iv) coupling of cell-free expressed GPCRs with G-proteins. The ligand binding properties of the endothelin receptors could be analyzed directly in the cell-free reaction mixture and opens new avenues for the development of throughput drug screening processes.

STP04
The EGF Receptor in Cholinergic Signaling of Human Keratinocytes

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Acetylcholine (ACh) is an important neurotransmitter and effector of the parasympathetic nervous system. However, ACh and its receptors do not only play an essential role in the nervous system but also act as mediators of cell communication in non-neuronal cells. Two classes of receptors are known to perform signal transduction after cholinergic stimulation. The nicotinic acetylcholine receptors are ion channels, whereas the muscarinic receptors belong to the group of G protein coupled receptors (GPCR). Their activation can lead to the initiation of the mitogen-activated protein (MAP) kinase cascade which contributes to cell survival, differentiation and other important cellular responses.
Different ACh receptors can be found in epithelial cells like HaCaT cells, a human keratinocyte cell line. We show that the stimulation of the HaCaT cells with cholinergic agonists leads to the activation of the MAP kinase cascade and the protein kinase Akt via the muscarinic receptors. This pathway seems not to involve the canonical activation of protein kinase C but is mainly dependent on the transactivation of the epidermal growth factor (EGF) receptor. EGF receptor transactivation may be the major pathway of GPCRs to facilitate MAP kinase activation in these cells. Furthermore, we have here analyzed the involvement of the Src tyrosine kinase family, phosphatidylinositide 3-kinases and matrix metalloproteinases in this process in HaCaT cells.

STP05
A role for flotillins in cell-cell adhesion

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Cell-cell adhesion is essential for the formation an the integrity of epithelial structures like the skin or mammary gland. Different types of adhesion structures do not only ensure the tight contact between neighboring cells but are crucial platforms for communication and exchange in a tissue. Two of these adhering junctions are cadherin based, the belt-like adherens junctions and the spot-like desmosomes. Both structures have in common that they are composed of single membrane spanning proteins, the cadherins, which accomplish adhesion in a calcium-dependent manner. The intracellular parts of classical as well as desmosomal cadherins bind to different adaptor proteins of the armadillo-protein family and others which build a protein plaque underneath the membrane and link the cadherins to the actin or intermediate filament cytoskeleton.
Here we show that both classical and desmosomal cadherins colocalize and coimmunoprecipitate with flotillin-2, a protein that is associated with membrane rafts. In vitro, flotillin-2 and its homologue flotillin-1 both directly bind to the armadillo protein γ-catenin, so far the only protein known to be present in AJs as well as DSMs. Furthermore, depletion of both flotillins alters the morphology of the adherens junction in epithelial cells and changes the association of E-cadherin and γ-catenin with membrane rafts. In addition, keratinocytes depleted of flotillins exhibit an increased cell adhesion. Taken together, this indicates a functional role of flotillins in cell-cell adhesion in epithelial cells.

STP06
Pain modulators regulate the dynamics of PKA-RII phosphorylation in subgroups of sensory neurons

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Knowledge about the molecular structure of PKA isoforms is substantial. In contrast, the dynamics of PKA isoform activity in living primary cells has not been investigated in detail. Using a High Content Screening microscopy approach, we identified the RIIβ subunit of PKA-II to be predominantly expressed in a subgroup of sensory neurons. This subgroup expressed nociceptive markers (TRPV1, NaV1.8, CGRP, IB4) and responded to the pain eliciting compound capsaicin. Isoform-specific PKA reporters showed in sensory neuron-derived F11 cells that the inflammatory mediator PGE_2 specifically activated PKA-II but not PKA-I. Accordingly, pain sensitizing inflammatory mediators (e.g. PGE_2, PGI_2, 5-HT, and epinephrine) increased the phosphorylation of PKA-RII subunits (pRII) in subgroups of primary sensory neurons. The method allowed to analyze the involved receptor subtypes as well as the quantification of dose-response or kinetic relationships in primary sensory neurons. Basal pRII levels were regulated by the phosphatase PP2A. Increase of pRII was followed by phosphorylation of CREB in a PKA-dependent manner. Thus, we propose RII phosphorylation to represent an isoform-specific readout for endogenous PKA-II activity *in vivo*, suggest RIIβ as a novel nociceptive subgroup marker, and extend the current model of PKA-II activation. Moreover, our data reveal a novel subgroup-specific mechanism involved in pain sensitization signaling.

STP07
Calcium Dependent Processes in Plant Mitochondria

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Calmodulins (CaMs) are eukaryotic calcium sensors that have no intrinsic activities on their own. Instead, they bind to a number of protein targets (CaMBPs) in a calcium-dependent manner, regulate their function and affect a number of cellular processes. A large variety of metabolic processes essential for plant viability take place in mitochondria. Preliminary results indicated the presence of calcium-dependent processes in mitochondria and to understand this regulation it is important to identify the organellar calcium sensors and final targets involved in this signaling circuit. As an initial step, potential CaMBPs from Arabidopsis mitochondria were isolated by affinity chromatography on Calmodulin-Agarose. Potential candidates were identified by LC MS/MS including Tom22-V (Translocase of Outer Mitochondrial Membrane). Mitochondrial localization of Tom22-V was confirmed using YFP-tagged protein *in vivo* and the interaction between Tom22-V and calmodulin was supported by further *in vitro* experiments. Preliminary results indicate that the CaM-binding site is located within the cytosolic domain but the exact CaM-binding site is yet to be determined. This potential CaMBP and the role of CaM binding are now being functionally characterized.

STP08
Is the Ecto-5'-Nucleotidase CD73 a Suitable Mesenchymal Stem Cell Marker?

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The Ecto-5'-Nucleotidase CD73 is a membrane-bound enzyme that catalyses the hydrolysis of extracellular adenosine monophosphate to adenosine and phosphate. In addition, CD73 has been suggested by the International Society for Cellular Therapy as a marker defining mesenchymal stem cells (MSCs). For the last few years CD73 has been accepted by the scientific community working with MSCs.
Mesenchymal stem cells can be isolated from various tissues such as bone marrow, adipose tissue, and umbilical cord. They possess the ability of self-renewal and are characterised by the three positive markers CD73, CD90, and CD105 and several negative markers such as CD19, CD34, and CD45. Furthermore they have to show plastic adherence and the ability to differentiate towards adipogenic, osteogenic, and chondrogenic lineage.
In this study, human adipose tissue-derived mesenchymal stem cells were differentiated towards adipocytes, osteoblasts, endothelial, and smooth muscle cells. The differentiation was confirmed via semi-quantitative RT-PCR for cell

lineage specific markers and staining. The gene expression of CD73 on MSCs and differentiated cells was investigated.
As expected CD73 was expressed in all MSCs derived from female and male donors of different ages. Interestingly it could be shown that the gene expression of CD73 was up-regulated in all investigated differentiations. This can be found in all donors independent of gender and age.
In conclusion CD73 is not a suitable mesenchymal stem cell marker. However the increasing expression of CD73 might indicate that it has an impact on purinergic signalling known to regulate mesenchymal stem cell differentiation.

STP09

NLRC5 Controls Basal MHC Class I Gene Expression in an MHC Enhanceosome-Dependent Manner

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Nucleotide-binding domain and leucine-rich repeat (NLR) proteins play important roles in innate immune responses as pattern recognition receptors. Although most NLR proteins act in cell autonomous immune pathways, some do not function as classical pattern-recognition receptors. One such NLR protein is the MHC class II transactivator, the master regulator of MHC class II gene transcription. In this article, we report that human NLRC5, which we recently showed to be involved in viral-mediated type I IFN responses, shuttles to the nucleus and activates MHC class I gene expression. Knockdown of NLRC5 in different human cell lines and primary dermal fibroblasts leads to reduced MHC class I expression, whereas introduction of NLRC5 into cell types with very low expression of MHC class I augments MHC class I expression to levels comparable to those found in lymphocytes. Expression of NLRC5 positively correlates with MHC class I expression in human tissues. Functionally, we show that both the N-terminal effector domain of NLRC5 and its C-terminal leucine-rich repeat domain are needed for activation of MHC class I expression. Moreover, nuclear shuttling and function depend on a functional Walker A motif. Finally, we identified a promoter sequence in the MHC class I promoter, the X1 box, to be involved in NLRC5-mediated MHC class I gene activation. Taken together, this suggested that NLRC5 acts in a manner similar to class II transactivator to drive MHC expression and revealed NLRC5 as an important regulator of basal MHC class I expression.

STP10

Upregulation of growth associated genes and increased basal activity of mitogen activatedprotein kinase pathway in flotillin-2 knockout mice

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Flotillins are highly conserved and widely spread proteins that function in receptor tyrosine kinase signaling and membrane trafficking processes. Flotillin-1 and flotillin-2 have been shown to form both homo- and hetero-oligomers, and their cellular localization changes during signaling. Increased expression of flotillins has been detected in certain cancers, and flotillin-2 knockout mice show a reduced formation of metastases in a breast cancer animal model. Our recent data have shown that flotillin-1 depletion results in diminished activation of the epidermal growth factor receptor and impairs its downstream signaling towards the mitogen activated protein kinases and the respective transcriptional response. Here we show that genetic ablation of flotillin-2 in a mouse model or its knockdown in cultured cells increases extracellular signal regulated kinase (ERK) activation. Furthermore, the downstream transcriptional targets of ERK are upregulated at both mRNA and protein level. These data suggest that opposite effects are obtained upon ablation of one of the two flotillins, implying a distinct role for flotillin homo- and hetero-oligomers in signaling. Due to their overexpression in cancers, flotillins may be considered as cancer therapy targets. However, our findings suggest that care needs to be taken when interfering with flotillin function, as unexpected effects may emerge in certain cell types.

STP11

Regulation of immediate-early gene transcription following activation of Gaq-coupled designer receptors

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We have analyzed the regulation of gene transcription following activation of Gaq-coupled designer receptor (Raq). Stimulation of HEK293 cells expressing Raq with the designer drug clozapine-*N*-oxide (CNO) induced the expression of biologically active Egr-1, a zinc finger transcription factor. Expression of a dominant-negative mutant of the ternary complex factor (TCF) Elk-1 prevented Egr-1 expression. Stimulation of Raq with CNO increased the transcriptional activation potential of Elk-1 and enhanced transcription of a serum response element-regulated reporter gene. In addition, AP-1 transcriptional activity was significantly elevated. AP-1 activity was controlled by TCFs and c-Jun in cells expressing an activated Gaq-coupled designer receptor. Pharmacological and genetic experiments revealed that the protein kinases Raf and ERK were essential to connect Raq stimulation with enhanced Egr-1 and AP-1 controlled transcription. In contrast, MAP kinase phosphatase-1 functioned as a nuclear shut-off device of stimulus-transcription coupling. The fact that Raq stimulation activates the transcription factors Egr-1, Elk-1, and AP-1 indicates that regulation of gene transcription is an integral part of Gaq-coupled receptor signaling.
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STP12

High content screening microscopy based analysis of endogenous signaling kinetics suggests strong differences in regulation between primary neurons and glia

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Primary cell cultures mostly contain large numbers of functionally diverse cells. Averaging mixes all signals from different cell types in the culture as commonly done by cell-homogenization and western blot analysis. This is prone to misinterpretation. High content microscopy provides a solution to this problem by allowing single cell based analysis even of cells in the same culture. Here we investigated differences in responses of endogenous Erk1/2 between dorsal root ganglia (DRG) neurons and glia in vitro, focusing on the kinetic aspects of the response.
We established criteria for object identification to differentiate between the neurons and glia present in the primary DRG cultures. This allowed the separate analysis of these cell types even if cultured in the same dish. We tested growth factors, cytokines, neurotransmitters, proinflammatory lipid mediators and artificial stimuli as PMA or forskolin. Our results show that neurons exhibit a mixture of transitory, long lasting, or biphasic responses. In contrast, glia tend to strong, fast, and short lived responses in the ERK pathway independent of the receptor class activated. Subsequent pharmacological analysis will allow the identification of cell-specific regulation of signaling pathway kinetics in primary cells.

STP13

Investigation of the G protein signaling in the prototypic aGPCR latrophilin

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Latrophilins, highly conserved members of the adhesion-G protein-coupled receptor (aGPCR) family, became first interesting to scientists as the receptor for the toxin of the black widow spider. Physiologically, latrophilins have implications in essential developmental and neurological processes, rendering them promising drug targets. However, little is known about its activation or signaling mechanism as it is for the whole class of aGPCR. For the *C. elegans* latrophilin homolog LAT-1 two signaling modes have been shown: a C-terminus dependent and an -independent one. To shed light on the C-terminus-dependent mode, *in vitro* assays for GPCR signaling including cAMP and CRE SEAP assays identified $G\alpha_s$ coupling as the dominant G protein for LAT-1. These *in vitro* results are supported by an *in vivo* assay in *C. elegans lat-1* null mutant which displays various morphological defects. Using forskolin to stimulate signaling these defects could be ameliorated. To further understand signaling of LAT-1 the structure of the C-terminus was investigated utilizing an in vivo complementation assay in the *lat-1* null background. Several constructs were tested such as point mutations and sequence exchanges in highly conserved regions. These comprehensive examinations allow for a better understanding of latrophilin signaling. As latrophilins can be considered a prototypic aGPCRs, these insights are the first step towards a better understanding of latrophilins as well as the class of aGPCR in general.

STP14

Cerulein-induced Egr-1 expression in pancreatic cancer cells requires a rise in intracellular Ca2+ and the activation of the PKC, Raf, ERK and ternary complex factors

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The injection of supraphysiologically concentrations of cerulein, an analog of the pancreatic secretagogue cholecystokinin, induces acute pancreatitis in mice, that is accompanied by the synthesis of the proinflammatoric zinc finger transcription

factor Egr-1. The objective of this study was to analyze the signaling cascade that connects cerulein stimulation with enhanced Egr-1 biosynthesis. AR42J rat pancreatic acinar cells were used as a model system to measure cerulein-induced Egr-1 biosynthesis. Pharmacological and genetic methods were used to identify signaling molecules required for the upregulation of Egr-1 in cerulein-treated AR42J cells. Stimulation of AR42J cells with cerulein induced a robust and transient biosynthesis of Egr-1. The signaling cascade connecting cerulein stimulation with Egr-1 gene expression required elevated levels of cytosolic Ca2+ and the activation of the protein kinases PKC, Raf and ERK. Expression of a dominant-negative mutant of Elk-1 prevented Egr-1 expression in stimulated AR42J cells, indicating that Elk-1 or related ternary complex factors connect the intracellular signaling cascade elicited by activation of CCK receptors with transcription of the Egr-1 gene. Expression of MAP kinase phosphatase-1 prevented Egr-1 biosynthesis in cerulein-stimulated AR42J cells. In addition, Egr-1 biosynthesis induced following stimulation of Gαq-couled designer receptors with CNO In AR42J cells required the same signaling molecules. The identification of the signaling molecules connecting cerulein treatment with enhanced transcription of the Egr-1 gene may open new fields for pharmacological interventions. Moreover, experiments perfomed with Gαq-coupled designer receptors implies that overstimulation of the Gαq-induced signaling pathway is crucial to induce actute pancreatitis.

STP15

Identification and characterization of a novel interacting partner of LRRK2

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Mutations in the leucine-rich repeat kinase 2 (LRRK2) are the major cause of genetically inherited Parkinson´s disease (PD). It has been shown extensively from previous literature that some of these mutations, such as G2019S and R1441C, result in increased kinase activity of LRRK2 and subsequently in neuronal toxicity in neuronal secondary cell lines and primary neuronal cultures. LRRK2 also contains Serine and Threonine residues in its Roc GTPase and MAPKKK kinase domain, which are essential for its kinase further activation. In our initial data, we demonstrate that LRRK2 is associated with the Protein Phosphatase 2A protein (PP2A), which is a Serine/Threonine phosphatase, already implicated in Parkinson's Disease. We intend to perform additional experiments to confirm the interaction, but we will also test the effect of overexpression or silencing of the PP2A catalytic subunit in SH-SY5Y cells which will be transiently transfected with wild-type LRRK2 but also a mutant version of LRRK2 (R1441C) as well as transgenic LRRK2-overexpressing mice.

STP16

Dehydroepiandrosterone sulfate mediates activation of transcription factors CREB and ATF-1 via a Ga11-coupled receptor in the spermatogenic cell line GC-2

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Dehydroepiandrosterone sulfate (DHEAS) is a circulating steroid produced in the adrenal cortex, brain, and gonads. Whereas a series of investigations attest to neuroprotective effects of the steroid in the brain, surprisingly little is known about the physiological effects of DHEAS on cells of the reproductive system. Here we demonstrate that DHEAS at low concentrations acts as a hormone on the spermatogenic cell line GC-2. DHEAS induces a time- and concentration-dependent phosphorylation of c-Src and Erk1/2 and activates the transcription factors ATF-1 and CREB. These actions of DHEAS are consistent with the non-classical signaling pathway of testosterone and suggest that DHEAS is a pro-androgen that is converted into testosterone or other steroid hormone in order to exert its biological activity. The fact, however, that steroid sulfatase mRNA was not detected in the GC-2 cells and the clear demonstration of DHEAS-induced activation of Erk1/2, ATF-1 and CREB after silencing the androgen receptor by siRNA clearly contradict this assumption and make it appear unlikely that DHEAS has to be converted in the cytosol into a different steroid in order to activate the kinases and transcription factors mentioned. Instead, it is likely that the DHEAS-induced signaling is mediated through the interaction of the steroid with a membrane-bound G-protein-coupled receptor, since the silencing of Gna11 leads to the abolition of the DHEAS-induced stimulation of Erk1/2, ATF-1, and CREB. The investigation shows for the first time a hormone-like activity of DHEAS on a spermatogenic cell line. Since DHEAS is produced in male and female reproductive organs, these findings might help to define new roles for DHEAS in the physiology of reproduction.

STP17

AMPK-independent anti-angiogenic and Endothelial Barrier Stabilizing effects of AMPK inhibitor compound C

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Background
Compound C is a cell-permeable pyrrazolopyrimidine derivative, widely used as adenosine monophosphate-activated protein kinase (AMPK) inhibitor to characterise the role of AMPK in various physiological processes. We observed some AMPK-independent effects of compound C on endothelial function. In the present study we investigated the effects of compound C on in vitro angiogenesis and endothelial barrier function both in vitro in cultured HUVEC and in situ in isolated perfused rat hearts.

Methods and Results
Compound C inhibited endothelial cell proliferation, migration, tube formation, and endothelial cell sprouting in a conc.-dependent manner (0.1-10 µM; n =5; P<0.05, for all further exps.). At these concentrations it did neither blocked basal nor A-769662-induced AMPK activation. Moreover, these effects of compound C were not mimecked by specific knockdown of AMPK. Interestingly, specific activation of AMPK using A-769662 also caused a reduction in proliferation (18±5%), migration (24±6%), tube formation (20±9%), and sprouting. Combining compound C (1 µM) either with metformin or A-769662 had additive effect on endothelial cell proliferation, migration, and sprouting. Likewise, compound C inhibited RhoA/Rock signalling and antagonised thrombin-induced increase in HUVEC monolayer permeability in a conc.-dependent manner and abrogated ischemia-reperfusion (IR)-induced increase in myocardial water content in isolated perfused rat hearts.

Conclusion
The data of present study demonstrate that compound C antagonises VEGF-induced angiogenesis and thrombin/IR-induced endothelial hyperpermeability in AMPK-independent manner. Moreover, the study warns that this agent should be used with caution to demonstrate the effects mediated by AMPK activation.

FREE TOPIC

PKV05

The innate immune response to *Streptococcus pneumoniae* in the lung is influenced by immunoproteasomes

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The ubiquitin-proteasome system is responsible for degradation of most intracellular proteins and consists of two steps: ubiquitination and proteasome-mediated degradation. The proteasome is a large protease composed of multiple subunits that is present in all eukaryotic cells and is essential for many basic cellular processes such as protein homeostasis, cell growth and antigen presentation. Following infection and subsequent IFN-g release, three proteasome subunits are replaced by the inducible subunits LMP7, LMP2 and MECL1, producing immunoproteasome (IP). The observation that IP are constitutively present in macrophages and/or granulocytes which constitute the first line of defence against invading pathogens suggests a potential role for IP in the initial phase of infection. To clarify the contribution of IP to the innate response, we performed studies in a *Streptococcus pneumoniae* infection model using mice lacking LMP7. Interestingly, our data show that the bacterial burden in lung and blood in LMP7-/- mice after infection was substantially higher than that observed in wild-type mice. The phenotype of such LMP7-/- mice was further characterised by a stronger body weight loss, reflecting a more severe course of illness. Taken together, these findings demonstrate that IP are required for recovery from *Streptococcus pneumoniae* in mice and tend to highlight a yet unsuspected role for the inducible subunits in modulating the innate response to bacterial infection.

PKV14

'Traceless' Tracing of Proteins: High-affinity *Trans*-splicing for Protein Labeling in Living Cells

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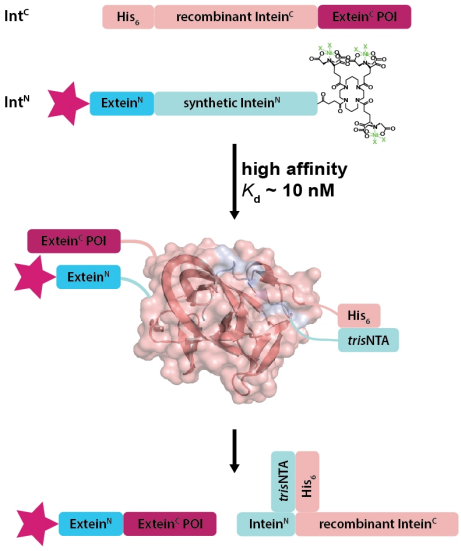
Site-specific and efficient incorporation of chemical probes into proteins remains a major challenge in chemical biology. Chemical probes can be introduced into proteins via split inteins. Protein *trans*-splicing allows the chemoselective ligation of a synthetic peptide with a recombinant protein at the N- or C-terminus depending on the intein system. Here, we use the characterized *Ssp* DnaB M86 intein [1] to develop an intein system to promote protein *trans*-splicing at

nanomolar concentrations triggered by small high-affinity pairs. To reach nanomolar affinity we used the *tris*NTA/His-tag interaction as a lock-and-key pair [2,3]. In combination with super-resolution microscopy and single-molecule analysis, these 'traceless' approaches will provide insights into conformational dynamics, clustering, and trafficking of macromolecular complexes in living cells.



A library of different Int^N-fragments modified by *tris*NTA as well as His_6-tagged Int^C-constructs was generated to allow *tris*NTA/His-tag interaction. The small high-affinity pair increases the affinity for the Int^N-fragments 100-fold, whereas the linker shows no significant impact on the intrinsic affinity. Based on the high-affinity interaction, protein *trans*-splicing is observed at nanomolar concentrations. The potential for a specific *in vivo* labeling of His-tagged proteins at ultralow concentrations will be demonstrated by confocal and super-resolution microscopy.

References

[1] Wasmuth *et al. Bioorg. Med. Chem.* 2013, 21, 3495-3503.
[2] Labòria *et al. Angew. Chem. Int. Ed.* 2013, 52, 848-853.
[3] Lata *et al. JACS* 2006,128, 2365-2372.

PKV23

The effect of hypoxia during vertebrate development

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Hypoxia evokes a number of responses in the organisms during development. It has been shown that PHDs (prolyl hydroxylases) target HIFs (hypoxia inducible transcription factors) to degradation in normoxia whereas in hypoxia, PHDs are gradually inactivated thereby stabilizing HIFs and evoking response to hypoxia via transcriptional initiation of a number of pro-angiogenic genes. It is of special interest to study how organisms respond to continuous hypoxia during development as this has correlations with the formation and progression of cancers. In this study, we used frog (*Xenopus laevis*) embryos to study the effects of hypoxia on hematopoiesis during development. Hypoxia conditions were created *in vitro* in small aquaria wherein two cell stage embryos were introduced into four different chambers with 2, 4, 6 and 8 % oxygen respectively and raised until tadpole. Whole mount *in situ* hybridization against various hematopoietic markers showed significant differences in the differentiation & formation of hematopoietic markers. Development is also greatly decelerated by continuous hypoxia conditions. Chronic hypoxia even interferes with the morphology of the embryos in addition to a cease development beyond early tadpole stage. Introducing embryos at different time points of development showed a stressing importance of oxygen at late tadpole stage. Interfering with PHD-2 via knockdown also resulted in similar effects suggesting large amounts of HIFs stabilized during continuous hypoxia inhibit rather than promoting angiogenesis.

PKV24

Localization of amyloid-beta 1-42 ($Aß_{1-42}$) peptide in cells and their influence on mitochondrial function

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Alzheimer's disease is the most common form of dementia, which is caused by amyloid-beta peptides with 38 to 43 amino acids. These peptides are processed from the amyloid precurser protein (APP). According to the oligomer hypothesis, amyloid-beta peptides aggregate to form oligomeric complexes which are assumed to cause the neurodegenerative disease [1, 2]. However, even Aß monomers are bioactive [3]. Most toxic is the amyloid-beta 1-42 ($Aß_{1-42}$) peptide [4].
At first, FITC-$Aß_{1-42}$ incubated cells were analyzed with a flow cytometer to demonstrate the association of the peptide with the cells. Thereafter, localization of the peptide inside the cells was shown by confocal microscopy.
Adherent cells were incubated with 2 µM $Aß_{1-42}$ for 24 h and then analyzed. Due to focusing on mitochondrial function, ATP concentration and generation of reactive oxygen species were determined. Additionally, solubilized mitochondrial proteins from $Aß_{1-42}$-treated cells were separated on blue-native gels and in-gel activity tests [5] of OxPhos complex I and IV as well as their supercomplexes were performed.
Experiments were conducted with the human neuroblastoma cell line SH-SY5Y which is often used as model system for Alzheimer's disease research. Since own experiments showed high endogenous Aß, the rat oligodendroglia cell line OLN-93 was also used to ensure that the results obtained were caused by the experimental treatment of the cells with $Aß_{1-42}$.

References

[1] Hardy, J. and Selkoe, D.J., *Science*, 2002. 297: 353-356
[2] Vestergaard, M., et al., *Biotechnol Bioeng*, 2008. 99: 753-763
[3] Buchsteiner, A., et al., *Soft Matter*, 2012. 8: 424-429
[4] Burdick, D., et al., *J Biol Chem*, 1992. 267: 546-54
[5] Wittig, I. et al., *Mol Cell Proteomics*, 2007. 6: 1215-1225

FTP01

- WITHDRAWN -

FTP02

Identification of probiotic properties of Iranian native Bacillus, isolated from poultry farms of Arak

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Introduction and objective: the oral probiotic bacteria with ability to change the intestinal microbial flora play an important role as health supplement in body. After ingestion they are residing in intestinal tract and exhibit beneficial effect. Also probiotic bacteria are suitable alternative for (natural) antibiotics. The purpose of this study was screening of Arak native Bacillus isolated from poultry farms and evaluation of their probiotic properties.
Materials and Methods: In this study 41 samples of fecal material were collected from 8 different poultry farms. After enriching and heat treatment, spore-forming bacteria were screened. The probiotic properties of the isolates (Acid, bile, salt, pepsin, and chicken gizzard extract resistance and antimicrobial compound production) were determined.
Results: In this study 140 isolates of bacillus were screened. 14 strains were subjected to more research base on negative haemolysis. Investigation of probiotic properties of isolates showed that most of them could growth in the presence of 10% NaCl, stain 7 was resistance to HCl (up 91.7%) while stains 9 and 10 were completely resistance to bile salts (cholate and deoxycholate sodium). The selected strains were tolerated to chicken gizzard extract pepsin (up to 80%) and their adhesion ability to intestinal cells in under evaluation. All the strains showed antimicrobial activity against the common poultry pathogens such as Salmonella sp.and Ecoli.
Conclusion: This study showed that the isolates are potential probiotics and after complementary and filed experiment they can be used as poultry probiotics with the ability of inhibition of poultry common disease.

FTP03

Surface Plasmon Resonance imaging and Fluorescence spectroscopy solutions to explore molecular interactions

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Proteins play an essential role in the functioning of living organisms. Their activity is mostly controlled by other molecules that bind to proteins in order to stimulate or activate them. As a result, the analysis of binding events or conformational changes using biophysical techniques (label-based and label-free) is being routinely used in scientific research.
Fluorescence spectroscopy is a standard technique used in Life Sciences for the characterization of biomolecules. More precisely, Förster fluorescence resonance energy transfer (FRET) is a useful tool for the determination of intra- and inter-molecular distances. It is based on the non-radiative energy transfer from an excited donor fluorophore to an acceptor fluorophore. It can be used to analyze molecular interactions at the single-molecule level.
Surface Plasmon Resonance (SPR) is a label-free technique used to follow the binding of molecules onto ligands immobilized on a biochip surface by

monitoring changes of the refractive index. Molecular interactions are followed in real-time, providing information on kinetic processes (association and dissociation rates), affinity and specificity. Surface Plasmon Resonance imaging (SPRi) is adding an imaging capacity to SPR. It combines the strength of SPR to monitor label-free biomolecular interactions to the throughput of microarrays. It is thus possible to measure several dozen to several hundred interactions in parallel (multiplexing).
A technical overview of both techniques will be given and illustrated by recent applications in the field of chemical biology.

FTP04
The Relationship Between Antioxidant and Anti-ulcer Activity in Saudi Honey Samples Harvested From Various Regions in Different Seasons

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The main chemical components of the 13 Saudi honey samples (compared of winter and summer honeys) were identified by Phytochemical and chromatographic analysis, beside anti-ulcer and antioxidant activity. Phytochemical screening of ethyl acetate and water extracts provides the presence of carbohydrates, flavonoids, amino acids and phenolic acids. HPLC of sugar contents provides that galactose was present in all honey samples. Other sugars demonstrated were sucrose, fructose, and arabinose. Fifteen amino acids were detected in all honey samples they are glutamic acid, proline, valine, histidine, serine, tyrosine, arginine, alanine, isoleucine, leucine, threonine, aspartic acid, phenylalanine, lycine and glycine. Citric and tartaric acid were also detected in addition to faint traces of free oxalic acid. The free amino acids were compared in those of winter and summer honey; both samples were tested for the amino acids, phenolic compounds and sugars. The most important result in this study is the affect of summer kind of honey on ulcer.
The anti-ulcer activities were also evaluated, it was noticed that oral medication with the honey samples reduced the intensity of ulcer scores compared to control group. Similarly, there is a high significant reduction in the values of the ulcer indices and areas in rats that received the same sample ($p<0.05$ & $p<0.01$) in comparison with those in the ulcer control rats. Finally antioxidant activity of the honey samples were evaluated, we found that there is a proportional relationship between the anti-oxidant activities and anti-ulcer activity. The study found that the more activity of honey as an anti-ulcer whenever, antioxidant activity are high.

FTP05
In Vitro Regenerative Tubulogenesis using a Novel Adult Stem Cell Culture Model System

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Introduction
unraveling the mechanisms of tubular morphogenesis is a key to comprehending basic tissue phenotypes, and key to effectively treating or ameliorating many common diseases and disorders. We have recently introduced a novel human adult stem cell type: Small, Mobile Stem cells (SMS cells), exhibiting characteristics conducive to morphogenesis in standard media conditions.

Aim
The aim of this study is to describe landmark events of cell processes that relate to de novo tubulogenesis.

Material & Methods
To achieve this aim, SMS cell type was applied as an in vitro cell culture model system. Long term in vitro SMS cell cultures were established. Extensive imaging of cells and emerging macrostructures were obtained using multimodal microscopy and tissue staining techniques.

Results
We have demonstrated, in a simple parametrically "poor" set up, the extraordinary ability of SMS cells to effect complex organization and differentiation events that lead to specific, regenerative, highly reproducible, tubular macro-structures. Details of several complex newly described processes that appear to relate to de novo tubulogenesis were outlined and defined. Relevancy to in vivo tubulogenesis was suggested by comparison to images obtained from analyzed tissue samples of Sheep heart.

Conclusion
SMS cells exhibit a high capacity for self-organization in an in vitro simple setup. In vitro and de novo established macrostructures demonstrate high regenerative potency. The observed structures appear to be highly relevant to a selected in vivo model system. SMS cells are therefore an important cell culture model system for obtaining mechanistic insight into tubulogenesis.

FTP06
Angiotensinogen (AGT) gene, M268T polymorphism in coexistent diabetes, hypertension and nephropathy

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Introduction
Diabetic nephropathy is the leading cause of deaths due to end stage renal disease. The AGT gene has got strong correlation with plasma AGT level and blood pressure. This study investigates the possible association of M268T polymorphism of AGT gene in diabetic, hypertensive and nephropathy patients.

Materials & Methods
Blood samples from 115 diabetic patients, 115 diabetic hypertensive patients, 110 diabetic nephropathy patients and 110 normal healthy subjects as control were collected followed by informed consent. ARMS-PCR was used to genotype the DNA isolated from subjects for AGT M268T using specific primers.
Results
The frequency of AGT genotypes for CC, CT and TT in diabetic patients was found to be 08%, 86% and 06% respectively. In diabetic hypertensive patients genotype CC was 2.7%, CT was 91.2% and TT was 5.3%. In diabetic nephropathy patients the frequency of genotype CC was 9.5%, CT was 40.5% and TT was 50%. The CC, CT and TT genotypes, and C and T alleles distributions were significantly different in diabetes (c^2=8.606, p=0.00081), diabetic hypertensive (c^2=6.245, p=0.0014) and diabetic nephropathy (c^2=10.674, p=0.000022) patients as compared to controls. The allele distributions were significantly different in diabetic vs. nephropathy patients (c^2=24.254, P=0.00000128). The nephropathy patients have significantly higher prevalence of T allele and TT genotype.

Conclusion
The frequency of TT genotype was found prevalent in diabetic nephropathy. Results indicated a clear relationship of T allele polymorphism in AGT gene with nephropathy in diabetes mellitus. It is suggested to take a close look to frequency of T allele polymorphism while screening diabetic patients to get them rid of progressive renal impairment leading to end stage renal disease.

FTP07
Phytochemical analysis and antioxidant activity of *Marrubium vulgare L. from Iran*

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Introduction
Marrubium vulgare (Lamiaceae) is a popular medicinal herb growing wild in Iran. Previous studies have demonstrated its anti-inflammatory, anti-edematogenic and antioxidant properties.

Aim
The present study was designed to investigate antioxidant activity of the methanolic extract of *M. vulgare* from Iran and to quantify the marrubiin, the main active compound of this plant, using a densitometer.

Materials & Methods
The *in vitro* free radical scavenging activity of methanol extract was determined using 1,1-diphenyl-2-picryl-hydrazil (DPPH) test. Quantitative evaluation of marrubiin was performed by HPTLC analysis and subsequent chromatogram scanning using a TLC Scanner. Furthermore, the penolics and flavonoids content of methanol extract of aerial parts of *M. vulgare* was determined to spectrophotometrically.

Results
The RC50 (reduction concentration of 50%) values of methanol extract of *M. vulgare* and quercetin (as a standard free radical scavenger) were using DPPH assay and obtained as 8.24 and 3 µg/ml, respectively. Marrubiin was quantified as 156 mg per gram of *M. vulgare* extract and the total phenolic and flavonoids contents for *M. vulgare* were determined as 60.4 mg gallic acid equivalent and 12.05 mg quercetin equivalent per each gram of the extract, respectively.

Conclusion
The present study demonstrated that the methanol extract of *M. vulgare* has considerable scavenging of DPPH and the activity could be due to the presence

of phenolic and flavonoids along with marrubiin that may exert a synergistic effect.

FTP08

Towards White Biotechnology by Exploring of Industrial Enzymes From The Metagenome of Hydrothermal Vent Microbes at Kawio Island - Indonesia

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Recently, much attention has been paid on the utilization of thermostable enzymes as potential catalyst for industrial bioprocess or white biotechnology. Thermostable glycerol kinase as an ATP-dependent enzyme can be used as a biocatalyst in the production of glycerol-3-phosphate (G3P) from biodiesel waste. G3P is an important intermediate precursor for medicine, an inducer of systemic immunity in plants and an intermediate for other value-added chemicals. The thermostable polyphosphate kinase is considered as a potential enzyme for ATP regeneration system in bioprocess. The thermostable enzyme is an enzyme usually derived from microorganisms that live at temperatures 60-100ºC. One of thermophiles' habitats is in the hydrothermal vent of deep sea. The sample mixture of water and sediment from hydrothermal vent surounding (1500-3000 m, 35-80ºC) might contain thousands of heat-resistant bacteria species. This study aims to isolate the thermostable genes of glycerol kinase (*gk*) and polyphosphate kinase (*ppk*) from hydrothermal vent sample mixture using metagenomic approach. In order to isolate the genes from the whole genome of hydrothermal microbes, it was designed two pairs of degenerate primers for *gk* and *ppk*. The isolated genes of *gk* showed the similarity of the gene *gk* from *Thermus thermophilus, Thermus aquaticus, and Geobacillus sp*., while the isolated *ppk* had similarity to *ppk* from *Shewanella denitrificans*. After cloning and expressing the genes to *Escherichia coli* BL21, the enzyme activities were checked.

Keywords: thermostable enzyme, glycerol kinase, polyphosphate kinase, metagenomic.

FTP09

Comparison of DNA extraction methods from cocoa leaves and cocoa beans

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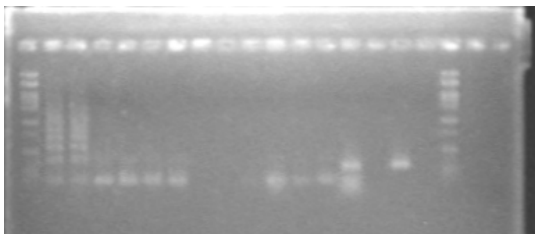
This research describes a applicable approach to the evaluation of DNA isolation methodology using cocoa leaves and cocoa beans. Six DNA extraction methods comprise DNeasy kit with and without PVPP, Stool kit, Wizard kit and two CTAB-based methods. DNA extraction procedures were analysed based on quality, quantity and PCR amplification. PCR amplification was evaluated by using a set of designed primers amplifying fragments of plant c/d of increasing size ranging from 500 bp to 600 bp. It is demonstrated that CTAB modified by Gryson et al, 2004 method performs perhaps the best methods for both cocoa leaves and cocoa beans.



FTP10

Changes in mitochondrial respiratory chain complexes associated with age, calorie restriction and age-dependent diseases

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ATP is mainly produced by mitochondrial respiratory chain complexes. These complexes exist as individual entities and as so called supercomplexes, which are stoichiometric assemblies of different respiratory chain complexes[1]. Quantitative analysis is feasible by 2D-BN/SDS PAGE[2]. Blue native (BN) PAGE preserves protein-protein interactions and therefore separates supercomplexes intact and according to their size. The subunit composition is visible after the denaturing second dimension, SDS PAGE. Sensitive and quantitative analysis of the protein-pattern is performed by fluorescent staining of 2D-gels or labelling the native proteins with fluorescent dyes before gel run. Protein spots are identified by MALDI-MS peptide mass fingerprinting. The activity of several respiratory chain supercomplexes and individual complexes can be quantitated by activity tests[3] in BN-gels. Analyses are carried out with rat mitochondria, isolated from different brain areas. Tissues of different age or of calorie restricted animals exhibit variations concerning the abundance and composition of the (super)complexes. Rat brain mitochondria from with 6-hydroxydopamine treated animals, triggering Parkinson's disease, were analysed to get a better understanding of this age-dependent disease. Our studies shall elucidate the processes occurring during ageing and age-related diseases[4].

Supported by the Statutory Funds of the Institute of Pharmacology, PAS, DAAD to KK and by DFG GRK 1657 to NAD and MK.

References

[1] H. Schägger, et al. *EMBO J.* 2000, 19, 1777.
[2] N.H. Reifschneider, et al. *J. Proteome Res.* 2006, 5, 1117.
[3] I. Wittig, et al. *Mol. Cell. Proteomics* 2007, 6, 1215.
[4] C. Wernicke, et al. *Pharmal. Reports* 2010, 62, 35.

FTP11

Distinct Affinities of human Histone Deacetylase 8 for Mono-Acetylated Histones

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The class I histone deacetylase (HDAC) family catalyzes the deacetylation of ε-N-acetyl lysine side chains of histones increasing their affinity to DNA. Due their central influence on transcription control, HDAC's dysfunctions are related to genetic diseases [1]. In contrast to the other classic HDAC members the catalytic process of HDAC8 is only partially revealed. HDCA8 mediated deacetylation of histones is not clearly demonstrated and protein-protein interactions seem to be restricted to a small group of tissue-specific targets [2].
We present the recombinant production of site-specific acetylated histones for the investigation of HDAC8 substrate specificity. Incorporating the amber codon TAG via site directed mutagenesis combined with an ε-N-acetyl lysyl-tRNA synthetase [3] enabled the production of mono-acetylated histones. Westernblot analyses (anti-HisTag as well as anti-AcK antibody) after an IMAC purification [3] showed a verifiable protein amount for H2A (acetylation at position 6, 10), H2B (13, 14, 17, 22, 29, 110), H3 (5, 10, 15, 19, 24, 28, 37, 57, 80) as well as the proof of the acetylation. A competitive enzyme activity assay using the fluorogenic Boc-Lys (TFA)-AMC substrate and mono-acetylated histones revealed a different substrate selectivity of HDAC8.
In summary we report for the first time a specific interaction between HDAC8 and mono-acetylated histones. In addition, binding experiments proved distinct differences in the affinity of HDAC8 to histones with single acetyl-modifications. Further experiments are ongoing to identify the cellular function of HDCA8 mediated histone deacetylation.

References

[1] PMID: 18824292 [2] PMID: 23175386 [3] PMID: 18278036

FTP12

Differences in protein expression and protein activities in mitochondria of OLN-93 cells after irradiation with X-ray

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Our proteome studies focus on ionizing radiation induced changes in the mitochondrial proteome, with the key aspect on the abundance, composition, structure and activity of inner mitochondrial membrane proteins with the oxidative phosphorylation (OXPHOS) complexes as well as water soluble proteins.
These studies were conducted on oligodendrocytes from rat brain (OLN-93 cells) irradiated with 8 Gy (X-ray). Isolation of mitochondria from cell cultures was performed on ice following a modified protocol by Rickwood et al. (1987). The activity of the complexes I and IV were analyzed using "In-solution activity tests". The determination of intracellular ATP amount served as an indirect test of especially the activity of complex V. In order to solubilize the membrane proteins, the mild detergent digitonin was used at a ratio of 1 to 8 (w/w, protein/detergent). Solubilized proteins were analyzed by 2D BN / SDS PAGE. In this way, proteins can be separated and quantified in different assembly states. For quantitative analysis, the 2D gels were stained with a fluorescent dye, detected protein spots were analyzed by Delta 2D software und also identified by MALDI-mass spectrometry or antibodies.
Rickwood, D., Wilson, M.T., Darley-Usmar, V.M., 1987. Isolation and characteristics of intact mitochondria. In Mitochondria. (Hrsg.: Darley-Usmar, V.M., Rickwood, D., Wilson, M.T.R.), 1- 16.

Acknowledgements: Supported by the DGF in the framework of GRK 1657: Molecular and cellular responses to ionizing radiation.

FTP13

Genetic evidence for a role of the MLL3/MLL4 histone H3K4 methyltransferase complexes in bipolar disorder

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Studies of epigenetics suggest a prominent role for chromatin regulation in the aetiologies of various psychiatric disorders, as evidenced by differential epigenetic profiles in blood samples or post-mortem brain samples of patients. However, it is not clear whether such alterations are related to environmental modification or mutations in the epigenetic machinery. To test the latter hypothesis, we implemented a permutation-based approach aimed at identifying chromatin-regulatory (CR) gene networks involved in the pathogenesis of schizophrenia (SCZ), bipolar disorder (BPD), and major depressive disorder (MDD). Using the publicly available Psychiatric GWAS Consortium dataset, we investigated whether SNPs in a set of 473 genes known to regulate chromatin function showed associations with SCZ, BPD, and MDD. For the combined set of CR genes, empirical p-values were calculated separately for the three disease outcomes using an adaptive permutation procedure (100,000 permutations per analysis). The set of CR genes was significantly associated with BPD ($p = 0.049$), showed a trend for SCZ ($p = 0.058$), but was not associated with MDD ($p = 0.48$). Within the entire set of CR genes, 21 categories of genes belonging to distinct pathways can be distinguished. Subsequent stratified analyses for these categories showed that particularly the MLL3/MLL4 histone H3K4 methyltransferase complex was significantly associated with BPD (corrected $p = 0.003$), but not with SCZ and MDD. Based on their biological action, MLL3/MLL4 complex genes were further subdivided, yielding significant associations with BPD for two subcategories: (1) genes unique to MLL3 and MLL4 (corrected $p = 0.002$) and (2) genes shared with the SET1/MLL complexes (corrected $p = 0.012$). Collectively, our findings indicate a role for aberrant chromatin regulation through the MLL3/MLL4 complex in the aetiology of BPD.

FTP14

Autophagy - pro-survival mechanism with impact on aging and development in the filamentous fungus *P. anserina*

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The mitochondrial free-radical theory of aging proposes that accumulation of oxidative damage over time is caused by reactive oxygen species (ROS), which are primarily generated during mitochondrial respiration. Different pathways including ROS scavenging, mitochondrial protein quality control and mitophagy are coping with the damaging potential of ROS and are active in keeping mitochondria functional over time. In recent years, we have studied various mutants impaired at different stages of these pathways in the filamentous fungus *Podospora anserina*. The characterization of these mutants revealed unexpected effects on aging and lifespan. For example, the loss of mitochondrial SOD, PaSOD3, in *P. anserina* leads to increased paraquat sensitivity whereas lifespan is not affected suggesting, that the lack of components of the ROS scavenging pathway may be compensated by the induction of other pathways like autophagy. In order to demonstrate this for *P. anserina*, we developed or adopted appropriate tools: a PaATG8 reporter strain allowing the microscopic tracking of autophagy, the measurement of the degradation of GFP fusion proteins via autophagy and a strain lacking a central component of the autophagy machinery, PaATG1. With these tools we were able to demonstrate that during aging of *P. anserina*, the number of autophagosomes strongly increases as well as the autophagy-dependent degradation of a GFP fusion protein. The *PaAtg1* deletion strain is characterized by a significant reduction in lifespan, stressing the role of autophagy as pro-survival mechanism during aging. This is also relevant in the *PaSod3* deletion mutant and demonstrates that autophagy is able to compensate for impairments of the ROS scavenging pathway.

FTP15

A FRET-based competition assay suitable for thermodynamic characterisation of histone deacetylase inhibitors

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Histone deacetylases (HDACs) are proven protein targets for the therapy of cancer (1). Although a number of isoform selective HDAC inhibitors are known the synthesis of novel highly potent and selective compounds is still challenging. Adding thermodynamic parameters to the process of rational drug design can provide useful information for the development of selective lead structures (2). Using a FRET-based competition assay and a van't Hoff approach the thermodynamic parameters for the binding of fluorescent dansyl-hydroxamates and non-fluorescent compounds to the HDAC homologue histone deacetylase like amidohydrolase (HDAH) from *Bordetella/Alcaligenes* are investigated. Binding of the dansyl-hydroxamates reveals increasing affinities with longer aliphatic spacers (n = 4-7) between the dansyl moiety and the hydroxamic acid. Van't Hoff plots of the binding to HDAH indicate that mostly entropic contributions are favourable for the binding of the investigated compounds. Two compounds, one with a six carbon unit aliphatic spacer and a psammaplin A derivate show also favourable enthalpic contributions. Inhibition studies with the dansyl-hydroxamates and HDAC1, 8 and 6 provide evidence for a possible application of the FRET assay to human class I and IIb HDACs.

References

1. Marks P, Xu W-S. Histone deacetylase inhibitors: Potential in cancer therapy. *Journal of cellular biochemistry*. 2009;107(4):600-8.
2. Ladbury JE, Klebe G, Freire E. Adding calorimetric data to decision making in lead discovery: a hot tip. *Nature Reviews Drug Discovery*. 2009;9(1):23-7.

FTP16

Modification of human trypsinogen reduces autoactivation

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Trypsinogen is the inactive precursor of trypsin, a serine protease cleaving peptides after arginine and lysine residues. Trypsinogen is active only after its eight-amino acid long activation peptide has been cleaved off by another protease, the enteropeptidase. However, trypsinogen can also cleave off the same activation peptide by itself, a process called autoactivation. In biotechnological and biomedical applications, this autoactivation mechanism mostly leads to an undesired background signal. Therfore, a trypsinogen mutant with a lower degree of autoactivation is desirable.
Based on a sequence alignment with trypsinogen variants of different species, a human trypsinogen mutant was generated. Several residues were changed to identify important residues which may be involved in the autoactivation mechanism. After insoluble expression in *E. coli* cells and subsequent refolding, trypsinogen was purified via an ecotin affinity chromatography. The autoactivation of the modified trypsinogen was characterized in comparison to the recombinant wild type enzyme and a mutant known from the literature. The new mutant of the human trypsinogen shows a significantly decreased autoactivation compared to human wild type trypsinogen and is potentially interesting for biotechnological applications where a low degree of autoactivation is needed.

FTP17

Bioproduction of prostaglandins in recombinant *E. coli* cells

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Prostaglandins (PG) act as local hormones in nearly all tissues of a mammalian organism and are involved in numerous physiological and pathophysiological processes. Due to their diverse physiological functions PG are important active pharmaceutical ingredients for a variety of therapeutic applications. Difficult chemical synthesis of these highly complex and unstable molecules raises the price and shortens the application in medical treatment.
Prostaglandin endoperoxide H_2-synthases (PGHS) catalyze the bis-oxygenation of arachidonic acid forming PGH_2. A severe limitation in biotechnological production of PG is the complexity of the enzyme. Due to the necessity of e. g. subcellular membrane organelles for posttranslational modifications of the enzyme, expression is limited to eukaryotic systems. Extensive research in the field revealed PG formation also in lower organisms like corals or algae. A PGHS variant of the red alga *G. vermiculophylla* which can easily be expressed in *E. coli* was described very recently. In our lab we demonstrated that this enzyme shares basic features with the mammalian forms like a high specificity for C_{20} substrate fatty acids. Aside from that considerable differences were shown regarding the inhibition of this enzyme by typical non-steroidal anti-inflammatory drugs like Aspirin®. Due to distinct amino acid substitutions these analgetics have no effect on the algal enzyme.

Prior to combining the initial enzyme PGHS of the arachidonic acid cascade with a downstream synthase to generate for example PGF_{2a} or PGE_2 several parameters such as enzyme expression levels, substrate delivery and product analysis will be optimized to increase product formation in whole-cell biotransformation experiments.

FTP18

Structural and Functional Investigation of the Phosphopantetheinylation Reaction in Nonribosomal Peptide Synthetases from *Bacillus*

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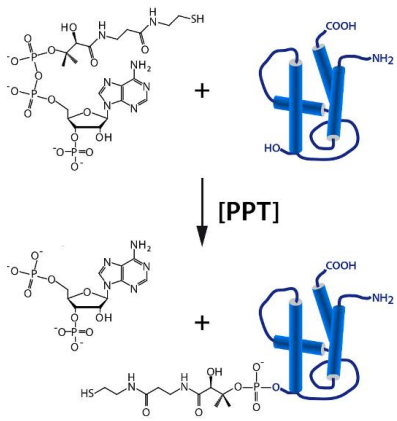
Carrier proteins play a crucial role in the metabolism of every organism. The topology of a four helix bundle is universal among the carrier proteins of fatty acid and polyketide synthases (FAS/PKS) as well as nonribosomal peptide synthetases (NRPS). Shuttling of substrates and intermediates (e.g. ketone bodies, growing fatty acid or peptide chains) by the carrier protein is facilitated by a phosphopantetheine cofactor attached to a conserved serine side chain making the phosphopantetheinylation a basic prerequisite for their proper function.
Two groups of phosphopantetheine transferases (PPTs) catalysing the transfer of the cofactor derived from coenzyme A are known. Group I PPTs consist of a single domain and are active as homotrimers. In PPTs from group II two similar domains are linked intramolecular and the enzyme is active in its monomeric state.
Previous work on a peptidyl carrier protein from *Bacillus* suggested that it undergoes a massive conformational change during its interaction with the group II PPT Sfp. Combining liquid state NMR and crystallization experiments we could show that a conformational change seems to play no role in the carrier protein/transferase interaction. Analysis of carrier protein mutants by isothermal calorimetry as well as a transfer assay revealed residues important for the productive interaction. Remarkably, the mode of interaction is conserved from the *Bacillus* NRPS to the human FAS carrier protein/PPT complex.



FTP19

FUNCTIONAL AND STRUCTURAL CHARACTERIZATION OF THE p63 ISOFORMS TA*p63α AND GTAp63α

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The transcription factor p63 is a homolog of the well-known tumor suppressor p53. The p63 isoform TAp63α was shown to play the key role in quality control of the genome in the female germ line. It is strikingly high expressed in undamaged oocytes and thus under a very tight regulation by being kept in an inactive dimeric state. Upon γ-irradiation induced DNA damage TAp63α gets phosphorylated and forms transcriptionally active tetramers, which are able to inducing apoptosis in the damaged oocytes.
Additionally to the TA and ΔN isoforms there are two other, poorly understood N-terminal extended isoforms of p63, TA*p63α and GTAp63α. The TA*p63α isoform is encoded in exon1 of the p63 locus, whereas the extension of GTAp63α corresponds to insertion of a LTR. GTAp63α was recently identified as the testis-specific counterpart of TAp63α, shown to be involved in protecting the genomic integrity of the male germ line. In contrast TA*p63α is apparently expressed in certain cancer cell lines, where its function is still unknown.
TA*p63α and GTAp63α are N-terminally elongated at the transactivation (TA) domain. As the TA domain is responsible for the transactivation ability and involved in dimer formation, these additional domains seem to have influence on the activity.
Here we will show a functional and structural characterization of TA*p63α and GTAp63α regarding their oligomeric state as well as their transactivation potential.Both isoforms still form dimers, even when the interaction of the TA domain with the oligomerization domain (OD), necessary for the inactive dimer in TAp63α, is destroyed. The observed stabilizing effect might inhibit phosphorylation as a putative activation process, which was demonstrated for TAp63α in the female germ line.

FTP20

Influence of external factors to mitochondrial respiratory chain complexes and chloroplast proteins of *Chlamydomonas reinhardtii* and characterisation of the chloroplast-ATP-synthase from *Spinacea oleracea*

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Nature´s universal energy currency is ATP. In mammalian cells it is predominantly produced in the mitochondria by the respiratory chain complexes. These complexes are arranged to large extent as structural and functional assemblies, the so called supercomplexes[1], and the ATP-synthase as homooligomers. In plant cells, ATP is also produced in the chloroplasts by light harvesting complexes and the ATP-synthase.
The green algae *Chlamydomonas reinhardtii* contains both of these organelles. A proper tool for separation of native mitochondrial and chloroplast proteins is the blue-native PAGE[1], thate we apply to determine native protein masses, oligomeric states and activity of respiratory chain complexes. Followed by a SDS electrophoresis in the second-dimension, changes in the protein composition of metabolic states under different cultivating conditions are analysed.
To structurally characterise the ATP-synthase we are focusing on crystallisation of the chloroplast-ATP-synthase from *Spinacea oleracea*. Therefore, detergents are needed to substitute the in vivo lipids to cope with the hydrophobic surfaces of this protein. But detergents interfere with classical crystallization methods like vapour-diffusion. The lipidic cubic phase crystallization represents an interesting approach to bypass this problem[2]. This method involves an artificial lipid bilayer incorporating the protein for crystallisation. For initial trials we are using the well studied transmembrane protein bacteriorhodopsin[3].

References

[1]I. Wittig, et al. *Proteomics* 2008, 8, 3974
[2]M. Caffrey, *J. Struct. Biol*. 2003, 142, 108
[3]E. Landau, *Biophysics* 1996, 93, 14532

FTP21

Dynamics of Tissue Specific Localisation of human Histone Deacetylase 8 fused to the Photoswitchable Protein mOrange

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Histone deacetylase (HDAC) 8, an unusual member of class I HDACs, shows a broad but tissue-specific behaviour, including cAMP Response Element Binding Protein (CREB) mediated gene transcription, its role in normal Human Smooth Muscle Cells (HSMC) and the induction of apoptosis in T-cell lymphomas [1]. There are contradictory reports about the localization of HDAC8 prominently in the nucleus or in the cytoplasma with fluctuating expression levels. Particular for proteins with not completely understood function like HDAC8, the determination of protein localization can provide crucial indications for their cellular function.
We observed a predominantly cytosolic incidence after fractionated cell lysis in the cell lines Hek293 (human embryonal kidney) and K562 (B-lymphoma). However, overexpression of HDAC8 as a fusion protein with GFP resulted in a homogeneous localization in the whole cell. The divergent profile led us to investigate the movement pattern change. Here we present the photo conversion of mOrange fused to HDAC8. Using Confocal Laser Scanning Microscopy (CLSM) as the imaging and detecting technique enables the time-resolved observation of potentially HDAC8 shuttling events. Laser irradiation at 488 nm results in an emission wavelength shift from 514 to 633 nm.
In conclusion, we observed a strong impact of the applied method on the preferred HDAC8 localisation strongly implying an essential functionality for HDAC8 in both compartments. Moreover, we measured for the first time the shuttling kinetics of a HDAC8 fusion protein between cytoplasma and nucleus.

References

[1] Wolfson, N., Pitcairn, C., Fierke, C. HDAC8 substrates: Histones and beyond. Biopolymers Volume 99, Issue 2, 112-126 (2013)

FTP22

Structure and functional aspects of *Rhodobacter sphaeroides* Cryptochrome B

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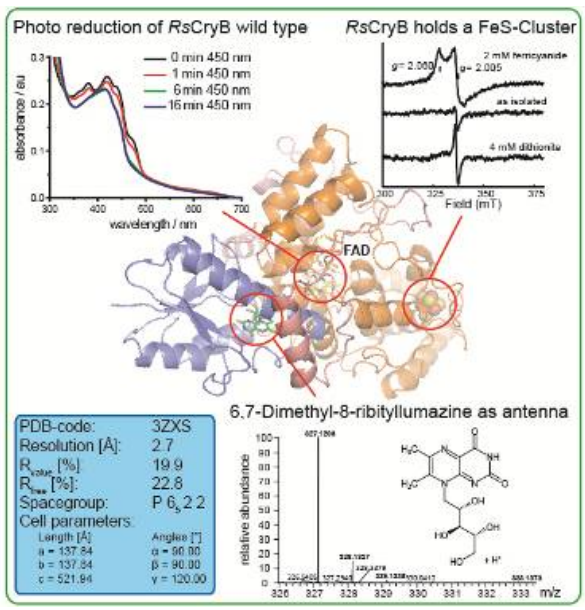
Cryptochromes are blue light photoreceptors highly related to DNA photolyases. Together they form the photolyase/cryptochrome superfamily (pcsf). They are responsible for a variety of photo responses in eukaryotes. Despite these important functions in eukaryotes there are only few cyanobacterial cryptochromes known with assigned signalling functions.[1]
Recently Cryprochrome B (*Rs*CryB), a photoreceptor from *Rhodobacter sphaeroides*, was shown to interact with nucleic acids and to trigger responses to blue light and oxidative stress.[2] *Rs*CryB defines a separate clade of proteobacterial cryptochromes (crypro) inside the pcsf. Biochemical analyses showed a [4Fe-4S]-cluster in the C-terminal region of *Rs*CryB, with a conserved binding motif in the crypro-family. The X-ray structure was solved at 2.7Å resolution and showed interesting new features around the binding moiety of the FAD chromophore which distinguish the crypro-family from the pcsf: (I) A water molecule next to the N5-nitrogen of the FAD is important for the photochemistry of *Rs*CryB. (II) Two tyrosines next to the FAD form the initial point of the photoreduction cascade, which takes a novel route via two tryptophans. Mutagenesis analysis showed that these two tryptophans are essential for photoreduction, in contrast to the tyrosines.[3]
Through the structure and analysis of *Rs*CryB we gathered deeper insights into the molecular mechanisms of this photoreceptor. Further studies focus on identifying possible *Rs*CryB interaction partners and their relevance *in vivo*.

References

[1] Chaves I., et al., *Annual review of plant biology* 62 (2011): 335-364.
[2] Hendrischk A-K., et al. *Molecular microbiology* 74 (2009): 990-1003.
[3] Geisselbrecht Y., et al. *EMBO reports* 13 (2012): 223-229.



FTP23

N^{α}-terminal protein acetylation by NatA is essential in Arabidopsis and confers tolerance to drought.

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N^{α}-terminal protein acetylation (NTA) is one of the most common protein modifications in eukaryotes, affecting up to 90 % of Arabidopsis and human cytosolic proteins. Despite the high abundance of NTA, the overall biological function of this modification is still enigmatic. NTA is known to alter the stability, activity, association and localization of some proteins *in vitro*. In yeast, NTA can serve as a built-in quality control mechanisms that marks misfolded proteins for degradation upon stress. NTA occurs in a co-translational manner and is catalyzed by N^{α}-terminal acetyltransferases (Nats), which are composed of a catalytic and an auxiliary subunit. Regarding the number of predicted target substrates, NatA is the main Nat complex in yeast and human. Database analyses reveal that the plant *Arabidopsis thaliana* contains orthologous for all subunits of all Nats. Isolation of T-DNA insertion lines that represent loss-of-function mutants for both subunits of NatA show an arrest at the globular stage of embryo development. This points to significant functions of NTA during embryogenesis in *A. thaliana*. An artificial microRNA approach allows to specifically down-regulate NatA activity to overcome the embryo-lethal phenotype and to investigate further functions of NTA during the vegetative and generative growth phase. To challenge a possible NTA quality control mechanism in Arabidopsis the NatA depleted mutant plants are analyzed during drought stress. Interestingly, the reduction in NatA activity results in an increased tolerance to drought. The transpiration rate of leaves from NatA depleted mutants is significantly reduced and stomata aperture observation shows a decrease in the stomatal opening of NatA knock-down mutants compared to wild type. Both together can explain at least partially the higher drought tolerance of the NatA knock-down mutants.

FTP24

Significance of N^{α}-Acetyltransferase B During Plant Development and Stress

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N^{α}-terminal protein acetylation (NTA) affects up to 50 % of cytosolic proteins in yeast and up to 90 % in human and Arabidopsis. It has been shown to influence localisation, activity, association and stability of proteins. In yeast this modification is catalysed by three major N^{α}-acetyltransferases, NatA, NatB and NatC. All three complexes differ in their composition of subunits as well as in their substrate specificity. NatB consists of the GNAT-type catalytic subunit Naa20p and the ribosome anchoring subunit Naa25p which allows a co-translational mode of acetylation.
The NatB complex seems to be conserved from yeast to Arabidopsis regarding substrate specificity and subunit composition. Although in plants the biological relevance of NTA is still enigmatic, its importance is illustrated by the retarded growth phenotype of *Atnaa20* and *Atnaa25* loss-of-function mutants. Even though viable and fertile, they hardly reach 60% of wild type size and show slightly twisted rosette leaves. The significance of NTA under non-stressed conditions in combination with the high number of substrates may indicate that NTA participates in fundamental protein biochemistry. In fact, the adaptation to stress situations includes a rapid alteration of the proteome in order to fulfil the new requirements. Interestingly a loss of NatB leads to a sensitivity of plant development towards different kinds of stresses including osmotic stress and DTT induced reductive stress. Remarkably, NatA and NatC mutants behave like wild type under these conditions, which hints towards different stress related roles of the major Nat complexes. Summarised, this indicates a function of NTA not only during normal plant development but also in counteracting specific stress situations.

FTP25

Insight into the hetero-oligomeric interaction of ΔNp63α and TAp73 by structural and functional analysis

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p63 and p73 are homologs of the well-known tumor suppressor protein p53. Some of their isoforms have been shown to be important for DNA damage control, although others have different functions e.g. ΔNp63α plays a role in epithelial differentiation.
The coexpression of p63 isoforms lacking the TA domain and proapoptotic p73 isoforms has been shown to occur frequently in squamous cell carcinomas (SCC). ΔNp63α effectively suppresses the transactivation properties of p73 isoforms by either direct promoter binding, thus blocking p73 binding, or direct interaction with p73 through hetero-oligomerization via the highly homologous tetramerization domain (TD). As we have shown previously, the isolated TDs of both p63 and p73 possess a strong tendency to form hetero oligomers in vitro, accounting for up to 40%of all tetramers in.
By introduction of several mutations into the TD of ΔNp63α we were able to construct proteins which can no longer interact with p73 isoforms in vitro and in vivo while retaining their normal tetrameric form. This allows us to study the properties of p73 isoforms in presence of ΔNp63α while effectively removing the suppression of p73 transactivation due to direct p73/p63 interaction.
Furthermore we show that a single point mutation in each TD is sufficient to stabilize the interaction between both proteins in vitro, shifting the equilibrium to nearly 100% of heteromers. This enabled us to solve a solution NMR structure of the complex between both TDs. We will elaborate why there is a preference towards the formation of hetero-TDs between p63 and p73. Suppression of hetero-TD formation and therefore partial restoration of p73 transactivation ability could prove beneficial in SCC patients, due to diminished cancer cell survival.

FTP26
Analysis of p63 mediated oocyte regulation upon γ-irradiation induced DNA damage

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TAp63α, the longest isoform of p63, belongs to the p53 transcription factor family and is a key player in maintaining the genomic integrity of the female germline. Once activated by γ-irradiation induced DNA-double strand breaks, it is known to be responsible for elimination of damaged oocytes. Surprisingly, the protein level of TAp63α in undamaged oocytes is already strikingly high, suggesting the need for a tight regulation mechanism. In a diverse set of experiments we could previously demonstrate that TAp63α is kept in an inactive dimeric conformation maintained by an inhibitory domain-domain interaction network until disrupted by DNA-damage induced phosphorylation. Here we could show that activation of TAp63α is characterized by a tight dose response relationship. Irradiation with high dosages of γ-irradiation leads to a faster activation of TAp63α. The timeline of TAp63α induced apoptosis and the caspase dependency was analyzed in detail by immunohistochemistry using TUNEL staining as well as activated caspase-3 staining. Furthermore a combination of RNA sequencing (RNA-seq) and chromatin-immunoprecipitation and sequencing (ChIP-seq) was used to characterize the complete transcriptome and the DNA-binding sites of TAp63α upon DNA damage induced activation. Taken together, oocyte transcriptome analysis, TAp63α-DNA binding data, as well as the characterization of TAp63α´s oligomeric state and the timeline of apoptosis illustrate TAp63α´s pivotal role in response to DNA damage in oocytes.

FTP27
Purification and Kinetic Studies of Pectinases from *Aspergillus niger* Isolated from Hot Water Sources

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Fungal species of the genus *Aspergillus* are filamentous ubiquitous saprophytes that are very important at industrial enzyme production (Adav et al., 2013). *Aspergillus niger* is used for the industrial production of pectinases.
Pectinases are today one of the most important enzymes at industrial scale (Kashyap et al., 2001). They are used at fruit juice industry, coffee and tea fermentation (Jayani et al., 2005). Analysis of extracellular biomass degrading enzymes producing by fungi with using agro-industrial residues is very important due to its environmental benefits.
In this study, pectinases were produced in submerged culture containing agro-industrial residues by *Aspergillus niger* . Pectinases were purified with $(NH_4)_2SO_4$ fractionation, gel filtration and ion-exchange chromatography and kinetic parameters were determined.

Keywords
Pectinases, *Aspergillus niger*, Gel filtration chromatography, Ion-exchange chromatography.

References
Adav, S. S., Ravindran, A., Kwan, S.S., 2013. Proteomic Analysis of Temperature Dependent Extracellular Proteins from *Aspergillus fumigatus* Grown under Solid-State Culture
Condition. Journal of Proteome Research, 12, 2715-2731.
Kashyap, D.R., Vohra, P.K., Chopra, S., Tewari, R., 2001. Applications of pectinases in the commercial sector: a review. Bioresource Technology, 77, 215-227.
Jayani, R. S., Saxena, S., Gupta, R., 2005. Microbial Pectinolytic Enzymes: A review. Process Biochemistry, 40, 2931-2944.

FTP28
Role of microvesicle-associated CD30 in the crosstalk between $CD30^+$ Hodgkin cells and $CD30L^+$ cells in the lymphoma microenvironment

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The microenvironment of non-transformed cells plays a crucial role in the establishment and progression of the tumor. In Hodgkin lymphoma (HL) a few $CD30^+$ tumor cells (HRS) cells are isolated by regulatory T cells but essentially supported by non-adjacent immune cells that express the ligand for the CD30 receptor (CD30L; CD153), such as mast cells, macrophages, eosinophils and neutrophils.
In this work we investigate the CD30/CD30L interaction between tumor and immune cells *in trans*. HL cell lines released full-length CD30 on microvesicles. The vesicle fraction contained a mixture of 40-100 nm exosomes and larger budding vesicles. These vesicles and CD30 on artificial particles were functional and able to stimulate a CD30-dependent release of IL-8 in granulocytes. In 3D co-culture experiments CD30 vesicles were released and guided by a network of HL cell-derived tubules towards $CD30L^+$ mast cells, where they caused a polarization of CD30L. A similar CD30 vesicle network was detected in HL tissue. This network may provide a functional link between tumor and distant immune cells, thus supporting the proinflammatory microenvironment in HL.

FTP29
Purification of Pectin lyase and Determination of Molecular Weight

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Pectic substances are essential for plants primary cell wall and middle lamella (Hoondal et al., 2002). They are prominent in pollen tubes, where they control the structure and yielding characteristics of the cell wall at the growing apex of these rapidly expanding cells (Hepler, 2005).
Pectic substances need complex enzyme systems due to their complex structure. These enzyme systems are termed as pectinolytic enzymes. Pectinolytic enzymes are divided three main groups:
a) Protopectinases: degrade insoluble protopectin and give highly polymerized soluble pectin.
b) Esterases: responsible for the de-esterification of pectin
c) Depolymerases: catalyze the hydrolytic cleavage of the α-1,4-glycosidic bonds in the galacturonic acid parts of pectin (Jayani et al., 2005).
In this study, pectin lyase was purified with ammonium sulphate fractionation, gel filtration chromatography and ion-exchange chromatography, respectively. Proteins were analyzed on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel. Then, molecular weight of protein was determined.

Keywords
Pectin lyase, $(NH_4)_2SO_4$ fractionation, Gel filtration chromatography, Ion-exchange chromatography.

References
Hoondal, G.S., Tiwari, R.P., Tewari, R., Dahiya, N., 2002. Microbial alkaline pectinases and their industrial applications: a review. Appl Microbial Biotechnol, 59, 409-418.
Hepler, P.K., 2005. Pectin Methylesterases and Pectin Dynamics in Pollen Tubes. The Plant Cell, 17, 3219-3226.
Jayani, R. S., Saxena, S., Gupta, R., 2005. Microbial Pectinolytic Enzymes: A review. Process Biochemistry, 40, 2931-2944.

FTP30
Pectinolytic Enzyme Production by *Aspergillus niger* Isolated from Hot-Water and Evalution of Metal Ions Effect on Enzyme Activity

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Plant cell wall is composed by pectin, cellulose, hemicellulose and lignin which are most abundant polymers in nature. Many microorganisms degrade these substances by secreting pectinolytic and cellulotic enzymes (Kashyap et al., 2001).
Pectinolytic enzymes are one of the upcoming enzymes at industrial scale. Action mechanisms of these enzymes are shown in Figure 1 (Gummadi and Panda, 2002). Microbial productions of pectinases are prominent for many

decades. Many microorganisms such as bacteria, yeast and fungi can produce pectinases. They can be produced by these microorganisms with different carbon sources (Gummadi and Panda, 2002).
In this study pectic enzymes were produced with different carbon sources in submerged culture. Then, they were purified with 3 steps. Effects of several chemical substances like ascorbic acid, $MnSO_4$, $ZnSO_4$ were evaluated.

Keywords
Pectinases, Purification, Metal ions.

References
Gummadi, S.N., Panda, T., 2002. Purification and charecterization of microbial pectinases-a review. Process Biochemistry, 38, 987-996.
Kashyap, D.R., Vohra, P.K., Chopra, S., Tewari, R., 2001. Applications of pectinases in the commercial sector: a review. Bioresource Technology, 77, 215-227.

FTP31
Highly retarded thermal *cis*-to-*trans* relaxation of an azobenzene-based photoswitch enables photocontrol of the enzyme activity of a histone deacetylase-like amidohydrolase from *Bordetella*/*Alcaligenes* strain FB188 by a single light pulse

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The photocontrol of specific enzyme reactions by use of an external stimulus provides novel and promising opportunities for consecutively triggered biotechnological applications. In particular, the regulation by light offers precise temporal and spatial control of the defined enzyme reaction. In this regard, azobenzene and its derivatives feature well defined properties to act as robust photoswitches. Azobenzene can be converted to its ***cis***- and ***trans***-state reversibly, via irradiation by ultra violet or visible light, or by thermal relaxation of the less stable ***cis***- to the more stable ***trans***-state in a time scale from milliseconds to hours and days. Especially, the thermal ***cis***-to-***trans*** relaxation process of azobenzene derivatives plays an important role and characterizes highly efficient photoswitches. In this work, the thermal ***cis***-to-***trans*** relaxation of an azobenzene-based photoswitch (4-phenylazomaleinanil; 4-PAM) conjugated to single cysteine variants of a bacterial histone deacetylase-like amidohydrolase (HDAH) from ***Bordetella***/***Alcaligenes*** strain FB188 was investigated. The thermal ***cis***-to-***trans*** relaxation of the covalently attached azobenzene moiety was highly retarded with respect to unbound 4-PAM allowing to maintain the thermodynamically less stable ***cis***-state after a single light pulse. The highly retardation of the thermal relaxation process is apparently caused by stabilization of the ***cis***-state due to interactions with adjacent hydrophobic amino acids at the HDAH surface leading to a half-life period of several hours. In contrast to unbound 4-PAM the ***cis***-to-***trans*** relaxation kinetic of the attached azobenzene to the HDAH surface had to be fitted to a two exponential model with a dominating slow process.

FTP32
Identification of threonine and tyrosine residues important for human P2X4 receptor activity by site-directed mutagenesis.

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The P2X4 receptor is an ATP-gated cation channel that is composed of three subunits. Each subunit has two transmembrane domains linked by a large extracellular loop and intracellularly located N- and C-termini. The receptors have been implicated in the modulation of membrane excitability, calcium signaling, neurotransmitter and hormone release, and pain physiology. We have studied the participation of highly conserved aminoacids residues located intracellularly in the N and C temini of P2X4 subunits as a critical determinant for receptor activation by using site-directed mutagenesis and electrophysiological characterization of recombinant human P2X4 receptors transiently expressed in HEK-293T cells. We have found that the mutant receptors P2X4E14A, and D16A exhibited properties not different from wild-type P2X4 receptors. However, in contrast, substitution of alanine for Tyr^{15}, and Thr^{17} produced non-functional receptors expressed at the plasma membrane. Flow cytometry analysis in the presence of an antibody against phosphotyrosine residues indicated that, among the cells that express the P2X4 receptor, the percentage of phosphotyrosine-positive cells was the same for Y372A (86 ± 10%) and Y378A (79 ± 6.9%) mutants, however, substantially lower for Y15A (35 ± 12%), Y367A (48 ± 6.4%) and Y372F (31 ± 1.7%) mutants when compared with cells expressing the wild-type receptor (76 ± 5.6%). Western blot assays revealed that the T17A mutant was phosphorylated at threonine residues, suggesting that the human P2X4 receptor also contains further phosphorylation sites. However, no phosphotyrosine-antibody signal was detected in the wild-type receptor and mutants in which tyrosine residues were replaced by alanine or phenylalanine. The present work indicates that tyrosine phosphorylation of intermediate signaling proteins regulates P2X4 receptor activity.

FTP33
Milk Adulteration - Problems and Remedies

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Introduction
Milk contains 3.4% protein, 3.6% fat, and 4.6% lactose, calcium, potassium, magnesium, iodine and various vitamins. India is the largest producer of Milk in the world followed by US.

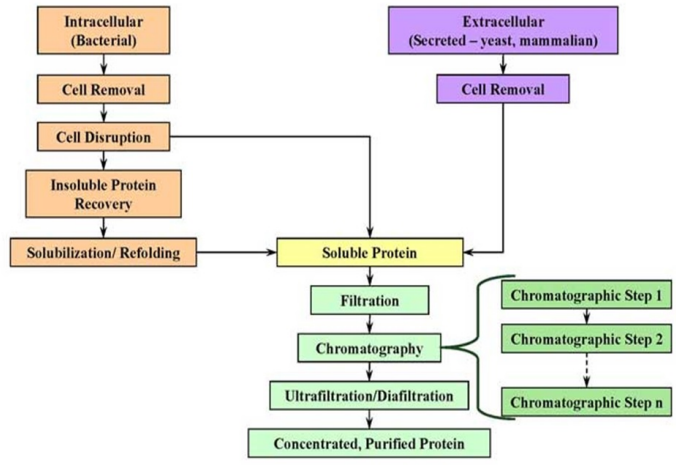
Harmful effects of Adulteration
The detergent in milk can cause food poisoning and other gastrointestinal complications. Urea is particularly harmful for the kidneys. Formalin can cause more severe damage to the body like liver damage The health impact of drinking milk adulterated with these chemicals is worse for children.

Objectives
Selection of anti-microbial proteins from milk Use of biotechnology to develop potent anti-microbial recombinant proteins Commercial Production Use of such proteins to prevent spoilage of milk Thereby, preventing adulteration with lethal compounds like Urea Eliminating the problem at the grass root level

Selection & Spectrum of Anti Microbial Agents
Lactoferrin: Gram positive, gram negative, enveloped virus, naked & enveloped virus, yeast and fungi, etc Lactoperoxidase: Depending upon the pH of the system, the mode of action is recruited. For medium pH systems, Gram negative bacteria is targeted while Gram positive for higher pH. Lysozyme: Major food borne Gram negative bacteria (like Salmonella sp.) N-Acetyl-ß-D-glucosamindase (NAGase): Wide bacteriocidal functions

Challenges to be considered for Revombinant Proteins
Protein Size
Protein Complexity
Post translational Modifications
Protein Aggregation
Refolding
Proteolytic degradation
Disulfide bond generation
Contamination

What we aim to do with Recombinant Proteins?
Identification of the active domains of the four molecules Constructing a supermolecule which should express all the four active domains Producing the supermolecule through a suitable host.



FTP34

Functional analysis of dopaminergic reprogramming factors designed for protein transduction in neural reprogramming

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The dysfunction or loss of meso-diencephalic dopaminergic neurons is associated with several neuropsychiatric disorders and Parkinson's disease. Mouse and human fibroblasts can be converted into functional dopaminergic neurons by viral-based expression of the three transcription factors Lmx1a, Mash1 and Nurr1 (Caiazzo, M., *et al*., Nature 476, 224-227, 2011). Viruses cannot be used in cell replacement therapies for humans because of their adverse effects but protein transduction could be an alternative approach. Protein transduction comprises the cellular uptake of a heterologous expressed protein with the help of a fused cell penetrating peptide like the basic peptide TAT (trans-activator of transcription).
Here, we show the expression of the three human transcription factors Lmx1a, Mash1 and Nurr1 as N-terminal fusion proteins to a His-tag, the TAT-domain and a nuclear localization sequence obtaining HTN-Lmax1a, HTN-Mash1 and HTN-Nurr1. The cellular uptake of these proteins was investigated. Furthermore, we verified the biological activity of the transduced transcription factor proteins by quantifying the mRNA levels of their respective downstream targets by real-time qPCR in secondary cell lines and their functional efficacy in promoting fate changes in primary neuronal and neural stem cell cultures.
In summary, we show the generation of cell penetrating transcription factors proteins for cellular reprogramming.

FTP35

Preparation and characterization of Gadolinium-loaded PLGA nanoparticles as magnetic contrast agents for diagnosis of hepatocellular carcinoma

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Human hepatocellular carcinoma (HCC) is one of the most common malignancies and major causes of cancer-related death. It is of great importance to achieve an early diagnosis of HCC. Gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) is extensively used as a potential magnetic resonance imaging (MRI) contrast agent for cancer diagnosis. Biodegradable PLGA nanoparticles loaded with Gd-DTPA were prepared for this perpose. Gd-based PLGA nanoparticles with additionally encapsulated rhodamine B (PLGA-(Gd-DTPA)-Rh) were developed to combine the visualization of HCC with MRI and fluorescence spectroscopy. Different ratios of lactide to glycolide prepared by a double emulsion solvent evaporation method and polyvinyl alcohol as a surfactant were employed. The optimum formulations for the polymer, Gd-DTPA, and surfactant concentration were 9.1 mg/mL, 10 mg/mL, and 1 mg/mL, respectively at a homogenization speed of 17,000 rpm yielded 34.7 ± 0.7 mg/mL particle content and 274.5 ± 1.9 nm particle size. With the Gd-chelated PLGA nanoparticles, an in vitro cell uptake study revealed intracellular uptake of nanoparticles into the HCC cell line. Furthermore, a MRI contrast enhancement was observed in tumor tissue of c-myc-transgenic mice bearing endogenously formed HCC. This study demonstrated the efficacy of PLGA-(Gd-DTPA)-Rh nanoparticles as an effective MRI contrast agents for the detection of HCC.

FTP36

Spontaneous chromosomal instability related to aging and gender

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Data from the literature confirms that spontaneous chromosomal instability increases with age, but correlation between spontaneous chromosomal instability and gender are controversial. The aim of our study was to quantify the effect of aging and gender on spontaneous chromosomal instability measured by micronucleus (MN) frequency in human peripheral blood lymphocytes.
Twenty nine healthy newborns (12 female and 17 male) and 19 healthy persons age over 80 years (85.18 ± SD 3.33) (13 female and 6 male) participated the study. For evaluation of MN frequency citokinesis-blok method -Fenech and Morley modification was used. DNA concentration in the peripheral blood was measured spectrophotometrically.
Mann- Whitney U - test revealed statically significant differences in MN frequency (p<0.001), concentration of DNA extracted from their peripheral blood (p=0.007), and number of nuclear buds (p=0.058) between analyzed samples of newborns and persons above 80 years of age. Statistically significant difference in the frequency of nucleoplasmic bridges between analyzed samples (p=0.839) was not found. People above 80 years of age had significantly higher values of MN frequency and number of nuclear buds, but lower concentrations of DNA were isolated from their peripheral blood. Analysis of covariance showed that MN frequency is related with age (p=0.001) but not with gender (p=0.269), related with nuclear buds (p=0.026) and nucleoplasmic bridges numbers (p=0.006).
Key words: micronucleus frequency, age, gender, DNA concentration, peripheral blood lymphocytes

FTP37

Haldane's substrate inhibition model: A reinterpretation as a stochastic service-interrupting system

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Substrate inhibition is a universal and long-known phenomenon in enzymology. To our knowledge the first (and still widely used) mathematical model for its explanation has been put forward by Haldane (1930) who supposed the reaction: ES (active) + S → ESS (inactive); when reaction velocity is plotted against the logarithm of substrate concentration, a strictly symmetrical bell-shaped curve is obtained. Haldane's mathematical treatment is based on classical mass-action theory, whereas we apply a more general, mass-service based theory (Kühl & Jobmann, 2006, 2007). Replacing mass action by mass service ensues that, for instance, nonexponential interevent times and the various possible modes of interrupting the ongoing "service" in ES are taken account of. Excess substrate molecules are considered as interruptors. By using stochastic matrix-analytic methods we have defined the stochastic conditions and diverse interruption scenarios that either genuinely reproduce Haldane's result (i.e. a symmetrical bell curve) or give rise to more or less "deformed" bell curves, e.g. asymmetrical ones or those whose maximal reaction velocity paradoxically exceeds that of an uninterruptible system. Our theoretical results open up new ways of explaining old experimental data.

References

Haldane JBS (1930) *Enzymes*. London: Longmans, Green and Company, pp. 84-85.
Kühl PW & Jobmann M (2006) *Journal of Receptors and Signal Transduction* **26**, 1-34.
Kühl PW & Jobmann M (2007) in: *Mathematical Modeling of Biological Systems, Volume I* (eds: Deutsch A et al). Boston: Birkhäuser, pp. 59-67.

FTP38

Quantitative proteome profile of Astrocyte reprogramming to neurons

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Meso-diencephalic dopaminergic (mdDA) neurons play a key role in cognition, motor control, arousal and motivation. Their dysfunction or loss is known to cause Parkinson's disease (PD), depression, schizophrenia and addiction. In efforts to treat PD, direct cellular reprogramming is a powerful tool for regenerative medicine. Caiazzo *et al*. showed the direct conversion of human and mouse fibroblasts into functional mdDA neurons by induced expression of the transcription factors Lmx1a, Mash1 and Nurr1 (Caiazzo, M. *et al*. Nature 476, 224-227, 2011). Astrocytes constitute the predominant cell type in the central nervous system, and apart from being neural in origin and which comparative ease in availability from brain invasive procedures. It is an ideal population for de-differentiation into mdDA neurons towards cell replacement therapies.
Here, we show for the first time a quantitative proteome profile of Astrocytes reprogrammed to neurons by transfection of the transcription factors Sox2, Lmx1a, Mash1 and Nurr1. The mass spectrometry data was obtained from an LTQ-Orbitrap Velos coupled to Proxeon nano-HPLC. Our preliminary data indicates a significant number of proteins, which are specifically expressed in astrocytes reprogrammed cultures. The biological activity of the transduced transcription factors was verified by analysis of downstream target mRNA by quantitative RT-PCR. Furthermore, we evaluated the expression of key candidate genes by immunocytochemistry and western blot.

FTP39
Bioactive Flavonoid Pigments From Yemeni Dragon's Blood with Therapeutic Effects

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From Dragon's blood (Loc. Called brother's blood) of Dracaena cinnabari, a medicinal Plant, growing endemically on Socotra Island of Yemen und is traditionally used for wide spectrum of human remedies, have been isolated. Towelf Flavonoid derivatives from ethylacetate extract seven biflavonoid structures and six triflavonoid pigments from alkaline methanol extract by repeated chromatographic methods including reverse phase and lon exchange techniques.
Their structures were confirmed by MS. UV. ID- and 2D - NMR studies. The H- an C- resonances were quite assigned by using of ATP, HMQC, HMBC and cosy experiments. The most of the isolated compounds showed asignificant antimicrobial activities (against C.herbarium, C. albicans, S. aureus and E. coli) especially the Bi- and Triflavonoid Pigments with new structure types showed strong activity in the primary antibiotic screen. The antimicrobial activities were evaluated as minimum Inhibitory concentration (MIC). This interest resinous product has got several therapeutic effects and uses: antiulcer, antiviral, antitumor, anti-inflammatory, antiseptic and haemostatic as well as additive material in food and cosmetic industry. So it should be further examined for safely exploiting by means of most acceptable commercial application on natural basis consequently.

FTP40
Development of an Enzymatic *In Vivo* Redox Cascade for Asymmetric Synthesis

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One-pot syntheses are often used to improve the efficiency of chemical reactions. The reactants are subjected to at least two consecutive reactions in one reactor, so that the time-consuming isolation of intermediates is not necessary anymore. The biocatalytic equivalent of this synthetic strategy is an enzymatic cascade, where at least two enzymes catalyze subsequent reactions. These cascades can either be carried out using isolated enzymes, crude cell extract or whole cells expressing the desired enzymes recombinantly. In this work we present an *in vivo* redox cascade for asymmetric synthesis combining alcohol dehydrogenase, enoate reductase and a Baeyer-Villiger monooxygenase in *E. coli* BL21 (DE3). This combination of enzymes from different origins led to a modular and flexible cascade, so that we were able to convert a diverse set of substrates into a range of enantiopure lactones.

FTP41
Polymorphism of SOD2 Ala/Val gene in patient with multiple sclerosis

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Multiple sclerosis (MS), a prevalent inflammatory disease of the central nervous system with autoimmune component is associated with some genetic risk factors. However, with a large proportion of disease heritability, the list of genes and their genetic causal alleles is still incomplete. Because of its autoimmune component, genetics variants of enzymes of oxidative stress could be of great interests. The aim of our study was to test if polymorphism of SOD2 Ala/Val gene is related to multiple sclerosis and if TNF-308α polymorphism in MS is related to SOD2 Ala/Val gene.One hundred thirty nine (78 patients with multiple sclerosis and 61 healthy control) were included in the study. Statistically significant differences in SOD2 Ala/Val genepolymorphism between patients with MS and healthy controls was found (Contingency Coefficient 0.475, p<0.001). 83.3% of patients with MS have SOD2 Ala/Val variant. 59.0% of healthy controls have SOD2 Ala/Ala variant. SOD2 Val/Val variant is 4.8 times and SOD2 Ala/Val is 2.1 times more frequent in patients with MS than in healthy controls. SOD2 Val/Val genotype could be more important for MS. Relation in polymorphism of SOD2 Ala/Val variants and TNF-308α polymorphism in-patient with MS were not found.
Key words: multiple sclerosis, SOD2 Ala/Val gene, TNFα gene, polymorphism

FTP42
Isolation of Polyketides Producing Actinomycete Isolates v*ia* PCR-Based Genome Screening for *PKS* Gene

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Polyketides include macrolides, tetracyclines and polyenes, a diverse family of secondary metabolites with diverse biological activities and pharmacological properties; produced through polyketide synthase pathway. As a part of a research program whose aim is to assess the antibiotic biosynthetic potential of the actinomycete isolates. The actinomycete isolates genomic DNA was screened by PCR for the polyketide biosynthetic gene cluster. In this study 341 bacterial isolates were screened by *PKS*-specific PCR. Twenty-four were characterized based on the presence of the expected size of the PCR-amplified DNA fragment in the genome. The nucleotide sequencing of the PCR-amplified DNA fragments showed that each of the 24 bacterial isolates contained the polyketide gene. Phylogenetic analyses using the *PKS* gene nucleotide sequences were conducted. Culture extracts from 4 of these isolates showed a typical polyketide-like high-pressure liquid chromatography (HPLC) chromatogram profile, and also strong antibacterial activity against the test organisms.
PCR screening of the genomes of actinomycetes for specific antibiotic biosynthetic gene clusters allows for the rapid determination of the antibiotic biosynthetic potential of these actinomycete isolates. This also gives advanced knowledge of the type of antibiotic(s) to expect and the appropriate methods of antibiotic purification to apply.

Keywords: actinomycetes; polyketides, *PKS* gene; antibacterial activity

FTP43
Absence of GSTT1 gene deldtion among diabetic retinopathic patients

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Objectives
Diabetic retinopathy (DR) is a common and specific microvascular complication of diabetes, and remains the leading cause of preventable blindness in working-aged people. Pathogenesis of the disease is extremely complex involving many different genes and environmental factors. Glutathione-S-transferases (GSTs), are members of a multigene family that work as one of the endogenous antioxidants in human. The aim of this study was to evaluate the association of GSTT1 gene deletion with diabetic retinopathy.

Methods
The study included 80 patients with DR and 80 healthy volunteers. Genomic DNA was extracted from peripheral blood leukocytes. Genotypes were determined by polymerase chain reaction (PCR). Statistical analysis was performed using the MedCalc program for Windows version 12.

Results
The genotype frequencies did not differ significanthy between diabetic retinopathy and control samples (P>0.05).

Conclusion
The results from this study suggest that the GSTT1 null genotype does not confer susceptibility to diabetic retinopathy..
Key words: Diabetic retinopathy, GSTT1, Gene deletion

FTP44
Nature and Position of the Binding Modules are Important for the Activities of Cellulases and Xylanases

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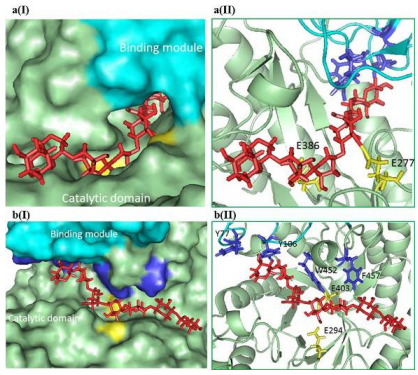
Glycosyl hydrolases commonly contain the carbohydrate binding modules (CBMs), through which they bind to the substrates thus facilitating the catalytic domain (CD) to act. However, the role of the CBMs in the activities of cellulases and xylanases seems quite varied. For example, in the case of the endoglucanase CelA of *Clostridium thermocellum* the binding module CBM3a when attached to the n-terminal of the CD is more than twice as active as the variant having the binding module attached to the c-terminal of the CD. In the case of xylanase XynZ of *C. thermocellum* deletion of CBM6, which is found in association with the CD in the native state, enhances the activity manifold. However, the addition of CBM22 to the CD enhanced the activity. Molecular modeling study showed that in the case of CelA, n-terminal attachment of the CBM allows favorable orientation of the binding residues of the CBM and the active site residues of the CD, but not when the CBM is attached to its c-terminal. In the case of XynZ, CBM6 seems to create a closed tunnel structure on attachment to the catalytic domain, making more difficult for the substrate to interact with the active site residues located inside the tunnel. CBM22 on the other hand on attachment with the CD produces a more open structure allowing accessibility of the substrate

and its breakdown. Thus the CBMs are not only specific for binding to the substrate but their orientation in association with the CD is also important for activity.



Relative positions of binding module (cyan) and catalytic domain (green) of XynZ-BC (a) and XynZ-B'C (b).

FTP45

Differences between akirin protein levels in imatinib resistant and sensitive K562 cell lines

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Akirin is a newly discovered highly conserved protein which is thought to function in immune response pathway. It has been thought that akirin regulates the transcription of NFκB dependent genes critically and it binds to NFκB via an undefined protein. It has been shown that NFκB activity increases in solid cancers, myeloid leukemia and chronic myelogenous. İmatinib resistant K562 cell line has higher NFkB activity than imatinib sensitive K562 cell line. Chronic myeloid leukemia (CML) is a malign hematopoietic stem cell disease which is characterised by the Philadelphia chromosome created by t (9;22) (q34;q11), and in which malignant leukemic cells replace the normal bone marrow and that results in fusion of BCR and ABL genes.
In this study, we investigated the differences of akirin protein expression between imatinib sensitive and resistant K562 cell lines. For this purpose, we performed western blot experiments in cytosols of K562 imatinib resistant and sensitive cells. We found that akirin protein level is significantly lower in imatinib resistant K562 cells than imatinib sensitive K562 cells. It can be concluded that akirin protein may play important roles in developing imatinib resistance.

FTP46

Design of novel artificial metalloenzymes based on semisynthetic protein assembly using the RNase S system

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The main idea behind artificial metalloenzymes is the combination of chemical transition metal catalysis with the more eco-friendly enzyme catalysis. Therefore several strategies have been employed, where a non-native metal cofactor is anchored into a macromolecular scaffold. Strategies for designing hybrid catalysts include dative, covalent and supramolecular anchoring of the metal cofactor to a macromolecule (Heinisch & Ward, 2010). Frameworks based on cofactor-protein interactions, including the intensively studied systems based on the streptavidin technology, peptides or DNA have been exploited (Creus & Ward, 2007; Lu, Yeung, Sieracki, & Marshall, 2009; Rosati & Roelfes, 2010; Wilson, Whitesides, 1978). Still, the search for novel protein scaffolds feasible for the design of bio-inspired transition metal catalysis gains much attention (Köhler, Wilson, Lo, Sardo, & Ward, 2010; Lu et al., 2009; Rosati & Roelfes, 2010).
We intent to expand the field of artificial metalloenzymes by building semisynthetic enzymes based on the ribonulcease S scaffold (RNase S). RNase S is the proteolysis product of the well characterized ribonuclease A (Raines, 1998). The RNase S is a unique system consisting of the S-peptide part (aa 1-20) and the S-protein part (aa 21-124). The S-protein can be purified from the RNase S system. Afterwards the RNase S complex can be reassembled from a synthetic S-peptide and the purified S-protein. The synthetic S-peptide contains non-canonical amino acids for creation of a transition metal center

FTP47

Therapeutic proteins with extended plasma half-life via PASylation: prolonged action of the satiety hormone leptin

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Leptin, a 16 kDa peptide hormone, plays a central role in the control of metabolism and appetite. However, due to its small size leptin is rapidly eliminated by renal clearance; this would require daily injections for therapy. We have engineered mouse leptin with an expanded hydrodynamic volume using the PASylation technology, which involves the genetic fusion with a nucleotide sequence encoding a conformationally disordered polypeptide comprising Pro, Ala and/or Ser (PAS) residues. Size exclusion chromatography and dynamic light scattering indicated a 22-fold enlarged hydrodynamic volume for PAS(600)-leptin if compared to the unmodified recombinant protein. High receptor-binding activity of the PASylated leptin was confirmed both in Biacore measurements (K_D = 4.2 nM) and in cell culture-based dual-luciferase assays (EC_{50} = 6.7 nM). Pharmacokinetic studies revealed a plasma half-life of 19.6 h for the PAS(600)-leptin. The *in vivo* activity of PAS(600)-leptin was investigated in C57BL/6J mice that were injected i.p. with a single dose of the leptin variant. A considerable reduction in food consumption and also body weight of up to 10 % indicated the high pharmacodynamic efficacy of the PASylated leptin. This effect was approximately tenfold stronger than for the unmodified protein, demonstrating the applicability of PASylation as a promising alternative to PEGylation for generating therapeutic proteins with increased bioactivity by prolonging their plasma half-life.

FTP48

Characterization of subunit Q in *cbb*$_3$-type cytochrome *c* oxidase from *Pseudomonas stutzeri* ZoBell

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Cytochrome *c* oxidases (C*c*Os) are the terminal enzymes of the respiratory chain and are members of the heme-copper oxidase superfamily (HCO). C*c*Os catalyze the four electron reduction of molecular oxygen to water and couple this exergonic reaction to transmembrane proton pumping. The *cbb*$_3$-type cytochrome *c* oxidase features a distinctly different subunit composition as compared to family A und B CcOs [1]. Furthermore, the *cbb*$_3$-type oxidases are characterized by reduced proton pumping and a higher catalytic activity at low oxygen concentrations [2]. The presence of the *cbb*$_3$-type cytochrome *c* oxidase is considered to be essential for the pathogenicity of many bacterial species.
The genome of *Pseudomonas stutzeri* ZoBell contains two independent *cbb*$_3$-operons, encoding the 1. (CcoNOP) and 2. isoform (CcoNOQP*)* of the *cbb*$_3$-oxidase. Instead of *ccoQ* in operon 2, the operon 1 contains a small non-coding nucleotide sequence (NT) between *ccoO* and *ccoP*. We generated two deletion strains to replace each version of both chromosomal *cbb*$_3$-operons. These deletion strains were used for the homologous recombinant expression of the two *cbb*$_3$-isoforms. To investigate the structural and functional differences between both isoforms, several variants were produced with focus on *ccoQ* and the NT. After purification by affinity chromatography the variants were compared with the wildtypes by ultraviolet-visible (UV-vis) spectroscopy, blue native (BN)- and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), size exclusion chromatography and oxygen reductase activity measurements. The results show that the deletions of *ccoQ* and NT have an influence on a *b*-type heme in the binuclear-reaction center. In addition, the stability of the *cbb*$_3$-oxidase is reduced in strains without *ccoQ* and NT. Besides the bands referring to the three subunits, the SDS-PAGE of the *cbb*$_3$-wildtypes and the variants shows additional bands in the low molecular weight range, which are currently under investigation with electrospray ionization mass spectrometry (ESI-MS). The oxygen reductase activity is significantly decreased in the variants from strains without *ccoQ* and NT as compared to the wildtypes. For further studies and direct identification of subunit Q, antibodies will be generated.

References
[1] RS. Pitcher, NJ. Watmough, The bacterial cytochrome *cbb*$_3$ oxidase. Biochim Biophys Acta, 1655(1-3):388-99 (2004).
[2] O. Preisig, R. Zufferey, L. Thöny-Meyer, C. A. Appleby, H. Hennecke, A high-affinity *cbb*$_3$-type cytochrome oxidase terminates the symbiosis-specific respiratory chain of *Bradyrhizobium japonicum*. J. Bacteriol.**178**, 1532 (1996).

FTP49

Clinical Relevance of Serum Vascular Endothelial Growth Factor in Patients with Colorectal Cancer

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Aim
Certain biological factors play a role in the stimulation of primary cancer growth - angiogenesis and subsequent metastases. However, the clinical significance remains controversial in terms of prognosis of the disease. We evaluated the clinical significance of preoperative serum vascular endothelial growth factor in patients with colorectal cancer (CRC).

Materials and Methods
We analyzed preoperative levels of vascular endothelial growth factor (VEGF), CEA and CA 19-9. We detected the serum levels by ELISA in 66 patients with colorectal carcinoma. We measured the same serum factors in 40 healthy controls, too.

Results
Preoperative levels of VEGF in patients with carcinoma of the colon and rectum are significantly higher as the levels in healthy controls (P <0,001). There is a significant correlation with the levels of vascular endothelial growth factor in the cancer group patient's clinical stage (P <0,001). Serum levels of VEGF remain independent of the other, investigated, prognostic factors. Diagnostic accuracy is characterized with 83% specificity and 79% sensitivity respectively. We have determined cut off value of 240 pg / ml.

Conclusion
The preoperative levels of VEGF are noninvasive, statistically significant, prognostic indicator in patients with CRC This prognostic factor can be used in prediction of desease - free survival and overall survival in CRC.

FTP50

Hsp90 dynamics regulated by molecular switch points

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The conserved abundant molecular chaperone Hsp90 is essential for cell viability in eukaryotes. Hsp90 promotes protein folding and has an important role in activation and maturation of a wide range of proteins. To master its functions, Hsp90 transforms chemical energy, gained by ATP hydrolysis, into large conformational changes. The Hsp90 ATPase cycle is fine-tuned by a set of co-chaperones and further regulated by posttranslational modifications. Comprehensive structural and biochemical studies have contributed to our current knowledge of the mechanism of Hsp90. However, central issues such as the location of conformational switch points and the propagation of conformational changes through Hsp90 are still elusive. Here, we analyzed these possible switch points regarding their effects on conformational changes within Hsp90. To address the importance of these hot spots, we carried out a combination of mutagenesis and biophysical approaches. The Hsp90 mutants that showed effects *in vivo* and *in vitro* were further investigated by fluorescence resonance energy transfer and analytical ultracentrifugation, which allowed us to follow and determine the kinetics of the structural rearrangements within the Hsp90 dimer as well as complex formation of Hsp90 and certain co-chaperones.

GLYCOBIOLOGY OF HUMAN DISEASES

PKV18

Green beards, beer & Candidiasis: Fungal adhesion & its molecular base

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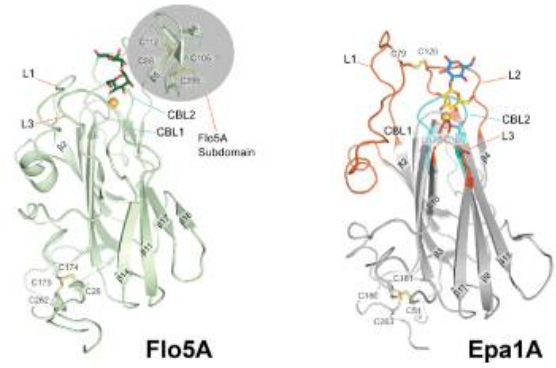
One hallmark of fungi is their unusual cell wall that provides not only a formidable physical barrier against host defense systems, but also the basis for fungal cell-to-cell communication. In *Saccharomyces cerevisiae* aggregation of yeast cells into protective flocs by self-recognition is mediated by a family of cell-surface adhesins, the flocculins (Flo). Although flocculation plays an eminent role in the food industry (e. g. for beer & wine), the mode of flocculin-mediated surface recognition and the exact structure of cognate ligands have remained elusive. We present structures of the adhesion domain of a Flo5 complexed to its cognate ligands derived from yeast high-mannose oligosaccharides at atomic resolution [1]. Besides a PA14-like architecture, the Flo5A domain shows a novel lectin fold that utilizes a unique *DcisD* calcium-binding motif for carbohydrate binding and is found from bacteria to Man. For host-cell adhesion the pathogenic yeast *Candida glabrata* utilizes 23 surface-exposed, epithelial adhesins (Epa), which are all related to *S. cerevisiae* flocculins. These adhesins recognize host glycans and discriminate between target tissues by their Flo5/PA14-like domains. We solved structures of the Epa1A domain complexed to different carbohydrate ligands. These data show how mucin-type O-glycans are recognized to different extent by the various Epa subtypes [2]. Further characterization of subtype-switched Epa1 variants reveals that specificity is governed by two inner loops, CBL1 and CBL2, involved in calcium binding, as well as by three outer loops, L1, L2 and L3.

References
[1] Veelders, M. et al. (2010). *PNAS* **107**: 22511-22516.
[2] Maestre-Reyna, et al. (2012), *PNAS* **109**: 16864-16869.



GLV01

Biomarker identification for Medullary Cystic Kidney Disease Type I (MCKD1) a chronic tubulointerstitial nephropathy

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The tubulointerstitial kidney disease MCKD1 is an autosomal dominant inherited disorder. Actually a frame shift mutation within the tandem repeat domain of the *muc1*- gene (Chr. 1q21) was identified to be the cause of the nephropathy. The *muc1*-gene encodes the type 1 transmembrane glycoprotein Mucin1 (MUC1) that is well characterized as a ubiquitously expressed protein on epithelial surfaces and overexpressed in a variety of carcinomas. In renal epithelium MUC1 is similar to the glycoprotein uromodelin (THP) shed/secreted into the urine and could potentially be targeted as a biomarker for early diagnosis of the disease. The frame shift mutation in the VNTR-domain (variable number of tandem repeats) of MUC1 induces the formation of a hybrid form with regular and variant repeat sequences that could be targeted immunochemically. The aberrant MUC1-hybrid is expected to enter the secretory pathway and to become glycosylated, however due to the use of an alternative-reading-frame-dependent stop codon a truncated mucin is formed that lacks the transmembrane domain. Preliminary data will be presented referring to early diagnosis on the basis of urinary MUC1 isoforms. Moreover, we present data of a differential proteomic study on urothelial membrane rafts that aim at the identification of other biomarkers associated with MCKD1.

GLV02

Polysialic acid is involved during “on/off mechanisms” of seasonal spermatogenesis

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The Roe deer are seasonal breeders and cyclic structural changes of their testis come along with a totally arrested (winter) and a highly activated spermatogenesis (summer). For this reason, roe deer represents an interesting

model to study general mechanisms of initiation and determination of spermatogenesis. We investigated if polysialic acid (polySia) - a linear homopolymer of α2,8-linked sialic acids which could act as a negative regulator of cell-cell adhesion - might represent one of the mediators during the activation and/or inactivation of spermatogenesis.
To address this point, testis samples of adult male roe deer were collected after castration. Intriguingly, we observed that polySia attached to NCAM was enhanced during the up regulation of spermatogenesis in April and polySia was predominantly located in the basal compartment between Sertoli and germ cells. In addition, polySia was highly expressed in December. Here, polySia was prevalently detectable between Sertoli cells and spermatogonia as well as in the adluminal regions of Sertoli cells.
Thus, polySia is expressed during key steps of the "on/off mechanisms" of seasonal spermatogenesis. However, the precise role of polySia during the up as well as down regulation of spermatogenesis needs to be further investigated.

GLP01

Development of a bioprocess for the high yield expression of glycoconjugated vaccines against *Shigella flexneri* 2a in *E. coli*

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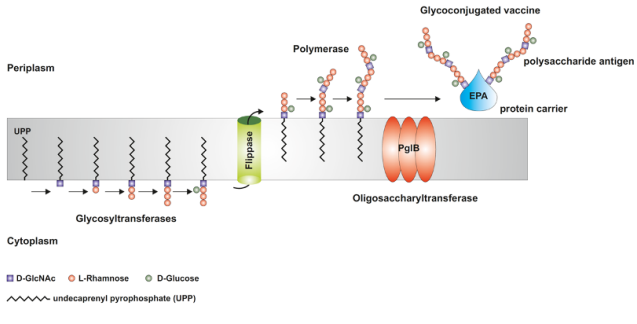
Shigellosis is a severe bacterial infectious disease. According to the WHO, shigellosis is a major public health concern worldwide with approximately 165 million annual cases leading to more than 1.1 million deaths. Vaccination is a powerful strategy to combat infectious diseases. Glycoconjugates composed of O-specific polysaccharides (O-PS) covalently bound to a recombinant, immunogenic protein carrier, have been demonstrated to be safe, immunogenic and efficacious. However, large scale production of these vaccines is challenging by the conventional chemical conjugation approach due to the labor intensive, expensive and complex process. Glycoconjugate vaccines can also be produced *in vivo* by appropriately engineered, non-toxic *E. coli* cells. The carrier protein is glycosylated with specific O-PS and processed in the periplasmic space. Factors and parameters influencing the transfer of the heterologous expressed oligosaccharides to the immunogenic carrier protein are still poorly understood. Therefore the aim of this project was to develop a cost-effective bioprocess for the expression of a glycoconjugated vaccine candidate against *Shigella flexneri* 2a, one of the clinically relevant *Shigella* serotypes. Critical parameters for glycoconjugate production were screened and identified in 96-deepwell plates using a Design of Experiment (DoE) approach and subsequently successfully validated and transferred to high cell density bioreactor fermentations hence increasing the overall yield. This bioprocess can serve as a blueprint for production of other clinically interesting *Shigella* serotypes and will presumably lead to the manufacturing of a cost-effective, multivalent *Shigella* vaccine in the near future.



GLP02

In-gel O-glycomics reveals structural conservation of O-Mannose glycans among vertebrates

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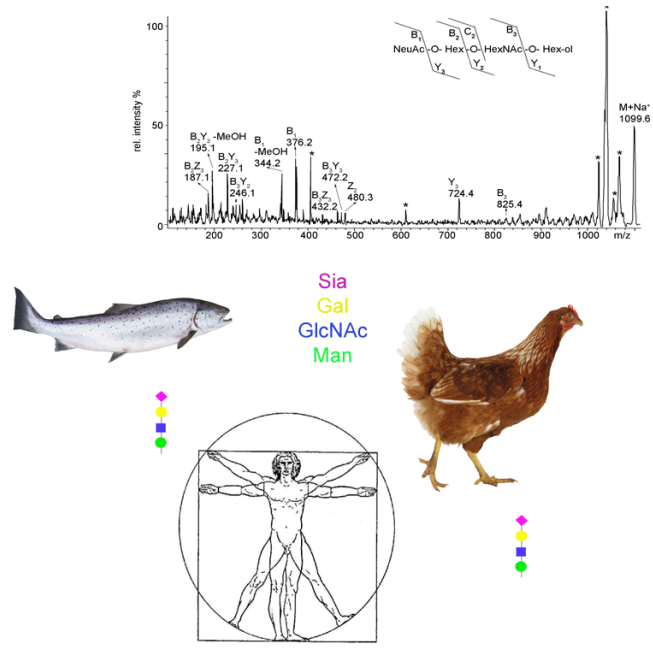
We developed a method to analyse O-glycans from proteins immobilized in a standard polyacrylamide gel matrix.To analyse O-glycans from in-gel protein samples of high complexity we applied a 3-phase-discontinuous SDS-gel. With this method proteins are enriched at the border between gel layers of different percentages while low-molecular mass impurities, like salts and detergents, either stay in the stacking gel or run through.Applying this method we analysed the complete brain O-glycoproteome of a variety of organisms with focus on O-mannose-based glycans. We found the linear O-mannose based tetrasaccharide and the brain-specific branched O-mannosyl heptasaccharide being structurally highly conserved. MALDI-MS/MS of the permethylated oligosaccharides revealed identical structures in organisms as different as fish and mouse, showing conservation among all vertebrate organisms analysed so far. This reveals an important but still to be discovered role of these unique structures.

Figure 1



GLP03

Evidence for core 2 to core 1 O-glycan remodeling during recycling of MUC1

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The apical transmembrane glycoprotein MUC1 is endocytosed to recycle through the *trans*-Golgi-network (TGN) or Golgi complex to the plasma membrane. We followed the hypothesis that not only the known follow-up sialylation of MUC1 in the TGN is associated with this process, but also a remodeling of O-glycan core structures, which would explain the previously described differential core 2- *vs*. core 1-based O-glycosylation of secreted, single Golgi passage and recycling membrane MUC1 isoforms (Engelmann *et al.*, 2005 Glycobiology 15, 1111-24). To address this novel observation we used recombinant epitope-tagged MUC1 (MUC1-M) and mutant forms with abrogated clathrin-mediated endocytosis (MUC1-M-Y20,60N) or blocked recycling (palmitoylation-defective MUC1-M-CQC/AQA). We show that theCQC/AQA mutant transits the TGN at significantly lower levels, concomitant with a strongly reduced shedding from the plasma membrane and its accumulation in endosomal compartments. Intriguingly, O-glycosylation of the shed MUC1 ectodomain subunit changes from preponderant sialylated core 1 (MUC1-M) to core 2 glycans on the non-recycling CQC/AQA mutant. The O-glycoprofile of the non-recycling CQC/AQA mutant resembles the core 2 glycoprofile on a secretory MUC1 probe that transits the Golgi complex only once. By contrast, the MUC1-M-Y20,60N mutant recycles *via* flotillin-dependent pathways and shows the wild-type phenotype with dominant core 1 expression. Differential radiolabeling of protein with [^{35}S]Met/Cys or glycans with [^{3}H]GlcNH$_2$ in pulse-chase experiments of surface biotinylated MUC1 revealed a significantly shorter half-life of [^{3}H]MUC1 when compared to [^{35}S]MUC1, while the same ratio for the CQC/AQA mutant was close to one. This finding further supports the novel possibility of a recycling-associated O-glycan processing from Gal1-4GlcNAc1-6(Gal1-3)GalNAc (core 2) to Gal1-3GalNAc (core 1).

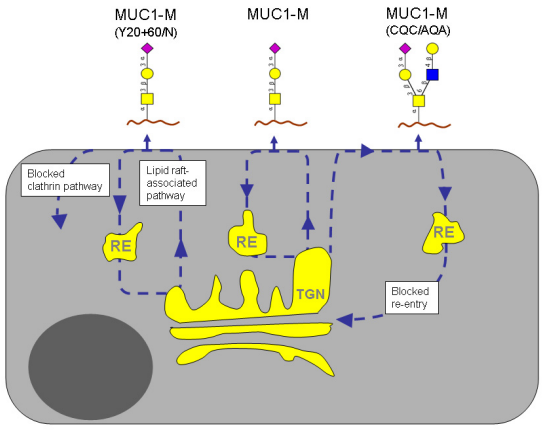

Fig. 9

GLP04

Trefoil domains represent highly efficient conformational determinants for N -linked LacdiNAc synthesis

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The disaccharide N,N'-di-N-acetylhexosediamine (LacdiNAc, GalNAcb1-4GlcNAcb) is found in a limited number of extracellular matrix glycoproteins and neuropeptide hormones indicating a protein-specific transfer of GalNAc by the glycosyltransferases b4GalNAc-T3 and -T4. Whereas previous studies have revealed evidence for peptide determinants as controlling and transferable elements in LacdiNAc biosynthesis we here report on an entirely independent conformational control of GalNAc transfer by single trefoil domains. The model protein human trefoil factor 2 (*h*TFF2) was recombinantly expressed in HEK-293 cells as a wildtype full-length probe (consisting of two trefoil domains P1 and P2), as single P1 (naturally N-glycosylated) or P2 domain (designed N-glycosylation site), and as a series of Cys/Gly mutant forms with partially abrogated domain structures. The N-glycosylation probes were analysed by mass spectrometry for their glycoprofiles. In agreement with the natural gastric *h*TFF2 the recombinant forms (full-length and single domain probes) expressed nearly exclusively fucosylated LacdiNAc on bi-antennary complex-type chains, whereas the Cys/Gly mutated variants showed preponderant LacNAc instead. Alignment of *h*TFF2 sequences with those of the neuropeptide hormones FSHb, LSHb, and TSHb revealed striking homologies of the Cys patterns relative to the N-glycosylation sites and conformationally stabilized hydrophobic patches formed by aromatic residues that may contribute to the formation of a common pattern-like determinant. These features are discussed on the basis of crystal structures of porcine spasmolytic protein, the neuronal glycopeptide hormones and a series of ECM-related LacdiNAc-positive glycoproteins detected as novel candidate proteins in the secretome of HEK-293 cells. (...)

GLP05

Characterization of the polysialylation status of the neuronal cell adhesion molecule NCAM during folliculogenesis of felis catus

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An ovarian follicle passes through a number of differentiation stages during the folliculogenesis: the primordial (resting), primary, secondary (pre-antral), tertiary (antral) and the Graafian (pre-ovulatory) follicle.
Since Campbell and co-workers mentioned that maturated oocytes express a not in detail specified isoform of the neuronal cell adhesion molecule NCAM after ovulation (1) we wanted to investigate if the polysialylated form of NCAM plays a role during the development of oocytes. This post-translational modification of NCAM, consisting of α2,8-linked Neu5Ac units, promotes *inter alia* changes in cell-cell interactions and plays an essential role during the development of the brain (2).
Using feline ovaries we were able to detect the polysialylated form of NCAM. Interestingly polySia-NCAM was primarily located on the surface of granulosa cells of tertiary and Graafian follicles. These cells are mainly responsible for an accurate development and nutrition of oocytes.
Functional studies to investigate the precise role of polySia on the surface of granulosa cells are under present investigations using isolated cumulus-oocytes-complexes (COC's) consisting of oocytes and granulosa cells *ex vivo*.

References
(1) Campbell et al., "Cell adhesion molecules on the oocyte and preimplantation human embryo", *Hum Reprod*, 1995
(2) Mühlenhoff et al., "Polysialic Acid: Versatile Modification of NCAM, SynCAM 1 and Neuropilin-2", Neurochem Res, 2013

GLP06

Sialic Acid Metabolic Engineering as a Novel Therapeutic Strategy for the Treatment of Neuroblastoma Cancer

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Neuroblastoma is a heterogeneous and highly metastatic solid malignant tumor in infants. Neuroblasoma cancer cells utilize multiple mechanisms to suppress host immune defense and escape [1,2]. There is an urgency to identify and apply novel strategies for attacking this kind of cancer and the aim is to implement a therapy to treat the aggressive forms of neuroblastoma cancer successfully.
We employed a sialic acid metabolic engineering (SME) strategy to modulate the cell surface sialylation through the application of natural *N*-acetylmannosamine (ManNAc) and semi-synthetic sialic acid precursors *N*-propionyl- and *N*-pentanoylmannosamine (ManNprop, ManNPent) [3]. We initiated our investigation with the expression profiling of polysialylation and cell-based functional assays on SME applied to SHSY5Y cells. The cells underwent stringent assessment, by real time (xCELLigence RTCA) and conventional assays for their susceptibility towards chemo- and combination therapies. We identified that the semi-synthetic sialic acid precursors make the SHSY5Y cells more vulnerable for chemo- and combination therapies compared to the controls. Detailed examinations of the mechanism responsible for the sensitization and application in mouse models are in progress.

References
[1] Maris, JM., *N Engl J Med.* **2010**, *362*, 2202-11
[2] Cheung, NK., Dyer, MA., *Nat Rev Cancer.* **2013**, *13*, 397-411.
[3] Seifert, A., Glanz, D., Glaubitz, N., Horstkorte, R., Bork, K.; *Arch Biochem Biophys* **2012**, *524*, 56-63.

GLP07

Characterization of polysialylated proteins in human breast milk

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In vertebrates, the highly negatively charged carbohydrate polysialic acid (polySia) is known to influence e.g. the regulation of cell-cell contact and repulsion. In mammalians, polySia consists of α2,8-linked *N*-acetylneuraminic acid (Neu5Ac) residues and chain length of these polymers can exceed 60 sialic acid residues. Interestingly, in human breast milk polySia chains attached to O-glycans of CD 36 were described (Yabe *et al.* 2003). Based on this, we became interested in the function of polySia in breast milk.
In a first set of experiments we wanted to reproduce the described findings. Using human colostrum Western blot analysis against polySia was performed. However, in contrast to the published data we did not observe a sharp band at 86 kDa. The immunostaining displayed a typical diffuse band for polysialylated proteins in a region between 150 and 230 kDa. Furthermore, after the release of N-glycans with PNGaseF the polySia immunostaining was abolished indicating that polySia chains were linked to N-glycans and not to O-glycans. Because of the described differences we also want to re-examine if polySia is attached to CD36 using a glycoproteomic approach. The identification of the polysialylated proteins as well as the identification of potential interaction partners of polySia are under present investigation using a glycoproteomic approach.
Yabe U, Sato C, Matsuda T, Kitajima K. 2003. *Polysialic acid in human milk. CD36 is a new member of mammalian polysialic acid-containing glycoprotein.* J Biol Chem, 278: 13875-13880.

GTPases in Health and Disease

PKV02

The chemoattractant cluster - studying GPCR ligand binding properties in a nutshell

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Chemogenomic analyses of G protein coupled receptors (GPCR) can be utilised to sort receptors according to conserved residues in their ligand binding cavity. The chemoattractant cluster mainly groups the angiotensin, kinin, anaphylatoxin, apelin and f-MLF receptors [1]. In contrast to the conserved ligand binding pocket, these receptors show a high variability in the length and architecture of the intra- and extracellular loops. For all chemoattractant ligands, high molecular weight precursors are proteolytically processed, yielding a set of peptides that differs strongly in length as well as amino acid composition and bind with different affinities to their respective receptors. In order to study mechanisms underlying the different ligand binding properties as well as ligand bias, selected chemoattractant cluster GPCRs are overproduced in insect cells. Comparing ligand affinities of receptors coexpressed with G protein subunits or arrestins, reveals a regulatory effect of the effector molecules on the affinity state of the receptors. Coexpression of chemoattractant cluster GPCRs results in the formation of heterodimeric complexes with cooperative binding properties. Together, these findings underline the complexity of GPCR regulation and signalling bias. [1] J.-S. Surgand, J. Rodrigo, E. Kellenberger, and D. Rognan, "A chemogenomic analysis of the transmembrane binding cavity of human G-protein-coupled receptors.," Proteins, vol. 62, no. 2, pp. 509-538, Feb. 2006.

PKV06

Conformational equilibria in Ras superfamily members as potential target to modulate signalling activity

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In addition to the structure of proteins there is an increasing interest in their dynamic properties because of their importance for function. Beside the general switching of small GTPases between the GDP-bound „off"-state and GTP-bound „on"-state several conformational substates can be detected for both forms. This phenomenon is expected for proteins acting in a regulation cycle in which a variety of different interaction states with regulators and effectors are essential. Depending on the phase within the activation/inactivation cycle single conformations predominate, whereas the others exist only in low population according to higher Gibbs free energies. We present data on different members of Ras superfamily of small GTPases in terms of conformational equilibria together with the functional consequence. Typical functional properties are the affinity to effector molecules or regulators, as well as GTPase activity. The conformational equilibria can be modulated e.g. by insertion of selective modifications in the amino acid sequence, the nature of the bound nucleotide, high pressure or the binding of small molecules. We have investigated members of the Arf, Ran, Rho and Ras families in terms of these dynamic equilibria and resulting functional properties. Conformational equilibria are found in the active state of all investigated Ras-like proteins, but with different equilibrium constants. It seems that these equilibria play a regulatory role in protein activity, which can be targeted to modulate signalling activity of these molecular switches.

PKV08

Dictyostelium Roco proteins to study LRRK2-mediated Parkinson disease

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Parkinson Disease (PD) is a neurodegenerative disorder affecting more than five million people worldwide. Recently a number of genetic factors causing PD have been discovered. Mutations in human leucine-rich-repeat kinase 2 (LRRK2) have been found to be thus far the most frequent cause of late-onset PD. LRRK2 belongs to the Roco family of proteins, which are characterized by the presence of a Ras-like G-domain, called Roc and a kinase domain. Importantly, pathogenic mutations in LRRK2 result in decreased GTPase activity and enhanced kinase activity, suggesting a possible PD-related gain of abnormal function. Here we show that *Dictyostelium discoideum* Roco4 is an excellent model to study the structural and biochemical characteristics of the LRRK2 kinase and can be used for optimization of the current and identification of new LRRK2 inhibitors. We have solved the structure of Roco4 kinase wild-type, PD related mutants G1179S and L1180T (G2019S and I2020T in LRRK2) and the structure of Roco4 kinase in complex with the LRRK2 inhibitor H1152 (Gilsbach et al., 2012). Together our data give important new insight in the LRRK2 activation mechanism and most importantly it explains the G2019S related increased LRRK2 kinase activity.

Gilsbach, B.K., Ho, F.Y., Vetter, I.R., van Haastert, P.J.M., Wittinghofer, A. & Kortholt, A. Roco kinase structures give insights into the mechanism of Parkinson disease-related leucine-rich-repeat kinase 2 mutations. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 10322-7 (2012).

PKV12

Structural and functional studies on Cp*Rh derivatives of GPCR peptide ligands

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The bioconjugation of organometallic complexes with peptides has proven to be a novel approach for drug discovery. We report the facile and chemoselective reaction of tyrosine-containing G-protein-coupled receptor (GPCR) peptides with $[Cp^*Rh(H_2O)_3](OTf)_2$, in water, at room temperature, and at pH 5-6. We have focused on three important GPCR peptides; namely, [Tyr^1]-leu-enkephalin, [Tyr^4]-neurotensin(8-13), and [Tyr^3]-octreotide, each of which has a different position for the tyrosine residue, together with competing functionalities [**1**]. Importantly, all other functional groups present, i.e., amino, carboxyl, disulfide, phenyl, and indole, were not prominent sites of reactivity by the Cp*Rh tris aqua complex. Furthermore, the influence of the Cp*Rh moiety on the structure of [Tyr^3]-octreotide was characterized by 2D NMR, resulting in the first representative structure of an organometallic-peptide complex. The biological consequences of these Cp*Rh-peptide complexes, with respect to GPCR binding and growth inhibition of MCF7 and HT29 cancer cells, will be presented for $[(\eta^6\text{-Cp*Rh-Tyr}^1)\text{-leu-enkephalin}](OTf)_2$ and $[(\eta^6\text{-Cp*Rh-Tyr}^3)\text{-octreotide}](OTf)_2$. Furthermore, the three-dimensional solution structure of $[Rh^{III}(\eta^5\text{-Cp*})(\eta^6\text{-Tyr}^1)]$Leu-enkephalin was solved by using 2D NMR spectroscopic techniques [**2**]. In addition, the binding of Leu-enkephalin and $[Rh^{III}(\eta^5\text{-Cp*})(\eta^6\text{-Tyr}^1)]$Leu-enkephalin to the recently published crystal structures of the μ- and δ-opioid receptor is studied. Docking of free Leu-enkephalin reveals two preferred conformations, one of which suggests an alternative binding site for the tyrosine residue.

References

[**1**] Albada, B. *et al.* (2012) *J. Am. Chem. Soc.*, **134**, 10321-10324.
[**2**] Wieberneit, F. *et al.* (2013) *Dalton Trans.*, **42**, 9799-9802.

GTV01

A farnesyl switch regulates the dynamic membrane binding of human guanylate binding protein 1

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Guanylate-binding proteins (GBPs) are interferon-inducible large GTPases of the dynamin superfamily. Recent data show that human and murine GBPs mediate antimicrobial resistance against intracellular pathogens at multiple levels. Several GBPs are subjected to C-terminal isoprenylation. In the case of human GBP1 the CaaX motif serves as a signal for farnesylation, which is necessary for the recruitment to cellular membranes.
Our research revealed that the lipid modification changes the nucleotide binding and GTP hydrolysis activity of hGBP1. Furthermore we found that both, the lipid modification as well as the GTPase activity, are important for the association with membranes, which only occurs in the activated state of the protein. Therefore, we have established a fluorescence assay to monitor this transient membrane association of lipid-modified hGBP1 during GTP hydrolysis in real time. Using this assay we show that solvent exposure of the farnesyl anchor is significantly increased in the activated state and during GTP hydrolysis enabling transient membrane binding of hGBP1. This membrane interaction of hGBP1 changes the hydrolytic activity in return. Fluorescence microscopy of hGBP1 together with several endo- and lysosomal markers revealed that human GBP1 localizes to diverse endolysosomal compartments and is recruited to phagosomes during the uptake of latex beads. Fluorescence recovery after photobleaching (FRAP) revealed a fast and constant exchange of hGBP1-molecules on endo- and phagolysosomal membranes. Point mutants of hGBP1 which display an activation-independent membrane binding in vitro also showed a slower exchange on endosomal membranes. In summary, our data indicate that the transient switch of the farnesyl anchor during GTP hydrolysis occurs also in living cells.

GTV02
Biochemical modulation of small GTPases by Legionella proteins
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Small GTPases are molecular on/off-switches that temporally and spatially coordinate crucial intracellular signaling pathways of eukaryotic cells. The activities of GTPases are frequently hijacked by bacterial pathogens (e.g. *Legionella pneumophila*) that subvert host biology to create a favorable intracellular environment. In addition to the manipulation of the activity states of these G-proteins, bacterial proteins also utilize posttranslational modifications that impair and reroute signaling in a highly specific manner. We currently analyze the structural and biochemical effects of two novel posttranslational modifications caused by Legionella enzymes, i.e. adenylylation and phosphocholination of small GTPases. The covalent attachment of AMP and/or phosphocholine to the Rab1 interferes with the on-off-transitions of the GTPase on a biochemical and structural level. The in depth investigation of the consequences of PTMs have transformed our understanding of the biology of small GTPases and allows us to formulate new hypotheses about -activation and specific -membrane-targeting of individual G-proteins.

GTP01
Mechanism in metastasis: Regulation of E-Cadherin mediated cell-cell contacts
Characterization of the Rho-interacting domain in p120ctn
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Reduced cell-cell adhesion is one hallmark of the metastatic process. The E-cadherin-mediated adherens junctions represent the best characterized intercellular adhesion modules. Their assembly and maintenance is influenced by numerous regulatory events, which enable single or groups of tumour cells to detach from the original tumour to initiate metastasis.
One regulator is p120 catenin (p120ctn), a member of the *armadillo-repeat*-protein family which stabilizes the cadherin-catenin-complex by binding to the cytoplasmic tail of classical cadherins. On the one hand p120ctn increases the recycling of cadherins when attached to cadherins, but also interacts with the small Rho GTPases in the cytoplasm thereby affecting their activity. Third, p120ctn influences the expression of genes due to regulation of transcription factors. GTPases of the Rho family play a key role in the regulation of actin cytoskeleton organization, which influences the attachment of the E-cadherin-complex with the cytoskeleton.
In the present study we characterized the binding site of p120ctn to the Rho GTPases RhoA and RhoC by co-immunprecipitation and *in vitro* precipitation experiments. Immunhistocheminal studies were performed to investigate the subcellular localization of p120ctn and different N-terminal-fragments. Using p120ctn deletion constructs encoding the first N-terminal 234 amino acids or encoding the amino acids 101-234, the interaction domain of p120ctn with the RhoA and RhoC was localized to the amino acids 101-234. Furthermore, it was shown that the p120ctn isoform 1A, but none of the p120ctn deletion mutants, was able to induce dendritic-like extensions (*branching phenotype).* These results emphasize the importance of the C-terminal region, possibly the *Armadillo-repeats*, in inducing these phenotypical alterations in epithelial cells, in addition to changes mediated by interacting to Rho GTPases.

GTP02
Galectins regulate Ras-induced signal transduction in adenocarcinoma cells
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Ras isoforms are localised in different nanodomains of the plasma membrane. Their localisation within the plasma membrane is regulated by their interaction with galectins (Gal), which affects the intensity of downstream signal transduction. So far, 15 mammalian galectins, which belong to the lectin family of proteins, are known. It is established that Gal-3 specifically interacts with K-Ras and our group identified Gal-8 as a new and specific interacting protein of K-Ras. In order to analyse the function of galectins, especially Gal-1, -3 and -8 in adenocarcinoma cell lines, different cell lines were studied for their protein content. Most of the tested cell lines expressed Gal-1, -3 and -8. The localisation of Gal-1, -3 and -8 was analysed in cell fractionation experiments, by using the SNAP-/CLIP-tag technology (New England Biolabs) and fluorescence microscopy. Gal-1, -3 and -8 were localised predominantly at the plasma membrane and to a lesser extent in the cytoplasm. Moreover, pancreatic carcinoma cells stably expressing oncogenic EGFP-K-Ras(V12) exhibited decreased expression of Gal-3 on protein and mRNA level, whereas expression of Gal-8 was unaffected. Next, the effects of downregulated Gal-3 and -8 protein expression on Ras-induced signal transduction pathways involved in malignant transformation, such as the PI3K/Akt or the Raf/MEK/ERK pathway were investigated in different adenocarcinoma cell lines. Our results indicate that decreased Gal-3 and -8 expression by siRNA differentially modifies the phosphorylation of Akt and ERK. Moreover, in some cell lines changes in the expression of the corresponding signalling proteins were observed. In summary, Gal-3 and -8 alter Ras-induced signal transduction by modulating the activity and the content of the signalling proteins and thus might affect Ras-induced malignant transformation.
This work is supported by UKGM, Forschungförderung

GTP03
- WITHDRAWN -

GTP04
Biophysical characterization of mGBP5 (murine guanylate binding protein 5)
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Guanylate-binding proteins (GBPs) are induced by interferons and belong to the dynamin superfamily. GBPs are involved in the immune response in a still not understood manner. Seven members of the human GBPs (hGBP1-7) and eleven members of the murine GBPs (mGBP1-11) have been identified. All GBPs characterized to date show biochemical characteristics similiar to dynamins or dynamin-related proteins such as low nucleotide affinities and cooperative GTP-hydrolysis. The best characterised GBP-family member is hGBP1 which hydrolysis GTP not only to GDP but also to GMP. In contrast, hGBP5 cleaves GTP exclusively to GDP (Wehner and Herrmann, 2010) though the residues that have been shown to be essential in hGBP1 for hydrolysing GTP to GDP and GMP are conserved in hGBP5.
We have purified and characterized the murine GBP5, which has been suggested to be the functional orthologue of human GBP5, based on multiple sequence alignment of the GBP family (Olszewski et al., 2006). Murine GBP5 was analysed with respect to its nucleotide binding and GTP-hydrolysis. Using multi angle light scattering (MALS) and analytical ultracentrifugation (AUC) the ability of self-assembly was investigated for mGBP5 and also for its human homologue, hGBP5. The biochemical characteristics are highly similar between mGBP5 and hGBP5 confirming the functional orthology proposed by Olszewski et al. (2006).
Recently, Shenoy et al. have described an important role of human and murine GBP5 in NLRP 3 inflammasome assembly in response to soluble bacterial products. This report also suggested a direct interaction of GBP5 with the pyrin domain of NLRP3. We are currently investigating this interaction *in vitro* using the purified proteins.
M. Wehner and C. Herrmann. *Biochemical Properties of the Human Guanylate Binding protein 5 and a tumor-specific truncated splice variant. FEBS Journal,* 277:1597-1605, 2010

References
M. A. Olszewski, J. Gray, and D.J. Vestal. *In Silico Genomic Analysis of the Human and Murine Guanylate-Binding Protein (GBP) Gene Clusters.* J. Interferon Cytokine Res., 26:328-352, 2006.
A. R. Shenoy, D. A. Wellington, P. Kumar, H. Kassa, C. J. Booth, P. Cresswell, and J. D. MacMicking. *GBP5 Promotes NLRP3 Inflammasome Assembly and Immunity in Mammals.* Science, 336:481-485, 2012.

MEMBRANES AND TRANSPORT

PKV07
A lipid E-MAP identifies Ubx2 as a critical regulator of lipid saturation and lipid bilayer stress
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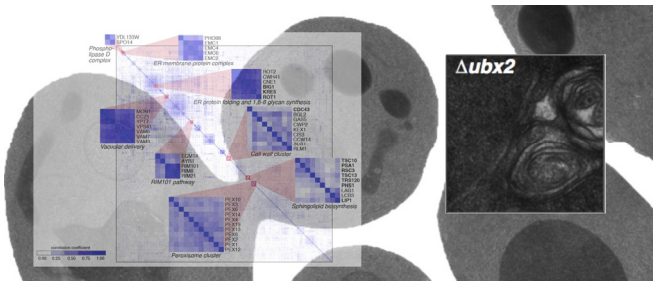
Biological membranes are complex and the mechanisms underlying their homeostasis are incompletely understood. Here, we present a quantitative genetic interaction map (E-MAP) focused on various aspects of lipid biology including their metabolism, sorting, and trafficking. This E-MAP contains ~250,000 genetic interaction scores, both negative and positive, and identifies a molecular crosstalk of protein quality control pathways with lipid bilayer homeostasis. Ubx2p, a component of the ER-associated degradation (ERAD) pathway, surfaces as a key upstream regulator of the essential fatty acid desaturase Ole1p. Loss of Ubx2p affects the transcriptional control of ***OLE1*** resulting in impaired fatty acid desaturation and a severe shift towards more saturated membrane lipids. Both the induction of the unfolded protein response and aberrant nuclear membrane morphologies observed in cells lacking ***UBX2*** are suppressed by supplementation of unsaturated fatty acids. Our results point

towards the existence of dedicated bilayer stress responses for membrane homeostasis.



PKV11

Intercellular Signaling Via Cell Surface Proteins - Stimulus-Induced Processing and Release of the Extracellular Nucleotidase, CD73, from Brain and Adipose Tissues in Various Assembly States

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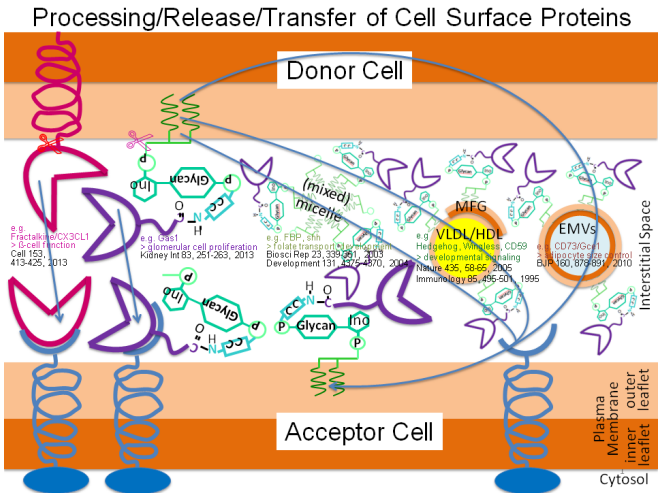
The paracrine and endocrine transfer of biological information by secretory proteins for the control of proliferation, differentiation and metabolism in multicellular eukaryontes has been known for decades. Recently proteomics nourished the hope for full description and functional understanding of the secretome. However, subsets of transmembrane and glycosylphosphatidylinositol (GPI)-anchored cell surface proteins which become released from plasma membranes of donor cells in response to certain nutritional or hormonal stimuli upon incorporation into phospholipid (mixed) micelles, lipoproteins or exosomes and microvesicles (EMVs), escape detection by proteomic procedures. In a first step to study the putative transfer of the GPI-anchored extracellular nucleotidase, CD73, from donor adipocytes to acceptor brain cells or vice versa and its role for the (dys)regulation of glucose and lipid metabolism, a novel dynamically operating biosensor was introduced for the discrimination of CD73 in its various assembly states, i.e. as soluble or detergent-solubilized dimer lacking or harbouring GPI, respectively, as soluble multimer in phospholipid micelles, lipoprotein particles and EMVs. Biosensing of the culture medium from mouse primary adipocytes and astrocytes as well as adipose tissue and brain extracts revealed the presence of CD73 in the various assembly states in differential stimulus-dependent fashion (e.g. fatty acids, reactive oxygen species, cytokines; chow vs. high fat diet). Future studies will address the relationship between the differential processing/release of CD73, its putative systemic availability and the pathogenesis of diabesity using transgenic manipulation of diabetic mice. Causality would argue for the use of CD73 in its various assemby states as functional biomarker and its processing/release as drug target for diabesity.



PKV17

Modification of self-organizing Min surface waves through protein engineering

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The emergence of macroscopic, observable properties from local, molecular interactions is a fascinating feature of biological systems. One intriguing example are the Min proteins of *E. coli*, which self-assemble into spatiotemporal patterns *in vivo* and *in vitro* (Loose et al., 2008;Raskin and de Boer, 1999).
In vivo, the membrane-binding ATPase MinD and its regulator MinE oscillate from pole to pole and constrict the site of division to the cell middle through the inhibitory action of MinC, which closely follows the oscillations.
The *in vitro* reconstitution approach has shown that MinD and MinE form planar surface waves on supported lipid bilayers in the presence of ATP. The observed patterns were already found to be sensitive to the surrounding geometry (Schweizer et al., 2012). A recent breakthrough was the reconstitution of Min oscillations in microcavities *in vitro* (Zieske and Schwille, 2012).
We now aim to engineer the properties of Min protein self-organization, such as the wavelength and velocity by functionally modifying the Min proteins. Our initial approach is to modulate the MinD ATPase activity through production of different MinD and MinE mutants. Furthermore, the manipulation of membrane association and dissociation of both MinD and MinE is expected to produce patterns with modified properties.
The *in vitro* behaviour of mutant Min proteins should yield new mechanistic insights into the Min system and have important implications for bottom-up synthetic biology.

PKV25

Find Out to Apply - The Hemolysin A Type 1 Secretion System

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The Hemolysin A (HlyA) transport machinery of *E. coli* represents the paradigm of Type 1 secretion systems (T1SS) and previous studies revealed many insights into its function. Nevertheless, many aspects remained mysterious. What, for example, is the function of the non-canonical domain of the ABC transporter HlyB, the C39-like domain (CLD)? Or how is the unfolded state of HlyA stabilized prior to transport? Structural and biochemical analyses of the CLD demonstrated an essential interaction with HlyA, importantly only with its unfolded state. Moreover, the binding surface was mapped onto the CLD revealing a novel mode-of-action. These data suggest a mechanism by which unfolded HlyA is protected in the cytoplasm from aggregation and/or degradation without the help of external chaperones[1,2].
Besides being characterized, the HlyA T1SS was considered as an interesting tool in biotechnology for the secretory production of polypeptides. However, its application was limited and most passengers failed to be secreted[3]. The identification of a secretion enhancer allowed the engineering of an optimized system that succeeds in the secretion of all expressed polypeptides. Secretion levels reach up to 1 g/l and secreted polypeptides are bioactive. In our opinion, the optimized system represents a valuable platform technology for the secretory production of functional polypeptides.

References

1. Lecher, J., Schwarz C. K. et al. An RTX Transporter Tethers Its Unfolded Substrate during Secretion via a Unique N-Terminal Domain. *Structure* (2012).
2. Kanonenberg, K., Schwarz, C.K. & Schmitt, L. Type I secretion systems - A story of appendices. *Res Microbiol* (2013).
3. Schwarz, C.K. et al. Using an E. coli Type 1 secretion system to secrete the mammalian, intracellular protein IFABP in its active form. *J Biotechnol* **159**, 155-161 (2012).

PKV27

Flotillins functionally organize the bacterial membrane.

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Proteins and lipids are heterogeneously distributed in biological membranes. The correct function of membrane proteins depends on spatiotemporal organization into defined membrane areas, called lipid domains or rafts. Lipid microdomains are therefore thought to assist compartmentalization of membranes. However, how lipid and protein assemblies are organized and whether proteins are actively involved in these processes remains poorly understood. We now have identified flotillins to be responsible for lateral segregation of defined membrane domains in the model organism Bacillus subtilis. We show that flotillins form large, dynamic assemblies that are able to influence membrane fluidity and prevent condensation of Laurdan stained membrane regions. Absence of flotillins in vivo leads to coalescence of distinct domains of high membrane order and, hence, loss of flotillins in the bacterial plasma-membrane reduces membrane heterogeneity. We show that flotillins interact with various proteins involved in protein secretion, cell wall metabolism, transport and membrane-related signalling processes. Importantly, maintenance of membrane heterogeneity is critical for vital cellular processes such as protein secretion.

PKV28
Optochemical biology: *In-situ* receptor clustering triggered by light

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Membrane organization and receptor clustering have emerged to be key processes in cell communication. Since these protein interactions span several dimensions in time and space, it is of great interest to manipulate and organize them in functional systems by molecular tools. Stimulus-driven protein assembly emerged as a very powerful tool to investigate cellular pathways. Controlling interaction by molecular lock-and-key elements is a major challenge in chemical biology to connect protein activity with macroscopic response. At present, no tools are available to specifically modulate membrane trafficking of receptors in cells.
Here, we present new optochemical tools to control receptor networking with superior spatiotemporal resolution in living cells. We designed novel photoactivatable small lock-and-key elements and membrane scaffolds for the controlled assembly of receptor networks.[1] A library of photoactivatable *tris*NTAs was generated to allow light induced *tris*NTA/His-tag interaction. The small high-affinity pair was used for a spatiotemporal labeling of proteins and to build photoactivatable hybrid hydrogels for *in-situ* protein assembly. Combined with two-photon activation, super-resolution microscopy and single molecule techniques, these tools provide valuable insights in the correlation of the receptor clustering and dynamics. With our approach, we will explore the fate and function of selected receptors and the implication of receptor surface trafficking in cellular function with utmost spatiotemporal control. The non-invasive manipulation of receptor surface trafficking will help to understand complex biological processes of receptor assembly and disassembly at the molecular level and allow to unravel how this type of regulation is used to orchestrate global cellular responses.

References

1. Labòria N, Wieneke R, Tampé R, Angew. Chem. Int. Ed. 2013, 52, 848-85

PKV29
Identification of new cargo proteins that require clathrin adaptor complexes for intracellular transport

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The family of heterotetrameric adaptor protein complexes (APs) comprises five members (AP1-5). They are key players in the formation of transport vesicles and bind to sorting signals of membrane proteins thereby incorporating them as cargo into nascent vesicles. Furthermore, APs recruit numerous other factors to the sites of vesicle formation, including the major coat protein clathrin.
A number of membrane proteins have been characterized until today whose sorting depends on APs, however it remains unclear how APs contribute to the protein composition of a whole organelle. In order to address this issue, we applied RNAi for AP-1 in RAW 264.7 macrophages and analyzed the process of phagocytosis. We purified phagosomes from AP1-depleted cells after SILAC (stable isotope labeling with amino acids in cell culture) followed by quantitative proteomics.
Nearly 1.400 phagosomal proteins were identified, of which 308 had a reduced abundance in the AP1-depleted macrophages. Based on this data we identified several membrane proteins as novel AP cargo proteins and characterized their sorting signals: We showed direct interactions between the APs and the putative cobalamin transporter LMBD1, Mpeg-1 (macrophage-expressed-gene 1 with unknown function) and the TLR4 regulator Rp105

PKV30
Semisynthetic Lipidated LC3 Protein Mediates Membrane Fusion

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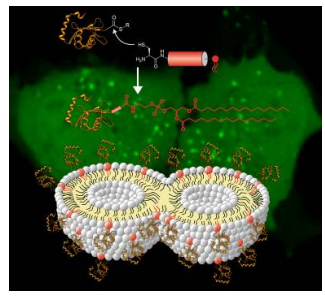
Autophagy is an evolutionarily conserved catabolic mechanism in eukaryotes. Autophagosome formation is the key process in autophagy. The ubiquitin-like protein Atg8 is required for the biogenesis of autophagosomes and needs to be C-terminally conjugated to phosphatidylethanolamine (PE) for correct membrane localization and function. In mammalian cells, lipidated mammalian Atg8 orthologs, microtubule-associated protein light chain 3 (LC3) protein family, are also essential for autophagosome biogenesis and have been used as a bona fide marker of autophagosome and autophagy progression. Despite extensive studies on the role of LC3 in autophagy, the molecular mechanism of LC3 function remains elusive. For the studies of LC3 function and the mechanism regulating LC3 localization and activity in autophagy, preparative amounts of lipidated LC3 would be invaluable tools. However, recombinant production of post-translationally modified proteins is usually challenging in terms of homogeneity and output. Herein we present the first synthesis of a lipidated LC3 using a combination of lipidated peptide synthesis and expressed protein ligation (EPL) technique. This strategy enabled us to prepare native LC3-PE under folding conditions without reconstitution of the conjugation system including mammalian Atg7 and Atg3 proteins, which are usually difficult to obtain recombinantly. The functionality of the synthetic lipidated LC3 protein has been proven by recognition and cleavage by Atg4. We show that lipidated LC3 is able to promote membrane tethering and fusion at physiologically relevant PE concentrations. This study provides insights into the function of lipidated LC3 protein in autophagosome biogenesis.



PKV32
The nucleo-mitochondrial GTPase NOA1 is stimulated by G-quadruplex nucleotide ligands and is a substrate of the mitochondrial matrix protease ClpXP.

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The GTPase NOA1 is essential for mitochondrial ribosome biogenesis[1-4] and OXPHOS function[5,6]. The impact of NOA1 on mitochondrial respiration and ribosome assembly is GTPase dependent[1,5]. The bacterial NOA1 homolog YqeH has a conserved RNA binding domain[7,8]. However, no RNA ligand has been identified. Although predominantly localized in mitochondria, NOA1 contains a nuclear localization signal that becomes functional when the mitochondrial targeting pre-sequence is deleted.
We aimed to characterize the subcellular dynamics of NOA1, identify its RNA ligand and explore the degradation pathway.
To assess the dynamic subcellular localization of NOA1 we used mutant and truncated versions of wild type NOA1 with fractionation assays and fluorescence microscopy analyses. To pinpoint a consensus motif of NOA1's native nucleotide ligand we performed SELEX. We used recombinant NOA1 protein for *in vitro* studies and C2C12 muscle myoblasts for *in vivo* experiments.
We have shown that NOA1 is a nucleo-mitochondrial shuttling protein. A nucleolar localization of the precursor protein is essential for the subsequent mitochondrial localization and function of mature NOA1. We found that the nucleotide ligand of NOA1 contains an even numbered G-quadruplex motif. G-quadruplex binding and GTP hydrolysis are coupled since a generic G-quadruplex was sufficient to significantly stimulate the GTPase activity of NOA1. Finally, we identified that NOA1 is degraded by the mitochondrial matrix protease ClpXP.
NOA1 makes a detour through the nucleus that is crucial for mitochondrial localization. Binding to G-quadruplexes is coupled to the GTP hydrolysis activity of NOA1. NOA1 is the first reported mammalian substrate of the ClpXP complex.

PKV35
Conformational flexibility of VDAC and the influence of effectors

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Since the solution of the molecular structures of members of the voltage dependent anion channels (VDACs)[1-3], the N-terminal α-helix has been the main focus of attention. Its strategic location, in combination with its putative conformational flexibility, could define or control the channel's gating characteristics.[4-6] By engineering of two double-cysteine mVDAC1 variants we achieved fixing of the N-terminal segment at the bottom and midpoint of the pore. Through black lipid membrane measurements we proofed that conformational restriction of the N-terminal α-helix dynamics strongly affects the gating characteristics of mVDAC1. Therefore the conformational plasticity of the N-terminal segment is indeed a major part in the control of the channel's switching behavior. Different biological responses can be elicited through the

regulation of these transitions triggered by the many effectors which interact with mVDAC1 and its N-terminus, offering thus a control point in mitochondrial apoptosis. Interaction between mVDAC1 and the anti-apoptotic protein Bcl-x_L was recently shown to require the N-terminal mVDAC1 helix and resulted in a ~2.5-fold reduction of the channel's conductance.[7] Here we investigate the putative interactions between mVDACs-Hexokinase and mVDACs-Bid, believed to be pivotal in the early stages of apoptotic signaling.

References

1. Bayrhuber, M., et al., PNAS, 2008. **105**(40): p. 15370.
2. Hiller, S., et al., Science's STKE, 2008. **321**(5893): p. 1206.
3. Ujwal, R., et al., PNAS, 2008. **105**(46): p. 17742.
4. Shoshan-Barmatz V., Biochem. J. 2012 **444** p. 475
5. Hiller, S., G. Wagner, Cur. op. struct. biol., 2009. **19**(4): p. 396.
6. Mertins, B et al., PlosONE 2012 **7** : e47938.
7. Shoshan-Barmatz V., JBC, 2012. **287**(27): p. 23152.

MTP01

Polyethylene glycol-400 (PEG-400) inhibitis P-gp activity and expression in Caco-2 cells

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Introduction

P-glycoprotein (P-gp) is an efflux integral membrane protein in intestinal cells believed to be responsible for descending in drug bioavailability (BA) [1]. It is suggested that some excipients may inhibit P-gp activity resulting in increase in drug BA.

Aim

The objective of this study was to determine the influence of a routine pharmaceutical excipient, polyethylene glycol-400 (PEG-400) on P-gp protein activity and expression in human colon adenocarcinoma cells (Caco-2).

Material and Methods

Non-cytotoxic concentrations of PEG-400 were determined using MTT test assay. Measuring the activity of P-gp was performed with Rhodamine-123 (Rho-123, a P-gp fluorescent substrate) uptake test assay in presence of PEG-400 and compared to control samples and verapamil 0.3 Mm, as positive control. Moreover, increase in Rho-123 uptake was shown by Fluorescent microscopy qualitatively. Furthermore, the expression of P-gp was investigated by Western blotting using P-gp specific antibody.

Results

Compared with the normal control, PEG-400 2% and 1% (w/v) showed up to 2-fold increase in Rho-123 uptake. Increased intracellular fluorescent light confirmed soar in Rho-123 accumulation when treated with sub-toxic concentrations of PEG-400. Moreover, western blotting data confirmed Rho-123 uptake data as well.

Conclusion

The results showed that PEG-400 in concentrations of 2% and 1% (w/v) is able to inhibit P-gp. The idea behind this study was to develop a way to improve drug absorption and introduce a new efficient concentration of excipients in drug formulations to improve drug BA.

1. Sachs-Barrable K et al. (2007) J Pharm Sci 10(3):319

MTP02

The diversity of mitochondrial protein import in eukaryotes

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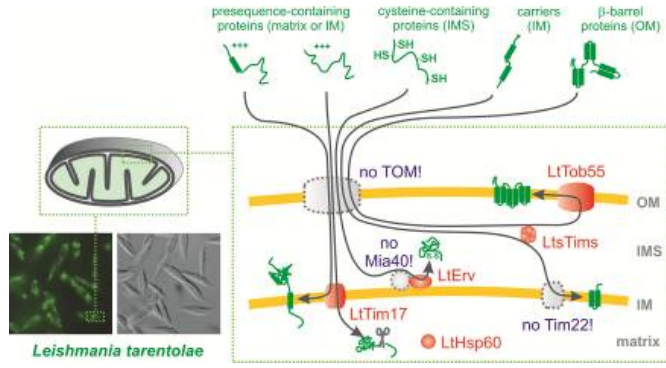
Mitochondrial protein import (MPI) is essential for the biogenesis of mitochondria in all eukaryotes. Prevalent models of MPI are predominantly based on experiments with one group of eukaryotes: the opisthokonts. Although fascinating genome database-driven hypotheses on the evolution of MPI machineries have been published, previous experimental research on non-opisthokonts usually focused on the analysis of single pathways or components. We therefore established the parasitic protist *Leishmania tarentolae* as a model organism for the comprehensive analysis of MPI into all four mitochondrial compartments. Our studies demonstrate that MPI pathways are functionally conserved among eukaryotes despite significant differences of the MPI machineries between eukaryotic lineages. Such differences include not only mechanistic variations but also drastically altered and even novel MPI components, for example, in kinetoplastid and apicomplexan parasites. In summary, the current knowledge on MPI in yeast and other opisthokonts cannot be generally transferred to all eukaryotes and the corresponding pathways, components and mechanisms remain to be analyzed.

References

Eckers E, Cyrklaff M, Simpson L, and Deponte M. (2012) Mitochondrial protein import pathways are functionally conserved among eukaryotes despite compositional diversity of the import machineries. Biol. Chem. 393:513-24.
Deponte M*, Hoppe HC*, Lee M*, Maier AG*, Richard D*, Rug M*, Spielmann T*, and Przyborski JM*. (2012) Wherever I may roam: Protein and membrane trafficking in P. falciparum-infected red blood cells. Mol. Biochem. Parasitol. 186:95-116.
Eckers E, Petrungaro C, Groß D, Riemer J, Hell K, and Deponte M. (2013) Divergent molecular evolution of the mitochondrial sulfhydryl:cytochrome c oxidoreductase Erv in opisthokonts and parasitic protists. J. Biol. Chem. 288:2676-88.



MTP03

Subcellular trafficking of flotillins: impact of dynamin and clathrin

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Endocytosis - the cellular uptake of cargo - can be accomplished by different pathways. The mechanism of clathrin-dependent endocytosis is well established, while several less understood clathrin-independent pathways exist. In addition, some endocytosis pathways depend on the large GTPase dynamin while others do not. Endocytosis that is independent of both clathrin and dynamin has been suggested to be mediated by membrane rafts. Both members of the flotillin protein family, flotillin-1 and -2 associate with the inner leaflet of membrane rafts and have been suggested to support their own flotillin-dependent endocytosis pathway under conditions of constitutive endocytosis (Glebov et al., 2006; Frick et al., 2007). However, it still is under debate whether flotillins are mechanistically involved in endocytosis or if they are cargo molecules. Our group has established that the translocation of flotillins from the plasma membrane to late endosomes can be induced by stimulation with epidermal growth factor (EGF) (Neumann-Giesen et al., 2007; Babuke et al., 2009) and seems to be independent of the EGF receptor kinase activity. In contrast to the constitutive flotillin turnover, we here show that the growth factor induced translocation of flotillins seems to depend on dynamin since chemical inhibition of dynamin impaired flotillin uptake. In addition, retrograde trafficking of flotillins from late endosomes to the plasma membrane depends on clathrin, since both the siRNA-mediated depletion of clathrin heavy chain and the expression of a dominant-negative clathrin Hub resulted in constitutive localization of flotillins to late endosomes. Thus, we here reveal novel insights into the subcellular trafficking of flotillins.

MTP04

Heterologous Expression, Solubilization and functional Purification of the human ABC transporter MDR3 in the yeast *Pichia pastoris*

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The human multidrug resistance protein 3 (MDR3/ABCB4), present only in the canalicular membrane of hepatocytes, was classified as a MDR transporter based on the amino acid sequence identity of over 75% to the MDR mediating ABC transporter P-glycoprotein. Despite this identity in primary sequence, up to date MDR3 shows no phenotype with respect to MDR. The function of MDR3 is the translocation of phosphatidylcholine (PC) from the inner to the outer leaflet of the membrane. PC is one major component of bile and is essential to protect the biliary duct from bile salts translocated by the bile salt export pump.
To investigate the function of MDR3 *in vitro*, we expressed *MDR3* in *Pichia pastoris* in amounts suitable for a detailed structure-function analysis. Beside the wildtype protein, we were able to express *MDR3* with a GFP tag and used

fluorescence-detection size exclusion chromatography (FSEC) to determine suitable detergents for solubilization and purification.
The purification yielded amounts of ~6 mg per 100 g wet cells with high homogeneity and the functionality was determined by measuring the ATPase activity of purified MDR3 in detergent solution. Purified MDR3 exhibited significant ATPase activity that can be stimulated specifically by PC compared to an ATPase deficient mutant. So far our work shows that heterologously expressed MDR3 can be purified in a functional state and might provide the foundation to address important questions like: Why is MDR3 a PC floppase, whereas the homologue MDR1 is involved in multidrug resistance?
Ellinger P, Kluth M, Stindt J, Smits SHJ, Schmitt L (2013) Detergent Screening and Purification of the Human Liver ABC Transporters BSEP (ABCB11) and MDR3 (ABCB4) expressed in the yeast *Pichia pastoris*. *PLoS One*. **8 (4)**.

MTP05
Approach in characterization of the membrane fusion protein HlyD

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Type I secretion system consists of an ABC transporter and a membrane fusion protein (MFP). The ABC transporter haemolysin B (HlyB) secretes the 110 kDa toxin haemolysin A in one step from the cytoplasm directly into the extracellular space. To achieve this a complex with the MFP HlyD and the outer membrane protein TolC is formed.
HlyD is a single-spanning membrane protein. The N-terminal 62 residues are located in the cytosol which adopt an unknown function and a single alpha helix predicted to contain twenty amino acids spans the inner membrane. The remaining 396 residues reside in the periplasmic space forming a domain, which closes the gap between TolC and HlyB. So far, no structural information of HlyD is known. The oligomeric state of HlyD is currently unknown but thought to be hexameric, which bridges a dimeric HlyB with the trimeric TolC.
Different tagged and untagged protein variants were designed and purification strategies were established. We are able to purify high homogenies amounts of HlyD, which show a monomeric state in solution. To improve proteins crystallization behavior, a thermofluor-based stability assay was performed to determine a more stable buffer condition. Initial crystalisation results will be shown.

MTP06
Cell-type specific expression of ZnT8 in the porcine pancreas

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Introduction
ZnT8 is a member of the slc30A gene family and expressed in few endocrine organs such as thyroid gland, adrenal gland and pancreas. In the pancreas ZnT8 is thought to be functionally involved in insulin metabolism and release of this molecule in B-cells. While in primates and rodents ZnT8 was found to be expressed in B- as well as in A-cells of pancreatic islets only little information exists about the cell-specific expression, distribution and potential role of this molecule in the pig species.

Materials and Methods
Pancreatic probes of 6 pigs ranging in age from 6 to 10 months were used to determine the precise cellular location of ZnT8 in the islets and consequently the co-expression with other islet hormones by immunohistochemical methods. Additionally was checked the local expression of ZnT8 by RT-PCR techniques.

Results
Serial sectioning followed by double-immmunostaining of pancreatic tissues clearly show that ZnT8 is exclusively co-expressed in insulin- but not in glucagon or somatostatin-producing cells. Moreover, the RT-PCR experiments confirm the local ZnT8 expression in the pig pancreas and indicate that its genomic DNA translation is probably tightly regulated.

Conclusions
Our results show for the first time the exclusively expression of ZnT8 in insulin-containing B-cells of the pig pancreas. The absent of ZnT8 in other pig islet cells such as glucagon- and somatostatin-producing cells indicates that the homeostasis of zinc in these cells is mediated by other cellular mechanism.

MTP07
ATP-binding cassette transporter G1 (ABCG1) prevents accumulation of atherogenic oxysterols

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Oxysterols are oxidized derivatives of sterols that have cytotoxic effects. Efficient oxysterol removal by the sub-family G member 1 of the ATP-binding cassette transporters (ABCG1) is essential for cell survival. However, the specific role of ABCG1 in the transport of various oxysterol species has been not systematically investigated to date.
We examined the involvement of ABCG1 in oxysterol metabolism by studying oxysterol tissue levels in a mouse model of *Abcg1*-deficiency by gas-liquid chromatography coupled to mass spectrometry (GC-MS) analysis. For functional analysis our stable regulable ABCG1 expressing cell lines were used.
Analysis of lung tissue of *Abcg1*$^{-/-}$ mice on a standard diet revealed that 3β,5α,6β-cholestanetriol (CT) and 25-hydroxycholesterol (HC) accumulated at more than 100-fold higher levels in comparison to wild-type mice. A [^{3}H]-labeled assay employing regulable ABCG1-expressing HeLa cell lines revealed that 25-HC export to albumin was dependent on functional ABCG1 expression and could be blocked by an excess of unlabeled 25-HC or 27-HC. In this cell line, 25-HC at low doses triggered mitochondrial membrane potential, and reactive oxygen species production, which are both indirect indicators of cellular energy expenditure.
Our results suggest that 25-HC and CT are physiologic substrates for ABCG1 mediated export from cells and that primary function of ABCG1 is protection against oxysterol-induced cell death, an anti-atherosclerotic property.

MTP08
Interaction of Alzheimer's amyloid beta with oxysterol-containing membranes

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The interaction of amyloid beta (Aβ) with cell membranes is the key event in pathogenesis of Alzheimer's disease, leading to channel/pore formation, interference in ligand/receptor binding, and loss of membrane integrity. Aβ/membranes interaction is controlled by conformation of the peptide and lipid composition including cholesterol (Vestergaard et al., 2008). It has been reported that some oxidized derivatives of cholesterol, called oxysterol, play as protectors of the brain from Aβ's neurotoxicity, while the others facilitate Aβ to insert into membranes because of their ability to change membrane properties (Papadopoulos et al., 2011; Kim et al., 2008). However, the effect of oxysterols on Aβ/membranes interaction has not been well understood.
Biomimetic membranes without protein are considered as an ideal platform for investigating the mechanism by which lipid component controls the interaction of external factors with membranes. In this study, Aβ-induced dynamics of cell-sized liposomes were used to investigate the interaction of Aβ with 7keto- or 25OH-containing membranes. We have demonstrated that cholesterol inhibited Aβ association with membrane and stabilized membranes. Conversely, oxysterols mediated Aβ localization and Aβ-induced membrane transformation. Moreover, we have shown that 7keto, a product of sterol ring oxidation, and 25OH, a side-chain-oxygenated oxysterols, have different influence. The former oxysterol increased Aβ localization, while the latter enhanced membrane transformation by facilitating the peptide insertion into the bilayer (Phan et al, 2013). These findings are useful for understanding the effect of cholesterol and its oxidation on the Aβ-induced Alzheimer's neurotoxicity.

MTP09
Evolutionary maturation: The Survivin-CRM1-axis is required to maintain chromosomal stability in higher eukaryots

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Survivin functions as a mitotic regulator and cytoprotective effector. Together with the Aurora B kinase, INCENP and Borealin, Survivin constitutes the *Chromosomal Passenger Complex* (CPC), active in guarding chromosomal segregation and genome integrity. To perform its (patho)biological activities, Survivin contains functional domains, including a nuclear export signal (NES) mediating the interaction with the export receptors CRM1. To understand the functional maturation of Survivin during evolution, we analyzed the biological activity of the Survivin-like protein ($Surv_{SD}$) identified in the metazoan sponge *Suberites domuncula*.
Whereas protein sequence alignment with vertebrate Survivin homologs revealed a conservation of the characteristic BIR domain in $Surv_{SD}$, no functional NES was predicted.
Ectopic expression studies in mammalian cells not only confirmed the absence of a NES, but also showed that the sponge $Surv_{SD}$ did not correctly localize

during mitosis, and failed to interact with the other CPC members in human cells.
As *Suberites domuncula* cells are characterized by a very low proliferation rate, our results suggest that the NES-mediated Survivin-CRM1 interaction evolved in vertebrates to meet the needs for ensuring proper chromosomal segregation even at a high proliferation rate. Thus, besides the deep evolutionary conservation of Survivin's cytoprotective function, a complementary mitotic effector function might have arisen later during evolution, concurrent with increased organismal complexity.

MTP10
The ubiquitous peptidoglycan-binding protein TsaP functions in surface assembly of type IV pili

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Type IV pili (T4P) systems are multiprotein complexes that assemble pili which are involved in adhesion to host cells, biofilm formation, motility and DNA uptake. The pili cross the outer membrane (OM) through the oligomeric secretin complex. In the β-proteobacterium *N. gonorrhoeae,* this complex is formed by PilQ and other proteins and forms a complex that in electron microscopy (EM) studies resembles a double ring structure with extending spikes. We identified a hypothetical protein, TsaP, which is conserved in bacteria containing T4P systems. TsaP mutants show a non-piliated colony morphology on solid media and EM studies showed that the deletion strain forms membrane protrusions containing pilus filaments. Lack of TsaP affected neither the stability nor the oligomeric state of PilQ, however, the stability and OM association of TsaP strongly depended on PilQ. The secretin complex of the deletion strain showed loss of the peripheral secretin ring. Biochemical characterization of TsaP showed that it binds to peptidoglycan via its LysM domain. In the δ-proteobacterium *Myxococcus xanthus*, TsaP is also involved in T4P surface assembly and accumulates and localizes in a PilQ-dependent manner to the cell poles. We propose that TsaP forms a ring around PilQ, anchors the secretin complex to the peptidoglycan layer and align the inner and outer membrane components of the T4P assembly system.

MTP11
E.coli sugar transporters of the major facilitator superfamily - electrophysiological investigations on LacY, FucP and XylE

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Sugar-proton symporters of the major facilitator superfamily (MFS), e.g. LacY, FucP and XylE from Escherichia coli, potentially share a universal transport mechanism. Fortunately for those three transporters 3D structures are available, showing each a different conformation. Possibly these conformations represent different states during the transport cycle, making these transporters ideal candidates for comparison studies.
Here we analyze the electrogenic steps during the transport cycle of these transporters using solid supported membrane (SSM) based electrophysiology. Our aim is to develop an overall kinetic model describing the behavior of LacY, FucP and XylE.
Whereas LacY clearly shows two electrogenic steps during the transport cycle, we only observe one electrogenic step for FucP and XylE. The main electrogenic step observed for all transporters is assigned to the proton release. Our working hypothesis is that the electrogenic step only observed for LacY is due to a conformational transition upon sugar binding. This conformational transition is not observed for FucP and XylE because charged key residues are missing.
In addition there are huge differences in the pH dependence of the transport reactions in the pH range between 4.5 and 9.0. Whereas LacY shows no turnover at highly acidic pH and the turnover increases up to pH 9.0, FucP clearly show a pH optimum at pH 8.0, possibly due to a shift in the pH dependence. In both cases the minimal turnover is less than 10% of the maximal rate. In the case of XylE the pH dependence is not as prominent as in the case of LacY and FucP. The minimal turnover in the observed pH range still is about 50% of the maximal transport rate, which supports our idea of multiple proton binding sites having regulatory effects. Nevertheless, for all transporters the KM of sugar binding is not altered by pH.

MTP12
Cooperation of Arf and flotillins in membrane trafficking

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The flotillin protein family consists of two ubiquitously expressed and highly conserved members, flotillin-1 and flotillin-2. Both flotillins are constitutively associated with membrane microdomains enriched in cholesterol and glycosphingolipids, also known as membrane rafts. Flotillins are involved in growth factor and insulin signaling, actin organization and in membrane transport processes. The ADP-ribosylation factors (Arfs) belong to the Ras-superfamily of small GTPases. The active, GTP-bound Arf6 is localizes to the plasma membrane as well as endosomal compartments and has a variety of cellular functions including endocytosis and recycling. In this study, we show that Arf6 and flotillins interact with each other. In addition, Arf6 influences the subcellular localization of flotillins, and overexpression of Arf6 leads to a constitutive endosomal localization of flotillin-2. Furthermore, we observed an altered localization of the dominant negative mutant of Arf6 in flotillin-depleted cells. Additionally, a depletion of flotillins interferes with the intracellular trafficking of Arf6-cargo proteins such as BACE1. Altogether, we here unveil a novel functional connection of flotillins and Arf6 in the context of membrane transport processes.

MTP13
The antigen translocation complex TAP in nanodiscs as a platform for mechanistic studies

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The ATP-binding cassette (ABC) transporter TAP is a key player in the major histocompatibility complex (MHC) class I mediated antigen processing pathway. The heterodimeric protein complex composed of TAP1 and TAP2 transports proteasomal degradation products from the cytosol into the lumen of the ER driven by the hydrolysis of ATP. The peptides are loaded on MHC class I molecules and finally delivered via the *Golgi* network to the plasma membrane where CD8+ T-lymphocytes screen the MHC-I-peptide complexes to eliminate virally infected or malignantly transformed cells.
To analyze the translocation mechanism of TAP in more detail we reconstituted the transport complex in Nanodiscs (Nds). Nds are small sized membrane particles formed by a scaffold protein with the advantage of full accessibility to both sites of the protein. We firstly established an orthogonal purification, which leads to monodisperse complexes. TAP1 and TAP2 were fused to mVenus and mCerulean, respectively, which allowed to monitor the stoichiometry of the TAP complex. TAP was successfully reconstituted in Nds of two different sizes, leading to monodisperse and active complexes. TAP function in Nds was confirmed by substrate binding and peptide stimulated ATP hydrolysis as an indirect measurement of antigen translocation. In addition, the specific interaction and inhibition mechanism of viral factors was analyzed.

MTP14
Electrophysiological investigation of the electroneutral Na^+/H^+ antiporter NhaP1 from Methanococcus jannaschii.

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The archaeal transporter NhaP1 from *Methanococcus jannaschii* (MjNhaP1) is a model system for the electroneutral cation/proton antiporter family CPA1 that contains the pharmacologically relevant eukaryotic NHE transporters. MjNhaP1 can exchange either Na^+ or Li^+ for protons, with the highest activity at pH = 6.0, and no activity at pH ≥ 7.5.
Reconstitution of MjNhaP1 into proteoliposomes which were subsequently adsorbed to a solid supported membrane (SSM) allowed the recording of a fast transient current upon Na^+ or Li^+ concentration jumps using a rapid solution exchange. The amplitude of this transient current increased with pH, reaching a plateau at pH = 7.5, allowing us to assign it to electrogenic binding and/or translocation of the substrate Na^+ to the transporter. A transient current that was assigned to proton binding and/or translocation could also be recorded by performing proton concentration jumps. Using SSM-based electrophysiology we determined substrate affinities of MjNhaP1 wt and selected mutants at different pH values and a transport mechanism based on Na^+ and H^+ competing for a common binding site was proposed.
We propose that a similar approach using SSM-based electrophysiology could also be employed in the case of other members of the CPA1 family, thereby allowing electrophysiological characterization of electroneutral Na^+/H^+ exchangers.

MTP15
The production of defensive compounds in the exocrine glands of juvenile leaf beetles relies on the activity of ABC transporters and sugar porters

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Insects have an outstanding virtuosity when it comes to producing defensive compounds for repelling their omnipresent enemies. To circumvent auto-intoxicative effects, these natural products frequently originate in exocrine glands. The immature leaf beetles of the subtribe Chrysomelina, for example, evolved such glands on their back and release deterrent secretions upon disturbance. Each of the glands is composed of several secretory cells which are attached to a large reservoir. Within the reservoir the deterrents are produced by few enzymatic reactions from non-toxic precursors. These precursors have to be translocated from hemolymph into the secretions *via* the secretory cells. By proteomics analyses of the secretory cells we found ATP binding cassette (ABC) transporters and sugar porters classified into the SLC2 family. Here we present localization of the ABC transporter *Cp*MRP within the cell for shuttling pre-filtered metabolites into defensive secretions. Silencing of *Cp*MRP by RNAi created a defenseless phenotype in the larvae indicating its key function also for the secretion process. Because energy is used *inter alia* for the transport process in the defensive glands, sugars need to be delivered by porters to fuel ATP production in this tissue. RNAseq expression studies revealed a set of six putative SLC2 members highly expressed in the defensive glands of the immature beetles. Only concerted silencing of these sequences resulted in a decrease of defensive secretions indicating an adaptive backup system which stabilizes the sugar homeostasis in the defensive glands. This also underlines the importance of the chemical defense used by the insects in the competitive interactions in natural ecosystems.

MTP16
The heme *a* synthase Cox15 associates with cytochrome c oxidase assembly intermediates during Cox1 maturation

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Cox1, the core subunit of the cytochrome c oxidase, receives two heme *a* cofactors during assembly of the enzyme complex. The heme *a* synthase, Cox15, catalyzes the final step of heme *a* synthesis. However, it has remained enigmatic at which step of the assembly process and how heme is inserted into Cox1. We performed a comprehensive analysis of cytochrome *c* oxidase assembly intermediates containing Shy1, the yeast SURF1 homolog, which has been implicated in heme transfer to Cox1. Our analyses reveal that Cox15 forms protein complexes with Shy1 but also associates with Cox1-containing complexes independent of Shy1 function. These findings indicate that Shy1 does not serve as a mobile heme-carrier between heme *a* synthase and maturing Cox1 but rather cooperates with Cox15 for heme transfer in early assembly intermediates of cytochrome *c* oxidase.

MTP17
Characterisation of an Ent3p and Ent5p interaction partner in trafficking between TGN and endosomes

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Several adaptor proteins mediate cargo sorting and budding of clathrin coated vesicles in TGN (trans-Golgi network) to endosome transport in *Saccharomyces cerevisiae*. Two of these adaptors are the ENTH (epsin N-terminal homology) domain protein Ent3p and ANTH [AP (adaptor protein)-180 N-terminal homology] domain protein Ent5p, which have partially redundant functions and interact with phosphoinositides, clathrin and other adaptor proteins. Ent3p interacts with the N-terminal domains of SNAREs Vti1p, Pep12p and Syn8p involved in the SNARE complex at the late endosome.
The mammalian putative kinase CAVK104 binds the Ent3p ortholog epsinR and has been implicated in sorting of SNAREs related to Vti1p and Syn8p. CAVK104 is related to Scy1p, which has not been characterized. Here, we study the role of Scy1p. In yeast-two hybrid experiments we show the interaction of Ent3p with Scy1p and Ent5p with Scy1p. We generated deletion mutants *ent3Δscy1Δ*, *ent5Δscy1Δ* and *ent3Δent5Δscy1Δ* to analyse and understand the function of Ent3p, Ent5p and Scy1p. *ent3Δent5Δscy1Δ* cells display different defects in growth, endocytosis, transport between TGN and late endosomes as well as in morphology.

MTP18
Role of Vti1a and Vti1b in phagocytosis and phagosome maturation

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SNARE proteins (soluble *N*-ethylmaleimide sensitive factor attachment protein receptors) mediate membrane fusion in eukaryotic cells. Distinct sets of SNAREs are needed for different transport steps. Vti1p is an endosomal SNARE in *S. cerevisiae* and participating in different endosomal SNARE complexes. A knockout of Vti1p is lethal for the yeast cells. Therefore our special interest lies on the mammalian homologues of Vti1p, Vti1a and Vti1b. Knockout mice for either Vti1a or Vti1b are viable and fertile, whereas a doubleknockout (DKO) of both proteins leads to massive neuronal impairements during embryogenesis and to perinatal lethality.
Macrophages are professional phagocytes. Fulfilling their duty in phagocytosis and killing of microbes and pathogens they depend on intense membrane trafficking. As the trafficking steps in phagosome maturation are very parallel to the maturation of endosomes to lysosomes, we investigated the role of Vti1a and Vti1b in phagocytosis, phagosome maturation and killing of pathogens in bonemarrow derived macrophages from Vti1a$^{-/-}$ or Vti1b$^{-/-}$ mice in comparison to their heterozygous counterparts. We analysed the killing of *E.coli* as well as phagocytosis and maturation of phagosomes containing *E.coli* or *M. bovis BCG* by means of fluorescence based techniques and microscopy.

MTP19
Host-guest Reporter Pairs with 'Turn-on' Fluorescence Response to Monitor the Uptake of Cationic Analytes into Live Cells

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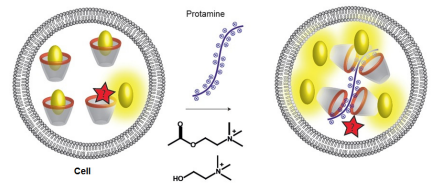
The *p*-sulfonatocalix[4]arene (CX4) host quenches the fluorescence of the cationic fluorescent guest lucigenin (LCG) upon forming the CX4·LCG complex. In this work, V79 and CHO cells were first incubated with the CX4·LCG complex, followed by incubation with acetylcholine, choline and protamine analytes. The CX4·LCG complex passed through the cell membrane and exists in the cytoplasm of the cells despite the presence of many biomolecules of various size and charge. The cationic analytes bind to CX4, displacing LCG, whose fluorescence recovery was followed by fluorescence microscopy. Additionally, to measure the LCG uptake, the cells were also incubated with CX4·LCG and LCG separately and lysed. The cells incubated with CX4·LCG were found to have a higher dye uptake. Therefore, CX4 can facilitate the cellular uptake of LCG and potentially be used as drug carrier.



MTP20
Functional and Structural Characterisation of the T1SS ABC Transporter HlyB

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Type I secretion systems (T1SS) in Gram-negative bacteria are composed of three indispensable membrane proteins, an ABC transporter, a membrane fusion protein and an outer membrane factor. Proteins secreted via a T1SS are translocated in one step across both membranes, without the occurrence of periplasmic intermediates.
HlyB is the T1SS ABC transporter involved in the secretion of the toxin HlyA. Apart from the canonical domains generally described for ABC transporters it contains a cytosolic C39-peptidase-like domain (CLD). Its structure resembles a C39 peptidase domain but is degenerated in respect to proteolytic activity.
So far, purification strategies for several tagged mutations of HlyB have been established and protein of high purity can be obtained. We are attempting further functional characterisation of the transporter in solution as well as incorporated into nanodiscs, with emphasis on the role of the CLD for the transporter's ATPase activity and secretion efficiency.

MTP21
Detecting substrates bound to the secondary multidrug efflux pump EmrE by DNP enhanced solid-state NMR

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E. coli EmrE, a homo dimeric multidrug antiporter has been suggested to offer a convenient paradigm for secondary transporters due to its small size. It contains four transmembrane helices and forms a functional dimer. We have probed the specific binding of substrates TPP^+ and MTP^+ to EmrE reconstituted into DMPC liposomes by ^{31}P-MAS NMR. Our NMR data show that both substrates occupy the same binding pocket but also indicate some degree of structural disorder in the substrate-binding pocket reflecting the promiscuous nature of ligand binding by multidrug efflux pumps. Direct interaction between ^{13}C labeled TPP^+ and key residues within the EmrE dimer have been probed by through-space ^{13}C-^{13}C correlation spectroscopy. This was made possible by the use of solid-state NMR enhanced by dynamic nuclear polarization (DNP) through which a 20-fold signal enhancement was achieved. Our data provide clear evidence for the long assumed direct interaction between substrates such as TPP^+ and the essential residue E14 in transmembrane helix 1. Our work also demonstrates the power of DNP-enhanced solid-state NMR at low temperatures for the study for secondary transporters, which are highly challenging for conventional NMR detection.

MTP22
Biochemical identification of SNARE complex interaction partners in the nematode *C. elegans*

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Most genes involved in neurotransmission are evolutionarily conserved. The SNARE complex with its coiled coil zippering of three subunits is the driving force for fusion of synaptic vesicles with the presynaptic membrane. The zippering is tightly controlled via a variety of known, and possibly unknown regulatory proteins. To identify such proteins we take a biochemical approach, i.e. tandem affinity purification (TAP) and mass spectrometry (MS) analysis. We fused a ProteinA tag to synaptobrevin (SNB-1), the vesicular SNARE, and a calmodulin binding peptide to UNC-64, the *C. elegans* orthologue of the target membrane SNARE syntaxin, to perform a split-TAP-tag purification, aiming to enrich for assembled trans- (and possibly cis-) SNARE complexes. Both proteins are expressed from a low-copy transgene, to avoid ectopic interaction as SNB-1/UNC-64 dimers, and in a snb-1 null background to enrich for SNARE complexes containing tagged subunits. We show the sequential purification of SNARE complex and interacting proteins directly from *C. elegans* under detergent solubilization, but non-denaturing conditions. In addition we prove the functionality of the tagged proteins and confirm the successful purification by western blot and silver-stain protein analysis. Currently, we prepare purified samples under different conditions for a comprehensive set of mass spectrometry analyses. With our TAP/MS approach we hope to identify unknown players in the control of vesicle fusion and possibly recycling.

MTP23
A novel miniaturized and automated biochip for multiplexed parallel single transport recordings

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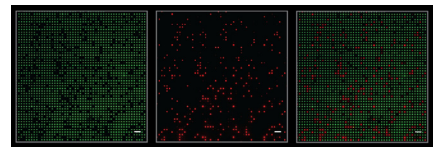
The detailed characterization of membrane proteins is crucial for the development of next generation pharmaceuticals. These omnipresent proteins found in all living cells are of upmost importance in the biochemistry of cellular function, making them the main target for the development of new pharmaceuticals. Especially passive and active membrane integral transporters are of particular interest, as they control most of the transport of ions and small molecules across the cell membrane. Techniques to elucidate their function are readily available for ion channels, but rarely for membrane proteins that facilitate transport of non-ionic substrates. Unfortunately especially those transporters play key roles in many widespread diseases.
We present the development of a new biochip platform to analyze especially those membrane transporters that are inaccessible with other methods until now.
A silicon-based biochip contains thousands of cylindrical cavities enclosed by a porous SiO_2 top layer with apertures in the nanometer range. Large unilamellar vesicles (LUVs) containing reconstituted membrane proteins can be directly fused to the chip surface. Membrane import and export are visualized *via* fluorescent read-out for each individual cavity. The highly parallel time-resolved detection of up to three fluorescent signals allows us to monitor the kinetics of the transport substrate, a control dye and a lipid dye to monitor bilayer integrity.
The presented biochip technique combines highly parallel, semi-automated analysis and small sample consumption with high sensitivity and a single transporter resolution, creating the first step towards a miniaturized system able to perform high-throughput screenings for pharmaceutical research.
Kleefen A, *et al*. (2010) Multiplexed parallel single transport recordings on nanopore arrays. *Nano Lett* **10**, 5080-5087



MTP24
The p7protein of Hepatitis C - characterisation of genotype-specific ion channel constructs and novel antiviral agents

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Hepatitis C is a major causes of liver disease worldwide, affecting more than 170 million individuals (~3 % of the world population). Prevalence in Egypt is ~18 % (15 million patients), and 70,000 to 140,000 new cases are reported annually. Secondary liver diseases following HCV infection, such as cirrhosis or hepatocellular carcinoma make hepatitis C one of the leading causes of morbidity in Egypt.
Nine different genotypes of hepatitis C have been identified to date. Genotype 1 responds poorest to chemotherapy, treatment for genotype 4 is considered more difficult than for the responsive genotypes 2 and 3. However, even combination therapies leave 20-50 % of patients without successful treatment.
In the search for novel treatment strategies, we chose the p7 viroporin as therapeutic target. p7 is a small 63-residue helix-turn-helix transmembrane protein, forming intracellular ion channels, likely acting as ER acidity regulator. Here, p7 cDNA constructs corresponding to consensus sequences of genotype 1, 2, 3, and 4 were generated. After expression in recombinant host systems (HEK 293, HuH cell lines), p7 channels were studied by patch-clamp electrophysiology. The pH-dependence of p7-mediated currents, as well as ion conductance and sensitivity to known inhibitors were studied. p7 channel properties were compared between different genotypes. Novel p7 inhibitors, based on amantadine, or isolated from marine extracts were tested and the sensitivity of different genotype-specific p7 channels to inhibitors was determined.
Supported by STDF (Science and Technology Development Fund Egypt), project ID 1785.

MTP25
(Re)constructing and Dissecting Membrane Proteins - Understanding Helix-Helix Interactions through Segmental Labelling and Solid-state NMR

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Proteorhodopsin (PR) is a seven-helix-transmembrane protein, which is homolog to bacteriorhodopsin (BR), working as a light-driven proton pump (Friedrich et al, 2002). Although a lot of structural information on PR has been discovered in recent years (Shi et al, 2009)(Reckel et al, 2011), less is known about the functional mechanism of the protein. In this context, one interesting issue is the importance of helix-helix interactions for the function of PR. Helix-helix contacts are generally difficult to predict or to measure but contribute significantly to the functional fold of a-helical membrane proteins. Previous studies showed a specific H-bond formation between H75 (helix B) and D97 (helix C) in PR (Hempelmann et al, 2011). Furthermore it has been observed that BR fragment can form spontaneously a retinal binding protein complex (Popot et al, 1986). To investigate helix-helix contacts in PR a segmental labelling approach in combination with solid-state NMR studies is applied. Segmental isotopic labelling is used to label only one or both parts of a protein with different isotopes. This method has already been successfully applied to soluble proteins for solution-state NMR (Zuger et al, 2005), but so far not for characterization of membrane proteins. For labelling a split intein-based approach is used here. Two segments of PR (AB- and C-F-helix) are fused with the N- and C-terminal parts of the *Npu* DnaE intein, respectively. Expressing the precursors separately (in *vitro* splicing) or under different promoters in the same cell (in *vivo* splicing) a “ligated PR” is obtained which shows similar characteristics than PRwt. With this method it will be possible to introduce different labels into one protein molecule to investigate interactions between the two parts of proteorhodopsin by solid-state NMR.

MTP26
Lipid and phase specificity of the pore forming toxin Hla from S.aureus

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The pore forming toxin Hla from *S.aureus* is secreted by the bacterium as a monomer and assembles up to heptameric oligomers on cellular und artificial membranes, which finally can undergo a conformational transition to a transmembrane pore. Different types of cells display very different levels of susceptibility. Since cholesterol depletion and removal of sphingomyelin was shown to abolish high susceptibility we proposed that lipid rafts play a role in keeping up sensitivity (Valeva et al., 2006, *JBC* 281). Also the involvement of ADAM10 in the lysis of cell upon Hla treatment could indicate an important role of lipid rafts (Wilke et al, 2010, PNAS, 107).In a detailed study with artificial membranes we now could show that a lipids in the liquid ordered phase as characteristic of lipid rafts is not the favoured interaction partner of Hla. Furthermore, fluorescence microscopy data also indicate the accumulation of toxin in liquid disordered phases. However, Hla forms more oligomers on membranes containing sphingomyelin than on those containing phosphatidylcholine (Schwiering et al., 2013, BBA 1828) and also increased levels of cholesterol increased oligomerisation. Thus, the reduction in susceptibility upon cholesterol and sphingomyelin removal as observed before is in line with the preference of Hla, without involving lipid rafts in the initial step of pore formation.
We thank the DFG (SFB 490) for financial support and Prof. M.Husmann (Institute for Medical Microbiology, Mainz) for help with the production of toxin.

MTP27
identification of a component of the import machinery at the inner membrane of mitochondria as a potential calmodulin-binding protein

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Mitochondria are organelles of endosymbiotic origin whose majority of proteins are nuclear-encoded and synthesized on cytoplasmic ribosomes. The proteins are transported by multi-heteromeric protein complexes into and across the outer and inner membrane of the organelle.While much is known about the different proteinaceous components involved in the import , very little is known, how the import process is regulated.The identification of a new regulatory mechanism for protein translocation into plant mitochondria affected by calcium/calmodulin led to the hypothesis this process could be integrated into the cellular calcium-signaling network of the cell. Using bioinformatical analysis of the available databases we could identified a component of the import machinery at the inner membrane of mitochondria as a potential calmodulin-binding protein as well as a calmodulin-like protein, CML30, as a mitochondria-targeted calcium sensor. Transient transformation of YFP-CML30 protein into tobacco leaf cells as well as *in vitro* import experiments confirmed the localization of CML30 in mitochondria. Cross-linking studies, affinity chromatography on calmodulin-agarose and the split-ubiquitin. system confirmed that the identified component of the import machinery at the inner membrane of mitochondria is a calmodulin-binding protein and the binding is calcium dependet. In addition we could show that this protein is able to bind specifically to CML30
These results lead to the suggestion that, these proteins together could be the mediators of the calcium regulation of the protein import process into plant mitochondria.

MTP28
Viromers: Safe delivery of siRNA using a bionic concept

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Viromers are carboxyalkyl modified polyamines. Unlike conventional transfectants, Viromers undergo a hydrophobic transition at low pH; a mechanism that facilitates endosome escape and delivery of payloads. Within the Viromer structure, the polyamine backbone binds the nucleic acid cargo molecules. Long-chain carboxyalkyl grafts maintain solubility of the materials at neutral pH, but mediate the membrane insertion upon acidification. The structure does also comprise additional alkyl groups to further promote membrane interaction. The balanced hydrophobicity of the Viromers is a functional equivalent to the fusion peptide of influenza hemagglutinin.
Here we show the development and performance of Viromer BLUE. Data for siRNA binding, delivery and toxicity were obtained in a high-throughput format and the identity of the novel carrier was determined by 1H-NMR. In solution, Viromer BLUE forms particles having a constant size of about 350nm and a neutral surface charge. Purified Viromer BLUE was used for the delivery of RNAi in hard-to-transfect cells, including adipocytes, primary skeletal myoblasts and iPS - cells of high interest when investigating cell differentiation & re-programming. Retaining full viability is a challenge with differentiating cells - which is a valuable confirmation of the safety features provided by Viromer BLUE.
In brief, Viromers are the first non-cationic polymers for the delivery of nucleic acids. The material provides a functional endosome escape inspired by and features effective delivery of siRNA into hard-to-transfect cells.

MTP29
The ionophores calcimycin and ionomycin extract iron and manganese from soil bacteria - a potent strategy against competitors

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Calcimycin and ionomycin produced by terrestrial *Streptomyces* species [1, 2] show potent antibacterial activity. Previously, the ionophore-mediated transport of individual metal cation species across model membranes was studied providing insights into transport mechanisms. The ionophores only cross membranes with cations bound [3]. Transport efficiency is determined largely by cation complexation, translocation, and release rates [4]. Two key questions remained: What is the antibacterial mode of action and the ecological role of these ionophores?
Calcimycin and ionomycin affect *B. subtilis* metal ion homeostasis in chemically defined medium - within 15 min, cells lost about 50% of the intracellular iron and manganese whereas calcium levels increased. Proteome analysis showed manganese and iron depletion coincide with oxidative stress, which was confirmed with a fluorescent probe. In soil, iron availability is generally very low, and manganese can also be growth-limiting. In medium lacking iron or manganese sensitivity to ionophores increased, likely because cation efflux depends on the concentration gradient. Conversely, with calcium omitted, *B. subtilis* tolerated higher ionophore levels, suggesting a lack of calcium either limits manganese and iron efflux or precludes harmful influx of excess calcium. Our findings suggest that ionophores act by extracting iron and manganese from bacteria, a potent strategy against competing microbes, particularly in soils rich in calcium and generally low in micronutrients.

References

[1] Wu *et al*. (2011) Antimicrob Agents Chemother 55:974-82
[2] Liu *et al*. (1978) J Biol Chem 253:5892-4
[3] Brasseur *et al*. (1982) Proc Natl Acad Sci USA 79:2895-7
[4] Erdahl *et al*. (1996) Biochemistry 35:13817-25

MTP30
AtCML4 and AtCML5 - Two calmodulin-like proteins of the endomembrane system in *Arabidopsis thaliana*

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Calmodulins (CaM) and calmodulin-like proteins (CMLs) are important signalling transducers in plant cells mediating the response to signal-induced changes in cellular calcium levels. These changes can be caused by environmental signals, e.g. abiotic and biotic stresses, or endogenous signals. The *Arabidopsis thaliana* (*A. thaliana*) genome encodes for more than 50 CaMs and plant-specific CMLs influencing the activity of a variety of downstream interaction partners like transcription factors, transporters, cytoskeletal proteins and metabolic enzymes. Whereas the different CaMs and their isoforms are exclusively localised in the cytoplasm, investigations on CMLs have shown their localisation to other cellular compartments as well, e.g. mitochondria and peroxisomes. In our study we focused on determining the subcellular localisation of the *A. thaliana* proteins CML4 and CML5 applying a self-assembling GFP assay and transient transformation of tobacco leaf cells. The results gained by this approach suggest a localisation of both proteins to the endomembrane system. The targeting is probably mediated by an extended N-terminus that differentiates CML4 and CML5 from their phylogenetically closely related CMLs in *A. thaliana*.

MTP31
Aspergillus nidulans nitrate transport inhibition by substrate structural analogues

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NrtA and NrtB permeases encoded by *nrtA* and *nrtB* genes respectively are the only nitrate transporters in *Aspergillus nidulans.* It has been established for a number transporters that their substrate structural analogues can inhibit the transport activity. Another thing is due to multiple permeases for a single substrate, it is difficult to study the inhibitory effect on an individual protein. In this present study, loss-of-function mutants in one or the other transporter-encoding gene was used to study the response of individual proteins to each

inhibitor. Growth response of fungus towards a number of nitrate analogues was evaluated for their potential as inhibitors of nitrate transport. Such growth tests showed that no inhibition of nitrate transport by NrtA or NrtB transporter proteins was observed in the presence of bicarbonate, carbonate, formate, malonate, oxalate or sulphite. Chlorate and chlorite inhibited fungal growth to a considerable extend with differential results for each transporter. Also chlorite showed greater growth reduction. Similarly, the cation, caesium proved to exert differential effects by strongly inhibiting the transport by NrtB, but not by NrtA. Chlorate and chlorite growth responses, coupled with net nitrate transport kinetic assays, clearly demonstrate differences in the properties of the two transporters. Kinetic analyses of nitrate uptake reveal that both chlorate and chlorite inhibit NrtA competitively, while these same inhibitors inhibit NrtB in a non-competitive fashion. The caesium ion appeared to inhibit NrtA in a non-competitive fashion, while NrtB was inhibited uncompetitively; further evidence of the distinct character of these transporters.

MTP32

Archaellum:The ancient nanomachine- a detailed biochemical and structural dissection.

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Unique to archaeal motility organelle Archaellum, formerly archaeal flagella is its structural similarity with bacterial type IV pilus and functional resemblance to flagellum. Extensive phylogenetic and molecular characterization has revealed that all the genes present in archaellum gene cluster are essential for its structural stability. In *Sulfolobus acidocaldarius*, a hyperthermophilic crenarchaeon the archaella gene cluster consist of seven genes namely, *flaBXGFHIJ*. FlaB encodes for the filament forming protein archaellins, while FlaX-FlaH encodes protein with unknown function, FlaI, a dual function ATPase and FlaJ is the polytopic membrane protein which is the structural homologue to PilC in type IV pilus assembly system.
While archaellum was studied extensively using physiological and genetic analyses but structural or biochemical characterization was limited. In this particular issue our group has initiated a systematic analysis to understand the precise assembly mechanism and physical properties of crenarchaeal flagella using *S.acidocaldarius* as a model organism. To this end we have crystallized ATP bound FlaI hexamer and also shown that the unstructured N terminal 29 amino acids holds its dual function in terms of archaellum assembly to motility. In a parallel study we have presented crenarchaea specific protein FlaX forms a ring like oligomer of 30 nm diameter in vitro and might acts as a membrane bound cytoplasmic scaffold in archaellum assembly. Furthermore, 1.5 Å resolution crystal structure of FlaF revealed a novel β sandwich fold. FlaF can bind to chitobiose and specifically, FlaF bound to the glycosylated S-layer which builds the only cell envelope component in *S. acidocaldarius*. Therefore FlaF is similar to MotB in the bacterial flagellum the stator that anchors the archaellum in the archaeal cell envelope.

MTP33

Functional and structural organization of the MHC I peptide loading complex

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The loading of antigenic peptides onto major histocompatibility complex class I (MHC I) molecules is an essential step in the adaptive immune response against virally or malignantly transformed cells. The ER-resident peptide-loading complex (PLC) consists of the transporter associated with antigen processing (TAP1 and TAP2), assembled among others with the auxiliary factors tapasin and MHC I. The N-terminal domain of each TAP subunit, TMD_0, bridges the interaction between the TAP translocation pore and, via tapasin, MHC I. Despite intensive work, multiple aspects of the interplay of TAP and tapasin and the organization of the PLC remained unclear. We demonstrated that TMD_0^{TAP1} and TMD_0^{TAP2} are both independently targeted to the ER and form autonomous interaction hubs for the interaction with tapasin, in a 1:1 stoichiometry. However, no structural data for TMD_0 is available so far. Interestingly TMD_0^{TAP1} and TMD_0^{TAP2} share only 16.75 % sequence homology. That raises the intriguing question about the nature of the interaction of TAP with tapasin, and how tapasin manages to bind these two very different interaction hubs. Here we demonstrate new insight into the organization of TMD_0^{TAP1} and the nature of its interaction with tapasin. By using a cysteine scanning and oxidative cross-linking approach, we provide insight into the structural understating of the TMD_0. Furthermore, we shed light on the tapasin-binding site of TMD_0^{TAP1}, and identified a residue in TMD_0^{TAP1}, which is important for tapasin binding. Taken together our data adds to the understanding of the structure of TMD_0^{TAP1} and the nature of the TAP - tapasin interaction.

MTP34

Ouabain interactions with the α4 isoform of the sodium pump induce a signaling cascade resulting in ATF-1 and CREB activation and stimulation of StAR and claudin 1 expression in Sertoli cells.

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The α4 isoform of the Na^+,K^+-ATPase (sodium pump) is known to be expressed in spermatozoa and to be critical for their motility. In the investigation presented here, we find that the rat-derived Sertoli cell line 93RS2 expresses considerable amounts of the α4 isoform in addition to the α1 isoform. Since Sertoli cells are not motile, one can assume that the function of the α4 isoform in these cells differs from that in spermatozoa. Thus, we assessed a potential involvement of this isoform in signaling pathways that are activated by the cardiotonic steroid (CTS) ouabain, a highly specific sodium pump ligand.
Treatment of 93RS2 cells with ouabain leads to activation of the c-Src/cRaf/Erk1/2 signaling cascade. Furthermore, we show for the first time that the activation of this cascade by ouabain results in phosphorylation and activation of the transcription factors CREB and ATF-1. The entire signaling cascade is mediated by the sodium pump α4 isoform, as it is ablated when cells are incubated with siRNA to the α4 isoform.
The ouabain-induced activation of the transcription factors ATF-1 and CREB within the nuclei leads to increased expression of steroidogenic acute regulator (StAR) and of claudin 1. While stimulation of StAR expression by ouabain might be associated with stimulation of steroidogenesis, the identification of increased expression of claudin 1 might indicate a ouabain-induced stabilization of the blood-testis barrier. Taking into consideration that CTS are most likely produced endogenously, the demonstrated induction of StAR and claudin 1 expression by ouabain may help to gain new insights into the physiological mechanisms associated with male fertility and reproduction.

MTP35

Structural and functional characterization of the eukaryotic SRP receptor.

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Membrane protein biogenesis is predominantly a co-translational process and relies on a universally conserved mechanism regulated by the signal recognition particle (SRP) and its cognate receptor. The prokaryotic SRP cycle has been studied in detail, but higher level of complexity and structural instability of the eukaryotic SRP cycle components has hampered the detailed evaluation of the system, specially the characterization of the receptor. The eukaryotic SRP Receptor (SR) is a heterodimeric complex of two GTPases: the SRP GTPase SRα and Sar1-type GTPase SRβ. SRα consists of two major domains: the SRX and the NG domain connected by a natively unfolded linker region of around 200 residues the function of which is unknown. The minimal SRX domain is responsible for interaction with SRβ and the NG domain possesses the GTPase acitvity. SRβ belongs to the canonical Sar1 family but its GTPase activity and mechanism of nucleotide exchange in context of the SRP cycle has not been evaluated in detail, primarily due to the instability of the human and yeast SR. In the present study we used the thermophilic fungus *Chaetomium thermophilum* for structural and functional characterization of both SRP receptor GTPases.

MTP36

Target-Nanoparticles for the Enhancer-Radiotherapy of Cancer - Lanthanide loaded Liposomes and Polymer particles

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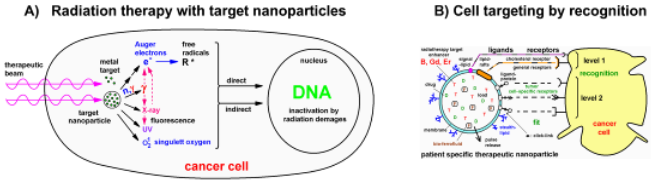
Nanoparticles can concentrate millions of drug molecules per unit and target them to specific cells, e.g. in a tumor. In enhancer radiotherapy the drug increases the radiation cross section and inactivates cancer cells by secondary radiation products (fig.1a). The absorber drugs are Lanthanides for photon therapy PT, or Boron and Gadolinium for neutron capture therapy NCT. The case- and person-specific cell targeting depends on surface modifications by 1)

signal lipids and 2) exposed ligands, both are recognized by cellular receptors (fig.1b). This is the focus of our development, which shall deliver case- and person-specific nanoparticles for cancer therapy.
Our therapeutic nanoparticles are liposomes, polymers, bio-Ferrofluids and combinations. The Nanoparticles of typically 100 nm size are loaded with enhancer drugs, e.g. Lanthanides or Boron. The enhancers inactivate the cancer cells by secondary radiation products upon external irradiation (only then, no radiotherapeutics). We have studied particle structure and dynamics, drug load, and surface modifications with SANS, neutron reflectometry (ILL), ASAXS (ESRF-ID01, BESSY), and DLS, while therapy tests were done with a) cold neutrons at the ILL reactor, thermal neutrons, at the TRIGA reactor Mainz, and b) photons at ESRF-ID17 (60 keV) and at the radiooncology clinics at Mainz using a clinical linear accelerator (8 MeV). For the therapy tests we have developed the high throughput EPN-test with kinetic cell cultures as tumor model. This can distinguish between the unfavourable direct cell inactivation and the clinically wished proliferation inhibition with delayed apoptosis. The system is a central part of a formulation test system for fast development (50,000 tests/week).



MTP37
Towards a structural understanding of 5S RNP biogenesis and the role of Syo1

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Ribosomal proteins (r-proteins) are synthesized in the cytoplasm and imported into the nucleus before their assembly with pre-ribosomal RNA to form nascent ribosomes. To date, little is known about the coordination between nuclear import and assembly of r-proteins in eukaryotic ribosome biogenesis. Previously, it was generally assumed that each ribosomal protein enters the nucleus individually. However, some r-proteins form functional clusters at the ribosomal surface or assemble at specific entry points during ribosome biogenesis, thus raising the possibility of coordinated import and assembly. Rpl5 and Rpl11, two proteins that bind to the 5S rRNA, need to assemble to the 5S RNP, which locates on the surface to the 60S subunit.
Biochemical analyses identified and characterized a novel import adaptor, the symportin Syo1. It concomitantly binds Rpl5 and Rpl11 in the cytosol and synchronizes the nuclear import of these r-proteins for pre-ribosomal 5S-RNP assembly. A karyopherin transport receptor (Kap104) is recruited to the Syo1-Rpl5-Rpl11 complex via an N-terminal PY-NLS on Syo1. Following transport across the nuclear pore complex (NPC), the Syo1-Rpl5-Rpl11 import complex is released from Kap104 by RanGTP, and as shown in vitro can be directly transferred onto the 5S rRNA. Finally, Syo1 may shuttle back to the cytoplasm on its own by interaction with FG-repeat nucleoporins. Following up on recent structural studies of the Syo1-Rlp5 complex, we now asked the question how Syo1 binds both Rpl5 and Rpl11, how the 5S RNA is recruited and how Syo1 is finally released from its cargo. New insights into the structure and function of Syo1 will be presented.

MTP38
Molecular Dissection of the Mitochondrial Protein Import Machinery

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A majority of the mitochondrial proteins are encoded by the nuclear genome. Upon synthesis in the cytosol, these proteins have to be translocated to their respective destinations within the mitochondria. The translocases of the outer membrane (TOM) and the inner membrane (TIM) facilitate the translocation of preproteins across both mitochondrial membranes. The TIM23 complex mediates the translocation of precursor proteins that are targeted to the mitochondrial matrix or to the mitochondrial inner membrane. Tim17 is an integral component of the TIM23 translocase containing four transmembrane domains. The sequence of Tim17 is highly conserved amongst fungi, plants and animals including humans. All Tim17 proteins contain two conserved cysteine residues that are located directly adjacent to the first and second transmembrane domains facing the intermembrane space. The relevance of these cysteine residues is not known. Various functions such as regulation and sorting during preprotein translocation, recruiting of the presequence assisted motor (PAM) machinery, regulation of the protein-conducting channel, and prevention of mitochondrial DNA loss have been assigned to Tim17. However, the precise role of Tim17 in these processes still remains unknown. Growth tests and *in vitro* import experiments revealed that the cysteine residues in Tim17 are crucial for cell growth under respirating conditions pointing at a critical mechanistic or regulatory function. Surprisingly, *in vitro* experiments on isolated mitochondria did not reflect these differences. Mitochondria harbouring cysteine mutants of Tim17 still efficiently import matrix proteins. They contain normal level of respiratory chain complexes and do not show respiration defects. Proteome analysis of these mutants after stable isotope labeling by amino acids in cell culture (SILAC) confirmed that wild type and mutant mitochondria contain basically unaltered protein compositions with very few characteristic differences. The implications for the role of Tim17 and its cysteine motif for mitochondrial protein import will be discussed on this poster. Supported by DFG (IRTG 1830).

Keywords
Yeast mitochondria, Protein import, Protein import regulation, TIM23-17 complex.

MTP39
Biogenesis of Dre2 Occurs Independently of Mitochondrial Oxidoreductase MIA 40

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The assembly of Fe-S-clusters in mitochondria gives the essential character to the organelles. The biogenesis of Fe-S proteins in eukaryotes is the interplay of three systems: the ISC assembly, the ISC export and the CIA machineries. Recently, a new component of the CIA machinery called Dre2 was identified in *S. cerevisiae*. It contains two Fe-S clusters and is implicated in an early step of cytosolic Fe-S-cluster synthesis. All so far identified members of CIA machinery are exclusively located to the cytosol. However, recently published studies claimed that the fraction of Dre2 was present in the IMS of mitochondria. In addition, the physiological interaction of Dre2 with the mitochondrial oxidoreductase Mia40 was proposed on the basis of *in vitro* experiments with purified proteins. It was suggested that Mia40 introduces two disulfide bonds between conserved cysteine residues of Dre2 in order to trap imported protein in the IMS. Here, we demonstrate that Dre2 is entirely localized in the cytosol associated with the cytosol-exposed surface of the mitochondrial outer membrane. This mitochondria-associated fraction further increased upon overexpression of *DRE2*, however, even then no Dre2 was found in the IMS. Dre2 is relatively resistant against proteases, at least as long as the membranes were not lysed with detergents. Moreover, upregulation or depletion of *MIA40* did not affect the level of mitochondria-associated Dre2, nor did Dre2 contain disulfide bonds *in vivo*. Taken together, we conclude that Dre2 is exclusively present in the cytosol where it contains reduced cysteine residues which coordinate Fe-S-clusters. Supported by DFG (IRTG 1830).

Keywords
Yeast, mitochondria, protein import, Mia40, protein homeostasis.

PROTEIN BIOSYNTHESIS AND FOLDING

PKV16
From structure to function of the mammalian SRP system

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Correct and efficient targeting of membrane and secretory proteins is of central importance for each living cell. This process is regulated in a GTP-dependent manner by the universally conserved signal recognition particle (SRP), which co-translationally recognizes hydrophobic signal sequences of its client proteins emerging from the ribosome. In eukaryotes, the SRP/ribosome-nascent chain complex (RNC) is targeted to the endoplasmic reticulum by interaction with the membrane-bound SRP receptor (SR) and the RNC is transferred to a vacant translocation channel. Mammalian SRP is a ribonucleoprotein complex (RNP) comprising an SRP RNA of about 300 nucleotides and six proteins (SRP9, SRP14, SRP19, SRP54, SRP68 and SRP72), while SR consists of the SRαβ heterodimer. The SRP GTPases SRP54 and SRα constitute the core within the targeting complex and regulate the entire process.
Although the structure and function of the SRP system has been extensively studied, especially for the most complex mammalian system many questions remain open. We present missing structural data for mammalian SRP system components and complexes and give insights into an additional level of structural and functional complexity in the regulation of co-translational targeting.

PKV33
A Strategy for the identification of mitochondrial CLPXP protease interactors and substrates

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Mitochondria are involved in various important biological processes. Functional decline of mitochondria is linked to numerous pathologies and to such complex phenomena as aging. Thus, a detailed understanding of the network of mitochondrial quality control pathways and its components is crucial[1].
In the mitochondrial matrix, two soluble proteases termed LON and CLPXP are present. While the LON protease's importance for mitochondrial protein quality control has been extensively characterised, the biological role of the CLPXP protease remains elusive and as yet none of its physiological interactors or substrates have been identified.
We have shown that deletion of the gene coding for the proteolytic component CLPP results in a pronounced longevity phenotype of the fungal aging model *Podospora anserina*. Significantly, it is possible to complement Δ*PaClpP* mutants by heterologous expression of human *ClpP*, demonstrating functional conservation between the human and fungal protein[2]. Purification of tagged variants of catalytically active and inactive fungal and human CLPP from isolated *P. anserina* mitochondria coupled with mass spectrometry analysis is currently being developed as a strategy to identify high confidence interactors and putative substrates of the mitochondrial CLPXP protease.

References

[1] Fischer, F., Hamann, A. & Osiewacz, H. D. *Trends Biochem. Sci.* **37**, 284-292 (2012).
[2] Fischer, F., Weil, A., Hamann, A. & Osiewacz, H. D. *Nat.Commun.* 4:1397 doi: 10.1038/ncomms2397 (2013).

BFP01
Biochemistry of the cell-free expressed human gonadotropin relasing hormone receptor: Towards targeted chemotherapy

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Many modern antitumor drugs have severe side effects due to the lack of selectivity. Targeted chemotherapy by engineering drug delivery systems with high specificity is therefore an important approach in order to optimize medical therapies. The gonadotropin releasing hormone receptor (GnRHR) is a key target being overexpressed in several tumor types (e.g. breast cancer).
A selection of GnRHR ligand derivatives have been synthesized and conjugated to the chemotherapeutical drug daunomycin in order to develop more specific antitumor agents. The effectivity of the conjugates was different which might be caused by their different GnRHR binding properties. In order to study GnRHR-ligand complexes on the molecular level, we have established an efficient protocol for the cell-free (CF) production of the human GnRHR. The quality of the receptor was modulated by its co- or post-translational solubilization in different hydrophobic environments such as detergents, amphipols or nanodiscs. Ligand interaction with the CF-expressed GnRHR was analysed by complementary techniques including surface plasmon resonance or microscale thermophoresis.
We report a new approach for the directed drug design against targets of the important family of G-protein coupled receptors. We further present the analysis of GnRHR-ligand interactions in artificial environments. The presented work might be a useful strategy for generating screening protocols towards selective drug delivery systems.

BFP02
Co-translational processing of glycoprotein 3 from equine arteritis virus:
N-glycosylation in the vicinity of the signal peptide prevents cleavage

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Signal peptide cleavage and N-glycosylation of proteins are co-translational processes, but little is known about their interplay if they compete for adjacent sites. We report two unique features for processing of glycoprotein 3 of equine arteritis virus. Gp3 contains an N-terminal signal peptide, which is not removed although bioinformatics predicts cleavage with high probability. Located just upstream of the signal peptide is the overlapping sequon NNTT which we show to be efficiently glycosylated at both asparagines. Deletion of the overlapping sequon and blocking glycosylation allows signal peptide cleavage indicating that carbohydrate attachment inhibits processing of a potentially cleavable signal peptide.

BFP03
Chaperoning a kinase under stress: v-Src and Hsp90

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Hsp90 is a major cytosolic chaperone crucial in eucaryotes for the activation of several hundred clients involving kinases and transcription factors. As it controls the activity of key regulatory proteins, Hsp90 is located at the hub of homeostasis. Despite extensive research, the central question however still remains unanswered: What makes a client a client?
Here, we determined the properties of a protein stringently dependent on Hsp90, the oncogenic kinase v-Src. Notably, its very close cellular isoform c-Src is only weakly affected by Hsp90. Based on the few sequence variations between c-Src and v-Src, mutants were generated that progessively turn c-Src into v-Src. Their *in vivo* activity and Hsp90 dependence were analyzed in *S. cerevisiae* and subsequently their biophysical properties were compared *in vitro*. Kinases found to be Hsp90 addicted differed in stability and unfolding characteristics.
Furthermore, we could reconstitute chaperone-dependent rescue of Src variants from thermal inactivation *in vitro*. This process was ATP-dependent and could be abrogated by Hsp90 specific inhibitors.
By generating a c-Src mutant that showed Hsp90 dependence, the elements within v-Src that define the protein as a client could be identified. Finally, we reconstituted the Hsp90 chaperone cycle required for activation of v-Src kinase *in vitro*.

BFP04
Growth induction of the translationally controlled tumour protein TCTP is regulated at the translational level through the Akt/mTORC1 signalling pathway

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Translationally controlled tumour protein TCTP is a highly conserved, anti-apoptotic protein involved in both physiological and disease processes, such as cancer. TCTP levels are tightly regulated in response to growth stimuli and to various stress conditions. For example, cellular growth induction leads to a rapid four-fold up-regulation of TCTP levels, but the signalling pathways involved are still largely unknown. Since the mRNA encoding TCTP contains a 5'-terminal oligopyrimidine tract, we hypothesised that its translation is under the control of the PI3K/Akt/mTORC1 pathway. Here we show by western blotting that serum-induced TCTP synthesis is inhibited by rapamycin and TOR kinase inhibitors, and by polysome profiling that these effects occur at the translational level. Consistent with this finding, interception of this pathway upstream of mTORC1 using an inhibitor of Akt prevented the induction of TCTP, whereas gene-knockout of TSC2 (a negative regulator upstream of mTORC1) led to derepression of TCTP synthesis in serum starvation. Overexpression of eIF4E, a downstream effector of mTORC1, enhanced the polysomal association of TCTP mRNA, but did not 'rescue' its translation from the effects of mTOR inhibition, indicating that additional regulatory mechanisms are involved in its control by mTOR.

BFP05
Lipid dependent activities of cell-free expressed MraY translocase homologues: Towards drug screening platforms in defined membrane environments

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Rapidly spreading multiple antibiotic resistances request new approaches for the screening of chemotherapeutic compounds. Although enzymes of bacterial cell wall biosynthesis have been among the first targets, drugs directed against the membrane integrated catalysts have been rarely found. The first membrane bound step of cell wall biosynthesis is catalysed by the integral membrane protein MraY. Expression of membrane proteins in cellular expression systems is still challenging and availability of the enzymes for biochemical characterization was limited.
We have developed efficient cell-free expression protocols for the preparative scale production of MraY homologues. The expression efficiency was dependent on the design of the mRNA and on optimization of translation initiation (1). The quality of the synthesized enzymes was modulated by (i) modifying the hydrophobic environment, (ii) by selecting conditions for the

post-translational solubilization and (iii) by screening of the lipid composition in preformed nanodiscs (2). The specific activity of the produced MraY samples was determined by *in vitro* lipid-I formation. We demonstrate that cell-free expression strategies as well as the solubilization conditions are specific to the individual MraY homologues (3). The *Bacillus subtilis* MraY can be synthesized as a stable enzyme with high activity in a variety of different conditions, whereas enterobacterial MraY homologues were inhibited by detergents and high quality protein samples could only be produced in presence of specific lipids. The complete biosynthetic pathway leading to Lipid-II formation could be reconstituted with cell-free expressed proteins and will provide the basis for new drug screening platforms.

BFP06

The efficiency and dynamics of the UGA recoding into selenocysteine *in vivo* and *in vitro*

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In bacteria, the biosynthesis and specific incorporation of Selenocysteine (Sec) into proteins requires the function of two *cis* (a UGA codon, which is usually recognized by the RF2 and the SECIS-element) and four *trans* elements (SelB, $tRNA^{Sec}$, SelD & SelA). The mechanism of UGA recoding into Sec on the ribosome remains unclear, largely because detailed mechanistic studies were so far hampered by the lack of reliable *in vivo* and *in vitro* assays. The efficiency of Sec incorporation into *E. coli* proteins *in vivo* was reported to be low and depend on the growth stage, raising the possibility that Sec incorporation is under translational control by auxiliary factors unknown so far or by changes in the concentration of RF2. To address these questions, we constructed a dual reporter system and validated its performance using Western blots and luciferase assays. In contrast to previous reports, which suggested a very low (3-5% efficiency of Sec incorporation) in rapidly growing cells, we found a robust 40% of Sec insertion. Overproduction of RF2 repressed synthesis of selenoproteins, indicating that RF2 competes with SelB·GTP·$tRNA^{Sec}$ for the UGA codon; however, the extent of inhibition is remarkably mild given the large excess of RF2 over SelB and $tRNA^{Sec}$ in the cell. To study the competition between RF2 and the Sec machinery, we described a well-defined fully reconstructed *in vitro* translation system to study the mechanism of selenoprotein synthesis.

BFP07

A Gateway-based high-throughput system for the optimization of recombinant protein expression in K. lactis

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Structural and functional studies require large amounts of a properly folded high purity protein. Identifying the most suitable heterologous system for a given protein target is usually a tedious trial-and-error process that often forms a bottleneck for subsequent experimentation. Yield, purity and functional properties of a heterologously produced target protein depend not only on an expression host, but also on the placement and physicochemical characteristics of purification tags.
We have implemented a high-throughput approach for heterologous protein expression in *Kluyveromyces lactis*. To this end we constructed a set of Gateway-compatible destination vectors featuring various combinations of His6, SA-StrepII, and GST tags that can be fused to both N- and C-termini of a protein of interest. We used mCherry fluorescent protein in the 96-wellplate format in our proof-of-concept study. In total, 20 different constructs for intracellular and secreted expression were cloned and evaluated. Data revealed substantial differences in expression levels between individual constructs as well as *K. lactis* clones.
The expression system presented here provides a robust approach to analyze a considerable amount of constructs and clones in order to find the best producer of a protein of interest within a relatively short time span.

BFP08

Cytosolic factors for oxidative protein import into mitochondria

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Mitochondria fulfill crucial functions such as the production of cellular ATP or the regulation of apoptosis. Most mitochondrial proteins have to be imported after their translation in the cytosol. The majority of these proteins depend on a mitochondrial targeting sequence (MTS) which facilitates their mitochondrial translocation in a post-translational manner. In comparison, a set of intermembrane space (IMS) proteins lack such an MTS but share conserved cysteine patterns such as twin-CX_3C or twin-CX_9C motifs. These motifs are required for their import which is coupled to oxidative folding. The oxidation of these proteins is mediated by the disulfide relay system in the IMS. The two main components of this system are the oxidoreductase Mia40 and the sulfhydryl oxidase ALR. The current knowledge about the disulfide relay system is mainly based on in vitro data and on yeast studies. We aim to reveal the function and regulation of the disulfide relay system in intact human cells. In our experiments we compared the MTS-mediated protein import pathway with the oxidation-dependent import pathway. We thereby found that protein import via the disulfide relay system is a much slower process compared to the MTS-dependent import [1]. We hypothesize that the slower protein import of twin-CX_3C or twin-CX_9C proteins requires stabilizing factors in the cytosol to maintain substrates in an unfolded and import-competent state until translocation. In this study we aimed to reveal these putative stabilizing factors and to characterize their interaction with IMS proteins in the cytosol.

References
[1] Fischer, Riemer (2013) Mol. Biol. Cell *May 15, 2013 mbc.E12-12-0862*

BFP09

Conformational dynamics of the ribosome recycling factor ABCE1

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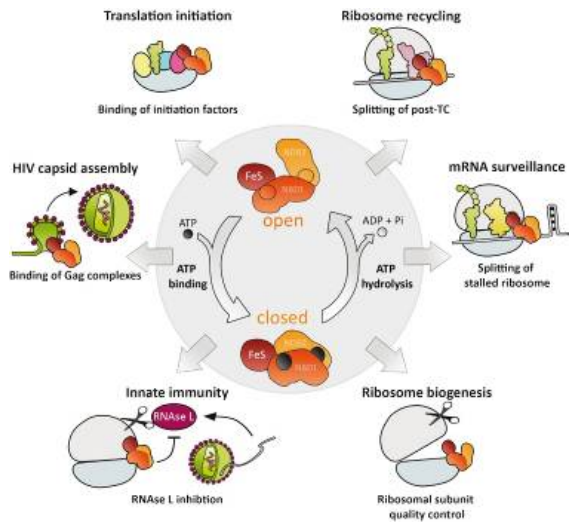
Protein biosynthesis is a central process in each living cell. It is tightly regulated and takes place at the ribosome, an RNA-protein machinery, which comprises of two macromolecular subunits. Ribosomal subunits split after translation termination, mRNA quality control and during ribosome biogenesis (1). The multi-domain twin ATPase ABCE1 has been identified as the major ribosome recycling factor in human (2), yeast (3) and archaea (4). ABCE1 is further proposed to participate in re-initiation of a next translation round, and is therefore regarded as the functional link between translation termination and initiation. Interestingly, ABCE1 also inhibits the RNAse-L pathway in innate immunity and assists HIV Gag-capsid assembly. We used the crenarchaeon *Sulfolobus solfataricus* as a model system to decipher the ancestral role of ABCE1 in ribosome recycling. Here, ABCE1 possesses an energy dependent ribosome splitting activity, which is enabled via a conformational switch of its nucleotide binding domains (NBDs) upon ATP binding. We focused on the structural organization of its unique FeS clusters by EPR spectroscopy (5) and determined the crystal structure of ABCE1 at 2.0 Å (4). We aim at identifying the conformational dynamics of ABCE1 during ribosome recycling. Therefore we arrest ABCE1 in different conformations by oxidative cysteine crosslinking to study their structure and their role in ribosome recycling. We gain insights into the movement of ABCE1 in solution by a combination of spectroscopic techniques.

References
1. Nürenberg & Tampé (2013) *TIBS* 38, 64-74
2. Pisarev *et al.* (2010) *Mol. Cell* 37, 196-210
3. Shoemaker *et al.* (2011) *PNAS* 108, 1392-1398
4. Barthelme *et al.* (2011) *PNAS* 108, 3228-3233
5. Barthelme *et al.* (2007) *JBC* 282, 14598-14607



Multitasking of cellular functions by ABCE1 (1).

BFP10
Expression and characterization of different fructose 6-phosphate aldolases

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Fructose 6-phosphate aldolases (FSA) belong to the class 1 aldolases which usually catalyze a stereoselective addition of a keto donor on an aldehyde molecule via a catalytic lysine. Among other things, aldolases are used for the biotechnological production of iminosugars what admittedly turns out to be extensive and difficult due to the number of available stereocenters. Furthermore, for the industrial production the so far used FSA from *E. coli* is of limited suitability because of their insufficient stability and inactivation by the aldehyde acceptor. Therefore the aim is to find alternative fructose 6-phosphate aldolases in the natural biodiversity with high thermo stability and the ability to tolerate high substrate concentrations.
The synthetic genes of the FSAs from *Lactobacillus plantarum* (Lp_FSA), *Clostridium beijerinckii* (Cb_FSA) and *Aeromonas veronii* (Av_FSA) were expressed in *E. coli* BL21 (DE3) and purified using sonication and heat treatment at 70 °C for 10 min. Subsequently the characterization of the enzymes and the comparison with the recombinantly expressed FSA of *Escherichia coli* W3100 (Ec_FSA) concerning temperature and pH optimum as well as temperature stability and kinetic parameters followed. The results showed that all four characterized FSA have the same temperature optimum at 60 °C. The Cb_FSA and Av_FSA have a pH optimum at 8.0 whereas the Lp_FSA showed the highest activity at pH 5 and the Ec_FSA at pH 6. After incubation at 37 °C all four FSA showed only a slight loss of their activity up to 10 %. After 8 hours of incubation at 60 °C the Cb_FSA, Lp_FSA and the Ec_FSA also showed only a slight loss of their activity while the Av_FSA showed about 50 % of its activity after 90 min and only about 0.3 % of its activity after 8 hours of incubation at 60 °C.

BFP11
Recombinant Expression of a Chimeric Antimicrobial ß-Defensin utilizing N^{pro} Fusion Technology

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Human β-defensin 2 (HBD2) / β-defensin 3 (HBD3) are small (2 to 6 KDa) antimicrobial peptides containing predominantly cationic amino acids. Chimeric molecules of HBD2/HBD3 show even enhanced antimicrobial properties for *E.coli* and *S.aureus* at physiological salt concentrations. This makes HBD2/HBD3 chimeric peptides promising candidates as therapeutic drugs and due to the low yield of expression, alternative expression strategies for efficient HBD2/HBD3 production become highly relevant (1).
Here we present the efficient expression of the chimeric peptide HBD2/HBD3 utilizing N^{pro} autoprotease fusion technology (NAFT) in *E.coli*. N^{pro} fusion allows autocatalytical cleavage of fusion proteins, which enables synthesis of proteins with an authentic N-terminus (2). High expression rates force the fusion protein into inclusion bodies. After refolding N^{pro} cleaves itself off and liberates correctly folded HBD2/HBD3. Antimicrobial activity was successfully proven by a microdilution assay according to the literature.
As conclusion we can say, that N^{pro} is a suitable tool for production of chimeric HBD2/HBD3 in *E.coli.* In principle defensins can be produced with N^{pro} technology and also other small antimicrobial peptides are suitable proteins for this expression system.

References

(1) Jung *et al*., 2011: Human β-Defensin 2 and β-Defensin 3 Chimeric Peptides Reveal the Structural Basis of the Pathogen Specifity of Their Parent Molecules, Antimicrob Agents Chemother. 55(3), 954-960
(2) Achmüller *et al.*, 2007: Npro fusion technology to produce proteins with authentic N termini in E.Coli, Nat methods, 4(12), 1037-43

BFP12
Adaptation of translation upon peptide deformylase inhibition in *Bacillus subtilis*

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Translation in bacteria makes use of N-terminally formylated methionine (fMet) as starting amino acid. From newly synthesized proteins the formyl residue is removed by peptide deformylase (PDF) followed by removal of Met from some proteins by Met amino peptidase (MAP). Both, removal of the formyl and Met can be crucial for correct protein folding and function. PDF has been identified as promising antibiotic target, complementing a whole suite of targets in bacterial translation. Actinonin is a potent inhibitor of PDF, yet it affects growth of *B. subtilis* only moderately. To study the bacterial adaptation we chose a global quantitative LC-MS-based approach, which allowed monitoring of the protein's N-termini as well as protein regulation. As expected, we found that PDF inhibition leads to accumulation of proteins retaining fMet at the N-terminus.
Actinonin-resistant *B. subtilis* mutants have previously been shown to be impaired in a pathway supplying the formyl donor for tRNA^{fMet} synthesis. These mutants are thought to bypass fMet-dependent translation initiation by initiating with Met. We found enzymes of the formyl donor supply pathway to be down-regulated in response to actinonin suggesting that this bypass is part of the metabolic adaptation. Interestingly, we observed that proteins normally processed by MAP accumulate as proteoforms with non-formylated Met. MAP being unable to remove the N-terminal Met from its regular substrates provides evidence that MAP acts PDF-dependently and co-translationally.

BFP13
Elucidating the Structure of an Autoproteolytic Expression Tool

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The N-terminal autoprotease N^{pro} encoded by some *Pestiviruses* represents a convenient tool for expression of recombinant proteins and peptides. By nature N^{pro} holds two interesting properties: on the one hand it generates authentic N-termini through self-cleavage, on the other hand it is deposited in inclusion bodies upon heterologous expression. N^{pro} Autoprotease Fusion Technology unites these two beneficial properties to produce proteins and peptides for pharmaceutical applications (Achmueller, 2007). We were able to elucidate N^{pro} 's detailed mode of action by defining its three dimensional structure.
Therefore, three autoprotease variants from strain BVDV-3 were refolded and purified combining affinity, ion exchange and size exclusion chromatography. Samples were concentrated by ultrafiltration or tangential flow filtration and crystallized. Crystals were measured at the European Synchrotron Radiation Facility (ESRF) in Grenoble.
The crystal structures reveal a two-domain protein with few secondary structure elements. The protease domain - responsible for the single in-*cis* cleavage event - harbors the catalytic core residues C69, H49 and a catalytically active hydroxide ion. The interaction domain contains a TRASH motif, an immunological interaction platform, in a Zinc-free and disulfide-containing conformation (Zoegg, 2013).
Elucidation of the three dimensional structure of the autoprotease N^{pro} sheds new light on its unusual mode of action and paves the way for optimization of N^{pro} Autoprotease Fusion Technology.

Achmueller et al. Npro fusion technology to produce proteins with authentic N-termini in E.coli. Nature Methods, 2007, 4(12), 1037-43.
Zoegg et al. Crystal Structures of the Viral Protease Npro Imply Distinct Roles for the Catalytic Water in Catalysis. Structure, 2013, 21, 1-10.

BFP14
Proteomic Analysis of the Yeast Mitochondrial Ribosome

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Eukaryotic cells contain two translation machineries, one in the cytosol and one in mitochondria. Whereas the cytosolic translation machinery is well studied and understood to very high resolution, only little is known on the structure and function of mitochondrial ribosomes. In fungi, the sizes of cytosolic and mitochondrial ribosomes are comparable and both structures are similarly complex. Mitochondrial ribosomes developed from bacterial ribosomes but were considerably changed during evolution. The mitochondrial genome codes almost exclusively for a very small set of proteins most of which are hydrophobic membrane subunits of respiratory chain complexes. Presumably as a consequence of its specialization on the synthesis of membrane proteins, mitochondrial ribosomes of fungi are stably associated with the inner membrane.
In order to analyze the composition of the yeast mitochondrial ribosome and to identify its interaction partners we used quantitative mass spectrometry. For this we purified mitochondria and isolated ribosomes on continuous sucrose gradients under very mild lysis conditions. Mitochondria were prepared from wild type cells and, for control, from cells which lacked mitochondrial DNA (and thus mitochondrial ribosomes) or in which mitochondrial ribosomes could be specifically depleted. Stable isotope labeling by amino acids in cell culture (SILAC) and subsequent mass spectrometric analysis of these complexes was performed. This strategy identified 68 of the 76 previously reported proteins of mitochondrial ribosomes and 13 novel proteins. Some of these ribosome-associated proteins appear to be involved in the assembly or the function of mitochondrial ribosomes and initial results on their function and relevance are presented on the poster.
This work was supported by the DFG (FOR967).

REDOXBIOLOGY

PKV09

The interactome of the mitochondrial oxidoreductase Mia40 in human cells.

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Cysteine oxidation either stabilizes the structure or modulates the activity of a protein. Hence, it is important to regulate protein oxidation since aberrant protein activities are often associated with severe cellular dysfunctions. One compartment where protein oxidation is enzymatically catalyzed is the intermembrane space (IMS) of mitochondria. Here, the oxidoreductase Mia40 imports substrates by forming an intermolecular disulfide (1, 2). The subsequent substrate oxidation leads to the stabilization of the substrate structure and retention in the IMS. Currently, it is not known whether Mia40 also oxidizes proteins to modulate their activity. In this study, we isolated Mia40-Substrate-Complexes from human cells and identified the Mia40 interactome by quantitative mass spectrometry. Thereby, we discovered novel interaction partners which do not share the structural features of so far known Mia40 substrates. Moreover, we find that these proteins do not rely on Mia40 for mitochondrial import and thus could in principle be redox-regulated substrates of Mia40.

References

[2] Mesecke, N, Terziyska, N, Kozany, C, Baumann, F, Neupert, W, Hell, K and Herrmann, JM (2005) A disulfide relay system in the intermembrane space of mitochondria that mediates protein import. Cell, 121, 1059-1069.
[3] Riemer, J, Bulleid, N and Herrmann, JM (2009) Disulfide formation in the ER and mitochondria: two solutions to a common process. Science, 324, 1284-1287.

PKV21

Cytosolic peroxidases protect African trypanosomes from lysosomal oxidative stress.

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Trypanosoma brucei species are the causative agents of human African sleeping sickness as well as Nagana cattle disease. In these parasitic protozoa, detoxification of hydroperoxides is achieved by 2-Cys-peroxiredoxins and non-selenium glutathione peroxidase-type enzymes (Px) which both obtain their reducing equivalents from the unique trypanothione/tryparedoxin system. Previous knockout studies revealed that the two cytosolic Px I and II are essential while the mitochondrial Px III is not. Bloodstream *T. brucei* lacking Px I and II are fully viable in the presence of Trolox but show severe lipid peroxidation and cell lysis within a few hours when the vitamin E analog is removed from the culture medium [Diechtierow and Krauth-Siegel, 2011, Free Radic Biol Med, 51, 4]. In the present work, using live cell imaging of the mutant parasites fed with fluorescent dextran, we observed that the cells undergo various states of lysosomal enlargement before they are completely stained and finally lyse which strongly suggested a direct link between the cytosolic peroxidases and this organelle. The removal of fetal calf serum from the medium prolonged the short-term lifespan of the mutant parasites after Trolox withdrawal, pointing to a serum component as origin of the lethal phenotype. Indeed, the cytosolic Px-type peroxidases seem to play an important role in preventing oxidative damage due to iron-related endocytosis. Interestingly, the results obtained so far emphasize the lysosome as main source of intracellular oxidative stress, not the mitochondrion. The minute role of the mitochondrial Px III observed for the mammalian bloodstream form of *T. brucei* in culture was recently corroborated by *in vivo* studies. Px III-deficient parasites were as infective as wild-type cells in the mouse model.

RBP01

Mechanism and regulation of glutaredoxin/glutathione-dependent peroxiredoxins

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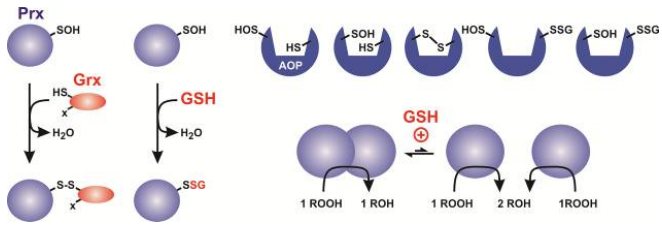
Peroxiredoxins (Prx) are ubiquitous thiol-dependent hydroperoxidases that are usually among the most abundant proteins in pro- and eukaryotes. Depending on the isoform, Prx do not only remove harmful reactive oxygen species but can also act as chaperones and redox sensors. Here I present recent advances regarding the comprehensive mechanistic characterization of glutaredoxin and glutathione as electron donors and redox regulators of a special class of Prx found in bacteria, plants and parasitic protists.

References

Deponte M. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. (2013) Biochim Biophys Acta 1830:3217-66.
Djuika CF, Fiedler S, Schnölzer M, Sanchez C, Lanzer M, and Deponte M. (2013) Plasmodium falciparum antioxidant protein as a model enzyme for a special class of glutaredoxin/glutathione-dependent peroxiredoxins. Biochim Biophys Acta 1830:4073-4090.



RBP02

Redox processes in mitochondrial respiratory chain assembly

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Complex I of the respiratory chain of mitochondria is crucial for cellular energy production. It is also a major source of reactive oxygen species, and dysfunctions of the complex have been implicated in the pathogenesis of a variety of neurodegenerative disorders. Dysfunctions often occur as a result of an impaired assembly, but so far only little is known about the biogenesis and maintenance of Complex I in mammalian cells. Seven of its subunits are encoded in the mitochondrial genome, while the remaining 38 subunits have to be imported from the cytosol. Subunits are sequentially assembled to give rise to the holoenzyme supported by assembly factors. We aim to characterize the function of one so far uncharacterized mitochondrial protein in the assembly/maintenance of Complex I - the flavoprotein FoxRed1. Homozygous mutations in this protein have been linked to isolated Complex I deficiencies. The cells of the patients expressed FoxRed1 point mutants, and the levels of Complex I were strongly reduced. The molecular function of FoxRed1, however, remains unclear. Here, we will present initial data on the characterization of FoxRed1 and its effects Complex I assembly.

RBP03

Molecular mechanisms of ROS production by mitochondrial complex II

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Introduction

The role of mitochondrial complex II (succinate:ubiquinone oxidoreductase) as a source of reactive oxygen species (ROS) has been underestimated for a long time. However, recent studies revealed that complex II can be a significant source of ROS. Nevertheless, there is an ongoing discussion whether ROS are mainly produced by the covalently bound flavin or by the ubiquinone (Q) binding site.

Material and Methods

Submitochondrial particles were prepared from bovine heart mitochondria and the ROS production was analyzed in presence of different succinate concentrations, respiratory chain inhibitors and TCA cycle intermediates. Hydrogen peroxide (H_2O_2) generation was determined with the Ampliflu Red™/HRP assay, while superoxide generation was measured by the SOD-sensitive reduction of acetylated cytochrome *c*.

Results

In presence of Q-site inhibitors (e.g. atpenin A5), complex II produced about 75% H_2O_2 and 25 % superoxide. ROS generation was attenuated by high succinate concentrations and other competitively binding dicarboxylates (including several TCA cycle intermediates) indicating that ROS were mainly generated by the unoccupied flavin site. In contrast, manganese ions induced a high rate of ROS-production that was suppressed by atpenin A5, indicating that ROS were generated at the Q-site.

Conclusion/Discussion

Our study provides mechanistic insight into the ROS production by complex II and defines conditions under which the flavin-site and the Q-site generate ROS. The two sites can be distinguished by their characteristic sensitivity towards different inhibitors and substrate concentrations. This might help to elucidate the role of complex II derived ROS in different pathological settings.

RBP 04

Functional characterization of single nucleotide polymorphisms in human lipoxygenases

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Lipoxygenases (LOX) are non-heme iron containing enzymes that catalyze the dioxygenation of polyunsaturated fatty acids. They are involved in the biosynthesis of inflammatory molecules like leukotrienes, lipoxins, maresins, and resolvins. Single nucleotide polymorphisms (SNPs) in different LOX isoforms have been implicated in clinical studies with a higher risk of developing atherosclerosis, colorectal cancer, ischtyiosis and other chronic diseases, but for most of the SNPs there are no allele frequency data available. The human ALOX15 Thr560Met variation is known to lead to an inactive enzyme variant and heterozygous allele carriers seem to have an increased risk in developing coronary artery disease (CAD). Catalytic silent variants in two other LOX genes (ALOX12B, ALOXE3) are known to be the molecular reason for a rare skin dysfunction (autosomal congenital ischtyiosis). In this study we have analyzed the functional effects of coding non-synonymous SNPs published in the 1000 genome database in different LOX isoforms *in vitro* and found that most of the SNPs, especially those with a more frequent distribution in the human population are mainly surface exposed and had only minor effects on enzyme function. Interestingly, we discovered with this approach two new near-null variants in the ALOX15 (Gly422Glu) and ALOX15B (Ala416Asp) and it seems that enzymatic inactivity is caused by a negative effect on enzyme folding.

RBP 05

Dissecting the catalytic mechanism of *Trypanosoma brucei* trypanothione synthetase by kinetic analysis and computational modelling.

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Trypanosoma brucei is the causative agent of human African trypanosomiasis, one of the most important neglected tropical diseases. The parasite possesses a unique redox metabolism which is based on the dithiol trypanothione [$T(SH)_2$]. This bis(glutathionyl)spermidine conjugate delivers the electrons for the synthesis of DNA precursors, the detoxification of hydroperoxides, methionine sulfoxide reduction and other thiol-dependent pathways. Trypanothione synthetase (TryS) catalyzes the synthesis of both glutathionylspermidine (Gsp) and $T(SH)_2$. We have done a thorough kinetic analysis of *T. brucei* TryS in a newly developed buffer system at pH 7.0 and 37 °C, mimicking the physiological environment of the enzyme in the cytosol of the parasites which multiply in the blood of the mammalian host. TryS displays K_m-values for GSH, ATP, spermidine and Gsp of 34, 18, 687, and 32 µM, respectively, as well as K_i-values for GSH and $T(SH)_2$ of 1 mM and 360 µM, respectively. To obtain a deeper insight in the molecular mechanism of TryS, we have formulated alternative kinetic models, with elementary reaction steps represented by linear kinetic equations. The model parameters were fitted to the extensive matrix of steady-state data obtained under the *in vivo*-like conditions. The best model describes the full kinetic profile and is able to predict time course data that were not used for fitting. This systems biology approach to enzyme kinetics led us to conclude that (i) TryS follows a ter-reactant mechanism, (ii) the intermediate Gsp dissociates from the enzyme between the two catalytic steps and (iii) $T(SH)_2$ inhibits the enzyme by remaining bound at its product site and, as does the inhibitory GSH, by binding to the activated enzyme complex.

RBP06

Stress-induced protein *S*-glutathionylation in trypanosomes

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African trypanosomes, the causative agents of sleeping sickness, lack a classical glutathione reductase system, but possess a trypanothione/trypanothione reductase-based thiol redox metabolism instead. Thus, the question arises whether free glutathione in the parasite acts only as precursor molecule for the synthesis of trypanothione or has distinct cellular functions. Protein *S*-glutathionylation, catalyzed by glutaredoxins, is a post-translational modification to protect cysteine residues from irreversible overoxidation and represents a mechanism for redox-regulation of proteins.
Bloodstream *T. brucei*, grown under standard conditions or exposed to oxidative stress, were lysed in the presence of NEM. Glutathione-protein mixed disulfides were cleaved by treatment with glutaredoxin-1 and newly generated free cysteine residues were labeled with HPDP-biotin. After affinity purification, proteins were identified by ESI-MS using stable isotope dimethyl labeling. In addition, a method was established to quantify free glutathione released from proteins by derivatization with 2,3-naphthalendicarboxaldehyde and subsequent fluorescence detection.
Our preliminary results strongly suggest that protein *S*-glutathionylation occurs in trypanosomes. The modification is induced by oxidative stress and is reversible after stress removal. So far, several proteins known to be glutathionylated in other organisms have been identified also in *T. brucei*. Future work will focus on the identification of parasite-specific proteins involved in the mechanism and the role of glutathionylation for a putative redox signaling.

RBP07

Methionine oxidation activates the hypochlorite-specific transcription factor HypT

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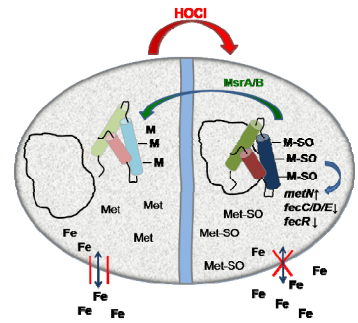
Reactive oxygen species are important components of the innate immune response. Hypochlorite (HOCl) is produced by neutrophils to kill invading microorganisms. The bactericidal activity of HOCl is due to proteome-wide unfolding and oxidation of proteins at cysteine and methionine residues. Little is known about how bacteria escape HOCl-inflicted damage. Recently, the transcription factor YjiE / HypT was identified that specifically protects *E. coli* from HOCl-killing. We unravelled that HypT is reversibly activated and reversibly by methionine oxidation to methionine sulfoxide (Met-SO). So far only inactivation of cellular proteins by methionine oxidation has been reported. Inactivation of HypT depends on the methionine sulfoxide reductases MsrA/MsrB. Mutational analysis revealed three methionines that are essential to confer HOCl resistance. Their simultaneous substitution by glutamine, mimicking the Met-SO state, increased the viability of *E. coli* cells upon HOCl stress. Triple glutamine substitution generates a constitutively active HypT that regulates target genes independently of HOCl-stress and permanently down-regulates intracellular iron levels. Thus, microbial protection mechanisms have evolved along evolution of antimicrobial control systems, allowing bacteria to survive within the host environment.



RBP08

Undiscovered ways of evolution in the peroxidase-catalase superfamily: hybrid heme peroxidases

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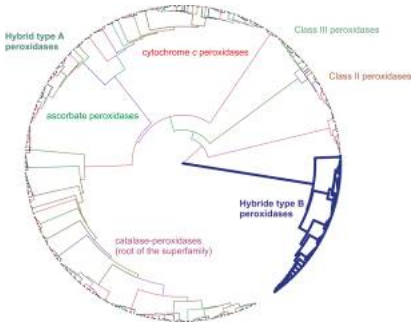
Peroxidase-catalase superfamily is the most abundant lineage of evolution of heme peroxidases comprising over 7,500 representatives of all kingdoms of life. All investigated representatives of this superfamily contain the prosthetic heme *b* group and a highly conserved domain architecture around the active site. Based mainly on structural homology, this superfamily was divided in three main classes by Welinder in 1992. A recent detailed phylogenetic reconstruction focused on Class I revealed the presence of two completely unknown subfamilies of hybrid peroxidases. Whereas hybrid type A peroxidases are positioned between ascorbate and cytochrome *c* peroxidases, hybrid type B peroxidases spread among various fungi have a common root with Class II and Class III peroxidases. We have studied the native and heterologous expression of selected hybrid type B peroxidases from mesophilic and thermophilic fungi. RT-PCR revealed induction of these peroxidases in stationary phase of growth in the presence of reactive oxygen species. Heterologous expression of engineered genes in *E. coli* and *P. pastoris* allowed us to investigate the properties of this subfamily and compare them with already known members of the peroxidase-catalase superfamily. We have applied a broad set of methods including UV-Vis spectroscopy, electronic circular dichroism, electronic paramagnetic resonance, differential scanning calorimetry and presteady-state and steady-state kinetics. It is demonstrated that heme is bound to the predominantly alpha-helical protein and enables one-electron oxidation reactions with various substrates. Biochemical/physical data will be compared with respect to our knowledge on related heme peroxidases.

References
This research was supported by the Austrian Funding Agency FWF (project P23855-B11) and with the project VEGA 2/0149/11 from Slovak Grant Agency.



RBP09
Which role do repair mechanisms play after oxidative damage of DNA, RNAand bases of the free nucleotide pool?

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The most abundant oxidative DNA base modification is 8-oxo-2'-deoxyguanosine (8-oxodG), which is mutagenic and used as a biomarker for oxidative stress. Not only DNA can be attacked by reactive oxygen species (ROS), but also RNA and the free nucleotide pool can be the aim of oxidative stress. 8-hydroxyguanosine (8-oxoGuo) is a marker for RNA damage and can be detected by HPLC-MS/MS. Another potentially damaged guanine base is 8-oxoguanine (8-oxoGua).
The aim of the study was to detect oxidative damage of DNA, RNA and bases of the free nucleotide pool in vitro and in vivo. Beyond that, the role of repair mechanisms after oxidative damage via the hormones angiotensin II (AngII) and aldosterone (Aldo) was investigated.
As methods to detect the damage, HPLC-MS/MS and comet assay were chosen. For the in vitro experiments, the pig kidney cell line LLC-PK1 was used. In vivo, kidneys and urine samples of treated mice and rats were analyzed.
In AngII-treated cells, increased amounts of 8-oxodG in DNA compared to the negative control were already detected after 10 minutes. The inhibition of the repair enzyme poly ADP ribose polymerase (PARP) via 3-aminobenzamide in LLC-PK1 cells after treatment with AngII showed that the DNA damage couldn't be repaired. By blocking another repair enzyme, ataxia telangiectasia mutated protein (ATM), with KU55933 similar results could be obtained.
In urine of AngII-treated mice and Aldo-treated rats, 8-oxoGua, 8-oxoGuo and 8-oxodG were increased compared to the urine of control animals. To detect the oxidative damage in the cytoplasm, treated cells were lysed with an organic based lysis solution. The treatment with AngII and Aldo showed different time points of increased oxidative damage.
In summary, the results show that AngII and Aldo induced oxidative damage in DNA and RNA. The inhibition of ATM and PARP showed that single and double strand breaks might play a role in oxidative DNA damage.

RBP10
Identification of a thiol-disufide redox switch in collapsin response mediating protein 2 (CRMP2/DPYSL2).

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This study addressed a new redox signaling mechanism that is essential for embryonic development of the brain. We have previously identified collapsin response mediating protein 2 (CRMP2) / dihydropriminidase realted protein 2 (DPYSL2), a mediator of semaphorin/plexin signaling, as a redox-regulated target of the thiol-disulfide oxidoreductase glutaredoxin 2 and demonstrated that this regulation is required for normal axonal outgrowth during neuronal development (Bräutigam et al. 2011). We thus proposeda thiol-disulfide switch in CRMP2 that regulates the protein's biological functions. We used recombinantly produced human and zebrafish CRMP2 to address this hypothesis. Both oxidised and reduced CRMP2 form homo-tetramers. Thiol-specific labelling and analysis by mass spectrometry demonstrated that, upon oxidation, CRMP2 forms an intermolecular disulfide between the cysteinyl residues 504 of two molecules. Oxidation profoundly altered the migration of CRMP2 in blue native gel electrophoresis, indicating significant conformational changes, i.e. exposure of hydrophobic surfaces. These reversible redox-dependent structural rearrangements were confirmed by circular dichroism spectroscopy and differential scanning calorimetry. Grx2c efficiently reduced CRMP2 and induced the conformational change both *in vitro* and *in vivo*. These results demonstrate the molecular basis for thethiol disulfide switch in CRMP2 that is essential for the development of the vertebrate brain.

References
Bräutigam L, Schütte LD, Godoy JR, Prozorovski T, Gellert M, Hauptmann G, Holmgren A, Lillig, C.H.*, and Berndt C.* Vertebrate-specific glutaredoxin is essential for brain development. Proc. Natl. Acad. Sci. USA 108: 20532-20537 (2011)

RBP11
The role of glutaredoxin 1 in the stress response of African trypanosomes.

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African sleeping sickness is caused by *Trypanosoma brucei* species. The parasites lack glutathione reductase and thioredoxin reductase, but depend on a unique trypanothione system for redox homeostasis. *T. brucei* possess two dithiol glutaredoxins (Grx1 and Grx2), located in the cytosol and intermembrane space of the mitochondrion, respectively. Both proteins are kept reduced by direct reaction with trypanothione. *T. brucei* Grx1 was shown to coordinate iron-sulfur clusters and to catalyze the reduction of GSH-protein mixed disulfides with greater efficiency than Grx2. RNA interference against each protein did not reveal any growth phenotype in bloodstream form of *T. brucei*, whereas depletion of Grx2 mRNA in procyclic cells resulted in a proliferation defect (Ceylan et al., 2010). Grx1 forms a GSH mixed disulfide at Cys 78 (Grx1-SSG). Grx1-SSG has about 50 % activity compared to unmodified Grx1 with glutathionylated BSA, hydroxyethyl disulfide or the redox sensitive green fluorescent protein 2 (roGFP2) as substrate. These results suggest that modification of the non-active site Cys may have a regulatory effect. Grx1 catalyses also the glutathionylation of roGFP2 (collaboration with R. Greiner and T. Dick, DKFZ, Heidelberg). Recently, we succeeded in generating Grx1-deficient bloodstream and procyclic parasites by replacing the two *grx1* alleles by blasticidin and puromycin resistance genes. Grx1-deficient procyclic parasites did not show any proliferation defect as compared to wild-type cells when cultured in the presence of diamide, hydrogen peroxide and the iron chelator deferoxamine. Work is in progress to investigate the effect of different stressors on Grx1-deficient bloodstream parasites.

RBP12
The Promotion of Megakaryocytic Differentiation of K562 Cells through Oxidative Stress Generated by Black Light Irradiation

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Introduction
Several reports showed the function of reactive oxygen species (ROS) in the regulation of signaling pathway as well as gene expression for various cellular activities. The importance of ROS has been reported in the megakaryocytic (MK) development in which the inhibition of ROS formation resulting in repressed cell differentiation. In the current study, we elevated the ROS level endogenously by means of black light (BL) irradiation and examined its effect on the MK maturation of K562 cells.

Methods
K562 cells were pre-treated with BL irradiation (~ 300 $\mu W/cm^2$) for 2 h and MK development was induced by phorbol myristate acetate (PMA; 10 ng/ml). Gene expression was checked by a real-time PCR method. Intracellular ROS content and DNA ploidy were analyzed using a flow cytometer.

Results
Pre-treatment with BL irradiation generated ROS accumulation in the K562 cells, and together with PMA, produced a higher level of intracellular ROS content than PMA alone. Under this condition, the percentage of high ploidy cells (DNA content >4N) at day 8 was promoted up to 150 % than non-irradiated cells. It was found that *gata2* (transcription factor for MK maturation) and *gp1ba* (encoding CD42b, platelet marker) were up-regulated confirming that BL irradiation promoted MK maturation in mRNA expression level as well. At day 1 after treatment, BL irradiation shifted the ratio of 4N:2N cells from 31:69 to 69:31. In this condition, *p21* (regulator for cell cycle arrest) was strongly up-regulated. An antioxidant will be introduced to the culture to elucidate this phenomenon.

Conclusion
BL irradiation elevated intracellular ROS content and subsequently promoted polyploidization and MK-associated genes expression in the PMA-induced K562 cells.

RBP13

Serine acetyltransferase-1, a key enzyme of the L-cysteine biosynthetic pathway, is a target of thioredoxin-mediated regulation in the human protozoan parasite *Entamoeba histolytica*

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In the human protozoan parasite *Entamoeba histolytica*, the causative agent of amoebiasis, L-cysteine plays an essential role in growth, survival, and defence against oxidative stress. Serine acetyltransferase-1 (EhSat1), a key enzyme in the L-cysteine biosynthetic pathway of the parasite, was captured by mutated thioredoxin affinity chromatography as a possible target protein of thioredoxin (EhTrx) in *E. histolytica* (Schlosser S., Leitsch D., Duchêne M. (2013) *Biochem J.* 451(2), 277-288). To further dissect this interaction, we aimed to determine whether EhSat1 can interact with EhTrx and whether its activity is redox-regulated. Far Western Blot analysis using wild-type EhTrx and different EhTrx cysteine mutants confirmed the interaction between EhSat1 and EhTrx but also revealed that binding is not solely based on disulfide bond formation involving the active site cysteines of EhTrx, as double mutant EhTrx$^{C31S/C34S}$ forms indeed a protein complex with EhSat1 but not with thioredoxin reductase. Furthermore, EhSat1 was found to be sensitive towards oxidation, as treatment of active EhSat1 with 0.5 mM of the thiol-oxidising agent diamide diminished its activity by 50%. The observed decrease in enzyme activity upon treatment with diamide was found to be associated with EhSat1 dimer- and oligomerisation via formation of intermolecular disulfide bonds as estimated by non-reducing SDS-PAGE. Only the addition of reduced wild-type EhTrx, but not the active site mutants EhTrx^{C34S} and EhTrx$^{C31S/C34S}$ increased the specific activity of the oxidised EhSat1, indicating that an intact active site motif of EhTrx is required for the reactivation of oxidised EhSat1. Taken together, these results suggest that EhSat1 is a redox-regulated enzyme in *E. histolytica*.

RBP14

Imbalance in redox regulation enhances Smac mimetic-induced cell death in acute lymphoblastic leukemia (ALL)

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Apoptosis resistance contributes to poor prognosis in pediatrics acute lymphoblastic leukemia (ALL). Smac mimetics that suppress Inhibitor of Apoptosis Proteins (IAPs) can overcome apoptosis resistance in leukemic cells. Here we identify a novel treatment strategy which is based on the combination of the Smac mimetic BV6 and the glutathione depletion agent Buthioninesulfoximine (BSO). BV6 alone induces apoptosis by degrading IAPs and promoting the assembly of a RIP1/FADD/caspase-8-containing cell death complex. This complex is critically required for BV6-induced apoptosis, since knockdown of RIP1, FADD or caspase-8 reduce caspase activation and cell death. Here we show for the first time that reactive oxygen species (ROS) are produced during BV6-induced apoptosis. Furthermore, depletion of glutathione levels by BSO in combination with BV6 induces an amplification of ROS production, especially of lipid peroxides. Consequently the combination of both drugs leads to strong caspase activation and a highly synergistic induction (CI ≤ 0.33) of apoptosis. Lipid peroxidation and subsequent apoptosis are prevented by the Vitamin E derivate α-Tocopherol, a pharmacological inhibitor of lipid peroxidation. Furthermore, overexpression of glutathione peroxidase 4 (GPX4), that specifically reduces lipid-membrane peroxides, abolishes lipid peroxidation and rescues cells from apoptosis. Taken together the combination of both drugs finally leads to a significant increase in lipid peroxidation and a highly synergistic induction of apoptosis. Findings from this study indicate that BV6-induced apoptosis is mediated via redox alterations, which represents a promising new treatment strategy to overcome apoptosis resistance in ALL.

RBP15

PDGF-BB induces protein kinase A signaling via production of reactive oxygen species rat glomerular mesangial cells

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Glomerular diseases are often accompanied with the generation of reactive oxygen species (ROS). These mediators may affect glomerular podocyte function and may lead to cell death of mesangial cells. However, ROS also trigger well-defined signaling processes that result in cell proliferation or migration. To analyze ROS-driven signaling, we performed a non- hypothesis driven proteomic approach to identify redox-dependent formation of homo- or heteromeric protein complexes. Protein lysates of human podocytes were treated with or without hydrogen peroxide (250 µM) for 10 min and cell lysates were subjected to redox diagonal 2D gel electrophoresis. We identified among others the regulatory (R) subunit of protein kinase A (PKA), which belongs to the family of serine/threonine kinases as a redox-modified protein. PKA is activated by the second messenger cAMP that binds to the PKA R subunits, a process that triggers kinase activity. To further analyze redox-mediated PKA-signaling in a more physiological setting, we treated rat mesangial cells with PDGF-BB to induce endogenous ROS formation and tested whether PDGF-BB mediated dimerization of PKA R subunits and PKA downstream signaling events. PDGF-BB treatment resulted in dimerization of PKA R subunits and caused serine 157 phosphorylation of VASP, a classical target of PKA. PDGF-BB-induced phosphorylation of VASP occurred independently of changes in cAMP levels. Furthermore, PDGF-BB-induced effects on PKA were blocked by diphenyljodonium, indicating that activation of a NADPH oxidase is essential for PKA activity. Taken together, we could show that PDGF-BB triggers PKA signaling in a redox-dependent manner and this may hint for a possible protective role of ROS in rat mesangial cells.

RBP16

Impact of oxidative stress on TCF11/Nrf1-dependent regulation of the ubiquitin-proteasome system

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The ubiquitin-proteasome system (UPS) is the most important non-lysosomal protein degradation machinery ensuring intracellular protein homeostasis. Therefore cells adjust their UPS to alterations in environment, for instance to oxidative stress. The UPS degrades oxidant-damaged and misfolded proteins, thereby preventing their aggregation and furthermore disorders like neurodegeneration or cancer.
The transcription factor TCF11/Nrf1 plays a central role in the regulation of UPS. We show that under normal conditions the transcription factor resides in the ER membrane and is degraded by the ERAD system. Proteasome inhibition causes the nuclear translocation of TCF11/Nrf1. Here, the factor mediates transcriptional activation of UPS-related genes by interacting with antioxidant response elements (ARE) of their promoter regions. This leads to an overall induction of proteasomal subunit expression as well as proteasome assembly and consequently to an enhanced proteasome activity.
The triggering of TCF11-dependent UPS upregulation is still under investigation. Proteasome inhibitors provoke the accumulation of ubiquitinated and oxidant-damaged proteins. Therefore we focus on the effect of the oxidant rotenone on TCF11/Nrf1-dependent UPS regulation. Likewise, rotenone seems to promote an upregulation of the proteasome expression and assembly in a TCF11/Nrf1-dependent manner, as shown by TCF11 siRNA depletion experiments in neuronal SH-SY5Y cells.

RBP17

Arabidopsis G6PD1 and PGL3 isoforms are targeted to chloroplasts and/or peroxisomes by interaction with Trx m2 in the cytosol

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During analysis of the oxidative pentose-phosphate pathway (OPPP) in Arabidopsis we found that plastidic glucose 6-phosphate dehydrogenase G6PD1 and 6-phosphogluconolactonase PGL3 localize to both plastids and peroxisomes and that the targeting switch is regulated at a post-translational level. Yeast 2-hybrid analyses indicated that thioredoxin *m2* (Trx*m2*) is also involved in regulating targeting of PGL3, as previously shown for peroxisome targeting of G6PD1 (via catalytically inactive isoform G6PD4). In planta bimolecular-fluorescence complementation confirmed that Trx*m2* interacts with PGL3 in chloroplasts and in the cytosol, but not in peroxisomes. Co-expression analyses further supported that chloroplast import of PGL3 involves Trx*m2*, and Cys-to-Ser changes in Trx*m2* showed that PGL3 binding occurs independent of redox-state. In leaves, dual-targeting of PGL3 to plastids and peroxisomes was detected in mesophyll cells but not in the epidermal cell layer, where Trx*m2* expression was undetectable. Thioredoxins were lately shown to exhibit chaperone function, acting as holdases (in multimeric form) but upon redox-activation as foldases (in monomeric form). Thus, interaction with Trx*m2* in the cytosol can explain coordinated subcellular targeting of G6PD1 and PGL3 by either preventing or promoting folding of PGL3 precursors, depending on cytosolic redox-state.

RBP18

Protein Disulfide Isomerase Mediates a Conformational Change of the Membrane Proximal Domain of ADAM17

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A disintegrin and metalloprotease-containing protein-17 (ADAM17) is a membrane spanning multi-domain protease with a zinc-dependent catalytic domain. Today, there are more than 75 known substrates which are shed by ADAM17. Aside from the substrates, various interaction partners affect this protease in multiple ways.
One interesting interaction partner is PDI (protein disulfide isomerase), which we found by co-immunoprecipitations. PDI is an oxidoreductase that is able to rearrange disulfide bonds and thereby serves as a chaperone.
Recently it was shown, that extracellular PDI has a negative effect on the shedding activity of ADAM17, probably through a conformational change [Willems et al, 2010].
Indeed our experiments show that the recombinant membrane proximal domain of ADAM17 (MPD17) changes its conformation when incubated with PDI. This finding proofs that two stable disulfide isomers of this domain exist. NMR-results showed that the isomer after purification is very flexible while the one treated with PDI is rather rigid. The hint that the PDI converts ADAM17 into an inactive conformation leads to the assumption that the rigid, PDI-treated disulfide isomer belongs to the inactive form of ADAM17.

References

Willems et al., (2010), Biochemical Journal, 428, p 439-450
Düsterhöft et al., (2013), JACS, 135, p 5776-5781

RBP19

Bleach turns a conserved *Escherichia coli* protein into a highly efficient chaperone

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The *Escherichia coli* Protein RidA is a member of the highly conserved YER057c/yjgf/Uk114 family of proteins. Members of this family have a multitude of assigned functions. Among others these include translational inhibition, activation of proteinases, and nuclease function. RidA in *Salmonella* has been recently shown to function as an enamine/imine deaminase in the branched chain amino acid pathway (Lambrecht *et al.*, 2012).
Here we show that the RidA enamine/imine deaminase gets efficiently converted into a chaperone with holdase function upon reaction with sodium hypochlorite (household bleach) or chloramines. The activated RidA holdase has a wide range of substrates and presumably helps stabilizing proteins that precipitate in the presence of chlorine species *in vivo*. Although thiols are a known target of chlorine, the activation we observe is independent of the amino acid cysteine: a cysteine to serine mutant of RidA is still activated by hypochlorite and chloramines. Nevertheless, the chlorine-based modification is reversible by reduction with ascorbic acid and DTT *in vitro*. A reconstituted thioredoxin/thioredoxin reductase/NADPH system in catalytic quantities can inactivate the RidA chaperone as well. To our knowledge, we describe here for the first time a chlorine-based mechanism of protein activation that is independent of cysteine and in principle reversible *in vivo*.

RBP20

Crystal structure of *Plasmodium falciparum* thioredoxin reductase in complex with its substrate thioredoxin - comparison with the human host proteins

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Thioredoxin reductase (TrxR) is a flavoprotein important for redox homeostasis and essential for the survival of *Plasmodium falciparum*, a parasite causing malaria in humans. Malaria parasites have rapidly developed resistance against most currently available antimalarial drugs, an observation that underlines the need for new antimalarial drug targets such as PfTrxR. Several selective inhibitors of PfTrxR were shown to inhibit *P. falciparum* growth *in vitro* thereby confirming PfTrxR as an antimalarial drug target. Here, we report the first crystal structure of *P. falciparum* TrxR bound to its substrate thioredoxin 1 (Trx1). Upon complex formation, only the flexible C-terminal arm and an insertion loop of PfTrxR are rearranged, suggesting that the C-terminal arm changes its conformation during catalysis similar to human TrxR. Striking differences between PfTrxR and hTrxR are a *Plasmodium*-specific insertion and the architecture of the C-terminal arm, which lead to considerable differences in PfTrx1 binding and disulfide reduction. Moreover, we present an in-depth biochemical analysis of residues involved in substrate binding and in the intersubunit cavity, a proposed inhibitor binding site. Differences between *P. falciparum* and human TrxR and details on substrate reduction revealed in this study provide the basis for structure-based drug development and inhibitor optimization.

RBP21

Smac mimetic primes FADD-deficient leukemia cells for ROS dependent TNFα-induced necroptosis

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Evasion of apoptosis contributes to treatment resistance, one of the major, yet unresolved obstacles in oncology. Searching for new strategies to bypass apoptosis resistance, we recently demonstrated that the bivalent Smac mimetic BV6 primes apoptosis-resistant acute lymphoblastic leukemia (ALL) cells deficient in FADD for TNFα-induced necroptosis [Laukens et al., 2011]. This alternative type of cell death occurs without caspase cleavage or DNA fragmentation. More detailed investigations reveal that BV6 and TNFα-induced cell death in FADD-deficient cells depends on the formation of the necrosome complex which consists of RIP1 and RIP3 kinase. Furthermore, we show that the recently identified downstream target MLKL is involved in necroptosis in FADD deficient Jurkat cells. Interestingly, ROS scavengers (i.e. butylated hydroxyanisole (BHA) and N-acetylcysteine (NAC)) significantly reduce necroptotic cell death. In line with this result, production of reactive oxygen species (ROS) precedes BV6 and TNFα-induced cell death in FADD-deficient cells. Of note, the RIP1 kinase inhibitor necrostatin-1 prevents ROS formation upon BV6 and TNFα combination treatment indicating an important role for RIP1 kinase in triggering ROS production. In conclusion, the Smac mimetic BV6 primes apoptosis-resistant leukemia cells lacking FADD to TNFα-induced necroptosis that critically depends on RIP1, RIP3, MLKL and ROS production. Future research on the molecular mechanisms involved in necroptosis will provide novel targets for innovative chemotherapy approaches for various apoptosis resistant tumor identities.

RNA BIOCHEMISTRY

PKV15

Tracking the cellular fate of siRNA via FRET

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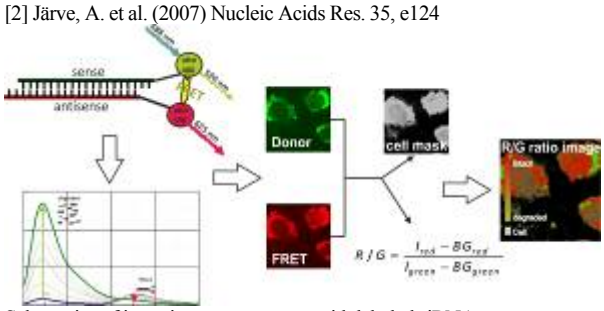
RNAi is a widespread tool for analyzing gene function and promises high potential for therapeutic application. Whereas in cell culture siRNA has become a common tool for silencing genes, an *in vivo* delivery remains still challenging and is one major draw-back in the development of RNAi therapeutics. Besides the question of delivery and uptake, the cellular fate of applied siRNA still remains unclear, including the question of their stability, availability and localization.
Labeled siRNA can be used for both the evaluation of cellular localization, as well as the analysis of the integrity level of the applied siRNA. Two dyes on the siRNA duplex, Atto488 on the sense and Atto590 on the antisense strand, create a robust system for determination the integrity level *via* the ratio of FRET to donor emission [1,2].
With the aid of double labeled siRNA, potential delivery systems can be evaluated for their protective efffect against nucleases, which is crucial for an *in vivo* application *via* the bloodstream (manuscript in preparation).
In this work live cell experiments were performed to investigate the uptake and release process of transfected siRNA in real-time. Simultaneously the integrity state of the applied siRNA can be determined. Thereby, an initial strong release phase of siRNA direct after the addition of the transfection mixture was observed. In live cell studies over 8 h cells showed a random but consistent siRNA release followed by a depletion from the cytosol (<2 h). Some cells thereby showed a second or third released event after some time.
The finding of multiple and consistent release might give an explanation why RNAi can be observed up to weeks as fresh and intact siRNA renews the population of active silencing complexes.

References
[1] Hirsch, M. et al. (2012) Biol Chem. 393, 23-35
[2] Järve, A. et al. (2007) Nucleic Acids Res. 35, e124



Schematics of integrity measurements with labeled siRNA.

PKV36
Mitochondriotropic liposomes for targeted delivery of therapeutic tRNAs

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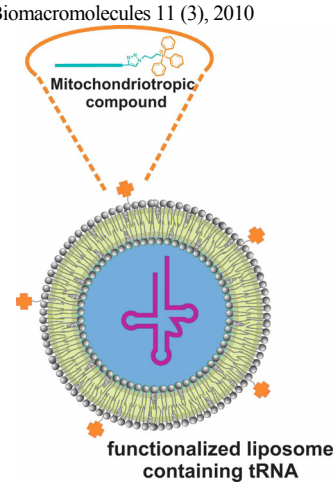
As the therapeutic potential of various RNA species is becoming more and more apparent, there is increasing need for *in vivo* carrier systems that warrant targeted delivery of RNA.
Our project focuses on an intracellular target, the mitochondria. This is of interest, as diseases like the mitochondrial encephalomyopathy, lactid acidosis and stroke-like episodes syndrome (MELAS) result in mutated and therefore dysfunctional mitochondrial tRNAs. Our therapeutic approach applies the replacement of these tRNAs by functional ones.
We want to achieve targeted delivery of tRNA to mitochondria by endowing fusogenic liposomes with targeting moieties. Liposomal formulations are prepared by Dual Asymmetric Centrifugation (DAC)[1] while functionalized lipids are incorporated by post-insertion. For this, Cholesterol-PEG-conjugates with terminal alkyne residues[2] are modified with mitochondriotropic compounds like the triphenylphosphonium cation or Cy5 via CuAAC Click Chemistry.
Fluorescent tRNA fragments as model payload are encapsulated into the liposomes and colocalization with mitochondria is investigated by confocal microscopy.
Functional studies will include the selection of tRNA species associated with mitochondrial diseases. Liposomes loaded with this cargo will be checked for their effects on *e.g.* respiration levels in MELAS cybrid cells.
By proposing this system for mitochondrial delivery of therapeutic RNAs that would be applicable *in vivo*, we want to contribute to finding a treatment of mitochondrial diseases.

References
[1] Hirsch et al., J Controlled Release 135 (1), 2009
[2] Hofmann et al., Biomacromolecules 11 (3), 2010



RNV01
Engineered guideRNA-Dependent Deaminases - A Tool to Reprogram RNA Function

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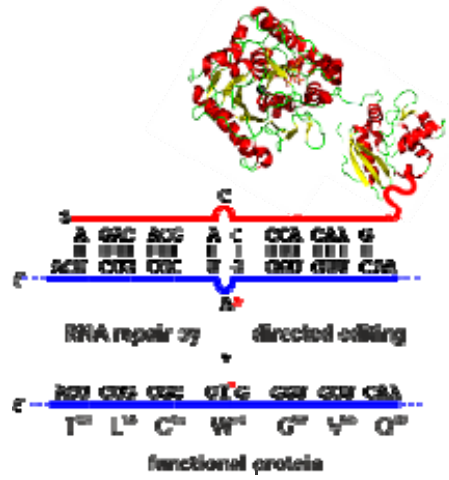
RNA editing has the power to reprogram genetic information on the RNA level.[1,2] The outcome of editing depends on the site at which a single adenosine to inosine (A to I) conversion occurs. Since inosine is read as guanosine, editing in the ORF results in substitution of single amino acids.[1] Editing in the introns or UTRs causes an alteration of RNA processing.[1] Thus, directing RNA editing activity to a user-defined site of an (pre)-mRNA allows to manipulate RNA and protein function with a high potential for application. Recently, we reported a strategy for the assembly of an artificial, guide-RNA-dependent RNA editing machinery that allows to apply simple Watson-Crick binding rules for the site-selective and highly rational targeting of any arbitrary codon.[3]
To re-direct RNA editing, we have re-engineered the protein-guided human ADAR1 into a guideRNA-dependent enzyme (Scheme).[3] The guideRNA moiety of such a tool fulfills two tasks. First, it steers the RNA-effector conjugate to the target site, and second, it forms the secondary structure motif required for highly efficient and selective editing of a single adenosine. Most appealing is the modular nature of our approach which allows us to program the machinery to target virtually any given codon by designing a respective guideRNA.
Today, we will present our newest results[4] on applying directed RNA editing for the repair of the disease causing Factor 5 Leiden gene mutation. The potential and limitations of this technique for practical application in medicine and basic Life science research will be discussed.

References
[1] K. Nishikura, *Annu. Rev. Biochem.* **2010**, *79*, 321.
[2] R. B. Emeson et al., *Science* **1995**, *267*, 1491.
[3] T. Stafforst, M. F. Schneider, *Angew. Chem. Int. Ed.* **2012**, *51*, 11166.
[4] M. F. Schneider, J. Wettengel, T. Stafforst, *manuscript in preparation.*



RNP01
Chemo-enzymatic modification of the mRNA 5'-cap using click chemistries

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Recent studies demonstrate that a large fraction of mRNAs is localized to distinct subcellular compartments to spatially and temporally restrict gene expression.[1] Therefore, the development of new methods for mRNA imaging seems to be a key step in elucidating the regulation and function of locally translated transcripts. We established a chemo-enzymatic approach to specifically modify the 5'-cap of eukaryotic mRNAs.[2] The *Giardia lamblia* trimethylguanosine synthase 2 (GlaTgs2), which catalyzes the methyl transfer from *S*-adenosyl-L-methionine (AdoMet) to the N2 atom of 7-methyl guanosine RNA-caps[3], could be engineered to introduce terminal alkene and alkyne moieties into the RNA. These reporter groups were harnessed for bioorthogonal labeling by Cu(I) catalyzed azide-alkyne (CuAAC) and thiol-ene click reactions (TEC). In this way any desired functional moiety could be attached to the cap demonstrated by the introduction of a biotin and a fluorophore. Futhermore, an *in vitro* transcribed and capped RNA could be fluorescently labeled making use of this method. These results suggest that our strategy can be used for selective labeling of mRNAs or the isolation and enrichment of mRNAs from total eukaryotic RNA. By extending the co-substrate profile of GlaTgs2 chemo-enzymatic modifications of the cap suitable for copper-free click chemistries could be achieved.[2]

References
1. Martin, K.C. and A. Ephrussi, *mRNA localization: gene expression in the spatial dimension.* Cell, 2009. **136**(4): p. 719-30.
2. Schulz, D.; Holstein, J. M.; Rentmeister, A., *A chemo-enzymatic approach for site-specific modification of the RNA cap.* Angew Chem, accepted.
3. Hausmann, S. and S. Shuman, *Giardia lamblia RNA cap guanine-N2 methyltransferase (Tgs2).* J Biol Chem, 2005. **280**(37): p. 32101-6.

RNP02

Nuclear Nucleosidetriposphate syntheses by newly detected chromatin bound Nucleoside-nucleotide-phosphotransferases.

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Which factors do influence the start of DNA or RNA-polymerases in eucaryotes? Allosteric nucleoside-nucleotide phosphotransferases detected in the chromatin of Morris Hepatoma 9121 and of regenerating rat liver had been isolated by DEAE-Cellulose chromatography and proved to be purified by polyacrylamide-gel-electrophoresis. The enzyme pattern of Morris Hepatoma 9121 and of regenerating rat liver differ from each other. The enzyme form C is Hepatoma specific and was also found in the cytoplasm and blood serum of early tumor bearing rats. All purified enzymes are able to synthesize the four ribonucleotidetriphosphates, ATP, GTP, CTP, UTP, and the four deoxyribonucleosidetriphosphates dATP, dGTP, dTTP, dCTP from the corresponding nucleosides being accepted as substrates in presence of a NTP as donor. Their molecular weights let conclude to be aggregates.The enzyme activities are dependent on different concentrations of poly d(A-T) and poly d(C-G) and of steroidhormons as cortisol, estradiol, progesterone. The corresponding enzyme-steroidhormon-complexes had K_D-values in the range of the steroidhormon-receptor-complexes. Then other poly d(A-T) and poly d(C-G) were neccessary to stimulate the enzyme activities. Incubation of purified nuclei with ^{3}H-steroidhormons resulted in ^{3}H-steroidhormon-nucleoside-nucleotide-phosphotransferase-complexes. RNA -and DNA - polymerases need different concentrations of NTPs in dependence on the DNA sequence being transcribed or replicated; these new enzymes might be important to synthesize the exact NTP concentrations for the start of RNA- or DNA polymerases in dependenc on the DNA-sequence. Cell growth in normal as well as in tumor cells might be controlled by the new enzymes.
Int.J.Biochem. 15, 1241 - 1247, 1983

References
1) Lenger, K.: Isolation of Nucleoside Phosphotransferases from chromatin of Morris Hepatoma 9121 nuclei, Int.J.Biochem. 14, 53-61, 1982.
2) Lenger, K.: Nucleoside triphosphate synthesis by chromatin bound enzymes from Morris Hepatoma 9121 nuclei, Int.J.Biochem. 14, 673- 677, 1982.
3) Lenger, K.: Characterization of six nucleoside-nucleotide phosphotransferases from the chromatin of Morris Hepatoma 9121 cells by physicochemical and biochemical techniques, Int.J.Biochem. 14, 955-960, 1982.
4) Lenger, K.: Assay of partially purified glucocorticoid receptor using both gel-electrophoresis and Dextran/charcoal technique, Int.J.Biochem. 15, 373 – 382, 1983.
5) Lenger, K.: Alteration of the enzyme activity pattern of nucleoside-nucleotide phosphotransferases in rat liver nuclei through the formation of steroid hormone-enzyme-complexes, Int.J.Biochem. 383-393, 1983.
6) Lenger, K.: Sensitive measurement of ^{3}H-glucocorticoid-receptor-complexes by gel-electrophoresis. In "Electrophoresis "82" (Stathakos,D. Hrsg.) 607-614, de Gruyter,Berlin, 1983.
7) Lenger, K.: Allosteric effects of cortisol, estradiol,progesterone and of the DNA-sequences poly d(A-T) and poly d(C-G) on the adenosine –and thymidine phosphorylating activity of the nuclear nucleoside-nucleotide phosphotransferases. Int.J.Biochem. 15, 1241 – 1247, 1983.
8) Lenger, K.: Verfahren zur Frühdiagnose maligner Tumoren bei Säugetieren und Menschen, Patentschrift Nr. 3336786, 1985, Deutsches Patentamt München, "Diagnostic method for identifying early malignant tumors in mammals and human beings," Patent-No: 3336786, 1985.

RNP03

Determination of the Conformation of the 2'OH Group in RNA by NMR Spectroscopy and Density Functional Theory Calculations

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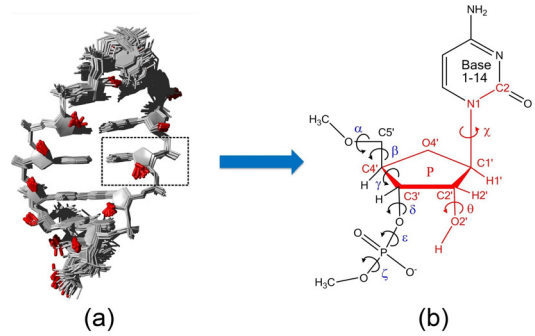
RNA hairpins are one of the building blocks of the secondary structure of ribosomal and messenger RNAs. The presence of the 2'OH group in RNA induces a change in the predominant sugar conformation and provides catalytic activity, but at the same time it reduces the chemical stability of RNA.[1] It is therefore of considerable interest to determine the conformation of the 2'OH group.



(a) (b)

Figure 1. (a) Solution structure of 14-mer RNA.[2] (b) Nucleotide-like structure as a model for density functional theory calculations.

We explored the 14-mer RNA hairpin, studied by Nozinovic et. al.[2] by NMR spectroscopy, using density functional theory (DFT) calculations at the B3LYP/TZVP(PCM) level of theory (Figure 1). We were able to show that (1) DFT produces ^{1}J(C1',H1') and ^{1}J(C2',H2') scalar coupling constants in excellent agreement with NMR spectroscopy and (2) knowledge of both ^{1}J(C1',H1') and ^{1}J(C2',H2') coupling constants is sufficient to distinguish between C3'-endo and C2'-endo conformations, as well as between *syn* and *anti* orientation of the base. Further, using these two calculated couplings, we were able to identify the most favorable orientation of the 2'OH group in a ribonucleotide, which still poses a great challenge to experimental techniques. This knowledge of 2'OH orientation will allow for a detailed exploration of the structural and chemical activity of RNA.

References
[1] M. Egli, S. Portmann, N. Usman, Biochemistry 1996, 35, 8489.
[2] S. Nozinovic, B. Fürtig, H. R. A. Jonker, C. Richter, H. Schwalbe, Nucleic Acids Res. 2010, 38, 683.
Note: The present work is published in Angew. Chem. Int. Ed. 2011, 50, 5397.

RNP04

First Contact Imaging of nanoparticular siRNA

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Delivery and intracellular release are major hurdles in the application of therapeutic nucleic acids, such as siRNA. Here, we address detection and imaging of a prodye-siRNA construct at the event of the intracellular release as the payloads "first contact" to the cytosol [1]. According to this aim, we have synthesized a non-fluorescent leukodyeazide derivative, which shows sensitivity to cellular esterases [2]. These were conjugated to terminal alkyne-bearing nucleic acids via theCu(I)-catalyzed azide-alkynecycloaddition (CuAAC). Our approach for the visualization of a release event is based on the action of lytic enzymes in the cell, which will hydrolytically cleave capping groups from the colorless leukodye, converting it to a fluorophore. The intracellular location of that event will thus become visible in live imaging of cells transfected with selected nanoparticular formulations, offering different degrees of accessibility of lytic enzymes to the siRNA. For example, formulations of siRNA in true liposomes provide nearly quantitative resistance against degradation [3]. We thus expect that in some nanocarriers, leukodye-siRNA will be more susceptible to esterase activity. This new method offers the opportunity to reveal potentially diverse uptake pathways of the various particle types and furthermore might offer an important readout to assess e.g. the influence of surface-tethered targeting moieties for targeted delivery.

References
[1] Vaishnaw et al. Silence (2010), 1:14
[2] Baba et al. Journal of Controlled Release 153 (2011) 278-287
[3] Hirsch et al. Journal of Controlled Release 135 (2009) 80-88

RNP05

Structure-function relationship of substituted bromomethylcoumarins in nucleoside specificity of RNA alkylation

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Selective alkylation of RNA nucleotides is an important field of RNA biochemistry, *e.g.* in applications of fluorescent labeling or in structural probing experiments, yet detailed structure-function studies of labeling agents are rare. Here, bromomethylcoumarins as reactive compounds for fluorescent labeling of RNA are developed as an attractive scaffold on which electronic properties can be varied by several substituents. Six different 4-bromomethyl-coumarins were tested for nucleotide specificity of RNA alkylation using tRNA from

Escherichia coli as substrate. Using semi-quantitative LC-MS/MS analysis, reactions at mildly acidic and slightly alkaline pH were compared. For all tested compounds, coumarin conjugates with 4-thiouridine, pseudouridine, guanosine, and uridine were identified, with the latter largely dominating. In addition, the performed experiments showed that selectivity of ribonucleotide alkylation depends on the substitution pattern of the reactive dye, and even more strongly on the modulation of the reaction conditions. Interestingly, the highest selectivity for labeling of a modified nucleoside, namely of 4-thiouridine, was achieved with a compound whose selectivity was less dependent on reaction conditions than the other compounds. In summary, bromomethylcoumarin derivatives are a highly interesting class of compounds, since their selectivity for 4-thiouridine can be efficiently tuned by variation of substitution pattern and reaction conditions.

RNP06
The multifunctional coumarin PBC displays extraordinary selectivity for 4-thiouridine

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Coumarines have been shown to be very useful for the labeling of nucleic acids. Thereby natural occurring modifications are of major interest, as they occur only in a minor extent and at defined positions[1].
We present a new compound named 4-bromomethyl-7-propargyl-coumarin (PBC) which is highly selective for the reaction with 4-thiouridine (s^4U). PBC could be successfully synthesized in a five step reaction. A Pechmann condensation was performed using 1,3-dihydroxybenzene and ethylacetoacetat. Acetylation of the hydroxyl-group in position 7, was followed by radical bromination of the obtained 7-hydroxy-4-methylcoumarin, using N-bromosuccinimide as bromine source. Finally, the intermediate product was alkylated with propargylbromide, providing PBC. This coumarin exhibits 2500-fold selectivity for s^4U under optimal reaction conditions, compared to all other nucleobases. The alkyne function can be used for further bioconjugations, such as the well-known CuAAC "click" reaction with azides. As these are available in many molecular structures, e.g. biotin or fluorescent dyes, RNA conjugates can be used for a huge range of analytic procedures, while the fluorescence of the coumarin scaffold itself can also be used for analytical manners. After exploring the selectivity of PBC on $tRNA^{Tyr}$ *E.coli* and total RNA *E.coli,* the incorporation of s^4U to HEK cell RNA[3] was investigated and the preference of PBC for s^4U hereby confirmed.

References

[1]Kellner S, Seidu-Larry S, Burhenne J, Motorin Y, Helm M. Nucleic Acids Res. 2011 Sep 1;39(16):7348-60

RNP07
The effect of base modification for click functionalization on duplex stability

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Chemical modification and fluorescent labeling of RNA contribute largely to the development in RNA therapeutics. Typically, after a chemical alteration of an RNA nucleoside, studies need to be conducted to evaluate such influence on RNA properties, as *e.g.* base pairing behavior[1].
Herein, we report a method for the synthesis of the new 5-methyl-N^4-propargylamino-dC phosphoramidite (PA) for site-specific incorporation into DNA-oligonucleotides (ODNs). Starting from thymidine, a propargyl-functionality was introduced at position 4 of the pyrimidine ring, leading to the conversion into a modified dC, which bears the ability to react with an azide in a copper mediated click reaction[2], having received particular interest as functionalization method. After formation of the PA and its subsequent application in solid-phase oligonucleotide synthesis, the ODNs were hybridized to selected antisense strands - DNA and RNA respectively. The impact of the attached propargyl-group and its interaction with the 5-CH_3-group, as well as the position of modification in the given sense strand, on duplex stability was studied *via* thermodynamic measurements and gel analysis. It was observed, that melting temperatures T_M correlated to the varying modification positions. Thus, understanding the influence of modified nucleosides on duplex stability can aid the tuning of *e.g.* the RNAi effect. However, reflected in the extent of T_M increase, our major serendipity was that particular ODNs showed extremely increased affinity to RNA than to DNA in competition experiments. Having this tool in hand gives us the possibility to fish for RNA with our functionalized DNA probes.

References

[1] Seidu-Larry *et al*., *Chem. Commun.*, **2012**.
[2] Kolb *et al*., *Angew. Chem. Int. Ed.*, **2001**.

RNP08
Analysis of Nucleotide Modifications in RNA based on Deep Sequencing

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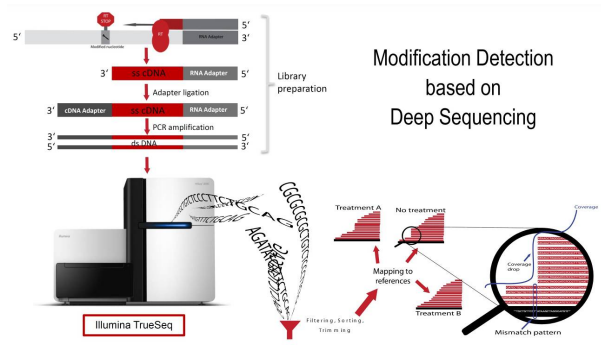
Modification of nucleotides plays an important functional role in gene expression of both, prokaryotic and eukaryotic organisms. It induces translational fidelity in tRNA, influences gene expression in mRNA and is essential for ribosome maturation. Quantification of modifications is feasible by LCMS, but information on positions and sequential context requires other techniques.
We developed a deep sequencing-based approach for modification detection in RNA nucleotides that relies on specific behavior of reverse transcriptase (RT) at non-standard residues. The corresponding positions exhibit increased rates of RT arrest and characteristic mismatch patterns becoming visible, when mapping the sequenced libraries to references. Chemical treatments highlight specific modifications and allow further differentiation of these RT signatures by altering the physico-chemical properties of the nucleotides. Machine learning approaches, such as Random Forests (Breiman, 2001), are then employed to determine regularities correlating the signatures with known modification events reported in databases or verified by LCMS. This results in rules that are used to predict nucleotide modifications from deep sequencing data of unknown RNA samples. Having applied a variety of six reagents with known molecular effects, we found characteristic RT signatures for a multitude of different modification types in *E.coli* and *S.cerevisiae*.



RNP09
Tetramolecular Fluorescence Complementation for Detection of Specific RNAs in Vitro

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Asymmetric localization of mRNA is a mechanism to regulate gene expression spatially as well as temporally and contributes to many important developmental processes. To study localization mechanisms various probes for RNA imaging have been established. These probes can be nucleic acid- or protein-based and give a characteristic fluorescent signal upon binding to target RNAs. Still, nucleic acid-based probes-*e. g.* molecular beacons or forced intercalation probes-cannot be produced inside cells, complicating *in vivo* imaging. Protein-based probes fused to split fluorescent reporters can be expressed by the cellular machinery. Sequence-specific binding of proteins to their target RNA and subsequent reconstitution of the fused fluorescent reporter allow RNA detection. However, the traditional fluorescent reporters based on split-GFP are large and self-assemble spontaneously, causing significant background.
To circumvent these limitations we used a three-body split GFP and developed a reporter system for detection of specific RNA *in vitro*. This system consists of four components: two Pumilio variants each fused to one β-sheet of GFP, a GFP detector, and the target RNA, which triggers assembly of the whole complex. We used this system to differentiate between closely related RNAs after as little as 10 minutes with a background fluorescence of merely 1.4 %. Since complex background of RNA and cell lysate did not prevent fluorescence complementation, detection of RNA with our system could become possible *in vivo*.
The ability to detect single stranded RNA sequence specifically might also be useful for monitoring of splicing events, detection of SNPs or discrimination of microRNAs. We currently extend our system to allow fast and easy detection of any given RNA of interest.

RNP10
The RNA methyltransferase Dnmt2 methylates DNA in the structural context of a tRNA

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A central question that may join the fields of DNA and RNA modification concerns the action of such rare enzymes that act on both types of nucleic acid. Ever since the discovery of its robust tRNA methylation,[1] Dnmt2 has been a paradigm in this respect, because all of its nearest neighbors in evolution are DNA-cytosine C5-methyltransferases and methylate DNA. The search for a Dnmt2 substrate had been exclusively focused on DNA until the discovery of tRNA methylation activity of Dnmt2 in the seminal paper by Goll [1]. With the declaration of $tRNA^{Asp}$ as the only substrate[1] the search for substrates appeared closed and reports on DNA methylation were now subject to highly controversial discussion.[2,3] We have set out to determine if the biochemical potential for DNA methylation is still present in the enzyme. Since linear double-strand DNA is a poor *in vitro* substrate at best, whereas $tRNA^{Asp}$ is efficiently methylated, we have constructed a series of $tRNA^{Asp}$ hybrids, in which RNA nucleotides were substituted with their corresponding deoxy-surrogates, starting with the single target nucleotide C38, which is methylated to rm^5C in native $tRNA^{Asp}$.[1] We found that DNA fragments, presented in the structural context of a tRNA, are more efficiently methylated than the corresponding natural tRNA substrate, testifying to the DNA methyltransferases ancestry of Dnmt2. Furthermore, we could show that the tRNA constructs were still a substrate of Dnmt2 after the introduction of a nick in the anticodon loop or even after the addition of further nucleotides to those new formed ends in the anticodon loop.

References

[1] M. G. Goll *et al.*, *Science* **2006**, *311*, 395.
[2] T. P. Jurkowski *et al.*, *PLoS One* **2011**, *6*, e28104.
[3] T. P. Jurkowski *et al.*, *RNA* **2008**, *14*, 1663.

RNP11
Investigating siRNA release from nanoparticular formulations

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Ongoing progress in the development of potential nucleic acid therapeutics highlights the relevancy of efficient and protective delivery systems. The release of formulated RNA from nanoscale polyplexes bears importance during all stages of therapeutic development. Association and dissociation of siRNA to and from the complex are affected by the ratio of positive to negative charge (N/P ratio), charge density and complex geometry. The kinetics of these processes however remain little understood so far.
Here, we address the complexation behavior by analyzing a panel of chemically diverse nanoscale polyplexes. Gel shift assays revealed particle-specific N/P ratios for a complete complexation, which were chosen for further analysis. We also conducted microscale thermophoresis as a fast and potent method to characterize complexation behaviour. A heparine competition assay gave first insights in the stability of the complexes.
To assess the stability and the dissociation kinetics in detail, we conducted an *in vitro* assay based on flourescence resonance energy transfer (FRET). The integrity of labeled siRNA can be continuously assessed by monitoring the FRET efficiency[1]. Upon addition of RNase V1 to a polyplex, tightly bound siRNA remains intact while dissociated siRNA degrades. This is manifested in a breakdown of the FRET signal that can be monitored in real time.
Testing of the various polyplex formulations by the FRET degradation assay revealed substantially diverging dissociation kinetics of siRNA from the various polyplexes. These measurements elucidate important properties of nanoscale siRNA polyplexes with strong implications for release during potential therapeutic applications.

References

[1]Hirsch et al., J Controlles Release 135 (1), 2009

RNP12
Isolation of small regulatory RNAs from E.coli and RNA modification analysis by LC-MS/MS

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RNA is known to be subject to extensive posttranscriptional modification with currently more than 150 naturally occurring, modified nucleosides known. For most RNA types, the modification pattern is well-investigated, however, only little information is available about modified nucleosides in small non-coding RNA. We are interested in investigating a number of small regulatory RNAs in *E. coli* for the appearance of modified nucleosides by highly sensitive LC-MS/MS.
Investigation of a specific RNA molecule for modified nucleosides by LC-MS/MS necessitates its isolation from total RNA in sufficient amounts. To address this, a hybridization based approach was applied, making use of a biotinylated oligodeoxynucleotide complementary to the target RNA. After hybridization of the capture probe to the RNA, the composed hybrid was separated from remaining RNA with the help of streptavidin-coated, magnetic beads and submitted to LC-MS/MS analysis. As a model RNA for optimization of the hybridization conditions and isolation procedure, $tRNA^{Met}$ was isolated from *E.coli* total tRNA.
With the isolation step established, we are now able to analyze small non-coding RNAs like those appearing in *E.coli*, which accomplish important regulatory functions for example in stress response. Here we chose the small RNA RybB, which is involved in the reaction to envelope stress, as target RNA for isolation and LC-MS/MS analysis.

RNP13
Dye-walk on human mitochondrial tRNALeu(CUN) to investigate tRNA recognition by Mouse Pseudouridine synthase 1 using Microscale thermophoresis

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Ribonucleotide modifications in tRNA have an exceeding influence on its functionality and stability [1]. Enzymatical isomerization of uridine to pseudouridine (Y) is an essential and the most common post-transcriptional modification in tRNAs [2]. Pseudouridine synthase 1 (Pus1p) is a well-studied site-specificYsynthasewhich modifies uridines in several regions of different tRNAs [3]. Missense mutation in Pus1p causes mitochondrial myopathy and sideroblastic anemia (MLASA) [3]. The minimal substrate for human Pus1p to modify the uridine at position 28 was found to consist of the Anticodon stem-loop (ASL) with substantial base pairing and the TYC stem-loop[3]. To determine which structural elements of tRNA are necessary for binding and modification by the murine equivalent mPus1p [4] we used various fluorescently labeled human mitochondrial tRNALeu(CUN)constructs as probes for a “Dye Walk”. Microscale thermophoresis (MST) assays enabled us to address possible variations in affinity of mPus1p for these constructs. With MST we have an elegant method which allows for quantitative analysis of protein interactions under previously challenging conditions [5].

References

[1] Y. Motorin and M. HelmBiochemistry2010, 49, 4934-4944.
[2]M. A. Machnickaet al.Nucleic Acids Res.2013,41,D1,D262-D26.
[3]Bryan S. Silbert and Jeffres R. Patton Nucleic Acids Res.2011, 1-12
[4] J. Chen and J. R. Patton RNA19995: 409-419
[5] Susanne A.I. Seidel et al.Methods2013, 59, 301-315.

RNP14
Aptamer mediated uptake of therapeutic agents

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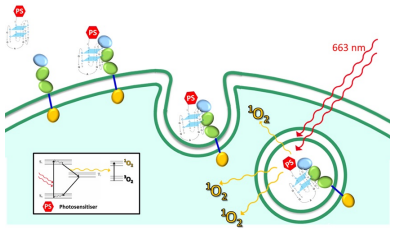
[2]*Rockefeller University, New York, United States*

Aptamers are single stranded oligonucleotides (DNA or RNA) with the ability to bind a certain target molecule due to their unique three-dimensional structure. Thus comparable, aptamers comprise significant advantages over antibodies such as convenient synthesis and feasibility in chemical derivatisation as well as non-immunogenicity.
We used the 19mer RNA-Aptamer AIR-3A for the delivery of therapeutic agents into cells bearing the target molecule, the human Interleukin 6 Receptor (hIL6R). AIR-3A bound to hIL6R is internalised via endocytosis and can therefore be harnessed for cargo delivery into the cell.
As a delivery cargo we conjugated a photosensitiser (PS), a chromophore that generates reactive oxygen species upon longwave irradiation, to AIR-3A. Photosensitisers are regularly used in photo dynamic therapy (PDT) for

the treatment of cutaneous lymphoma or surgically accessible solid tumors. One drawback of the conventional PDT is its lack of specificity for certain cell types, leading to adverse side effects on surrounding tissue or the withdraw of tumor cells from the PDT treatment.
When we exposed hIL6R positives cells to nanomolar amounts of the aptamer-PS conjugate, substantial uptake could be observed. Specific cell death could be induced by additional irradiation while hIL6R negative cells remained unharmed under these conditions. To induce the same extend of non-cell specific cell death with the free PS micromolar amounts are required.
Our results show that PS uptake by aptamer means can enhance the efficacy and the specificity of PDT.



RNP15
Microscale Thermophoresis and sophisticated fluorescence methods reveal no inhibition of Pseudouridine Synthase TruB by 5-Fluorouridine

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Nucleoside modifications sustain and optimize the functional conformation of tRNA [1]. The most abundant modification is pseudouridine (Y) [2], which is especially conserved at position 55 of the tRNA. Extensively studied are Y-55 synthases of the TruB family, for which tRNA's T-arm is a perfect minimal substrate [3]. The high propensity of these enzymes to co-crystallize with RNAs carrying 5-fluorouridine (5FU) at the substrate position, was attributed to 5FU being an irreversible inhibitor of Y synthases in general (*e. g.* [4]). We studied the affinity of TruB from *Thermotoga Maritima* for various fluorescently labeled $tRNA^{Phe}$*s. c.* constructs, both with and without 5FU55, using Microscale Thermophoresis [5], time-resolved (tr) fluorescence and tr anisotropy. The surprising result: whereas TruB tolerates various dye-labeled tRNAs very well, it binds U55 and 5FU55 tRNAs both reversibly and with similar affinity. Furthermore the binding is also detectable by changes in anisotropy or fluorescence lifetime using label sites far away from the substrate position. This implies global structural changes of the tRNA during TruB binding, which, in turn change dye-tRNA interaction.

References

[1] Y. Motorin and M. Helm *Biochemistry***2010**, 49, 4934-4944.
[2]M. A. Machnickaet al.Nucleic Acids Res.**2013**,41,D1,D262-D26.
[3] XiangrongGu et al.*Biochemistry**1998***,** 37, 1, 339-343.
[4] H. Pan, S. Agarwalla et al.*Proc Nat Acad Sci USA* **2003**, 100, 12648-12653.
[5] Susanne A.I. Seidel et al.*Methods***2013**, 59, 301-315.

RNP16
Structural dynamics in RNA modification enzyme complexes

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Enzymatic modification is a widespread mechanism to expand the functional and structural capabilities of RNA. These modifications serve distinct roles at specific positions. In eukaryotes, the most widespread sequence-specific mechanism is the isomerization of uracil to pseudouridine by a ribonucleoprotein complex formed around the central pseudouridine synthase, dyskerin/Cbf5. The bipartite complex recruits two of these sets of proteins to a H/ACA guide RNA, mostly formed from intronic splicing products. The guide RNA provides sequence specificity for the substrates by base-pairing.
Despite a body of structural data on H/ACA RNP complexes from various organisms, information on structural dynamics throughout the cycle of assembly, substrate recruitment and catalytic turnover are sparse.
Single-molecule spectroscopy in combination with fluorescence resonance energy transfer readout (smFRET) provides powerful means to study structural dynamics in RNA and RNP complexes. For this technique, RNA and/or proteins are covalently labeled with fluorophores and analyzed using fluorescence microscopy. Single molecule analysis provides data on conformational changes including kinetic parameters without ensemble averaging.
Here, a first approach to study structural dynamics in H/ACA RNA and RNP complexes will be presented. Methodology for labeling and complex formation will be presented, and the impact of these structural dynamics on the H/ACA RNP function will be discussed.

RNP17
Detection and Quantification of modified nucleic acids by LC-MS/MS analysis

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Nucleic acids like DNA and RNA are not only composed of the four major nucleosides but in addition of several chemical nucleoside alterations, *e.g.* methylations. Especially RNA is a heavily post-transcriptionally modified molecule and until today more than 150 different naturally occurring modified nucleosides have been discovered[1]. However, the distribution of these modifications varies from species to species and from RNA to RNA depending on the complexity of the required RNA function. One possibility for the detection and quantification of modified nucleosides is LC-MS/MS analysis, the successful combination of two powerful analytical methods. Liquid chromatography separates the analytes according to their physicochemical properties like hydrophilicity, basicity, or size, which can be used for compound identification. Mass spectrometry is a detection system that measures the mass to charge ratio of ionized analytes, allowing identification and sensitive detection. However, mass spectrometry is incapable of direct quantification due to fluctuations in *e.g.* analyte ionization and transmission, leading to the need of suitable internal standards and calibration measurements.
Here, we present a sophisticated LC-MS/MS method, which allows detection of over 20 modified RNA nucleosides in the subfmol range in a single run. In addition, the use of ^{13}C-labeled RNA digests from *E. coli* or yeast as an internal standard allows direct quantification of modified nucleosides from different RNA samples.

References

[1] Machnicka MA et al. MODOMICS: a database of RNA modification pathways: 2012 update Nucleic Acids Res 2013 Jan 1;41(D1): D262-D267

RNP18
Regulation of alternative splicing during differentiation

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Pre-mRNA splicing is one of the key steps of gene expression. During splicing, differential usage of alternative splice sites is regulated via different cis and trans acting elements. Chromatin and splicing are interwoven bi-directionally. Interestingly, changes in chromatin modifications are often observed during cell differentiation. We used mouse myoblasts (C2C12) as a model for differentiation. C2C12 differentiation is accompanied by chromatin changes[1]. Also, the role and significance of alternative splicing had been shown in myogenesis by splicing-sensitive microarray[2]. In order to find out the relationship between chromatin and alternative splicing changes during myogenesis, we analyzed ChIP-seq and microarray data. We compared 123 exons which are alternatively spliced during myogenesis with the same number of randomly sampled exons which had the same length. We observed that alternatively spliced exons gained more methylation marks (like H3K4met1, H3K4met2 and H3K36met3) and kept acetylation marks (like H3K9ac and H4K12ac) during myogenesis. Next, to compare alternative splicing and chromatin changes in more detail, we analyzed three genes during first week of differentiation. We found the significant change of histone marks such as H3K36met3 and H3ac at altenatively spliced exon of ITGA7 and Ncam1. In conclusion, our study suggests that chromatin has effects on splicing during muscle differentiation.

Refrences

1- Asp, P., R. Blum, et al. "Genome-wide remodeling of the epigenetic landscape during myogenic differentiation." Proc Natl Acad Sci U S A 108(22): E149-58.
2- Bland, C. S., E. T. Wang, et al. "Global regulation of alternative splicing during myogenic differentiation." Nucleic Acids Res 38(21): 7651-64

RNP19

Detection of RNA modifications via reverse transcription and Next Generation Sequencing

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RNA modifications have been found in a wide range of living organisms, from bacteria to humans. Currently, three general methods are used to locate and characterize RNA modifications: thin layer or liquid chromatography, mass spectrometry and enzymatic detection by Reverse Transcriptase or Rnase H.
In our lab, we extend the enzymatic detection via Reverse Transcriptase (RT) by combining it with the power of Next Generation Sequencing (NGS). Since certain RNA modifications are sensitive to specific chemical treatments, which alter their structure, a polymerase can act as a detector for these modifications via synthesis arrest. In order to exploit the possibilities of NGS for detection of modifications, the RNA molecule has to be converted into dsDNA first and multiple copies must be synthesized. Therefore, after the chemical treatment, an RNA adapter is ligated to the 3' end of the RNA molecule and the RT step is performed. After this, another DNA adapter is ligated to the 3' end of the newly synthesized cDNA strand. Following this, a PCR can be finally run to obtain the ready-to-be-sequenced, amplified DNA molecules. After sequencing and completed mapping of the reads to a reference sequence, a coverage profile is created, which is examined for significant drops and characteristic mismatch patterns by automated screening procedures. Machine learning approaches, such as Support Vector Machines, help in transcriptome-wide prediction of unknown modified positions.

TUMORIGENESIS AND PARANEOPLASTIC SYNDROMES

PKV22

Generation of a cell based system to visualize epithelial-mesenchymal transition (EMT) in tumor progression.

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Despite the fact that metastasis constitutes the major cause of cancer related mortality, therapeutic advancements in this process remain limited. As EMT is a considerable mechanism in the origin of metastasis it has moved into focus of cancer research during the last decade. However, visualization of this highly dynamic process in disease relevant models is still a bottleneck in the development of EMT modulating compounds. The application of fluorescent fusion proteins is restricted since overexpression may lead to mistargeting of critical EMT factors, whereas conventional antibodies do not trace dynamic changes of endogenous proteins in living cells. To overcome these limitations, we screened for fluorescent antibody fragments (chromobodies) derived from heavy-chain antibodies of camelids detecting epithelial and mesenchymal marker proteins. After analyzing target specific binding molecules in various biochemical applications, we expressed the chromobodies in living cells to visualize the distribution and dynamic changes of endogenous EMT markers. The aim of this study is to develop cellular models of metastatic cancer for high content analysis to screen for EMT modulators in real time. Identified compounds could then be used for the development of antimetastatic cancer therapy.

TPP01

p53 DNA Binding Cooperativity is Essential for Apoptosis and Tumor Suppression *In Vivo*

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Four molecules of the tumor suppressor p53 assemble to cooperatively bind DNA. The structural basis for cooperativity are interactions between adjacent DNA binding domains. Mutations at the interaction interface that compromise cooperativity were identified in cancer patients suggesting a requirement of cooperativity for tumor suppression.
Comprehensive ChIPseq profiling of p53 mutants with reduced or increased cooperativity highlighted a particular relevance of cooperativity for extending the p53 cistrome to non-canonical binding sequences. Furthermore, it revealed a striking functional separation of the cistrome: low cooperativity genes were significantly enriched for cell cycle and high cooperativity genes for apoptotic functions.
To address the role of cooperativity for apoptosis and tumor suppression *in vivo*, we generated cooperativity-mutant p53 knock-in mice. Apoptotic functions of p53 triggered by DNA damage and oncogenes were abolished in these mice, while functions in cell cycle control, senescence, metabolism and antioxidant defense were retained and sufficient to suppress development of spontaneous T-cell lymphoma. Cooperativity-mutant mice are nevertheless highly cancer-prone and susceptible to different oncogene-induced tumors.
Our data underscore the relevance of DNA binding cooperativity for p53-dependent apoptosis and tumor suppression and further strengthen the concept that the requirement for intermolecular p53 cooperation provides a crucial safeguard mechanism protecting against the accidental activation of apoptosis as the most final, irreversible cell fate decision.

TPP02

Statistical analysis of cell properties and distribution in Hodgkin Lymphoma whole slide images

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Hodgkin lymphoma (HL) is a type of B cell lymphoma which arises from germinal center B cells. HL differs from other lymphoma and cancer types in the morphology and distribution of the malignant cells. Most notably, no solid tumour is formed, and the malignant HRS cells make up only a small fraction of the cells in the affected tissue. Modern immunostaining protocols and the acquisition of high-resolution images for diagnostic purposes in pathological labs currently lead to large and growing databases of HL images. Exploring these images using automated image analysis is a hard task but may lead to a deeper understanding of HL. Here we present our current work on the analysis of a database of HL whole slide images. We performed pre-processing and identified regions of interest (ROI) that contain CD30-positive cells in the images as described before. A CellProfiler pipeline was used to detect and measure cells in the ROI. The cells were then classified using shape descriptors and stored in a database with their 2D coordinates. This allowed for a statistical analysis of the spatial distribution of HRS cells within the tissue.

TPP03

Characteristics of the CSN-CRL pathway during urological tumorigenesis

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Renal cell carcinomas (RCC) belong to the most frequently occurring tumors worldwide. Despite a lot of effort spent to personalize therapeutical approaches, a group of RCC patients still appears to be therapy-resistant. The COP9 signalosome (CSN)-cullin-RING ubiquitin (Ub)-ligase (CRL) pathway is a prominent segment of the Ub proteasome system (UPS). It specifically ubiquitinates regulatory proteins and is often deregulated in cancer. The exact mechanism of how deregulated CRL components are integrated in urological tumorigenesis is however unknown. Recently, our group revealed a post-transcriptional fine-tuning of COP9 signalosome (CSN) biosynthesis regulated by the c-myc/Lin28b/*Let-7* pathway. Interestingly, analysis of RCC patient samples revealed down regulated *let-7* depending on metastatic state. Based on these observations we think that the CSN-CRL pathway is an attractive subject for urological cancer research.
CSN-CRL pathway components were immunohistochemically stained in RCC samples to determine their expression pattern in urological tumor tissue. CSN subunit expression levels varied only slightly, whereas CAND1 staining revealed a deregulation in the intensity in urological cancer tissue nuclei. CAND1 is currently further examined in a TMA with an appropriate cohort. The CAND1-Skp2-p27 axis was deregulated in a RCC cell line as follows: Skp2 was overexpressed and p27 appeared as an atypical double band in immunoblots. Interestingly, no subunit of the CSN complex was expressed irregularly in 4 RCC cell lines. In order to study the interplay of particular CSN-CRL pathway components and a possible impact of the p27 double band during renal tumorigenesis further investigations are in progress.

TPP04

Glycine receptor autoantibodies recognize extracellular epitopes of the receptor complex

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Hyperekplexia is a neurological motor disorder also known as *startle disease*. Symptomatic issues are muscle seizures that can lead to uncontrolled falling without loss of consciousness. The symptoms can be triggered by unexpected tactile or acoustic stimuli. Recently, it became evident that similar phenotypes can be due to antibodies. So far, the major causes of hyperekplexia are mutations in the *GLRA1* or *GLRB* gene. These are either autosomal dominant or recessive inherited.
A similar hyperekplectic phenotype has also been reported in adult patients without a mutation in the described affected genes. Autoantibodies against GlyR were recently described. The underlying mechanism is not known yet. The glycine receptor (GlyR) is a pentameric, ligand-gated ion channel with high permeability for Cl^- anions. Upon ligand binding, the GlyR alters its conformation and opens the intrinsic ion channel. Defects at the GlyR protein lead to differences in ion channel properties or result in disturbed biogenesis.
In our study we are using immunocytochemical, proteinbiochemical as well as electrophysiological methods to determine the role of autoimmune antibodies on GlyR functionality. Plasmapherisates or sera from patients diagnosed with *stiff-man syndrome, stiff-limb syndrome* or *PERM* were used. GlyR variants were transfected together with GFP into HEK293 cells. Live staining experiments showed a clear signal of the glycine receptor extracellular domain independent from the α-subunit used. The sera also labeled specifically the GlyRs in primary spinal cord neurons. The effect of the autoantibodies on GlyR function was tested with whole-cell electrophysiological measurements.
These data will allow to understand the pathomechanism of GlyR-autoantibodies leading to hyperekplexia-like phenotypes in the adult human.

TPP05

Quinoxaline antitumor compounds that do not interact with the DNA

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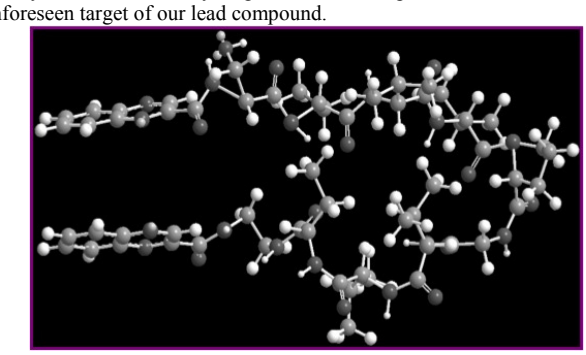
The potent activity of (bi)cyclic (depsi)peptides as antitumors, antivirals and antibiotics raised the need to identify novel analogues with improved pharmacokinetic properties for clinical applications. Nevertheless, none of the synthetic compounds have shown better biological activity than their parent natural compound.
We designed and synthesized a small library of simplified triostin A analogues. The peptide scaffold is defined by a β-hairpin motif, in which two strands are connected by a two-residue loop with two quinoxalines attached to both ends. The aromatic groups were thought to be oriented in parallel to interact with two adjacent DNA base pairs. The secondary structure was simulated using molecular dynamics and corroborated through CD and NMR.
Our compounds were tested on normal cells and several human cancer cell lines and the best of them (C2) displays better activity than doxorubicin in cervix and colon cancer cells. To overcome its low solubility, C2 was encapsulated in liposomes achieving an improvement in its biological activity. The hemolysis and stability in human serum essays showed that the compound does not affect human red blood cells and that C2 is stable to proteases.
Our drugs activate caspase-3 without previous loss of cell membrane integrity. The evaluation of the cell cycle arrest caused by our compounds as the apoptotic trigger was evaluated by flow cytometry. However, the characterization of their biophysical properties through fluorescence assays and DNase I footprinting showed that their mechanism of action is different from the quinoxaline antibiotics' one.
Finally, DNA microarray experiments were performed to address the unforeseen target of our lead compound.



TPP06

Switching Sides: How p53 takes out its brothers in arms

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In contrast to other tumor suppressors that are often either completely lost or show nonsense mutations in tumors, p53 frequently harbors missense mutations that cluster within the DNA binding domain (DBD). These mutations are commonly classified into DNA contact mutations (e.g. R273H) that directly affect residues involved in DNA binding and conformational mutations (e.g. R175H) that further destabilize the already rather unstable DBD of p53 causing it to unfold even under physiological conditions. In addition to losing their wildtype function (loss of function - LOF), most of these mutations have dominant-negative effect over the remaining wildtype protein. Moreover, it could be shown that some of these mutants also acquire new oncogenic properties, reffered to as 'gain of function' (GOF). As part of the GOF properties, it could already been shown, that many mutants interact with different other transcription factors that do not interact with wildtype p53. Two of the most important 'new' interaction partners are the other two p53 family members p63 and p73. Interaction between mutant p53 and p63 or p73 has been linked to chemoresistance, invasion and metastasis. Investigating the exact mechanism of how mutant p53 inhibits p63 and p73 might, therefore, open new ways for blocking this interaction and restoring tumor suppressor activity in cells with mutant p53. In contrast to previous studies suggesting that the p53 DBD co-aggregates with the highly conserved DBDs of p63 and p73, we could show that conformational p53 mutants only interact with the α-isoforms of p63 and p73 via their transactivation inhibitory (TI) domain. Introducing specific point mutations abrogated these interactions and restored functionality for p63 and p73.

TPP07

Translationally controlled tumour protein TCTP, a new biomarker in colon cancer?

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Translationally controlled tumour protein TCTP is a highly conserved, anti-apoptotic protein involved many physiological and disease processes. TCTP is overexpressed in many cancer cell lines and human tumours. We are addressing the question of whether TCTP is suitable as a potential biomarker in colon cancer. The following results will be presented: 1. Immunohistochemical staining on a panel of 90 human colon tumours demonstrated that TCTP levels are significantly higher in the adenomas and carcinomas, compared to the surrounding normal colon. 2. By employing the mTOR inhibitor rapamycin and an inhibitor targeting the protein kinase Akt, we demonstrate that in HT29 colon cancer cells TCTP levels are regulated through the PI3K/Akt/mTOR growth signalling pathway, which is frequently up-regulated in colon cancer. 3. TCTP as an anti-apoptotic protein has a range of cytoprotective properties. In order to investigate TCTP's potential role in cancer, we performed model investigations on MCF7 breast cancer cells, which showed that knock-down of TCTP partially sensitised these cells to treatment with oxaliplatin, an anti-cancer drug frequently utilised in colon cancer treatment.

LATE ABSTRACTS

SAP14

Misregulation of the Hsc70 Chaperone Cycle impairs Muscle Functionality in *C. elegans.*

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The molecular chaperone Hsc70 together with its cofactors assists in the folding of non-native proteins. ATP hydrolysis is used to perform structural rearrangements, which regulate cofactor and substrate binding. Two groups of cofactors influence this cycle: on the one hand the Hsp40 proteins, which deliver substrate to Hsc70 and accelerate ATP hydrolysis on the other hand the nucleotide exchange factors. In *C. elegans* UNC-23 is a weak homolog of the human Hsc70s nucleotide exchange factor Bag2. UNC-23 is expressed in muscle cells and several other tissues and knockout of UNC-23 induces a severe motility dysfunction. Using reporter strains, we find UNC-23 associated with the muscular attachment sites in muscle cells. We fluorescently labeled a shortened fragment of UNC-23 and verified its interaction with Hsc70 via analytical ultracentrifugation *in vitro*. UNC-23 binds to Hsc70 in a nucleotide-dependent manner and this binding is not influenced by the presence of Hsp40 proteins. By using this shortened fragment *in vivo* we could show that the N-terminal part of UNC-23 is crucial for the localization at the dense bodies in muscular tissues. The C-terminus supports all Hsc70-related functions, like Hsc70-binding, ATPase stimulation and regulation of folding activity. These findings indicate that the direction of Hsc70's ATP-driven cycle by UNC-23 may be important for the correct muscle function and attachment.

Index of presenting Authors

Notes

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Molecular
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ISBN-Nr. 978-3-9816002-4-7