

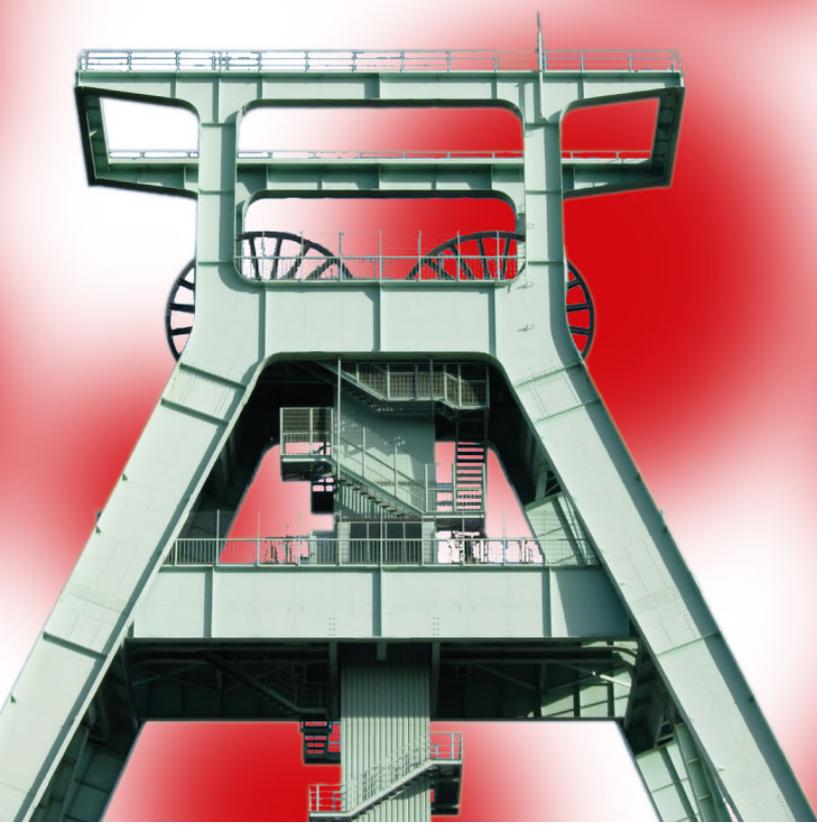


Molecular
Basis of Life

Molecular Basis of Life 2017

Ruhr University Bochum
[September 24 - 27, 2017]

Abstracts



Content

Abstracts - Talks	3
Abstracts - Poster/Short talks	35

Abstracts are sorted by presenting author!

Session/Abstract ID: Cancer & Inflammation / 16637

Drug action on the proteome

Presenting author:
Marcus Bantscheff

Author(s):
Marcus Bantscheff

The discovery of innovative, safe and efficacious drugs is hampered by a multitude of challenges. For example off-target effects or inefficient target engagement are frequent causes for late stage failure of candidate drug molecules. The discovery of innovative therapeutic approaches, however, critically depends on our ability to identify the mechanisms of action of bioactive molecules. In order to address some of these fundamental challenges in drug discovery, we have developed several (chemo-)proteomics approaches that allow the comprehensive analysis of cellular targets of bioactive compounds in live cells and correlation of target engagement with effects on the proteotype. This presentation describes experimental strategies in current chemical proteomics research, discusses recent examples of successful applications, and highlights areas in drug discovery where proteomics has impact.

Session/Abstract ID: RNA-Regulation und Transport / 16386

Novel Methods to Investigate RNA-Protein Interactions

Presenting author:
Benedikt Beckmann

Author(s):
Benedikt Beckmann

RNA-binding proteins (RBPs) and RNA form ribonucleoproteins (RNPs) which play major roles in post-transcriptional regulation of gene expression, e.g. as key proteins in RNA-transport or translation.

We will present 2 novel methods to study RNPs:

1. OligoPepSVM: a machine learning approach implementing a support vector machine (SVM) to predict RBPs from a taxon or single species. The SVM is trained solely on short amino acid motifs. We tested our novel machine learning algorithm first on established human datasets containing >1000 well established RBPs. Surprisingly and despite its simple training set, OligoPepSVM outperforms other prediction tools based on RNA-binding domain similarity in performance tests. Next, we searched for RBPs in bacterial species: We identify >90% of the known RBPs in Salmonella Typhimurium and predict many novel ones.

2. PTex - Phenol Toluol extraction: a novel technique for the unbiased purification of in vivo UV-crosslinked RNPs. Our method consists of three fast extraction steps thereby removing non-crosslinked RNA and proteins. Purification with PTex relies solely on physicochemical differences between crosslinked and "free" RNA/protein and thus enables analysis of non-poly(A) RNA interacting proteins, e.g. for interactors of ribosomal or tRNA. PTex can be performed in 2.5 - 3 hrs.

Session/Abstract ID: RNA-Regulation und Transport / 16146

Regulating neuronal granules in space and time

Presenting author:
Florence Besse

Author(s):
Florence Besse

Neuronal granules, comprised of RNAs and associated regulatory proteins, have been implicated in the long-distance transport of mRNAs to axons or dendrites, and in their local translation in response to external cues. Although it has become clear that the properties of these complexes are modulated in response to developmental and environmental cues as well as aging, how such changes are achieved at the molecular and cellular levels is currently poorly understood.

Using the fruitfly as a model organism, we have identified the conserved RNA binding protein Imp as a component of neuronal RNP granules and have shown that its transport to axons is tightly regulated during brain maturation. Furthermore, Imp function is essential for axonal remodeling (Medioni et al., 2014).

Our current work aims at understanding how neuronal RNP granules are assembled, and how their properties and transport are regulated during development. This is of particular importance in light of recent evidence linking non-functional RNA/protein aggregates to several age-related degenerative human diseases. To identify factors controlling RNA granule assembly and turnover, we are combining different approaches including structure/function analyses, purification of RNP granules, and high throughput microscopy-based RNAi screen. Recently, we have uncovered the importance of prion-like domains in the regulation of RNA granules, and have revealed their requirement for axonal transport and remodeling.

Session/Abstract ID: Emerging Analytical Methods / 16138

Dissecting the structure of protein complexes by sub-complex analysis and XL-MS

Presenting author:

Karsten Boldt

Author(s):

Karsten Boldt

To exert biological functions, proteins are not free-floating within cell but rather organized into molecular machines, the protein complexes. Therefore, to dissect the function of a given protein, the determination of its integration into complexes as well as the structure and modularity of those is essential. Affinity purification can define the protein repertoire of a given complex, yet, it remains difficult to gain knowledge of its substructure and modular composition. To get insights into the modular composition of a protein complex, we developed an approach that is based on the controlled decomposition of protein complexes in combination with quantitative mass spectrometry and bioinformatic analysis (EPASIS). This approach was applied to the complex of a disease-associated protein Lebercilin, which, when mutated, causes early childhood blindness. Thereby, starting from a raw list of potential interactors as determined by affinity purification, a modular model of the target complexes could be achieved. To gain insights into the interlinks that enable the assembly of the modular complex and into the structure of a sub-module, chemical cross-linking in combination with mass spectrometry was applied. Integration of results obtained by both approaches enabled us to generate a modular network

of the Lebercilin protein complex and to determine binding domains within the complex modules.

Session/Abstract ID: Molecular machines in Bioenergetics / 16310

Molecular Mechanism and Regulation of Mitochondrial Complex I

Presenting author:

Ulrich Brandt

Author(s):

Ulrich Brandt

In mammalian mitochondria, respiratory complex I (NADH:ubiquinone oxidoreductase) is composed of 44 different subunits with a total mass of ~1 MDa; it comprises 1 FMN and 8 iron-sulfur clusters as prosthetic groups. Defects of human complex I are the most frequent cause of inherited mitochondrial disorders and play a role in neurodegeneration and biological ageing. Converting the energy released by the transfer of two electrons from NADH to ubiquinone, complex I pumps four protons across the inner mitochondrial membrane. High-resolution structures of complex I obtained by us and others, now shed light onto the still elusive molecular mechanism of and provide important clues on the regulation of complex I by the active/deactive transition. Ubiquinone chemistry, which takes place in the peripheral arm of the L-shaped complex, plays a pivotal role in the catalytic mechanism by providing the energy that drives vectorial proton transport at the four putative pump sites in the membrane domain. Recent evidence corroborates the two-state stabilization change mechanism proposed earlier implicating that the stepwise reduction and protonation of ubiquinone results in concerted structural changes, which exert electrostatic strokes transmitted over a distance of ~200 Å to drive the proton pump modules in the membrane. The proposed mechanism inherently provides a thermodynamically feasible rationale for the reverse mode of the enzyme and its regulation by the active/deactive transition.

Session/Abstract ID: Lunch Sessions / 16660

Next generation antibody therapeutics: bispecific & multifunctional derivatives

Presenting author:

Ulrich Brinkmann

Author(s):

Ulrich Brinkmann

no abstract submitted

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16646

From understanding CRISPR immunity to CRISPR 2.0 applications

Presenting author:

Stan J.J. Brouns

Author(s):

Stan J.J. Brouns

not submitted

Session/Abstract ID: Emerging Analytical Methods / 16160

The art of delivery: labeling and manipulating intracellular targets

Presenting author:

M. Cristina Cardoso

Author(s):

M. Cristina Cardoso

Cell delivery of free molecules into the cytoplasm and nucleus using arginine-rich cell-penetrating peptides (CPPs) has been limited to small cargos, while big cargos like antibodies are taken up and trapped in endocytic vesicles. Functional antibody delivery in living cells would enable the labeling and manipulation of intracellular antigens, constituting a long-thought goal in cell biology and medicine. We have shown that the uptake efficiency of arginine-rich CPPs can be greatly enhanced by their cyclization. We have now tested the ability of cyclic CPPs to transport full-length proteins to the cytosol of living cells. We

will present a modular strategy for creating functional cell-permeable antibodies and other proteins capable of targeted labeling and manipulation of intracellular antigens in living cells. This technology constitutes a major step in the labeling, delivery and targeted manipulation of intracellular antigens. Ultimately, this approach opens the door towards immunostaining in living cells and the expansion of immunotherapies to intracellular antigen targets.

Session/Abstract ID: Plenaries / 16154

Cryo-EM shows how cytoplasmic dynein is auto-inhibited and activated.

Presenting author:

Andrew Carter

Author(s):

Andrew Carter

Cytoplasmic dynein-1 binds dynactin in the presence of cargo adaptor protein,s such as BICD, to form a transporter capable of long distance movement along microtubules. However, it is currently unclear why dynein-1 moves poorly on its own or how dynactin activates it. I will present a cryo-electron microscopy (cryo-EM) structure of the 1.4 MDa human dynein-1 complex in an inhibited conformation known as the phi-particle. This reveals the 3D structure of the cargo binding dynein tail and shows how self-dimerization of the motor-domains locks them in a conformation with weak microtubule affinity. Disrupting the phi-particle with structure-based mutagenesis drives dynein-1 into an open-form with a higher affinity for microtubules and dynactin and a tendency to mislocalize to the centrosome in cells. We find that open-dynein is not capable of moving on its own but requires dynactin binding to relieve its auto-inhibition by reorienting the motor domains to interact correctly with microtubules. Our model explains how dynactin binding to the dynein-1 tail stimulates its motor activity directly.

Session/Abstract ID: Plenaries / 15750

Reprogramming the Genetic Code

Presenting author:

Jason Chin

Author(s):

Jason Chin

The information for synthesizing the molecules that allow organisms to survive and replicate is encoded in genomic DNA. In the cell, DNA is copied to messenger RNA, and triplet codons (64) in the messenger RNA are decoded - in the process of translation - to synthesize polymers of the natural 20 amino acids. This process (DNA → RNA → protein) describes the central dogma of molecular biology and is conserved in terrestrial life. We are interested in rewriting the central dogma to create organisms that synthesize proteins containing unnatural amino acids and polymers composed of monomer building blocks beyond the 20 natural amino acids. I will discuss our invention and synthetic evolution of new 'orthogonal' translational components (including ribosomes and aminoacyl-tRNA synthetases) to address the major challenges in re-writing the central dogma of biology. I will discuss the application of the approaches we have developed for incorporating unnatural amino acids into proteins and investigating and synthetically controlling diverse biological processes, with a particular emphasis on understanding the role of post-translational modifications.

Session/Abstract ID: Lunch Sessions / 16627

Magnetic control of intracellular dynamics

Presenting author:

Maxime Dahan

Author(s):

Maxime Dahan

Investigating the dynamics of living cells requires not only imaging tools but also methods to perturb cellular activities with high spatio-temporal resolution. Here, we present an approach based on the use of functionalized magnetic nanoparticles (MNPs) inserted in the cytosol which can be displaced at a subcellular level with magnetic forces in order to trigger specific cellular events. I will first discuss the key parameters underlying the dynamics of nanoparticles

inside the cytosol, emphasizing the role of size and surface chemistry in the intracellular mobility. Next, I will show how MNPs can be used to activate Rac1/Cdc42 signaling pathways involved in cell migration, using sub-50 nm synthetic MNPs or engineered protein-based ferritin cages. Finally, I will describe our effort to target and manipulate organelle dynamics at the single cell level using magnetic actuation. Overall, we anticipate that the magnetic control of cellular behavior will become a vital field of research in the forthcoming years. With its successful advancement, it should establish as a key technology, complementary to optogenetic tools, and will find multidisciplinary applications in chemistry, bionanoscience, biology, biophysics, or neurosciences.

Session/Abstract ID: Plenaries / 16428

Membrane contacts sites and lipid dynamics

Presenting author:

Pietro De Camilli

Author(s):

Pietro De Camilli

A defining characteristic of eukaryotic cells is the presence of intracellular membranes. The most abundant endomembrane system is the endoplasmic reticulum (ER), which participates in a multiplicity of functions, including protein and lipid synthesis, a variety of metabolic reactions and intracellular signaling. While membranes of the ER are functionally connected to all membranes of the secretory and endocytic pathways via vesicular transport, they only physically fuse with each other and with vesicles involved in retrograde transport from the Golgi complex. However, close appositions between the ER and the membranes of virtually all other organelles including the plasma membrane play major roles in cell physiology. In my talk I will discuss the properties and functions of ER-localized proteins which contain lipid transfer modules (SMP domains or ORD domains) and whose binding in trans to other membranes helps control lipid homeostasis within cells.

Session/Abstract ID: RNA-Regulation und Transport / 16274

Long-non-coding RNAs in cardiovascular biology: from functions to mechanisms

Presenting author:
Stefanie Dimmeler

Author(s):
Stefanie Dimmeler

In the last years, non-coding RNAs gained increasing attention as multifactorial transcriptional and post-transcriptional regulators. Long non-coding RNAs (lncRNAs) comprise a heterogeneous class of RNAs, which act as epigenetic regulators of gene expression, modulator of transcription or by modulating splicing. Several lncRNA were shown to control vascular function. We have demonstrate that the lncRNA MALAT1, which is highly expressed and induced by hypoxia, controls angiogenesis preferentially by modulating endothelial cell proliferation (Michalik et al, Circ Res 2014). In addition, additional ncRNAs controlling vascular growth and function have recently been identified including the well-known lncRNA Meg3 (Boon et al, JACC 2017). The presentation will summarize two recent studies providing insights into the epigenetic regulation of gene expression by the hypoxia-induced antisense transcript GATA6-AS, and a newly identified non-coding RNA (NTARS), which interacts with hnRNPL, regulates splicing of tight junction proteins and controls endothelial cell proliferation and permeability.

Session/Abstract ID: Electron transport chains / 16246

Super-assembly of respiratory complexes

Presenting author:
José Antonio Enríquez Domínguez

Author(s):
José Antonio Enríquez Domínguez

Respiratory chain complexes can super-assemble in quaternary structures called supercomplexes (SCs) that optimize cellular metabolism. The interaction between complexes III (CIII) and IV (CIV) is modulated by COX7A2L, renamed SCAF1 (SC assembly factor 1). The discovery of SCAF1 represented strong genetic evidence that SCs are true entities in vivo. By combining deep proteomics and immunodetection

analysis we found that SCAF1 is always required for the interaction between CIII and CIV and that the respirasome is absent in most tissues of animals harbouring a SCAF1 mutant isoform with the exception of heart and skeletal muscle. By directed mutagenesis we characterized SCAF1 regions that interact with CIII and CIV. Furthermore, we found that the CIV subunit COX7A2 is substituted by SCAF1 in SCs containing CIII and CIV, or by COX7A1 in CIV dimers, that seemed to be stabilized by COX6A2 when compared with COX6A1 isoform.

Session/Abstract ID: Lunch Sessions / 16661

Fragment based screening at Boehringer Ingelheim: BRD4 BDI as an example

Presenting author:
Dennis Fiegen

Author(s):
Dennis Fiegen

no abstract submitted

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16363

Multiscale dynamics of heterochromatin revealed by single-molecule fluorescence

Presenting author:
Beat Fierz

Author(s):
Beat Fierz

Eukaryotic DNA is organized into chromatin which regulates gene transcription, DNA replication and repair. Local chromatin structure and dynamics control biochemical access to underlying DNA, e.g. for transcription factors and the gene expression machinery. The dynamic architecture of chromatin fibers in complex with effector proteins is however poorly understood.

We combine chemical chromatin synthesis and single-molecule imaging to study how multivalent chromatin effectors dynamically interact with modified chromatin. Employing single-molecule colocalization imaging, we could dissect the multivalent chromatin

interaction dynamics of heterochromatin protein 1a (HP1a) with modified chromatin fibers. Conversely, using a multimodal single-molecule Förster resonance energy transfer (smFRET) approach we revealed the underlying structural states and their interconversion kinetics in single chromatin fibers from microseconds to seconds. We found that individual nucleosomes engage in short-lived stacking interactions, resulting in tetranucleosomes with rapidly interconverting interaction registers. The underlying energy surface is modulated by HP1a, which induced a dynamically compacted state that exhibited structural fluctuations on its binding timescales. Together, our work reveals the fundamental dynamic hierarchy governing internal site exposure in chromatin fibers, which enables the gene regulation machinery to invade and remodel compact chromatin.

Session/Abstract ID: Electron transport chains / 16643

Dynamics of Biomolecules "in Action" studied with X-ray Free Electron Lasers

Presenting author:

Petra Fromme

Author(s):

Petra Fromme

not submitted

Session/Abstract ID: Systems biology and autophagy signal control / 16282

Molecular Targeting of Autophagic Cell Death

Presenting author:

Simone Fulda

Author(s):

Simone Fulda

Autophagy is a cellular stress response and quality control mechanism that in general acts in a pro-survival manner. In addition, it is now well established that autophagy can exert also cytotoxic functions depending on the context. However, the molecular determinants that are responsible for autophagic cell death are currently poorly understood. We recently discovered that excessive induction of autophagy by BH3 mimetics triggers the assembly of the necrosome,

a multiprotein complex essential for the execution of necroptosis, on autophagosomal membranes, thereby engaging necroptotic cell death. This demonstrates that autophagy can act as a backup cell death mechanism. These findings have important implications to bypass therapy resistance especially in apoptosis-refractory tumors such as glioblastoma.

Session/Abstract ID: Lunch Sessions / 16624

Label-free tissue classification by FTIR- and QCL-based IR-Imaging

Presenting author:

Klaus Gerwert

Author(s):

Klaus Gerwert

Infrared imaging is an emerging tool for label-free, non-invasive characterization of tissue, cells, and body fluids.(1) The approach is applied to fresh frozen and formalin fixed paraffin embedded tissue samples. For the entities colon, bladder, and lung, spectral data bases are established to characterize tissue in an automated workflow with sensitivity and specificity of over 95%.(2) However, the therapeutic decision of the clinician requires a differential diagnosis. Therefore, in the next step a predictive differential diagnostics is established. We were able to differentiate between the predictive subtypes of the adenocarcinoma in lung cancer.(3) Furthermore the grading of colon cancer could also be classified.(4)

In the next step the spatial resolution is combined with molecular resolution by -omics technologies. The label-free classified tissue is dissected by laser microdissection and the homogenous samples are analyzed by proteomics to provide beside spatial also molecular resolution. This is successfully applied to the mesothelioma subtypes.(5) All currently used biomarkers in immunohistochemistry in pathology-panels are identified. This validates the power of this approach.

In the next step a QCL-based microscope is implemented. It shows the same quality of spectra as the FTIR-Images, but the measuring-time is now drastically reduced by a factor of 90 below 30 min for a typical thin section used in pathology. This improvement in time opens up new avenues for clinical applications.

1 Gerwert K., Großerüschkamp F., Ollesch J. (2014), SPIE Newsroom, DOI: 10.1117/2.1201-312.005297.

2 Kallenbach-Thieltges A., Großerüschkamp F., Mosig A., Diem M., Tannapfel A., Gerwert K. (2013), J. Biophotonics, 6(1), 88-100.

3 Großerüschkamp F., Kallenbach-Thieltges A., Behrens T., Brüning T., Altmayer M., Stamatis G., Theegarten D., Gerwert, K. (2015), Analyst, 140(7), 2114-20.

4 Kuepper C., Großerueschkamp F., Kallenbach-Thieltges A., Mosig A., Tannapfel A. and Gerwert K. (2016), Faraday Discussion, DOI 10.1039/C5FD00157A.

5 Großerueschkamp, F., Bracht, T., Diehl, H. C., Kuepper, C., Ahrens, M., Kallenbach-Thieltges, A., Mosig, A., Eisenacher, M., Marcus, K., Behrens, Th., Brüning, Th., Theegarten, D., Sitek, B., Gerwert, K. (2017), Scientific Reports, 7, 44829, DOI:10.1038/srep44829.

Session/Abstract ID: Lunch Sessions / 16636

Whole slide, label free screening of tissue on clinically relevant time scales

Presenting author:

Matthias Godejohann

Author(s):

J. Rowlette, E. Fotheringham, B. Bird, B. Shine, Matthias Godejohann

Marker free chemical imaging for classifying cancer subtypes is known for a couple of years in the academic community (1), but it is not widely used in clinical practice.

Infrared Imaging based on Fourier-Transform-Infrared-Microscopes (FTIR-Microscopes) has its limitation in speed, leading to measurement times of a couple of days for characterizing one slide of tissue. The low speed prevented the technology from entering the clinical workflow so far.

Integrating a next generation, fast tuning external cavity quantum cascade laser (EC-QCL) as a light source into a commercial Spero infrared microscope resulted in an order of magnitude increase in data acquisition speed without compromising spectral data

quality. The new approach achieves measurement times, which are probably short enough for improving the clinical workflow.

1 Frederik Großerüschkamp, Angela Kallenbach-Thieltges, Thomas Behrens, Thomas Brüning, Matthias Altmayer, Georgios Stamatis, Dirk Theegarten and Klaus Gerwert

„Marker-free automated histopathological annotation of lung tumour subtypes by FTIR imaging“

Analyst. 2015, 140, 2114

Session/Abstract ID: Subcellular organisation / 16102

Mitochondrial protein import studied by cryoET

Presenting author:

Vicki Gold

Author(s):

Vicki Gold, Piotr Chroscicki, Piotr Bragoszewski, Agnieszka Chacinska

Protein import into mitochondria is a highly coordinated process, responsible for correct protein targeting and sub-cellular protein organisation. There is growing evidence that in living cells, protein import frequently follows the recruitment of mRNA molecules and cytosolic ribosomes to the outer mitochondrial membrane. In this work, we were able to visualise translation-arrested ribosomes bound to the surface of mitochondria by electron cryo-tomography and subtomogram averaging, revealing both membrane-bound and polysome populations. By complementary biochemical studies, we reveal that ribosomes interact specifically with the protein Translocase of the Outer Membrane (TOM) complex and that nascent chain binding is crucial for ribosome recruitment and stabilisation. The membrane-bound ribosomes were observed with their polypeptide exit tunnels correctly orientated towards the mitochondrial outer membrane for protein import, and were used to depict the clustered organisation of the TOM complex. The ribosome-TOM complex interaction may thus serve to couple protein synthesis with protein transport, highlighting the importance of spatial organisation for efficient mitochondrial protein import.

Session/Abstract ID: Age-related pathologies and autophagy / 16280

Sophisticated pathways link fatty acid metabolism and autophagy

Presenting author:

Martin Graef

Author(s):

Martin Graef

Macroautophagy is a critical stress response where autophagosomes form de novo as double-membrane vesicles in order to transport cytoplasmic cargo to vacuoles or lysosomes for degradation and recycling. Although our understanding of the core autophagy machinery has increased substantially, the exact events underlying the complex membrane rearrangements of autophagosome biogenesis are not fully understood. We have identified conserved proteins functioning in acyl-CoA metabolism that are components of the core autophagy machinery in *Saccharomyces cerevisiae*. These dedicated proteins localize to and accumulate on nucleated autophagic membranes via specific targeting sequences. Cells compromised in autophagosome-localized protein activity show severe defects in autophagy. Our work shows that mechanisms controlling FA fluxes drive key stages of autophagy and provides insights in the fundamental events of autophagosome biogenesis.

Session/Abstract ID: Cell signalling and membrane trafficking / 16070

Phospholipid nanodiscs as a tool to study the structure and function of membrane proteins in a native environment

Presenting author:

Franz Hagn

Author(s):

Franz Hagn

This talk will focus on our recent attempts to establish phospholipid nanodiscs - a patch of phospholipid bilayer membrane encircled by two copies of a so-called membrane scaffold protein (MSP) - as a membrane mimetic for structural studies of membrane proteins. We designed subsequently smaller nanodiscs for NMR structural studies and solved the first solution structure of a membrane protein in nanodiscs. Furthermore, we used SortaseA-mediated protein ligation to produce circular MSPs that show exceptional size homogeneity that is beneficial for NMR and electron microscopy applications. We applied this methodology to study functional aspects and interactions of a variety of membrane protein systems ranging from neurotensin receptor, a GPCR, and its complex with a heterotrimeric G-protein, the mitochondrial VDAC1 ion channel to single-spanning transmembrane helices that are part of membrane-anchored proteins, which are essential for mitochondrial apoptosis as well as other cell-signalling pathways. These examples will highlight the benefit of a native membrane environment in studying functional properties of membrane proteins and their complexes with soluble partner proteins that are often sensitive to detergents.

Session/Abstract ID: Lunch Sessions / 16628

Footprint-free gene editing using CRISPR/Cas9 ribonucleoprotein (RNP) delivery and long ssDNA donor templates

Presenting author:

Cornelia Hampe

Author(s):

Cornelia Hampe

While CRISPR/Cas9 is a powerful technique for gene editing, two significant challenges remain: obtaining efficient delivery of Cas9 and gene-specific sgRNA to a broad range of cell types, and leaving no additional footprint (i.e., persistent and elevated expression of Cas9 in target cells) that could lead to off-target effects.

To address these challenges, we have developed improved delivery tools:

1. Direct delivery of Cas9-sgRNA RNP complexes using novel Gesicle technology.
2. Gene knockouts and knockins via electroporation of Cas9-sgRNA RNP complexes.

Knockout mutations can usually be obtained with high efficiency, but knocking in longer sequences (>200 bp) via homology directed repair (HDR) is more difficult to achieve. Although single-stranded DNA (ssDNA) donor templates have recently been shown to have several advantages over double-stranded DNA (dsDNA) templates, the usefulness of long ssDNA templates is limited due to the difficulty and cost of producing them. We recently developed a simple method for generating long ssDNA donor templates up to 5 kb. Compared to dsDNA donor templates, ssDNA templates have a dramatically reduced tendency to randomly integrate into the genome and are far less toxic to cells when delivered via electroporation. Long ssDNA donor templates thus greatly help getting better specificity for knockin experiments.

About Takara Bio Europe

Takara Bio Europe is part of the Takara Bio Group, a leading supplier of tools for life scientists worldwide. Through our brand names TAKARA®, CLONTECH®, and CELLARTIS® we develop innovative technologies in the fields of Next-Generation Sequencing, Gene Delivery and Gene Function, Molecular and Cell

Biology, Protein Expression and Purification as well as Stem Cell Research.

Our Guide-it™ kits are excellent tools for genome engineering using CRISPR/Cas9 technology. The Guide-it™ portfolio includes kits for efficient in vitro production and screening of single guide RNAs (sgRNAs) as well as various options to deliver Cas9/sgRNAs into target cells. Our Mutation Detection, Genotype Confirmation, and Indel Identification kits allow to monitor the efficiency of genome editing and to determine the exact edits in target cells

Email address: infoEU@takarabio.com

Website: www.takarabio.com

Session/Abstract ID: Molecular machines in Bioenergetics / 16161

Dynamic interactions at the end of the linear electron transport chain

Presenting author:

Guy Hanke

Author(s):

Guy Hanke

In the last steps of photosynthetic linear electron flow (LEF), ferredoxin (Fd) is reduced by photosystem I, and transfers these electrons to ferredoxin:NADP(H) oxidoreductase (FNR) for the regeneration of NADP⁺ to NADPH. Very high control coefficients of FNR for photosynthesis of 0.94 (at saturating light) and 0.7 (at limiting light) were calculated from a study on antisense tobacco plants with variable FNR concentrations. FNR is present in the chloroplast both as a soluble enzyme, and also bound to several membrane complexes. Binding has been reported to the cytochrome b6f complex, leading to suggestions that FNR may also be involved in cyclic electron flow (CEF), and two specific FNR binding proteins, Tic62 and TROL. Localization of the FNR enzyme at the thylakoid, close to the site of Fd reduction, is necessary for efficient electron flow. However, the mild phenotype of tic62 and TROL Arabidopsis mutants lead to the suggestion that soluble FNR is actually responsible for NADP⁺ photoreduction. Although the catalysis and structure of FNR are well characterized, the relationship between its different locations and its function remains confusing. In a previous study we

produced Arabidopsis plants expressing different FNR genes from maize. We will present data describing how different FNR interactions affect enzyme catalysis and flux into different electron transport pathways

Session/Abstract ID: Public Lecture / 16317

Immer der Nase nach: Neue Wirkmechanismen von Duftstoffen im menschlichen Körper

Presenting author:

Hanns Hatt

Author(s):

Hanns Hatt

Seit 25 Jahren sind die Riechrezeptorproteine, die für die Bindung der Duftstoffe in den Riechzellen der Nase verantwortlich sind, identifiziert. Säugetiere wie Maus und Ratte haben bis zu 1000 verschiedene dieser Rezeptoren in ihrer Riechschleimhaut, Menschen dagegen „nur“ noch ca. 350. Es ist jedoch die größte Genfamilie in unserem Erbgut. Vor einigen Jahren konnten wir zeigen, dass ein Riechrezeptor sehr spezifisch empfindlich ist für eine bestimmte chemische Struktur (funktionale Gruppe). So gibt es Rezeptoren, z.B. für Vanilleduft, Moschusduft, Buttersäure. Riechrezeptoren gehören zu der Superfamilie der GPCR's. Die Struktur der Rezeptorproteine ist relativ homolog unterscheidet sich aber in der „Bindetasche“, in die die Duftstoffmoleküle hineinpassen müssen. Man kann dies mit dem Schloss- und Schlüsselprinzip vergleichen. Wir konnten die ersten Riechrezeptoren des Menschen hinsichtlich ihres Duftspektrums detailliert analysieren. Es gelang uns außerdem, für einen dieser Rezeptoren, der auf den Duft von Lilial reagiert, einen antagonistischen Duftstoff zu finden, der diesen Rezeptor blockieren kann, das Undecanal. Diese interessanten Forschungsergebnisse können z.B. dazu beitragen, unangenehme Düfte hoch selektiv „auszublenden“. Nach Aktivierung durch ein Duftmolekül wird ein G-Protein gekoppelter Signalweg angesteuert, der via Adenylatcyclase die cAMP Konzentration in der Zelle erhöht. Dies führt zur Öffnung von cAMP-aktivierten Ionenkanälen und dem Einstrom von Ca²⁺ (und Na²⁺) in die Zelle.

Interessanterweise findet man, wie wir vor einigen Jahren zeigen konnten, viele dieser Rezeptoren nicht nur in der Nase, sondern auf allen Körperzellen des Menschen, u. a. auch den Spermien. Spermien besitzen über 20 Riechrezeptoren aus der Nase und

reagieren auf Düfte, wie Lilial (Maiglöckchen) oder Myrac (Orange) positiv chemotaktisch. Da wir im Vaginalsekret auch entsprechend viele Duftstoffe gefunden haben, könnte dies zum Fertilisationsprozess entscheidend beitragen.

Inzwischen konnten wir Riechrezeptoren auch in Hautzellen (Keratinocyten, Melanozyten) nachweisen und zeigen, dass eine Aktivierung des Rezeptors für Sandelholzduft zu einer verstärkten Regeneration der Hautzellen führt und damit auch die Wundheilung beschleunigt. Ein Riechrezeptor im Herzen, der auf Fettsäuren aus dem Blut reagiert, reduziert die Herzfrequenz und -kraft oder Rezeptoren in den Bronchien beeinflussen die Dilatation der Bronchien der Lunge. Darüber hinaus gelang es vor allem, Riechrezeptoren in Tumorzellen zu detektieren, wie in Prostata-, Leber- oder Blutzellen, wo sie oft in großen Mengen zu finden sind. Hier führt eine Aktivierung des Rezeptors zu einer Reduktion des Zellwachstums und der Zellmigration und somit Hemmung des Tumorwachstums. In weiteren Geweben, wie Darm, Blase oder Magenzellen wird die Funktion der Riechrezeptoren zurzeit untersucht. Solche experimentellen Fortschritte werden schnell dazu beitragen, besser die zellulären Prozesse zu verstehen und dazu führen, Düfte vermehrt zur Diagnose und Therapie in der Klinik einzusetzen.

Session/Abstract ID: Emerging Analytical Methods / 16313

Probing protein assemblies and interactions by hybrid mass spectrometry approaches

Presenting author:

Albert Heck

Author(s):

Albert Heck

Mass Spectrometry based proteomics has played a pivotal role in revealing the plethora of protein interactions that take place inside a cell, wherein proteins form protein assemblies and/or signalling networks. Especially using affinity purification of a tagged proteins followed by mass spectrometric analysis of its binding partners a wealth of data has been gathered revealing the all-embracing protein networks present in cells. A next step will be to now gather more in-depth structural and functional information on these individual protein assemblies. Mass spectrometry may contribute to this next level

of protein interaction analysis. To contribute to this emerging area in proteomics, we develop new methods using native and cross-linking mass spectrometry with the aim to bridge the gap between interaction proteomics and structural biology. These new innovations and applications of them in interaction proteomics will be central in this presentation.

In the first part of the talk native mass spectrometry and its applications in probing

protein assemblies and interactions will be described, focusing on examples wherein the dynamic assembly of a protein complex involved in the circadian timing in cyanobacteria will be highlighted. The second part of the talk will highlight our recent work on proteome-wide cross-linking mass spectrometry.

Session/Abstract ID: Plenaries / 16632

Algal Sensory Photoreceptors, All Biochemistry at the End

Presenting author:
Peter Hegemann

Author(s):
Peter Hegemann

Biological sensory photoreceptors are families of proteins that can be studied with unprecedented precision in space and time. Excited state dynamics, chromophore isomerization and electron transfer reactions, as well as inactivation processes are studied on rhodopsins with retinal chromophores or LOV and BLUF-proteins with flavin chromophores by UV/Vis, Raman and IR spectroscopy on fs to ps time scales. Proton transfer reactions, hydrogen-network changes and structural changes can nowadays also be studied on fs to second time scales, whereas ion transport or catalytic activities are monitored on microsecond to second scales by biochemical or electrical methods. By employment of these technologies in conjunction with protein engineering and theoretical calculations my group in collaboration with many colleagues has deciphered or at least enlightened the reaction mechanism of light-gated ion channels, light-driven pumps, and photo-activated guanylyl/adenylyl cyclases. These proteins are widely applied in the neurosciences for activation or deactivation of selected neurons in large neuronal networks as the animal brain (Optogenetics).

#Hertie Senior Professor for Neuroscience

Session/Abstract ID: Plenaries / 16145

RNAi-dependent epimutations evoke transient antifungal drug resistance

Presenting author:
Joseph Heitman

Author(s):
Joseph Heitman

Max Delbrück was a founding member of the phage group that developed molecular biology as a discipline and shared the Nobel Prize in 1969 with Salvador Luria for their paradigmatic discovery that mutations occur randomly prior to selection (1). During his later career, Max Delbrück turned his attentions to *Phycomyces* as a model to elucidate mechanisms of sensory perception. This basal fungus is a member of the former zygomycete phylum, now known as the Mucoromycota. We study *Phycomyces* and *Mucor* species as models to understand light sensing, sexual reproduction, genomic evolution, and the nature of spontaneous mutations to drug resistance. Remarkably, ~70 years after Luria and Delbrück's seminal work, we discovered a novel mechanism of spontaneous drug resistance operating in these basal fungi.

Microorganisms evolve via a range of mechanisms that may include or involve sexual/parasexual reproduction, mutators, aneuploidy, Hsp90 and even prions. Mechanisms that may seem detrimental can be repurposed to generate diversity. Microbes are known to evolve resistance to antimicrobial agents via pathways that involve both stable and unstable genetic mechanisms, such as the recent discovery of aneuploidy underlying azole resistance in *Candida albicans* and *Cryptococcus neoformans*. We have discovered a new mechanism conferring antifungal drug resistance in the human fungal pathogen *Mucor circinelloides*. In this species, spontaneous resistance to the antifungal drug FK506 (tacrolimus) was found to evolve via two distinct mechanisms (2). One involves Mendelian mutations that confer stable irreversible drug resistance; the other occurs via an epigenetic RNA interference (RNAi)-mediated pathway resulting in unstable, transient drug resistance. The peptidyl-prolyl isomerase FKBP12 interacts with FK506 forming a complex that inhibits the protein phosphatase calcineurin. Calcineurin inhibition by FK506 blocks the

M. circinelloides dimorphic transition to hyphae and enforces growth as yeast cells. In some FK506 resistant isolates, mutations in the *fkbA* gene encoding FKBP12 or the calcineurin *cnbR* or *cnaA* genes have occurred and confer FK506 resistance and restore hyphal growth. In other drug resistant isolates, no mutations are found in any of the known drug target genes. Instead, RNAi has been spontaneously triggered to silence the *fkbA* gene, giving rise to drug-resistant epimutants. FK506-resistant epimutants readily reverted to the drug-sensitive wild-type phenotype when grown in the absence of FK506. The establishment of these epimutants is accompanied by generation of abundant *fkbA* small RNAs and requires several known components of the RNAi pathway whereas other RNAi components are dispensable. Surprisingly, epimutants occur at a higher frequency and are more stable in mutants lacking RNA-dependent RNA polymerase 1 (Rdrp1), revealing that some RNAi components serve to constrain or reverse the epimutant state. Silencing of the drug target FKBP12 appears to involve the generation of a double-stranded RNA trigger intermediate using the *fkbA* mature mRNA as a template to produce antisense *fkbA* RNA. Epimutational silencing may be stochastic, similar to Mendelian mutations, but differs in that the altered phenotypic state is fully reversible in response to fluctuating environmental conditions.

Our recent studies reveal several novel components required for epimutation, including orthologs of the *Neurospora crassa* quelling inducing protein (QIP) and Sad-3 helicase (RnhA); interestingly the *rnhA* gene is linked to the *Mucor* sex locus, suggesting that sexual reproduction may activate epimutation similar to sex induced silencing in *Cryptococcus neoformans*. We have also found epimutants occur at a higher frequency in two additional mutants lacking either RNA-dependent RNA polymerase 3 (Rdrp3) or the RNaseIII-like protein R3B2. The Rdrp1, Rdrp3, and R3B2 factors operate a non-canonical RNA degradation pathway that suppresses the RNAi-dependent epimutation pathway by competition for targets (3). We have further generalized these findings by showing that epimutations also occur in a second species of *Mucor*, and by identifying epimutations in the *pyrF* or *pyrG* genes that confer resistance to 5-fluoroorotic acid (5-FOA).

These studies uncover a novel, reversible, transient epigenetic RNAi-based epimutation mechanism controlling phenotypic plasticity, with possible implications for antimicrobial drug resistance and RNAi-regulatory mechanisms in fungi and other eukaryotes. The full impact of epimutations in this and

other genetic systems may have eluded discovery previously given their inherently unstable nature.

1. Luria, SE and Delbrück, M. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics*, 28: 491-511, 1943.

2. Calo et al, Antifungal drug resistance evoked via RNAi-dependent epimutations. *Nature*, 513: 555-558, 2014.

3. Calo et al, A non-canonical RNA degradation pathway suppresses RNAi-dependent epimutations in the human fungal pathogen *Mucor circinelloides*. *PLOS Genetics*, 13: e1006686, 2017.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16136

Recognition of biomolecules with aromatic foldamers

Presenting author:

Ivan Huc

Author(s):

Ivan Huc

Aromatic amide oligomers constitute a distinct and promising class of synthetic foldamers – oligomers that adopt stable folded conformations. Single helical structures are predictable, show unprecedented conformational stability, and represent convenient building blocks to elaborate synthetic, very large (protein-sized) folded architectures. They possess a high propensity to assemble into double,[1] triple and quadruple helices, or to fold into sheet-like structures.[2] Cavities can be designed within such synthetic molecules that enable them to act as artificial receptors[3] and molecular motors. Water soluble analogues of these foldamers show promise in nucleic acid[4] and protein[5,6] recognition. This lecture will give an overview of the design principles of these functional molecular architectures.

[1] J. Shang, Q. Gan, S. J. Dawson, F. Rosu, H. Jiang, Y. Ferrand, I. Huc, *Org. Lett.*, 2014, 16, 4992.

[2] L. Sebaoun, V. Maurizot, T. Granier, B. Kauffmann, I. Huc, *J. Am. Chem. Soc.*, 2014, 136, 2168.

[3] N. Chandramouli, Y. Ferrand, G. Lautrette, B. Kauffmann, C. D. Mackereth, M. Laguerre, D. Dubreuil, I. Huc, *Nat. Chem.*, 2015, 7, 334.

[4] P. K. Mandal, B. Baptiste, B. Langlois d'Estaintot, B. Kauffmann, I. Huc, *ChemBioChem*, 2016, 17, 1911.

[5] M. Jewginski, T. Granier, B. Langlois d'Estaintot, L. Fischer, C. D. Mackereth, I. Huc, *J. Am. Chem. Soc.*, 2017, 139, 2928.

[6] M. Jewginski, L. Fischer, C. Colombo, I. Huc, C. D. Mackereth, *ChemBioChem*, 2016, 17, 727.

Session/Abstract ID: Cell signalling and membrane trafficking / 15954

Dynamic interactions of proteins quantified by single molecule FRET

Presenting author:

Thorsten Hugel

Author(s):

Thorsten Hugel

Cellular analyses of signaling pathways often relies on population-averaged measurements of protein interactions or conformational changes. Such measurements are usually not sufficient to determine kinetic parameters in and out of equilibrium.

Here we show how single molecule Förster Resonance Energy Transfer (FRET) can be used to quantify protein dynamics and interactions simultaneously in real time in vitro. This enables a complete kinetic description and revealed a previously undescribed mechanism on how cochaperones can modify the ATPase cycle of the heat shock protein 90 (Hsp90). In addition, weak cooperativity in protein oligomers can be quantified.

Finally, we show how dynamic protein-protein interactions with a substrate protein can specifically suppress conformational dynamics in the Hsp90 system. These coupled dynamics are likely crucial to understand the role of Hsp90 in cellular signaling.

Session/Abstract ID: Prize Lecture / 16626

Suprises in RNA Biology - A new Role for an old Coenzyme

Presenting author:

Katharina Höfer

Author(s):

Katharina Höfer

A hallmark in prokaryotic gene expression was the absence of 5'-capped RNA. In eukaryotes, the m7G-cap protects mRNA from degradation. Recently, the cofactor nicotinamide adenine dinucleotide (NAD) has been found to be covalently linked to bacterial RNA (1). We discovered a subset of small regulatory RNAs in the bacterium *Escherichia coli* to be specifically 5'-modified with NAD in a cap-like manner. Biochemical studies revealed that analogous to a eukaryotic cap, 5'-NAD-cap stabilise RNA against processing by ribonucleases (1, 2). Moreover, the Nudix hydrolase NudC was found to act as a NAD-RNA decapping enzyme in vitro and in vivo. Crystal structures of *E. coli* NudC in complex with NAD reveal the catalytic residues lining the binding pocket and principles underlying molecular recognition of NAD-RNA. In in vitro competition experiments, NudC preferred NAD-RNA over NAD, suggesting that NAD-RNA may be its primary biological substrate. (3)

Given the central role of NAD in redox-biochemistry, protein-modification, and signaling, its attachment to RNA points to unknown roles of RNA in these processes and to undiscovered pathways in RNA metabolism and regulation.

(1) Cahová, H*, Winz, M.L.*, Höfer, K.*, Nübel, G., and Jäschke, A. (2015). NAD captureSeq indicates NAD as a bacterial cap for a subset of regulatory RNAs. *Nature* 519, 374-377.

(2) Jäschke, A., Höfer, K., Nübel, G., and Frindert, J. (2016). Cap-like structures in bacterial RNA and epitranscriptomic modification. *Curr Opin Microbiol* 30, 44-49.

(3) Höfer, K*, Li, S.*, Abele, F., Frindert, J., Schlotthauer, J., Grawenhoff, J., Du, J., Patel, D.J., and Jäschke, A. (2016). Structure and function of the bacterial decapping enzyme NudC. *Nat Chem Biol* 12, 730-734.

Session/Abstract ID: Plenaries / 16650

Membrane fusion mediated by SNARE proteins

Presenting author:

Reinhard Jahn

Author(s):

Reinhard Jahn

Eukaryotic cells are compartmentalized into membrane-enclosed organelles. Most of them are connected with each other by the regulated exchange of transport vesicles that bud from the precursor membrane and are transported to their destination membrane where they dock and fuse. In most (but not all) cases, fusion is carried out by SNAREs that represent an evolutionarily conserved superfamily of small and mostly membrane-anchored proteins. SNAREs are distinguished by a conserved stretch of 60-70 amino acids, termed SNARE-motifs, that are located adjacent to the membrane anchor domain. During fusion, four of such SNARE motifs, each belonging to a different subfamily, align with each other to form a highly stable coiled-coil of α -helices. Complex formation proceeds from the N-terminal end towards the C-terminal membrane anchors, thus pulling the membranes together and initiating fusion ("zipper" hypothesis of SNARE function). The steps of SNARE assembly are controlled by members of conserved protein families such as the SM- and CATCHR-proteins, with additional proteins being involved in regulated exocytosis.

In our own work, we have focused on understanding the mechanisms of SNARE assembly and SNARE-induced fusion using structural and biochemical approaches and in-vitro fusion reactions with native and artificial membranes. We hope to achieve a better understanding of the energy landscape of the fusion pathway, thus shedding more light on a reaction fundamental to all eukaryotic cells.

Session/Abstract ID: Lunch Sessions / 16663

Live cell super-resolution microscopy with switchable fluorescent proteins

Presenting author:

Stefan Jakobs

Author(s):

Stefan Jakobs

no abstract submitted

Session/Abstract ID: Lunch Sessions / 16629

*Modern Microscopes in Biomedical Research-
about Ultra-High Mag Optics, Spatial resolution
and Numeric Aperture (NA)*

Presenting author:

Andreas Kerstan

Author(s):

Andreas Kerstan

The main challenges for FTIR imaging in biomedical applications are:

-to image large samples at low or better medium spatial resolution in minutes, not hours or days

-readily obtain high quality data in a reasonable time analyzing features as small as 2 μ m in transmission mode

-obtain highest spatial resolution of large samples in hours, not days

In order to find the best set up one should ideally select an optical configuration (magnification) that provides just enough oversampling in the spectral region of interest, without sacrificing, unnecessarily, FOV, which impacts significantly on your time of collection and spectral sensitivity. So the challenge is to find the balance between a large field of view and small pixel size on the one hand and system magnification and working distance on the other hand. A further critical point was up to now that only synchrotrons IR beamlines were thought to provide the ability to analyze samples to the micron level of detail and there is a very restricted access to synchrotron sources world-wide.

This presentation gives an insight in the newest developments in FTIR imaging and describes ways to overcome the mentioned challenges in biomedical research and how to consider factors like magnification, spatial resolution, numeric aperture and analysis time in the most ideal way.

Session/Abstract ID: RNA-Regulation und Transport / 16406

The Role of the RNA-Binding Protein Staufen2 in Dendritic mRNA Localization and Memory

Presenting author:

Michael Kiebler

Author(s):

Michael Kiebler

Targeting of mRNAs to synapses and the subsequent regulation of local synaptic translation are essential for hippocampal synaptic plasticity and for learning and memory. A set of RNA-binding proteins (RBPs), amongst others the brain-specific double-stranded RBP Stau2 (Stau2) critically contributes to these processes, however, the precise machinery and the underlying mechanisms involved in dendritic mRNA localization remain poorly understood.

In my talk, I would like to present several independent lines of investigation that we are currently exploring to elucidate the role of Stau2 at the molecular, the cellular and at the organismic level. On the molecular level, we benefit of having recently identified physiologically relevant target mRNAs for Stau2 (Heraud-Farlow et al., 2013, Cell Reports). Amongst them is a set of transcripts including (1) Rgs4 (regulator of small G protein signaling) whose protein products all cluster in the signaling pathway of G-protein coupled receptors and (2) an intron-containing isoform of Calm3 (calmodulin 3) in its 3'-UTR. To delineate the *in vivo* role of Stau2, we generated – in collaboration with Dusan Bartsch (Mannheim) a transgenic rat model, in which Stau2 expression is conditionally silenced by Cre-inducible expression of a miRNA targeting Stau2 mRNA in adult forebrain neurons. Interestingly, this transgenic rat revealed synaptic strengthening upon stimulation together with deficits in hippocampal spatial working memory, spatial novelty detection and in associative learning for operant rewards. Together, our findings provide new insight into how Stau2 contributes to learning and memory.

Intense research focuses on mitochondrial maintenance, and in particular mitophagy has emerged as an exciting area for ubiquitin research. This process involves unstudied ubiquitin Lys6-linked chains, which are assembled by the RBR E3 ligase Parkin, and disassembled by the Lys6-selective deubiquitinase USP30. Using new affinity tools for Lys6-linked chains, we recently uncovered E3 ligases for this chain type, which may constitute a fast-track proteasomal degradation signal. Mitophagy induction also relies on PINK1, the first described ubiquitin kinase. PINK1 specifically phosphorylates ubiquitin Ser65, and we recently learned how this signal is distributed across mitochondria. In my talk, I will discuss our latest insights into the mechanisms of mitophagy and of the proteins involved.

Session/Abstract ID: Subcellular organisation / 16134

Degrading mitochondria by mitophagy - a new ubiquitin playground

Presenting author:

David Komander

Author(s):

David Komander

Ubiquitin signals come in many flavours, forming a complex 'ubiquitin code', in which eight chain types mix with other PTMs such as phosphorylation and acetylation. This leads to a mind-boggling potential architectural complexity that is currently being unravelled with new methods and approaches.

Session/Abstract ID: Systems biology and autophagy signal control / 16349

Dynamic fine-tuning of selective autophagy

Presenting author:

Roswitha Krick

Author(s):

Roswitha Krick

Autophagy avoids accumulation of deleterious materials, such as protein aggregates, damaged mitochondria, or invasive bacteria within cells. Therefore, autophagy has a direct relevance for human diseases. The autophagic cargo is selectively or non-selectively enwrapped by a unique double membrane layered transport vesicle, the autophagosome. The autophagosome fuses with the vacuole/lysosome and its content is degraded. The dynamic assembly and disassembly of the different autophagic protein complexes, required for the biogenesis of autophagosomes, has to be strictly regulated but the employed molecular mechanisms are mostly unclear. The best-characterized type of selective autophagy is the cytosol-to-vacuole (Cvt) pathway that serves as a prototype for selective autophagy in yeast. The Cvt pathway is active under nutrient-rich conditions and delivers specific enzymes to the vacuole where they are matured and execute enzymatic functions. We unraveled molecular mechanisms of complex assembly and disassembly that set the stage for phagophore formation under nutrient-rich conditions.

Session/Abstract ID: Molecular machines in Bioenergetics / 16644

CryoEM of mitochondrial membrane protein complexes

Presenting author:

Werner Kühlbrandt

Author(s):

Werner Kühlbrandt

The advent of direct electron detectors for electron cryo-microscopy (cryoEM) is having an enormous impact on structural studies of large, flexible protein complexes that were previously out of reach (Kühlbrandt, 2014). We use cryoEM to determine the structure and function of key membrane protein assemblies in mitochondria. Single-particle cryoEM of mitochondrial ATP synthase dimers revealed a pair of long, membrane-intrinsic helices in subunit a adjacent to the c-ring rotors of two different mitochondrial ATP synthase dimers (Allegretti et al, 2015; Hahn et al, 2016). The helices are a fundamental feature of all rotary ATPases and contain the strictly conserved arginine that is essential for function (Kühlbrandt & Davies, 2016). By electron cryotomography (cryoET) of mitochondria from a wide range of organisms (Davies et al, 2012; Mühleip et al, 2016; 2017) we found that rows of ATP synthase dimers along cristae ridges are a conserved, universal feature of inner membrane organization. By single-particle cryoEM we discovered an unexpected functional asymmetry in the respiratory supercomplex I1III2IV1 from bovine mitochondria (Sousa et al, 2016). CryoET revealed that the interaction of complex I with the complex III dimer in the supercomplex is conserved across animals, fungi and plants, and thus appears to be essential for effective energy conversion in mitochondria. Recently, we determined the cryo-EM structure of the twin-pore protein translocase TOM that transports more than 1000 different pre-proteins from the cytoplasm into mitochondria (Bausewein et al, 2017). In the TOM complex, two 19-strand beta barrels of the Tom40 subunit, which resembles the mitochondrial VDAC anion channel, are surrounded by six small, trans-membrane Tom subunits and held together by the tilted helices of the pre-protein import receptor Tom22.

References

Allegretti M, Klusch N, Mills DJ, Vonck J, Kühlbrandt W & Davies KM (2015). Horizontal membrane-intrinsic α -

helices in the stator a-subunit of an F-type ATP synthase. *Nature* 521: 237-240.

Bausewein T, Mills DJ, Langer JD, Nitschke B, Nussberger S & Kühlbrandt W (2017). Cryo-EM structure of the TOM core complex from *Neurospora crassa*. *Cell* 170: 693-700.

Davies KM, Anselmi C, Wittig I, Faraldo-Gómez JD, Kühlbrandt W (2012). Structure of the yeast F1Fo-ATP synthase dimer and its role in shaping the mitochondrial cristae. *PNAS* 109, 13602-13607.

Hahn A, Parey K, Bublitz M, Mills DJ, Zickermann V, Vonck J, Kühlbrandt W & Meier T (2016). Structure of a complete ATP synthase dimer reveals the molecular basis of inner mitochondrial membrane morphology. *Mol Cell* 63, 445-456.

Kühlbrandt W (2014). The resolution revolution. *Science* 343, 1443-1444.

Kühlbrandt W, & Davies K M (2016). Rotary ATPases: A new twist to an ancient machine. *Trends Biochem Sci*, 41(1), 106-116.

Mühleip AW, Joos F, Wigge C, Frangakis AS, Kühlbrandt W, & Davies KM (2016). Helical arrays of U-shaped ATP synthase dimers form tubular cristae in ciliate mitochondria. *PNAS* 113 (30), 8442-8447.

Mühleip AW, Dewar CE, Schnauffer A, Kühlbrandt W & Davies KM (2017). In situ structure of trypanosomal ATP synthase dimer reveals a unique arrangement of catalytic subunits. *PNAS* doi:10.1073/pnas.1612386114.

Sousa JS, Mills DJ, Vonck J, & Kühlbrandt W (2016). Functional asymmetry and electron flow in the bovine respirasome. *eLife* 5, e21290. doi:10.7554/eLife.21290.

Session/Abstract ID: Emerging Analytical Methods / 16139

Decoding Molecular Plasticity in the Dark Proteome

Presenting author:

Edward Lemke

Author(s):

Edward Lemke

The mechanisms by which intrinsically disordered proteins (IDPs) engage in rapid and yet highly selective binding is a subject of considerable interest and represents a central paradigm to nuclear pore complex (NPC) function, where nuclear transport receptors (NTRs) move through the NPC by binding disordered phenylalanine-glycine-rich nucleoporins (FG-Nups). I will present a single molecule based approach that paired with atomic simulations revealed that a rapidly fluctuating FG-Nup populates an ensemble of conformations that are prone to bind NTRs with diffusion-limited on-rates. Since site-specific labeling of proteins with small but highly photostable fluorescent dyes inside cells remains the major bottleneck for directly performing such high resolution studies in the interior of the cell, I will also demonstrate an approach how to overcome this limitation. We have now developed a semi-synthetic strategy based on novel artificial amino acids that are easily and site-specifically introduced into any protein by the natural machinery of the living cell. Expressed proteins only differ from their natural counterparts by very few atoms, constituting a ring-strained cyclooctyne or cyclooctene functional group. This allowed rapid, specific "click" labeling and even multi-color studies of living cells and subsequent super resolution microscopy of the permeability barrier of the nuclear pore complex in situ.

Session/Abstract ID: Cell signalling and membrane trafficking / 16319

Structural studies of human ABC transporters

Presenting author:

Ioannis Manolaridis

Author(s):

Ioannis Manolaridis

ABCG2 is a constitutively expressed ABC transporter that protects many tissues against xenobiotic molecules. Its activity affects the pharmacokinetics of commonly used drugs and limits the delivery of therapeutics into tumor cells, thus contributing to multidrug resistance. We present the structure of human ABCG2 determined by cryo-electron microscopy, providing the first high-resolution insight into a human multidrug transporter. We visualized ABCG2 in complex with two antigen-binding fragments of the human-specific, inhibitory antibody 5D3 that recognizes extracellular loops of the transporter. We observed two cholesterol molecules bound in the multidrug-binding pocket that is located in a central, hydrophobic, inward-facing translocation pathway between the transmembrane domains. Combined with functional in vitro analyses, our results suggest a multidrug recognition and transport mechanism of ABCG2, rationalize disease-causing single nucleotide polymorphisms and the allosteric inhibition by the 5D3 antibody, and provide the structural basis of cholesterol recognition by other G-subfamily ABC transporters.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16648

Molecules that generate 'Fingerprints': A new class of fluorescent probes for medical diagnosis and chemical biology

Presenting author:

David Margulies

Author(s):

David Margulies

Our group has recently developed a new class of molecular sensors, termed 'combinatorial fluorescent molecular sensors', which mimic the function of cross-reactive sensor arrays (the so-called chemical "noses/tongues") (1-6). In this talk I will explain how

these pattern-generating probes could expand the fluorescent toolbox currently used to detect and image proteins. Specifically, I will show how such systems can be used to identify combinations of specific protein families within complex mixtures and to discriminate among isoforms in living cells, where macroscopic arrays cannot access (6). The way by which these molecule-size 'noses' can be recycled and be used to track several binding interactions simultaneously will also be discussed (6).

Selected references:

- (1) Rout, B.; Unger, L.; Armony, G.; Iron, M. A.; Margulies, D. *Angew. Chem. Int. Ed.* 2012, 21, 12477.
- (2) Rout, B.; Milko, P.; Iron, M. A.; Motiei, L.; Margulies, D. *J. Am. Chem. Soc.* 2013, 135, 15330.
- (3) Rout, B.; Motiei, L.; Margulies, D. *Synlett* 2014, 25, 1050.
- (4) T. Sarker, K. Selvakumar, L. Motiei, D. Margulies, *Nature Commun.* 2016, 7, 11374-11382.
- (5) Hatai J., Motiei L., Margulies D. *J. Am. Chem. Soc.* 2017, 139, 2136–2139.
- (6) Pode, Z.; Peri-Naor, R.; Georgeson, J. M.; Ilani, T.; Kiss, V.; Unger, L.; Markus, B.; HM, B.; Motiei, L.; Margulies, D. *Nature Nanotechnol.* 2017, in press.

Session/Abstract ID: Lunch Sessions / 16370

Illuminating the serotonergic system - Modulation of GPCR signaling by light

Presenting author:

Olivia Masseck

Author(s):

Olivia Masseck, Katharina Spoida, Stefan Herlitz

G-Protein coupled receptors (GPCRs) integrate extracellular cues into intracellular signals to modulate the cellular state. Because of their diverse modulatory function GPCRs represent one of the major drug targets of the pharmaceutical industry. Until now, the characterization and control of GPCRs and their intracellular signaling cascades has mainly relied on chemical compounds, which either activate or inhibit GPCR pathways albeit with limited receptor and cell-type specificity and low temporal and spatial

resolution. Recently, serotonergic chimeric GPCRs, which can be activated by light, have been developed by our laboratory to control signaling cascades in a cell- and receptor type specific way. Serotonin (5-HT) has been shown to modulate emotional behavior and alterations in 5-HT levels have been related to different disease states including anxiety disorders and depression. We now use light activated serotonergic receptors to investigate neuronal circuitries, which are thought to be involved in the development and manifestation of anxiety and depression. Additionally, our research focuses on the development of genetically encoded sensors to illuminate the role of the 5-HT system in general.

Session/Abstract ID: Molecular machines in Bioenergetics / 16645

Induced production of H₂O₂ from chloroplasts: a bioengineering approach and its applications

Presenting author:
Veronica Maurino

Author(s):
Veronica Maurino

not submitted

Session/Abstract ID: Systems biology and autophagy signal control / 16321

Decision making in autophagy - How to loose specificity

Presenting author:
Peter Mayrhofer

Author(s):
Peter Mayrhofer, Nena Matscheko, Yijian Rao, Thomas Wollert

Autophagy is a pivotal recycling pathway that operates in all eukaryotic cells. More than 40 AuTophagy related (Atg) proteins are known in yeast and many of them are conserved in humans. In non-stressed, vegetative cells, autophagy mainly recycles unwanted or damaged cytoplasmic components by exclusively engulfing them into autophagic membrane sacks, termed phagophores. In stressed cells autophagy switches from a selective to a non-selective mode in

which the phagophore captures cytoplasm randomly and delivers it to lysosomes. The molecular basis for this switch has been unclear. We reconstituted this very important step in autophagy from purified components and revealed how this process is coordinated by cells.

Session/Abstract ID: Prize Lecture / 16623

Specificity in the ubiquitin system: molecular mechanisms and applications of OTU family deubiquitinases

Presenting author:
Tycho Mevissen

Author(s):
Tycho Mevissen, David Komander

Ubiquitination is a reversible posttranslational modification with key roles in a vast range of cellular processes. Eight distinct ubiquitin linkage types co-exist in polyubiquitin chains, which can feature highly complex mixed and branched topologies. This 'ubiquitin code' requires tight regulation, and ubiquitin signals are removed by enzymes called deubiquitinases (DUBs).

The human family of ovarian tumor (OTU) DUBs comprises 16 active members, most of which regulate cell-signaling cascades. We performed a comprehensive analysis of this family, revealing that the majority of human OTU enzymes are linkage specific, preferring one, two, or a defined subset of chain types including largely unstudied atypical ubiquitin modifications. Using biochemical and structural approaches, we uncovered distinct molecular mechanisms of linkage specificity. For instance, OTU DUBs utilize additional ubiquitin-binding domains in cis, defined S1' and S2 ubiquitin-binding sites within the OTU domain, or conformational dynamics of the catalytic domain to distinguish between the different ubiquitin chain types. The latter mechanism was revealed by a series of crystal structures of the Lys11-specific DUB Cezanne/OTUD7B alone, and in complex with Lys11-linked diubiquitin and monoubiquitin, respectively, which together reconstruct the catalytic cycle in great detail.

Finally, linkage-specific DUBs were exploited in a novel biochemical approach for the analysis of polyubiquitin modifications. Ubiquitin chain restriction (UbiCRest)

analysis is a rapid and qualitative assay useful for the characterization of ubiquitin chain type composition and architecture, and thus will contribute to deciphering the 'ubiquitin code'.

Session/Abstract ID: Public Lecture / 16639

Die Erleuchtung des Gehirns

Presenting author:
Gero Miesenböck

Author(s):
Gero Miesenböck

not submitted

Session/Abstract ID: Electron transport chains / 16163

Identification and characterization of novel intracellular glutathione transporters

Presenting author:
Bruce Morgan

Author(s):
Bruce Morgan

Glutathione fulfils multiple roles in the cell, including acting as an important redox co-factor and playing an essential role in Fe-S cluster biogenesis.

The development of genetically encoded sensors, which enable measurement of the glutathione redox potential inside living cells, has changed our view of cellular glutathione homeostasis. Cellular glutathione appears to be highly compartmentalized. From studies in yeast, we know that the cytosolic glutathione pool is extremely reduced and robustly regulated; any glutathione disulfide (GSSG) that is formed is either quickly reduced or actively transported to the vacuole. Whole cell GSSG levels are completely dependent upon changes in non-cytosolic GSSG and thus can be used as an indirect marker for changes in GSSG transport into or out of the cytosol.

We have targeted the glutathione biosynthetic pathway enzymes, Gsh1 and Gsh2 to alternative cellular compartments. Combining this system with our previous insights, we can employ growth assays and biochemical analyses of cellular GSH and GSSG

content to identify putative intracellular glutathione transporters. We have identified a strong candidate for an ER GSSG exporter. Current work is focused on determining the subcellular localization of our candidate protein, performing transport assays with recombinant transporter protein reconstituted into ER microsomes, and assessing the impact of changing ER glutathione redox homeostasis on oxidative protein folding and UPR induction.

Session/Abstract ID: Plenaries / 16625

The topology of cohesin's association with chromosomes and its regulation by HAWKs

Presenting author:
Kim Nasmyth

Author(s):
Madhu Srinivasan, Johanna Scheinost, Naomi Petela, Thomas Gligoris, James Rhodes, Kim Nasmyth

Cohesin is thought to regulate enhancer-promoter interactions during interphase as well as holding sister chromatids together during mitosis. Cohesin's Smc1, Smc3, and kleisin (Scc1) subunits form a tripartite ring within which sister DNAs are believed to be entrapped until they are released during anaphase through separase-mediated Scc1 cleavage. Cohesin's association with chromosomes is regulated by HEAT repeat containing proteins associated with its kleisin subunit (HAWKs), namely Scc2/Nipbl, Pds5, and Scc3/SA, a property shared by the related condensin complex. Both cohesin and condensin are capable of organizing chromosomal DNAs into chromatid-like structures, the former during interphase and the latter during mitosis. To explain this, we have suggested that both extrude large loops of DNA in a processive manner. Loop extrusion (LE) also provides an explanation for how CTCF insulates enhancers from non-cognate promoters. Continuous loop extrusion initiating throughout the genome facilitates interactions between enhancers and cognate promoters while actively preventing interactions arising from three dimensional diffusion.

According to this notion, CTCF insulates enhancers from non-cognate promoters by hindering LE beyond CTCF sites. We have used two techniques to explore the nature of cohesin's association with chromosomes in yeast. The first is calibrated

ChIP-seq to monitor loading at and translocation from CEN loading sites of cohesin rings and their HAWKs. The second is site-specific crosslinking between cysteine pairs introduced to the ring's Smc1-Smc3, Smc1-Scc1, and Smc3-Scc1 interfaces (6C), to measure DNA entrapment. We find that cohesin switches between two mutually exclusive states, one with Pds5 and a second with Scc2 bound to its kleisin subunit. We suggest that only the latter is capable of hydrolyzing ATP and it is in this state that cohesin loads onto and translocates along chromosomes. Occupation by Pds5 in contrast is accompanied, albeit rarely, by opening the Smc3-Scc1 interface and release of cohesin from chromatin, a process that is shut off upon acetylation of Smc3 by Eco1. 6C crosslinking shows that loading of cohesin onto chromosomes and establishment of sister chromatid cohesion are accompanied by entrapment of individual and sister DNAs inside cohesin rings respectively. Crucially, mutations that neutralize the positive charge inside the Smc1-Smc3 hinge interface greatly reduce DNA entrapment but not association with chromosomes as measured by calibrated ChIP-seq. This suggests that cohesin loading can also take place without topological entrapment *sensu stricto*. A key question is whether loading without topological entrapment involves insertion of loops inside rings and if so whether the processive extrusion of such loops through the cohesin ring is responsible for LE.

Session/Abstract ID: Cancer & Inflammation / 16647

Circulating tumor cells in cancer patients: Biology and clinical implications

Presenting author:

Klaus Pantel

Author(s):

Klaus Pantel

not submitted

Session/Abstract ID: Plenaries / 16254

Eating to live

Presenting author:

Linda Partridge

Author(s):

Linda Partridge

Ageing is malleable to genetic and environmental interventions. Dietary restriction (DR), a reduced food intake that falls short of nutritional deficiency, can extend lifespan in organisms ranging from yeast to rhesus monkeys. DR also induces a remarkably broad spectrum improvement in health and protection against ageing-related diseases. Short-term studies have found similar health improvements in humans who undertake voluntary DR. Although it is often referred to as calorie restriction, the effects of DR are mediated by specific dietary constituents, particularly protein. We find that balancing the essential amino acids in the diet allows flies and mice to maximise their efficiency of use of protein and hence reduce their protein intake. Responses to interventions that ameliorate ageing are often sex-specific, and we have found that the greater response of female lifespan to DR in flies is mediated through an improvement in the health of the gut. Although most humans cannot adhere to a DR regime, our results suggest that alteration of diet composition may be able to achieve some of the benefits of DR itself.

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16638

Catching macromolecules in the act: approaches for time-resolved structural biology

Presenting author:
Arwen Pearson

Author(s):
Arwen Pearson

One of the major aims of structural biology is to obtain a molecular level mechanistic understanding of biological processes. Ideally this means imaging the reaction or process of interest as it occurs and with near-atomic resolution. One option is the, now well established, use of mechanistic and cryo-trapping approaches that accumulate specific meta-stable intermediates in the crystal or solution long enough for them to be structurally interrogated. However, it can be difficult to find conditions to trap all intermediates in a reaction pathway, especially those that are very short-lived. For these reasons a true time-resolved experiment has long been the dream of many structural enzymologists.

However, such an experiment is not technically easy to achieve. Although 100 ps time-resolved experiments have been possible for decades on Laue beamlines and more recently fs time-resolved experiments have hit the headlines as the new ultra-bright free electron X-ray laser sources have come on line, sub-ms time-resolved structural studies have still only been carried out for a handful of proteins. The major bottle-neck is the ability to quickly and uniformly initiate the reaction throughout the sample. Our collaborative research team at the Hamburg Centre for Ultrafast Imaging is addressing this challenge in two ways. The first is the development of microfluidic rapid mixing devices. These are suitable for slower reactions that occur on the order of 100s μ s - ms. For faster reactions initiation with a laser flash is required. However, current photocages are not ideal for time-resolved structural experiments for a number of reasons. We are actively developing both new photocages with improved properties and alternate caging approaches to decouple the decaging and reaction chemistries. I will present an overview of the possible approaches to reaction initiation as well as some of our recent advances.

Involvement of vacuolar proteins in the biogenesis of peroxisomes.

Presenting author:
Harald Platta

Author(s):
Harald Platta

The vacuole is a central component for the regulation of cellular homeostasis. Here we demonstrate a novel, selective link between the vacuole and peroxisomes. The combination of specific vacuolar proteases as well as the GTPase cycle of the small GTPase Ypt7 are essential for the import of PTS1-proteins into the matrix of peroxisomes. The mutation of these factors triggers the polyubiquitination and proteasomal degradation of the PTS1-import receptor Pex5p and finally leads to an inhibition of matrix protein import and peroxisomal biogenesis.

Session/Abstract ID: Lunch Sessions / 16630

Raman 4 clinical applications

Presenting author:
Jürgen Popp

Author(s):
Jürgen Popp

Due to an aging society a large increase of cancer is observed. Besides, due to an increasing world-wide mobility a fast spread of infectious diseases and antibiotic resistant pathogens can be observed. For an effective and personalized therapy of these diseases new methods providing a differential diagnosis are required. In the last years, linear and non-linear Raman methods have shown their potential to be in a position to meet these aforementioned challenges. In this presentation, we will highlight our recent advances in translating Raman approaches towards routine clinical applications with focus on cancer and infectious diseases. We will start with highlighting the potential of Raman microspectroscopy for an early diagnosis and therapy of infectious diseases. Furthermore, it will be shown that the combination of linear and non-linear Raman approaches with other spectroscopic technologies provides a sensitive and selective tool to potentially solve challenges currently faced by clinical pathology.

Session/Abstract ID: Subcellular organisation / 16364

Overall, the presented examples highlight the potential of Raman-based approaches for clinical diagnosis like e.g. rapid diagnosis and therapy of sepsis or for a label-free morphochemical characterization of complex tissue samples for an intraoperative diagnosis of cancer potentially even in vivo.

Acknowledgements

Financial support of the EU, the "Thüringer Ministerium für Wirtschaft, Wissenschaft und Digitale Gesellschaft", the "Thüringer Aufbaubank", the BMBF, the DFG, the FCI and the Carl-Zeiss Foundation are greatly acknowledged.

Session/Abstract ID: Lunch Sessions / 16260

Remote Magnetothermal Deep-brain Neuromodulation in Freely Behaving Animals

Presenting author:

Arnd Pralle

Author(s):

Arnd Pralle

Remote activation or silencing of specific neuronal activity in vivo are valuable tools for unraveling connections between behavior and neurocircuits. If translatable they hold great potential for diagnosis and treatment. We discuss magnetothermal neuromodulation combining spatiotemporally controlled heating with temperature sensitive channels. Membrane targeted superparamagnetic nanoparticles are briefly heated by exposure to an external alternating magnetic field. We demonstrate activation of neurocircuits using a heat-sensitive cation channel. Magnetothermal genetic stimulation in the motor cortex evokes ambulation, deep brain stimulation in the striatum causes rotation around the body-axis, and stimulation near the ridge between ventral and dorsal striatum causes freezing-of-gait. The duration of the behavior correlates tightly with field application. Furthermore, using a temperature sensitive chloride channel, we demonstrate magnetothermal silencing of neurons in the Ventral Tegmental Area, leading to abolishing of a place preference. We evaluate duration and repetition rate of alternating field dosage required to generate memory retention of the demonstrated aversion. Magnetothermal neuromodulation provides genetically and spatially targetable, repeatable and

temporarily precise activation of deep-brain circuits without need for surgical implantation of any device. It also covers an otherwise difficult time-window of tens of seconds to minutes of simulation.

Session/Abstract ID: Lunch Sessions / 16481

Mechanotransduction of axonal growth: effect of pico-Newton forces

Presenting author:

Vittoria Raffa

Author(s):

Vittoria Raffa

Although the exact mechanisms involved in the growth of axons is still incompletely understood, it is clear that force is a crucial factor for both axonal guidance and lengthening. Experimental studies indicate that forces, when carefully controlled, act as powerful stimulators of axonal lengthening. Axonal elongation as a function of the applied tensile force has been investigated by several teams. It has been found that neurites start to elongate when the applied tension is above 1 nN [1]. Recently, we established a novel approach for the application of a controlled tensile force to neurons and axons in order to accelerate regeneration after peripheral nerve injury [2]. The approach we proposed is based on the use of superparamagnetic iron oxide magnetic nanoparticles (MNP). This methodology has been used to explore the effect of pico-Newton force on axon guidance and lengthening. In contrast with previous results, we found that there is no threshold for stretched growth and we observed an elongation rate of 0.2-0.3 $\mu\text{m}/\text{h}/\text{pN}$ (the same elongation rate previously reported for both central and peripheral nervous system [1]), even with the application of picoNewton forces. This finding supports the concept of "stretch growth model" of axonal growth, according to which axonal elongation is driven by tension, irrespective of its origin, i.e., from the traction exerted by the growth cone, the mass body growth or external force application.

[1] PMID: 2010807

[2] PMID: 24407149

Session/Abstract ID: Lunch Sessions / 16664

Small crystals, intense lasers, and living cells - Novel strategies for structural biology

Presenting author:

Lars Redecke

Author(s):

Lars Redecke

no abstract submitted

Session/Abstract ID: Cancer & Inflammation / 16087

The role of the glycoproteome alterations in gastrointestinal cancer and its clinical applications

Presenting author:

Celso Reis

Author(s):

Celso Reis

Glycosylation alterations are frequently found in cancer and specific glycan structures are a hallmark of cancer development and progression [1]. The characterization of glycosylation modifications occurring in cancer is of high interest and represents a source of biomarkers for cancer detection, therapeutic intervention and patient stratification. This presentation reports the application of glycomic and glycoproteomic approaches for: (a) the characterization of increased expression of terminal sialylated structures in cancer; (b) the effect of these alterations that lead to the activation of tyrosine kinase receptors, such as MET and RON [2,3]; and (c) the identification of altered glycosylated proteins, carrying the simple mucin-type carbohydrate antigen STn, in engineered cancer cells and in gastric cancer patients sera [4], including the demonstration that the primary gastric tumors are the source of the identified glycoproteins (CD44) expressing truncated O-glycans [4].

References:

[1] Pinho S.S. and Reis C.A. Glycosylation in cancer: mechanisms and clinical implications. *Nat Rev Cancer* 2015, 15, 540-555.

[2] Mereiter S. et al. *Biochim Biophys Acta* 2016, 1860, 1795-1808.

[3] Magalhães A., et al. *J Biol Chem* 2016, 291, 8308.

[4] Campos D. et al. *Mol Cell Proteomics* 2015, 14, 1616-1629.

Session/Abstract ID: Prize Lecture / 16649

The Way of the Electron - Oxidizing Proteins in Mitochondria

Presenting author:

Jan Riemer

Author(s):

Jan Riemer

Cysteine is unique among coded amino acids because it contains a reactive sulfhydryl (thiol) group. The thiol group can bind different cofactors and metal ions, and it can become oxidized to different oxidation states ranging from disulfide bonds (cystine) to sulfonic acid. Presumably, due to their high reactivity cysteines are underrepresented in proteins and if conserved usually serve specific functions. We are interested in the enzymatic formation of disulfide bonds and how they are used to stabilize protein folds, form multi-subunit assemblies, and regulate (mitochondrial) protein functions. We investigate disulfide formation in the mitochondrial intermembrane space (IMS) a tiny subcompartment of mitochondria that harbors a diverse set of proteins that fulfill important tasks e.g. in mitochondrial protein import, detoxification of reactive oxygen species and transport of metabolites. Moreover, the IMS takes a critical position in cellular signaling by relaying hydrogen peroxide signals, integrating cellular clues to initiate apoptosis and by supporting Ca²⁺ signaling. In the last years, we have unraveled the mechanisms of disulfide formation in the IMS and its physiological impact. In this lecture, I will give a guided tour through our findings, and their meaning for mitochondrial function.

Session/Abstract ID: Prize Lecture / 15939

Mass spectrometry - from folding proteins to rotating motors

Presenting author:

Carol Robinson

Author(s):

Carol Robinson

Two decades after the introduction of electrospray for the analysis of proteins the mass spectra of intact ribosomes have become almost routine. These 2.5 MDa particles remain intact during their flight through the mass spectrometer yielding new information about the stoichiometry of subcomplexes and the effects of modifications 1.

Knowledge of the intact mass of a protein or complex is only one part of the mass spectrometry information available however. Data from the disruption of protein complexes is leading to subunit interaction maps and architectural models 2. Such models are enhanced by coupling with ion mobility mass spectrometry in which the collision cross-section of a protein complex can be defined 3.

With the advent of mass spectra of membrane embedded macromolecular complexes new information and hypotheses are emerging about the effects of lipid binding 4,5. In this lecture I will trace the developments that have made possible the transition from determining the mass of an individual protein to elucidating the structures and dynamics of rotary ATPases, as well as other membrane embedded assemblies.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16233

Hierarchical assembly of DNA filaments with designer elastic properties

Presenting author:

Barbara Sacca

Author(s):

Barbara Sacca, Wolfgang Pfeifer, Pascal Lill, Christos Gatsogiannis

Many of the macromolecules of living organisms are filamentous and are often organized into bundle suprastructures fundamental for cellular scaffolding and transport processes. The elastic features of such beams are encoded in their component units and in the way they are connected. Using DNA origami design approaches, we here show the realization of synthetic DNA filaments with tailored mechanical stiffness, originated from a single DNA module, composed of two quasi-independent domains and four interacting interfaces. Whereas the length and flexibility of the intra-domains region can be regulated by switchable DNA motifs, the inter-domain interfaces feature mutually and self-complementary shapes, which allow for their connection into filaments of programmable flexibility and periodical pattern. The level of structural complexity was further increased by linking distinct domains through combined facial and lateral associations, resulting in rigid polymers with a persistence length almost 1000-fold higher than a double stranded DNA and more than twice the value of natural actin filaments (ca. 15 μm). Thus, applying hierarchical strategies of DNA self-assembly on a segmented and reconfigurable unit with shape-complementary interfaces, artificial polymer-like materials can be created, with a large variety of ultrastructures and mechanical strengths comparable, or even superior, to their natural counterparts.

Session/Abstract ID: Cell signalling and membrane trafficking / 16642

Structural elucidation of the G-protein coupled receptor allosteric machine

Presenting author:

Patrick Scheerer

Author(s):

Patrick Scheerer

not submitted

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16132

Going beyond nature: Using supramolecular binding motifs to modify the structure and function of peptides

Presenting author:

Carsten Schmuck

Author(s):

Carsten Schmuck

Proteins and Peptides are one of the most important classes of biomolecules. Their properties and functions critically depend on their amino acid sequence which controls both the folding of the peptides and provides chemically active functional groups as needed for binding of other molecules and catalysis. However, the chemical space and variety provided by the 21 proteinogenic amino acids is limited. The use of artificial amino acids with tailor-made functional groups in the side chain might open the way to obtain new peptides and proteins with new properties and functions.

We explore how the use of a tailor-made arginine mimetic, the guanidiniocarbonyl pyrrole cation (GCP), incorporated into peptides changes their properties. The GCP has superior binding affinity for oxoanions compared to both arginine as well as lysine, the two amino acid based anion binding motifs used by Nature. At the same time the GCP is less basic but also more hydrophobic than arginine or lysine. All these aspects significantly change the properties of peptides which contain this supramolecular binding site. For example, we developed the smallest peptide based artificial gene transfection vector known until now. A simple tetrapeptide with four GCP-groups in the side

chains is a nontoxic but highly efficient transfection vector. But also the folding and conformational properties of small GCP-containing oligopeptides are dramatically different from their natural counter parts.

Session/Abstract ID: Systems biology and autophagy signal control / 16315

Selective Microautophagy of the Endoplasmic Reticulum

Presenting author:

Sebastian Schuck

Author(s):

Sebastian Schuck

The accumulation of misfolded proteins in the endoplasmic reticulum (ER) in budding yeast, *Saccharomyces cerevisiae*, leads to the formation of multi-lamellar membrane structures called ER whorls. Subsequently, ER whorls are delivered to lysosomes (the yeast vacuole) as part of a highly selective microautophagic process we term micro-ER-phagy. The physiological functions of micro-ER-phagy may include the control of ER size and the degradation of misfolded proteins. Importantly, micro-ER-phagy does not require the core autophagy machinery that is indispensable for macroautophagy and other organelle-selective types of microautophagy. To identify the molecular machinery involved, we have developed a microscopy-based screen for mutants with defects at various stages of micro-ER-phagy. A preliminary analysis of this screen will be presented. This approach promises to shed light on the molecular underpinnings of how portions of the ER are reorganized into whorls and selectively degraded by microautophagy.

Session/Abstract ID: Plenaries / 15209

*You take the high road and I take the low road -
Uncovering new routes for secretory pathway
translocation*

Presenting author:

Maya Schuldiner

Author(s):

Maya Schuldiner

In eukaryotes, up to a third of cellular proteins are targeted to the endoplasmic reticulum (ER), where they undergo folding, processing, sorting and trafficking. ER targeting has been shown to occur co-translationally by the SRP (Signal Recognition Particle) pathway or post-translationally by the mammalian TRC40 (Transmembrane Recognition Complex of 40kDa) and its homologous yeast GET (Guided Entry of Tail-anchored proteins) pathways. Despite the wide breadth of proteins that can be catered for by these two pathways, many proteins are still known to be both SRP and GET independent, hence there must exist an additional dedicated pathway for ER relay.

We set out to uncover additional targeting proteins using high-content screening approaches. We performed a systematic visual screen in yeast and uncovered three uncharacterized proteins whose loss affected targeting. We show that these proteins function in parallel to both SRP and GET to target a broad range of substrates. The three proteins, which we now name SND1, SND2 and SND3 (SRP-independent targeting), can synthetically compensate for the loss of both the SRP and GET pathway, and act as a backup targeting system. This explains why it has previously been difficult to demonstrate complete loss of targeting for some substrates. Our discovery thus puts in place an essential piece of the ER targeting puzzle, highlighting how the targeting apparatus of the eukaryotic cell is robust, interlinked and flexible.

Session/Abstract ID: Electron transport chains / 16165

*How to stay flexible: Respiratory physiology in
plant cells*

Presenting author:

Markus Schwarzländer

Author(s):

Markus Schwarzländer

The energy conversion that occurs in cells requires tight surveillance and dynamic adjustment to meet demands, maintain efficiency and avoid dysfunction. Plant cells are exposed to pronounced changes in the environment particularly frequently, including dark-light transitions, seed germination or changes in oxygen availability, which makes flexible acclimation essential. Nevertheless our understanding of the dynamics in energy physiology and their regulation at the subcellular level in plants remains limited. We have been assessing transitions in energy physiology in vivo by quantitative confocal microscopy and fluorimetry using genetically-encoded fluorescent protein sensors. In this talk I will highlight recent progress that we have made in dissecting the mechanistic interplay of the plant mitochondrial transport machinery with the regulation of mitochondrial energy physiology, including calcium transport, ATP dynamics and thiol redox regulation.

Session/Abstract ID: Pain / 16164

Pain from the human perspective

Presenting author:

Petra Schweinhardt

Author(s):

Petra Schweinhardt

Pain and nociception are linked yet separate constructs. In this talk I will show evidence that nociception is just one contributing factor to pain and that in order to understand the human pain experience, other factors need to be taken into account. Modulation of pain occurs throughout the neuraxis and ranges from peripheral events, such as Abeta or C-tactile fiber stimulation, to supraspinal influences, such as cognitions and emotions. I will present several examples of cognitive-emotional pain modulation and their underlying neurobiology as investigated in humans. Specifically, it will be

demonstrated (1) how the controllability of a stimulus influences pain processing and (2) how competing motivations in the form of rewarding stimuli influence pain perception. Finally, I will describe a conceptual framework that accommodates many different types of pain modulatory influences. This framework helps to explain clinical pain where in many instances, the problem appears to be disproportional to the suspected degree of nociception.

Session/Abstract ID: Lunch Sessions / 16492

Design and bio-functionalization of magnetic nanoparticles for intra-cellular remote control of signaling pathways

Presenting author:

Emilie Secret

Author(s):

Emilie Secret

Cell replacement therapy is among the most promising approach to treat neurodegenerative disorder such as Parkinson's, Alzheimer's or Huntington's diseases. In the work presented here, part of the MAGNEURON European project, we aim at having magnetic nanoparticles (MNPs) that are stable inside the cytoplasm of cells and bio-functionalized to trigger neurons' differentiation and growth along the direction of use of the external magnet gradient.

To this goal, $\gamma\text{Fe}_2\text{O}_3@SiO_2$ core-shell nanoparticles and $\gamma\text{Fe}_2\text{O}_3$ nanoparticles coated with polymers such as poly(ethylene glycol) or poly(acrylic acid) were synthesized. The particles were optimized to have size, charge and magnetization to obtain a good colloidal stability, render them injectable in cells, and facilitate intracellular motion. These MNPs were then functionalized with a HaloTag ligand in order to interact specifically with proteins able to trigger different pathways in the cell. MNPs were microinjected in the cell and showed intra-cellular biased diffusion toward the micro-magnet. The magnet can then be used to displace target proteins attached to the MNPs inside the cell, and trigger signaling events such as actin polymerization at particular subcellular localizations. Finally the synthesis of Fe_3O_4 nanorods, coated with silica or polymers will also be presented. Nanorods are of interest for their potentiality to be internalized without endocytosis and for their higher magnetic hyperthermia capacities.

Session/Abstract ID: Pain / 16143

New roles of autoantibodies in arthritis-induced pain

Presenting author:

Camilla Svensson

Author(s):

Camilla Svensson

Joint pain in rheumatoid arthritis (RA) often precedes joint inflammation and may persist even after successful anti-inflammatory treatment. We have addressed the possibility that anti-citrullinated protein antibodies (ACPA), present in a major subset of patients with RA and used clinically as a serological marker for the diagnosis of RA, may be directly responsible for the induction of pain, independent of the type of inflammatory processes that antibodies are most commonly associated with. We found that mice injected with either human ACPA IgG purified from RA patients, or murinized monoclonal ACPAs generated from synovial B-cells from RA patients, developed long-lasting pronounced evoked and spontaneous pain-like behavior in the absence of visual and histological signs of inflammation. Non-ACPA IgG from RA patients, IgG from healthy controls and control monoclonal IgG were without pronociceptive effect. This presentation will highlight the role of autoantibodies and antibody receptors in arthritis-induced pain and focus on the mechanisms by which ACPA activate sensory neurons. The identification of novel contributions of autoantibodies to persistent pain may aid in the development of new treatment strategies, not only for pain in RA, but also for pain in other diseases associated with autoantibody production

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16312

Structural insights into CRISPR-Cas12a - a genome editing alternative to CRISPR-Cas9

Presenting author:

Daan Swarts

Author(s):

Daan Swarts

The CRISPR-associated protein Cas9 is an RNA-guided DNA endonuclease that can be programmed using short RNA molecules to generate double-strand breaks in the genomic DNA of eukaryotic cells. Repair of DNA breaks by non-homologous end joining or homology-directed DNA repair can be exploited to introduce genetic modifications in targeted genomic DNA. Cas12a, another CRISPR-associated nuclease has also recently been repurposed for genome editing in eukaryotic cells [1].

In contrast to Cas9, Cas12a processes its own RNA guide and generates staggered-end DNA breaks. Furthermore, Cas9 requires a GG-containing motif next to the targeted sequence (Protospacer Adjacent Motif, PAM), whereas Cas12a requires a T-rich PAM. Combined, these features make Cas12a a useful addition to the genome editing toolkit.

We have determined crystal structures of *Francisella novicida* Cas12a [2]. These structures shed light on the mechanisms of RNA guide processing, and RNA-guided target DNA binding and cleavage. Together, our structural observations and corroborating biochemical experiments advance our understanding of Cas12a endonucleases and establish a mechanistic foundation for their use in genome engineering applications.

[1] Zetsche, B., et al. (2015). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*.

[2] Swarts, D.C., et al. M. (2017). Structural basis for guide RNA processing and seed-dependent DNA targeting by CRISPR-Cas12a. *Molecular Cell*.

Session/Abstract ID: Age-related pathologies and autophagy / 16033

Mitophagy in neurodegeneration and ageing

Presenting author:

Nektarios Tavernarakis

Author(s):

Nektarios Tavernarakis

Mitochondrial function impinges on several signalling pathways modulating cellular metabolism, cell survival and healthspan. Maintenance of mitochondrial homeostasis requires both generation of new, and elimination of dysfunctional mitochondria. Impaired mitochondrial content homeostasis is a common characteristic of ageing and several human pathophysiological conditions, highlighting the pivotal role of the coordination between mitochondrial biogenesis and mitophagy. However, the cellular and molecular underpinnings of the relevant mechanisms remain obscure. We found that DCT-1, the *Caenorhabditis elegans* homolog of mammalian BNIP3 and BNIP3L/NIX, is a key mediator of mitophagy, promoting longevity under stress. DCT-1 acts downstream of the PINK-1/Parkin pathway and is ubiquitinated upon mitophagy-inducing conditions to mediate the removal of damaged mitochondria. Accumulation of damaged mitochondria triggers SKN-1 activation, which initiates a bipartite retrograde signaling pathway stimulating the coordinated induction of both mitochondrial biogenesis and mitophagy genes. Our results unravel a homeostatic feedback loop that allows cells to adjust their mitochondrial population in response to environmental and intracellular cues. Age-dependent decline of mitophagy both inhibits removal of dysfunctional or superfluous mitochondria and impairs mitochondrial biogenesis resulting in progressive mitochondrial accretion and consequently, deterioration of cell function.

Session/Abstract ID: Pain / 16640

Employing neurotoxins as potent tools for personalized pain treatment

Presenting author:

Alesia Tietze

Author(s):

Alesia Tietze

Venom of marine cone snails is found to possess a variety of biochemical activities and therefore became a research field for drug and therapeutic development, especially in chronic pain treatment. The major focus of studies in this field is to understand relationship between the structure and the activity of biologically active peptides and their targets, membrane proteins. In order to be able to determine the interaction mechanism, the function and structural information on membrane proteins, ion channels and transporters, is needed. Thus, we develop methods to facilitate the availability of these classes of molecules to improve solubility and stability, develop modifications and study their interactions.[1,2] Chemical synthesis is an advantageous method here primarily because synthetic peptides/proteins can be customized (i.e. isotopic labeling) and derivatized (i.e. incorporation of post-translational modifications). The amount of compound produced by chemical synthesis allows structural analysis and functionalization of those molecules.

Through affording the availability of selected membrane proteins, using novel strategies established our lab, i.e. the first fully synthetic structurally uncharacterized potassium channel and influenza protein fragment were synthesized in order to study interactions of potent neurotoxins as potent drug leads for pain treatment [3,4]

References:

1)Miloslavina, A. A.; Leipold, E.; Kijas, M.; Stark, A.; Heinemann, S. H.; Imhof, D., A room temperature ionic liquid as convenient solvent for the oxidative folding of conopeptides. *J. Pept. Sci.* 2009, 15, 72-77.

2)Böhm, M.; Tietze, A. A.; Heimer, P.; Chen, M.; Imhof, D., Ionic liquids as reaction media for oxidative folding and native chemical ligation of cysteine-containing peptides. *J. Mol. Liq.* 2014, 192, 67-70

3)Tietze, D.#, Leipold, E.#, Heimer, P., Böhm, M., Winschel, W., Imhof, D., Heinemann, S.H., and Tietze,

A.A.* Molecular interaction of δ -conopeptide EVIA with voltage-gated Na⁺ channels (2016), *BBA General Subjects*, 2016, 1860 (9), 2053-2063

4)Leipold, E., Ullrich, F., Thiele, M., Tietze, A.A., Terlau, H., Imhof, D., Heinemann, S. H* Subtype-specific block of voltage-gated K⁺ channels by μ -conopeptides (2016), *Biochem. Biophys. Res. Commun.*, 2016, DOI: 10.1016/j.bbrc.2016.11.170

Session/Abstract ID: Age-related pathologies and autophagy / 16285

Ubiquitin signaling in neurodegenerative diseases

Presenting author:

Konstanze Winklhofer

Author(s):

Konstanze Winklhofer

Neurodegenerative diseases are characterized by the accumulation of misfolded protein species in the brain. How protein aggregation and cellular handling of misfolded proteins is linked to neuronal dysfunction and cell death has become a central question that is crucial to develop causal therapies. We are interested in the role of ubiquitination as a highly versatile posttranslational modification in regulating cellular quality control and stress response pathways. Ubiquitin can be attached to substrate proteins as a single moiety or as polymeric chain. Depending on the type of ubiquitin linkage, polyubiquitin chains adopt different conformations and thereby influence various cellular functions. We recently identified a specific non-degradative type of ubiquitination that modifies the subcellular localization of mutant huntingtin and markedly decreases its toxicity.

Session/Abstract ID: Systems biology and autophagy signal control / 15946

Decision making in autophagy - How to loose specificity

Presenting author:
Thomas Wollert

Author(s):
Nena Matscheko, Yijian Rao, Thomas Wollert

Autophagy is a pivotal recycling pathway that operates in all eukaryotic cells. More than 40 AuTophagy related (Atg) proteins are known in yeast and many of them are conserved in humans. In non-stressed, vegetative cells, autophagy mainly recycles unwanted or damaged cytoplasmic components by exclusively engulfing them into autophagic membrane sacks, termed phagophores. In stressed cells, however, autophagy loses its selectivity and degrades bulk cytoplasm. The regulation of this dramatic loss in selectivity is intimately intertwined with the onset of human diseases such as neurodegeneration or cancer. Its molecular bases remained, however, not well understood. Through a combination of classical biochemistry and cell biology with cutting edge in vitro reconstitutions, we have been able to solve the long standing question how selectivity is regulated at a molecular level in yeast. We found that the decision is made at the earliest step in autophagy, i.e. nucleation of the phagophore.

Session/Abstract ID: Pain / 16641

Restoring the spinal pain gate: Subtype-specific GABA-A receptor ligands against chronic pain

Presenting author:
Hanns Ulrich Zeilhofer

Author(s):
Hanns Ulrich Zeilhofer

not submitted

Session/Abstract ID: Lunch Sessions / 16662

Antibody Hit Discovery using Yeast Surface Display

Presenting author:
Stefan Zielonka

Author(s):
Stefan Zielonka

no abstract submitted

Session/Abstract ID: Lunch Sessions / 16665

Intra-molecular regulation of nucleotide binding to the Ski2- like RNA helicase Brr2

Presenting author:
Karine dos Santos

Author(s):
Karine dos Santos

no abstract submitted

Session/Abstract ID: Cancer & Inflammation / 16128

A bitter sweet symphony: how tumor glycans orchestrate immune evasion

Presenting author:
Sandra van Vliet

Author(s):
Sandra van Vliet

Tumor cells often display altered glycan structures on their cell surface. The presence of these tumor-associated glycans is correlated to metastasis and a lower disease-free survival. We have previously demonstrated that the C-type lectin MGL can distinguish between healthy and cancerous tissue through its specific recognition of Tn antigen (α GalNAc-Ser/Thr) on the tumor. Triggering of MGL on macrophages by Tn antigens evoked MGL-dependent signaling and enhanced secretion of the anti-inflammatory cytokine IL-10. Using high throughput tissue microarray screening we showed that late stage colorectal cancer (CRC) patients have a strongly reduced survival if their tumors harbor Tn antigens.

Furthermore, the presence of MGL ligands was correlated to activating mutations in BRAF, providing a link between oncogenic transformation and the immune system. To decipher the immunoregulatory role of tumor glycans in vivo, we developed a panel of mouse MC38 CRC cell lines using CRISPR-Cas9 genome editing. We knocked out the sialic acid activator CMAS to prevent sialylation and developed a COSMC knockout to increase Tn antigen exposure. Strikingly, both the Tn high and the Sialic acid low variants displayed enhanced tumor growth in vivo and an altered immune infiltration profile compared to Mock-transfected cells. Together, our results imply a direct effect of aberrant tumor glycosylation on anti-tumor immunity, indicating that tumor glycans may act as a powerful immune evasion strategy.

Session/Abstract ID: Subcellular organisation / 16351

Functional Architecture of the Mitochondrial Inner Membrane

Presenting author:

Martin van der Laan

Author(s):

Martin van der Laan

Mitochondria are characterized by a peculiar membrane architecture. The outer membrane mediates the communication with other cellular compartments. The highly folded inner membrane harbors the oxidative phosphorylation machinery for ATP synthesis. Specialized membrane domains, termed cristae, protrude as tubular or disc-shaped invaginations from the inner boundary membrane. The narrow junctions that connect cristae to the inner boundary membrane are stabilized by the mitochondrial contact site and cristae organizing system (MICOS). Mutations that inactivate MICOS lead to the detachment of cristae and their accumulation as lamellar membrane stacks in the mitochondrial matrix. Moreover, MICOS subunits interact with protein machineries of the outer mitochondrial membrane leading to the formation of membrane contact sites. Genetic interactions with the ER-mitochondria encounter structure suggest that MICOS is part of an extended ER-mitochondria organizing network controlling organellar crosstalk. We have identified two distinct membrane-shaping mechanisms mediated by different MICOS subcomplexes that are responsible for the generation of the strong membrane curvature at crista junctions. These intricate

membrane structures recruit assembly factors that control the step-wise assembly of respiratory chain complexes in space and time. Altogether, our data indicate that MICOS represents a central hub for the sorting of proteins between different mitochondrial membrane domains.

Session/Abstract ID: Lunch Sessions / 16532

Attachment of protein-targeting molecules to the surface of ultrasmall gold nanoparticles (2 nm) by click chemistry

Presenting author:

Selina van der Meer

Author(s):

Selina van der Meer, Matthias Epple

Ultrasmall nanoparticles with a diameter of 2 nm or smaller were prepared in a one-pot synthesis by reducing tetrachloroauric acid in the presence of functional ligands. These nanoparticles are autofluorescent and permit a label-free tracking in biological setups like cells.

The ultrasmall particles were functionalized with alkyne groups either in situ or after a reduction step with azido groups carrying ligands. By click chemistry, molecules with an azido or an alkyne function were covalently bound to the particles under mild reaction conditions.

These surface-functionalized ultrasmall particles permit a specific targeting of epitopes on the surface of a protein as they are smaller than most proteins. This is of special interest to influence the function or conformation of a protein, e.g. to inhibit its function. The protein-binding affinity is increased by the multiavidity (more than one ligand on each nanoparticle) or heteroavidity (different ligands on one nanoparticle) of nanoparticles. The heteroavidity can be established by clicking several molecules simultaneously to the particle surface.

We thank the Deutsche Forschungsgemeinschaft (DFG) for funding in the framework of SFB 1093: Supramolecular Chemistry on Proteins.

Session/Abstract ID: Other (free) topics / 16654 / OT-22

Functional analysis of zebrafish specific opsin kinases

Presenting author:

Nicole Ahrens

Author(s):

Nicole Ahrens, Karl-Wilhelm Koch

Effective phototransduction requires efficient feedback mechanisms to restore the dark-state after light stimulation. One feedback loop involves recoverin that inhibits G protein-coupled receptor kinases (GRKs) at high Ca^{2+} -concentration and thereby controls the phosphorylation of rhodopsin. Ca^{2+} -concentration decreases after the light stimulus, recoverin loses its Ca^{2+} -ions setting the GRK free to inactivate rhodopsin. A widely used model for cone vision research is the zebrafish (*Danio rerio*), which has a cone-dominant visual system and expresses two paralogs of GRK1 (zGRK1a & zGRK1b) and GRK7 (zGRK7a & zGRK7b). Our aim is to investigate the different isoforms of the GRK in zebrafish with regard to their functional properties. The zGRK isoforms were functionally expressed in COS-1 cells. For analyzing post-translational modifications of zGRKs, we added mevalonolactone to facilitate an isoprenylation. zGRKs expressed in the presence of mevalonolactone showed surprising differences in the activity in comparison to the absence of mevalonolactone. For zGRK1a an effect of mevalonolactone was only detectable in the soluble protein fraction by increasing the activity, whereas there was no effect between the presence and the absence of mevalonolactone for zGRK7b. Further experiments are designed to investigate the different isoprenylation profiles of the zGRKs as well as the inhibition of the zGRKs by the different recoverin isoforms in a Ca^{2+} -dependent manner.

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16121 / GE-02

Biochemical Characterization of a Novel Nuclease present in the Mitochondria and in the Nucleus of Human Cells

Presenting author:

Natalie Al-Furoukh

Author(s):

Natalie Al-Furoukh, Annika Pfeiffer, Vimal Parkash, Mara Doimo, Tore Samuelsson, Md. Obaidur Rahman, Steffi Goffart, Sjoerd Wanrooij

The genetic information of cells is stored in the nucleus (nDNA). Yet, a small fraction of genes is encoded in the mitochondria. Mitochondrial DNA (mtDNA) is a circular, double-stranded (ds) molecule. The core protein machinery for mtDNA replication is known and unique to the organelle. Still, the mtDNA replication and repair processes are not fully understood, because numerous DNA-linked enzymes are shared between the nucleus and the mitochondria and many of them remain to be characterized.

We aimed to identify such factors and performed IPOND (Identification of Proteins On Nascent DNA) to isolate proteins that are associated with mtDNA. In a pilot study, we found a novel nuclease.

First, we confirmed the subcellular localization of the new protein. We show that it localizes to both DNA containing compartments, the nucleus and the mitochondria. To pinpoint the molecular function in cello, we generated an inducible T-Rex cell line and performed siRNA experiments.

Second, we purified a recombinant form of this protein to study the catalytic activity of the nuclease in vitro. We show that the nuclease forms homo-dimers and releases negative supercoiling tension from circular dsDNA in an ATP-independent but metal-dependent manner. Furthermore, the nuclease is capable of decatenating circular dsDNA resulting in a linear product in vitro.

Finally, we show that this novel mitochondrial nuclease targets supercoiled and catenated mtDNA.

Session/Abstract ID: Cancer & Inflammation / 16617 /CA-26

Resistance, selectivity, distribution, and metabolism of tyrosine kinase inhibitors in cancer cells by Raman spectral imaging

Presenting author:
Karim Aljakouch

Author(s):
Karim Aljakouch, Tatjana Lechtonen, Hesham Yosef, Mohammed Hammoud, Ibrahim Daho, Wissam Alsaidi, Carsten Kötting, Carolin Mügge, Robert Kourist, Samir El-Mashtoly, Klaus Gerwert

Targeting epidermal growth factor receptor (EGFR) is one of the effective strategies to suppress tumors. First-generation tyrosine kinase inhibitors (TKIs) such as erlotinib disrupts downstream signaling cascade, responsible for tumor growth and progression. Clinical studies have also shown that erlotinib therapy induces T790M mutation in EGFR that mediates resistance to first-generation TKIs. To overcome this problem, second-generation TKIs such as neratinib are used. Here, we have monitored the effect of erlotinib and neratinib TKIs on lung cancer (NSCLC) cells with and without T790M mutation in EGFR by Raman spectral imaging. Interestingly, erlotinib or neratinib induced large spectral changes in cells without T790M mutation. On the other hand, only neratinib causes large spectral changes in cells with T790M mutation in EGFR. These results are in agreement with the clinical trial results in which, NSCLC patients with T790M mutation in EGFR have shown response to only neratinib. Furthermore, we have investigated the distribution, and metabolism of neratinib in cancer cells. The Raman results also indicated that the drug in cells is metabolized. Two neratinib metabolites were identified using a combination of Raman microscopy, DFT calculations, and liquid chromatography-mass spectrometry. Therefore, Raman spectral imaging has a great potential as an in vitro assay for new anti-cancer drugs evaluation.

Session/Abstract ID: Cell signalling and membrane trafficking / 16553 / CS-41

In silico modeling of signaling pathways with focus on the characterization of signal flows

Presenting author:
Leonie Amstein

Author(s):
Leonie Amstein, Jennifer Scheidel, Jörg Ackermann, Ina Koch

Signaling pathways process diverse stimuli to orchestrate the appropriate cellular response. Dysfunctions in signal transduction may result in pathologies. Biological knockout experiments are often applied to reveal the function of signaling pathways like essential proteins for certain processes. These systematic knockouts support to disentangle the regulation and to construct a potential working model of the signaling pathway. In silico modeling aims to capture the signaling processes at different levels of abstraction. The simulation and analysis of the model support to monitor system-wide processes. One important aspect is to determine all possible signal flows in the model.

We developed a Petri net model of the TNFR1-mediated NF- κ B signaling pathway. We adapted the standard analysis of transition invariants and defined Manatee invariants [1] for the analysis of possible signal flows. Based on the determination of signal flows in terms of Manatee invariants, we studied in silico knockouts [2]. We showed that Manatee invariants reveal correct pathway dependencies compared to in silico knockouts based on transition invariants. Manatee invariants also detect system-wide process dependencies in complex network models like feedback loops and crosstalk. The complement approach of Manatee invariants and in silico knockout is beneficial to elucidate the regulation of a signaling system.

[1] Amstein et al., BMC Syst Biol, 2017, in press

[2]Scheidel et al., PLoS Comp Biol, 2016

Session/Abstract ID: Cell signalling and membrane trafficking / 16277 / CS-16

Identification of RBR E3 ubiquitin ligases that can compensate Parkin deficiency

Presenting author:
Lena Angersbach

Author(s):
Lena Angersbach, Maria Patra, Konstanze Winklhofer, Jörg Tatzelt

Mutations in the gene encoding Parkin are a major cause of autosomal recessive Parkinson's Disease. Parkin is a RBR (RING-between-RING) E3 ubiquitin ligase which promotes neuronal survival and maintains mitochondrial integrity. In contrast to the severe phenotype observed in Parkin-deficient *Drosophila melanogaster*, Parkin knockout (KO) mice do not show obvious phenotypic alterations. However, an acute deletion of Parkin in adult mice has been shown to induce degeneration of dopaminergic neurons, suggesting that the loss of Parkin can be compensated in conventional Parkin KO mice. To identify pathways implicated in this compensation, we analyzed the expression profile of RBR E3 ligases in primary neurons from Parkin KO mice. Notably, 14 human RBR E3 ligases have been identified, sharing evolutionarily conserved sequences within their RBR domains. We found a consistent pattern of RBR E3 ligases differentially regulated in the absence of Parkin. Candidates showing a more than twofold upregulation were tested for their ability to rescue phenotypes induced by an acute silencing of Parkin in cellular models. Our study identified certain RBR E3 ligases that contribute to compensatory strategies in Parkin-deficient mice and may at least partly account for the fact that patients with pathogenic mutations in the Parkin gene develop symptoms only after some decades.

Session/Abstract ID: Other (free) topics / 16491 / OT-37

*Protein S-bacillithiolation functions in thiol-protection and redox regulation of the glyceraldehyde-3-phosphate dehydrogenase Gap in *Staphylococcus aureus* under hypochlorite stress*

Presenting author:
Haike Antelmann

Author(s):
Marcel Imber, Nguyen Thi Thu Huyen, Agnieszka Pietrzyk-Brzezinska, Vu Van Loi, Melanie Hillion, Jörg Bernhardt, Lena Thärichen, Katra Kolsek, Lorenz Adrian, Frauke Gräter, Markus Wahl, Haike Antelmann

Bacillithiol (BSH) maintains the thiol-redox balance of the human pathogen *Staphylococcus aureus*. We have previously shown that BSH functions in protein S-bacillithiolation under hypochlorite stress in Firmicutes. Here, we have used the redox proteomics approach OxICAT to quantify hypochlorite-sensitive protein thiols in *S. aureus* USA300 and analyzed the role of BSH in protein S-bacillithiolation. The OxICAT analyses enabled the quantification of 228 Cys residues in the redox proteome of *S. aureus* USA300. Hypochlorite stress resulted in a >10% increased oxidation of 58 Cys residues (25.4 %). Among the highly oxidized NaOCl-sensitive proteins are five S-bacillithiolated proteins including the glyceraldehyde-3-phosphate dehydrogenase Gap as most abundant S-bacillithiolated protein. The active site Cys151 of Gap was very sensitive to overoxidation and irreversible inactivation by H₂O₂ or NaOCl in vitro. Treatment with H₂O₂ or NaOCl in the presence of BSH resulted in reversible Gap inactivation due to S-bacillithiolation, which could be regenerated by the bacilliredoxin in vitro. Molecular docking was used to model the S-bacillithiolated Gap active site suggesting that formation of the BSH mixed disulfide does not require major structural changes. In conclusion, our results show that S-bacillithiolation of Gap efficiently functions in redox regulation and protection of the active site against irreversible overoxidation in *S. aureus* under NaOCl stress.

Session/Abstract ID: Cancer & Inflammation / 16561 /CA-19

Interaction of pro-apoptotic spin-labelled BIM BH3 peptides with solvents and Bcl-2 proteins

Presenting author:

Tufa Assafa

Author(s):

Tufa Assafa, Sukhendhu Nandi, Enrica Bordignon, Bastian Kohl, Raphael Stoll, Andrzej Rajca, Hui Zhang, Laura Galazzo

The BIM BH3 peptide has potential as a pro-apoptotic protein-mimicking small molecule drug that has a high affinity towards antiapoptotic proteins and therefore can kick-start apoptosis in uncontrollably replicating cancer cells. We have produced single and double cysteine mutants of a 26 and a 20 amino acid version of the peptide, and labelled it with MTSL by adding cysteine residues at the N and C termini. We characterized by DEER spectroscopy complemented by CD and NMR spectroscopy the structure of the peptides in different solvents and unveiled the associated conformational changes in Bcl-XL upon peptide binding in the presence and absence of membranes.

The BIM peptides were labelled with reduction-resistant nitroxide spin labels as well as with functionalized GdIII or CuII chelators to enable reactions with Bcl-XL proteins at the outer mitochondrial membrane of freshly isolated mitochondria or insertion of the peptide into cells. The metal-based labels can allow orthogonal spin-labelling of peptides and proteins to distinguish between different interactions. BIM peptide is shown to remain active with several combinations of labels, which offers an excellent tool for calibration of the orthogonal dipolar techniques for differentially spin labelled protein complexes. The BIM peptide is shown to remain active with several combinations of labels, which offers an excellent tool for calibration of the orthogonal dipolar techniques for differentially spin labelled protein complexes.

Session/Abstract ID: Age-related pathologies and autophagy / 16084 /AP-03

The role of PP2A in the complex intramolecular activation of LRRK2

Presenting author:

Panagiotis Athanasopoulos

Author(s):

Panagiotis Athanasopoulos, Franz Ho, Rolf Heumann, Arjan Kortholt

Mutations in the gene coding for the multidomain protein leucine-rich repeat kinase 2 (LRRK2) are the leading cause of genetically inherited Parkinson's disease (PD). Initially we found that the protein phosphatase 2A (PP2A) is interacting with LRRK2. In more detail, the Ras of complex protein (ROC) domain is the main docking site of LRRK2, responsible for interacting with the three subunits of PP2A in human neuroblastoma SHSY5Y cells and in HeLa cells. The alpha subunit of PP2A is interacting with LRRK2 in the perinuclear region of HeLa cells. Silencing the catalytic subunit of PP2A by shRNA, aggravated cellular degeneration induced by the pathogenic R1441C-LRRK2 mutant expressed in neuroblastoma SH-SY5Y cells. A similar enhancement of apoptotic nuclei was observed by downregulation of the catalytic subunit of PP2A in cultured cortical cells derived from neurons overexpressing the pathogenic mutant G2019S-LRRK2. Conversely, pharmacological activation of PP2A by sodium selenate showed a partial neuroprotection from R1441C-LRRK2-induced cellular degeneration. Finally preliminary data show that some autophosphorylation sites of the ROC domain of LRRK2 could regulate the GTPase domain of LRRK2. All these data suggest that PP2A is a new interacting partner of LRRK2 and reveal the importance of PP2A as a potential therapeutic target in PD.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16366 /SM-02

Dissection and Modulation of (patho)biological Survivin Functions by supramolecular Ligands

Presenting author:

Sandra Bäcker

Author(s):

Sandra Bäcker, Christian Heid, Cecilia Vallet, Stefanie Sichelschmidt, Christine Beuck, Peter Bayer, Thomas Schrader, Shirley K. Knauer

Survivin plays an important role in cancer. While it is mostly absent in normal resting adult tissues, it is up-regulated in almost all cancer types. Its overexpression is associated with a resistance of tumors against chemo- and radiotherapy, making Survivin an attractive target for novel therapeutic strategies.

As a member of the IAP family, Survivin plays a role in the inhibition of cell death, but as part of the CPC it is also crucially involved in mitotic regulation. For both biological functions, an interaction with the nuclear export receptor Crm1 mediated by Survivin's NES is pivotal. Thus, interference with this interaction should lead to a loss of Survivin's functions and therefore to an inhibition of cancer cell proliferation.

Our project aims to characterize novel supramolecular binders targeting Survivin's NES by quantitative assessment of its interaction with Crm1 and a thorough analysis of the resulting biological effects.

Binding of a non- and a peptide-linked molecular tweezer molecule to Survivin could be demonstrated by ITC analyses and precisely linked at specific basic amino acids within or near Survivin's NES by NMR titration experiments. Biochemical pulldown assays show that both tweezers interfere with the Survivin/Crm1 interaction at effective concentrations of 10-50 μ M. The establishment of cellular assays for Survivin/Crm1 co-localization and first evidence for cellular uptake of molecular tweezers enable further investigation of biological effects.

Session/Abstract ID: Other (free) topics / 16545 /OT-42

Synthesis, purification and characterization of membrane proteins using in situ-cleavable solubilizing tail

Presenting author:

Andreas Baumruck

Author(s):

Andreas Baumruck, Lena Steinacker, Alesia A. Tietze

Membrane proteins are key drug targets since they are involved in essential processes in the cell. The study of membrane proteins leads to new and improved pharmaceutical treatments for a wide range of illnesses (i.e. migraine, multiple sclerosis, cancer). Synthetic production of membrane proteins is an advantageous method primarily because synthetic peptides can be customized.

We present a robust method for the synthesis of highly hydrophobic peptides on the example of membrane proteins, which uses an in situ self-cleavable solubilizing tail for the native chemical ligation. Our strategy combines the ligation of two peptide fragments where the N-terminal fragment, which is thioester-forming peptide and carries the self-cleavable solubilizing tail, and the second, is the Cys-peptide fragment. To demonstrate the feasibility of our anticipated strategy we synthesized small model peptides and an extended part of the membrane region of the influenza B proton channel (BM2), which is a highly hydrophobic peptide and prototypical for the class of small membrane-spanning ion channels. BM2 represents an important drug target for the treatment of the seasonal flu and its molecular structure has not been fully solved yet. An optimized ligation condition led to a nearly quantitative yield of BM2 (1-51).

Session/Abstract ID: Subcellular organisation / 16159 /SC-02

Connection of mitochondrial protein and metabolite transport

Presenting author:

Thomas Becker

Author(s):

Lars Ellenrieder, Martin P. Dieterle, Kim N. Doan, Christoph U. Mårtensson, Nikolaus Pfanner and Thomas Becker

Mitochondrial biogenesis depends on the import of precursor proteins that are synthesized on cytosolic ribosomes. The translocase of the outer mitochondrial membrane (TOM complex) forms the entry gate for almost all precursor proteins. After passage of the TOM channel two protein translocases sort precursor proteins into the inner membrane and the mitochondrial matrix. The presequence translocase (TIM23 complex) transports proteins into the inner membrane and the mitochondrial matrix. The carrier translocase (TIM22 complex) inserts hydrophobic carrier proteins into the inner membrane. Mitochondrial function depends also on the exchange of metabolites with the cytosol. Whether protein and metabolite transport are coordinated remain unknown. Unexpectedly, we identified a close connection of protein transport to the voltage-dependent anion channel (VDAC), which forms the channel for metabolite transport in the outer membrane. VDAC associates with the TOM complex and promotes the transport of the carrier proteins to the TIM22 translocase, which inserts these preproteins into the inner membrane. Thus, we report for the first time that mitochondrial metabolite and protein transport are connected.

Session/Abstract ID: Emerging Analytical Methods / 16296 /AM-03

Structure and dynamics of the neuronal calcium-sensor Synaptotagmin-1

Presenting author:

Julian Bender

Author(s):

Julian Bender, Caroline Haupt, Matteo Degiacomi, Carla Schmidt

Synaptic vesicles are small storage organelles for neurotransmitters. They are essential for neurotransmission including neurotransmitter import, docking, priming and fusion with the presynaptic membrane, neurotransmitter release into the synaptic cleft as well as vesicles recycling.

These processes are supported by the dynamic and on-demand formation of non-covalent protein-lipid assemblies.

Neurotransmitter exocytosis depends on the formation of SNARE-complexes to tether synaptic vesicles to the presynaptic membrane. However, membrane fusion in response to intracellular Ca^{2+} signals is regulated by the interplay between Complexin-1 and calcium-sensor Synaptotagmin-1 (Syt-1). Binding of Ca^{2+} ions to the calcium binding domains of Syt-1 is thought to induce conformational changes eventually leading to displacement of Complexin-1 and bridging of the presynaptic and vesicular membranes. Thereby, Syt-1 interacts specifically with lipids in the presynaptic membrane.

Here, we combine chemical cross-linking, native mass spectrometry and molecular dynamics simulations to study the effect of calcium and the presence of various lipids onto the structure and dynamics of Syt-1. Our first cross-linking experiments suggest a high flexibility with multiple distinct conformations in the absence of calcium. Currently, we investigate conformational changes in the presence of calcium ions or various lipids. Our studies therefore provide detailed insights into the mechanism of membrane fusion.

Session/Abstract ID: Cell signalling and membrane trafficking / 16275 /CS-14

A mitophagy-independent neuroprotective activity of the mitochondrial kinase PINK1

Presenting author:

Lena Berlemann

Author(s):

Lena Berlemann, Dominik Sehr, Lena Angersbach, Jens Meschede, A. Kathrin Müller-Rischart, Jörg Tatzelt, Konstanze F. Winklhofer

Protein aggregation, mitochondrial dysfunction and oxidative stress are pathophysiological alterations consistently found in the course of Parkinson's disease (PD), however, the etiopathogenesis of PD still remains enigmatic. Thus, the identification of genes which cause familial variants was a major breakthrough in PD research. Importantly, several PD-linked gene products have a direct or indirect impact on mitochondrial integrity, emphasizing a crucial role of mitochondria in the disease process.

Mutations in the genes encoding the mitochondrial kinase PINK1 and the E3 ubiquitin ligase Parkin cause autosomal recessive PD. Pioneering work in *Drosophila melanogaster* revealed that PINK1 and Parkin loss-of-function phenotypes are similar and characterized by dysfunctional mitochondria. Further research showed that PINK1 acts upstream of Parkin in a mitochondrial quality control pathway to induce removal of damaged mitochondria in a process called mitophagy. Albeit the PINK1/Parkin-induced mitophagy pathway is well established and has recently been elucidated in great detail, mounting evidence indicated that PINK1 has additional functions for example in regulating complex I activity and maintaining neuronal viability. We and others observed that PINK1 protects against neuronal cell death in various stress paradigms. Here we show that the pro-survival activity of PINK1 is mediated by phosphorylation of ubiquitin but independent of mitophagy.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16611 /SM-07

NMR investigation of supramolecular ligand binding to proteins

Presenting author:

Christine Beuck

Author(s):

Christine Beuck, Andrea Sowislok, Christian Heid, Tatjana Ruks, Sandra Bäcker, Shirley Knauer, Matthias Eppe, Thomas Schrader, Peter Bayer

Nuclear Magnetic Resonance (NMR) spectroscopy is ideally suited to monitor ligand binding to proteins with single-residue resolution. However, binding of large supramolecular ligands to proteins and protein-protein complexes poses additional challenges due to the size and complexity of the systems.

We present examples how NMR methodology has been extended to study supramolecular tweezers and nanoparticle binding to proteins.

Side chain specific experiment for lysine and arginine residues directly observe the atoms encapsulated by the ligand and allow a precise ranking of multiple ligand binding sites on a given protein. TROSY spectra extend the size range suitable for NMR and allow to map ligand binding sites on larger or multimeric proteins. Diffusion and relaxation experiments are used to determine the size of peptide-fused nanoparticles and their complexes with a model protein.

Session/Abstract ID: Other (free) topics / 16559 /OT-29

FLUORESCENCE IMAGING OF THE INTERACTIONS BETWEEN ULTRA-SMALL GOLD NANOPARTICLES AND BACTERIA

Presenting author:

Nataniel Bialas

Author(s):

Nataniel Bialas, Tatjana Ruks, Viktoriya Sokolova, Matthias Eppe

The interactions between nanoparticles (NPs) and bacteria are important in biomedicine. Particle size is the crucial parameter which determines the cellular

uptake of NPs. Gold NPs may be used as biomolecule-carrying agents. Fluorescence microscopy is an excellent tool to investigate the nature of interactions between NPs and bacteria.

Our aim was to study particle-cell interactions and the possible uptake of gold NPs by bacteria. The difference in size between the NPs (about 2 nm) and bacteria (about 1-3 μm) is significant.

Ultra-small gold NPs were synthesized by reduction of gold ions (HAuCl_4) with NaBH_4 in the presence of glutathione or fluorescently labeled hexapeptides for red and green fluorescence, respectively. The NPs were purified and characterized. Bacterial strains (*Escherichia coli* DH5 α and *Staphylococcus xylosum* DSM 6179) were transformed with a pGEX6P1 vector encoding the green fluorescence protein (GFP). Red-fluorescent gold NPs (autofluorescence) were incubated with green-fluorescent strains, whereas green-fluorescent gold NPs were incubated with *Escherichia coli* TOP10 strain which, due to DsRed2 protein expression, was red-fluorescent. The interactions of NPs with bacteria were studied by fluorescence microscopy.

Gold NPs had spherical morphology and an average size of 2 nm. Transformation of bacterial strains was successful. The interactions between NPs and bacteria were shown by following their fluorescence.

Session/Abstract ID: Other (free) topics / 16104 /OT-05

A new mechanism for clathrin stabilization of microtubules via adaptor-like interactions on the mitotic spindle

Presenting author:
Alexander Bird

Author(s):
Alexander Bird, Arnaud Rondelet, Yu-Chih Lin, Shweta Bendre

The precise regulation of microtubule (MT) dynamics during mitosis is critical for accurate chromosome segregation and genome stability. MTs are frequently hyperstabilized during mitosis in cancer cells, leading to chromosome missegregation and chromosomal instability (CIN). Clathrin has recently emerged as an unexpected player controlling mitotic MT stability. Clathrin is recruited to the mitotic spindle where it

stabilizes MTs and facilitates chromosome alignment. The mechanisms by which clathrin stabilizes MTs, however, have remained elusive. Here we show that clathrin directly recruits the MT-stabilizing protein GTSE1 to the mitotic spindle, in an analogous manner to clathrin-adaptor protein interactions: via clathrin adaptor binding sites within clathrin heavy chain (CHC) and conserved adaptor-like motifs in GTSE1. This interaction is required for efficient chromosome alignment. GTSE1 facilitates chromosome alignment by directly inhibiting the MT depolymerase activity of the kinesin MCAK and thereby stabilizing MTs. Cells lacking GTSE1 or CHC have defects in MT stability caused by excess MCAK activity. We show that reducing the high GTSE1 levels common to CIN cancer cell lines reduces chromosome missegregation defects, while artificially inducing GTSE1 levels in chromosomally stable cells elevates chromosome missegregation and CIN. GTSE1 overexpression leading to hyperstabilization of MTs via MCAK inhibition thus defines a new potential mechanism driving CIN.

Session/Abstract ID: Other (free) topics / 16219 /OT-13

Fam83F is a novel regulator of p53

Presenting author:
Christine Blattner

Author(s):
Christine Blattner, Mohammed Salama, Diego Benitez Riquelme, Leonel Munoz

p53 is one of the most important tumor suppressor proteins yet despite intensive research on this protein for more than 35 years, its regulation is still incompletely understood. Clearly, its most important regulator is Mdm2 which ubiquitinates the protein and targets p53 for proteasomal degradation. However, other proteins can alter Mdm2 activity by binding to p53 or Mdm2 and reduce or increase p53 abundance and activity.

By performing a cell culture overexpression screen using a Medaka cDNA library, we have identified FAM83F as a novel regulator of p53. FAM83F stabilizes p53 in an Mdm2-dependent manner and leads to a reduction in the ubiquitination of p53. This increase in p53 abundance is also reflected by an increase in p53 activity. In response to DNA damage, FAM83F is induced and activates the p53-mediated DNA damage

response. Most interestingly, downregulation of Fam83F strongly enhanced carcinogenesis in a xenograft model indicating that Fam83F may also contribute to tumor suppression. To further investigate this hypothesis, we are currently analyzing Fam83F expression in human tumors versus healthy tissue.

Session/Abstract ID: Cancer & Inflammation / 16562 /CA-20

Membrane-embedded Bcl-2 proteins: Insights into topologies and interactions

Presenting author:
Stephanie Bleicken

Author(s):
Stephanie Bleicken, Tufa Assafa, Markus Teucher, Enrica Bordignon, Ana J. Garcia-Saez

The proteins of the Bcl-2 family can have pro- or anti-apoptotic functions. Together, they form a complex interaction network that controls mitochondrial permeabilization and apoptotic cell death. Many Bcl-2 proteins capture not only soluble and but also membrane-embedded conformations with the latter ones regulating the mitochondrial permeabilization. Thereby, most studies on the structure and function of Bcl-2 proteins address the soluble conformation, while the membrane-embedded one are only partially understood. Our studies investigate the structure, the conformational changes and the interactions of three crucial player in the network (cBid, Bax, and Bcl-xL) both in solution and in membranes by means of various spectroscopic techniques ranging from fluorescence correlation spectroscopy to electron paramagnetic resonance. We discovered that the possible or preferred protein-protein interactions as well as the protein conformations are strongly influenced by the presence of a membrane bilayer, and that the lipid composition plays an important role in the fine tuning of those interactions (1, 2). Our work add new insights into how mitochondrial permeabilization and apoptotic cell death is facilitated.

References:

1. Bleicken et al. (2014) Structural Model of Active Bax at the Membrane. *Molecular Cell* 56, 496-505
2. Bleicken et al. (2017) Quantitative interactome of a membrane Bcl-2 network identifies a hierarchy of

complexes for apoptosis regulation. *Nature Communications* 8, 73

Session/Abstract ID: Other (free) topics / 16477 /OT-20

Gating Mechanism of the Mitochondrial Voltage-Dependent Anion Channel

Presenting author:
Raphael Böhm

Author(s):
Raphael Böhm, Giuseppe Federico Amodeo, Sruthi Murlidaran, Ishan Ghai, Shashank Chavali, Gerhard Wagner, Grace Brannigan, Mathias Winterhalter, Sebastian Hiller

The voltage-dependent anion channel (VDAC) is the most abundant protein in the outer mitochondrial membrane, where it forms the primary path for diffusion of ATP, ADP, other metabolites and ions. The structure of VDAC-1 comprises a 19-stranded β -barrel and two short N-terminal α -helices that extend into the pore. VDAC features a voltage gating function, allowing it to switch between a high- and several low-conductance states. The low-conductance state, which can be triggered by β -NADH binding or by an elevated membrane potential, is characterized by a reduced nucleotide flux through the VDAC pore. Here, we clarify the two mechanisms underlying the transition of VDAC into low-conductance states by a multidisciplinary approach of solution NMR spectroscopy, single-channel electrophysiology and molecular dynamics simulations. The high-resolution structure of human VDAC-1 shows that β -NADH binding reduces the pore conductance sterically without a structural change. NMR spin relaxation experiments identify the N-terminal helices as more dynamic than the barrel, implying a role of these structural elements in voltage gating. Consequently, affixing helix α 2 to the barrel wall leads to loss of the native gating characteristics. Finally, single amino acid mutations in helix α 2 lead to single channel conductances identical to the closed state. Taken together, our data resolve the mechanisms of VDAC gating to low-conductance states after 40 years of extensive research in this direction.

Session/Abstract ID: RNA-Regulation und Transport / 16162 /RR-02

METTL16 is a N6-methyladenosine (m6A) methyltransferase that targets a conserved region of the U6 snRNA required for pre-mRNA splicing

Presenting author:

Markus T. Bohnsack

Author(s):

Ahmed Warda, Jens Kretschmer, Philipp Hackert, Claudia Höbartner, Katherine E. Sloan, Markus T. Bohnsack

N6-methyladenosine (m6A) is a highly dynamic RNA modification that has emerged as a key regulator of gene expression as it can be recognised by specific m6A binding proteins ("readers") and can be reversed by the action of the demethylases ("erasers") ALKBH5/FTO. A methyltransferase complex of METTL3/METTL14 has been identified as an m6A "writer" in human cells, however, detection of METTL3/METTL14 binding sites on cellular RNAs suggests that some known m6A modifications are introduced independently of this complex and additional human m6A methyltransferases remain to be identified. Based on its homology to the E. coli rRNA m6A methyltransferase YbiN, METTL16 represents a putative human m6A writer protein. Using crosslinking and analysis of cDNA (CRAC), we show that METTL16 predominantly binds to the U6 snRNA, which forms the catalytic core of the spliceosome. Identification of METTL16 interaction partners revealed an RNA-dependent interaction with La, implying that METTL16 associates with an oligouridylated form of U6 during early stages of assembly of the U6 snRNP. The modification target of METTL16 is A43 that lies within the conserved ACAGAGA box of U6. This sequence base-pairs with 5' splice-sites of pre-mRNAs suggesting that METTL16-mediated modification of this site may be important for splicing regulation. We further identify METTL16-associated mRNAs and show that the methyltransferase often binds to their introns, suggesting that the enzyme has additional substrates.

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16022 /GE-01

Cryo-EM structure of a metazoan separase-securin complex at near-atomic resolution

Presenting author:

Andreas Boland

Author(s):

Andreas Boland

Separase is a caspase-family protease that initiates chromatid segregation by cleaving the kleisin subunits (Scc1 and Rec8) of cohesin, and regulates centrosome duplication and mitotic spindle function through cleavage of kendrin and Slk19. To understand the mechanisms of securin regulation of separase, we used single-particle cryo-electron microscopy (cryo-EM) to determine a near-atomic-resolution structure of the *Caenorhabditis elegans* separase-securin complex. Separase adopts a triangular-shaped bilobal architecture comprising an N-terminal tetratricopeptide repeat (TPR)-like α -solenoid domain docked onto the conserved C-terminal protease domain. Securin engages separase in an extended antiparallel conformation, interacting with both lobes. It inhibits separase by interacting with the catalytic site through a pseudosubstrate mechanism, thus revealing that in the inhibited separase-securin complex, the catalytic site adopts a conformation compatible with substrate binding. Securin is protected from cleavage because an aliphatic side chain at the P1 position represses protease activity by disrupting the organization of catalytic site residues.

Session/Abstract ID: Cancer & Inflammation / 16113 /CA-02

Role and regulation of translationally controlled tumour protein TCTP in colorectal cancer

Presenting author:

Ulrich Bommer

Author(s):

Ulrich Bommer, Valentina Iadevaia, Christopher Proud, Prianka Puri, Alistair Lochhead, Lisa Belfiore, Kara Vine

Translationally controlled tumour protein (TCTP) is a cytoprotective protein involved in diverse basic biological and disease processes. TCTP is

overexpressed in many human tumours, and it is often associated with a poor patient outcome. We have studied the regulation of this protein in colorectal cancer (CRC) and its potential involvement in the resistance to anti-cancer drugs.

Using immunohistochemistry on about 70 human CRC samples, we show that TCTP levels are significantly higher in tumor tissue compared to normal colon, and that they increase early in the development of CRC. We show that growth-stimulation of cancer cells results in the induction of TCTP synthesis via translational regulation and signalling through the PI3K/Akt/mTORC1 pathway.

We asked whether TCTP overexpression may contribute to anti-cancer drug resistance in cancer cells. Treatment of HCT116 colon cancer cells with two anti-cancer drugs, 5-fluorouracil (5-FU) or oxaliplatin, resulted in a 4- to 5-fold increase of TCTP levels. This increase is prevented by mTOR kinase inhibitors, indicating that the mTORC1 pathway is involved in this regulation. We also performed TCTP knock-down on HCT116 cells and found this to sensitise these cells to treatment with 5-FU or oxaliplatin.

In summary, our results indicate that TCTP could potentially serve as a biomarker in early cancer growth of CRC and that it may play a key role in the development of anti-cancer drug resistance in the later stages of cancer.

Session/Abstract ID: Systems biology and autophagy signal control / 16358 / SB-03

Rapamycin-induced peroxisome degradation (R.I.P.): a novel degradation mode for peroxisomes.

Presenting author:
Fahd Boutouja

Author(s):
Fahd Boutouja, Christina Reidick, Harald W Platta

The turn-over of peroxisomes in *Saccharomyces cerevisiae* is known to be mediated via pexophagy, which is a selective autophagic degradation pathway. It is induced, when the cells are shifted from oleate-medium to glucose-containing pexophagy-medium. In contrast, macroautophagy, which has been regarded as a non-selective pathway, degrades cytosolic components and has been thought to be

exclusively induced by the addition of the TOR-inhibitor rapamycin. While the classical pexophagy depends on the pexophagy-receptor Atg36 and an intact actin-cytoskeleton, macroautophagy occurs independently from both factors.

In this study, we demonstrate, that rapamycin also induces a novel mode of peroxisome degradation that is distinct from classical pexophagy as well as macroautophagy with respect to the involvement of Atg36, the cytoskeleton and myosin-related motor protein Myo2.

Session/Abstract ID: Cell signalling and membrane trafficking / 16360 / CS-22

Involvement of vacuolar proteins in the biogenesis of peroxisomes.

Presenting author:
Fahd Boutouja

Author(s):
Fahd Boutouja, Christina Reidick, Ralf Erdmann, Christian Ungermann, Harald W Platta

The yeast vacuole is a central component for cellular homeostasis. Recent work from mammalian cells shows, that peroxisome-lysosome contacts are involved in cholesterol transport. Here we demonstrate a novel link between these organelles, because a combination of specific vacuolar proteases as well as the GTPase cycle of the small GTPase Ypt7 are essential for the import of PTS1-proteins into the matrix of peroxisomes. The mutation of these factors triggers the polyubiquitination of the PTS1-import receptor Pex5p and leads to a block of matrix protein import and peroxisomal biogenesis.

Session/Abstract ID: Pain / 16096 /PA-01

Glucose - a potentiator of neuronal glycine receptors and possible modulator of nociceptive signaling

Presenting author:

Hans-Georg Breitinger

Author(s):

Ulrike Breitinger, Rama Hussein, Marwa Abdelhalim, Heinrich Sticht, Hans-Georg Breitinger

The inhibitory glycine receptor is a ligand-gated ion channel in the mammalian spinal cord, brainstem and higher brain areas. In humans, nociceptive signaling is modulated by spinal alpha 3 glycine receptors. Inhibition of glycine receptors leads to disinhibition of pain signaling, resulting in intensified nociception and allodynia, as observed in strychnine poisoning and prostaglandin PGE2-mediated inflammatory signaling.

We identified glucose and related sugars as potentiators of neuronal glycine receptors. Currents mediated by alpha1 homomeric, spinal alpha1/beta, and also alpha 3 glycine receptors were all augmented, with the EC50 for glycine left- shifted 3 – 5 – fold. A high-affinity alpha 3 receptor variant, a3(P185L) was not further potentiated by glucose, indicating that there is an upper limit of receptor activity. Glucose-related saccharides, such as fructose, mannose, and the disaccharides sucrose and lactose also potentiated glycinergic signals. A possible binding site for glucose on the receptor was suggested from mutagenesis and molecular modeling data.

Our data suggest that some of the analgesic effects that are observed for glucose may be mediated through glycine receptors.

Session/Abstract ID: Systems biology and autophagy signal control / 16359 /SB-02

Ubiquitination/Deubiquitination-dynamics of the PTS1-receptor Pex5p regulate the matrix protein into peroxisomes.

Presenting author:

Rebecca Brinkmeier

Author(s):

Rebecca Brinkmeier, Fouzi El Magraoui, Thomas Mastalski, Wolfgang Girzalsky, Bettina Warscheid, Ralf Erdmann, Harald W. Platta

Peroxisomal matrix proteins are posttranslationally imported into peroxisomes in an already folded state. Most peroxisomal matrix proteins harbor the peroxisomal targeting signal-type 1 (PTS1). The corresponding PTS1-receptor is Pex5p. After Pex5p has bound the PTS1-cargo protein, it associates with the peroxisomal docking-complex and releases the cargo via a transient import pore into the lumen of the organelle. The PTS1-receptor is monoubiquitinated on the conserved cysteine 6 in *Saccharomyces cerevisiae*. The monoubiquitinated Pex5p is recognized by the peroxisomal export machinery and is retrotranslocated into the cytosol for further rounds of protein import. In this study, we have analyzed a Pex5p truncation lacking Cys6 [(Δ6)Pex5p], a construct with a ubiquitin-moiety genetically fused to the truncation [Ub-(Δ6)Pex5p], as well as a construct with a reduced susceptibility to deubiquitination [Ub(G75/76A)-(Δ6)Pex5p]. While the (Δ6)Pex5p-truncation fails to support matrix protein import, the Ub-(Δ6)Pex5p chimeric protein facilitates matrix protein import. In contrast, the Ub(G75/76A)-(Δ6)Pex5p chimera exhibits a complete import defect. The data show that ubiquitination and deubiquitination rates are tightly regulated and that balanced ubiquitination/deubiquitination dynamics are essential for peroxisomal biogenesis.

Session/Abstract ID: RNA-Regulation und Transport / 16653 /RR-04

A de-ubiquitylating enzyme as new stress granule component

Presenting author:

Meike Brömer

Author(s):

Richa Das, Meike Brömer

Posttranslational modification of proteins with mono- or poly-ubiquitin influences an uncounted number of cellular processes by affecting e.g. protein stability, localization, protein-protein interaction or enzymatic activity. Ubiquitin gets covalently attached to target proteins by the action of a three-step cascade (E1, E2, E3). Specialized de-ubiquitylating enzymes (DUBs) counteract this process.

The DUB OTUD4 was found mutated in patients with Gordon Holmes syndrome, suffering from hypogonadotropic hypogonadism, ataxia and dementia. To date, knowledge regarding its biological function is rather limited, especially in a neuronal context. To identify new interaction partners and potential substrates of OTUD4, we performed pull-downs from mouse brain lysate and subsequent analysis by mass spectrometry. Interestingly, a high number of identified proteins were connected to RNA binding, translation, or localise to ribonucleoprotein granules. We show that OTUD4 localises to stress granules during various stress conditions. Stress granules are membrane-less organelles that form transiently under stress conditions, contain mRNAs and proteins and represent sites of stalled translation. Related to OTUD4's recruitment into these RNA-containing granules we found that OTUD4 is able to bind RNA. Currently we are assessing whether catalytic and disease-related mutations affect stress granule recruitment or dynamics. Additional studies aim to elucidate whether OTUD4 has RNA-related functions in unstressed cells and if its de-ubiquitylating activity contributes to this.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16589 /SM-05

Finding bioactive drug-like compounds with genetically tagged screening libraries

Presenting author:

Andreas Brunschweiler

Author(s):

Andreas Brunschweiler

The identification of bioactive compounds is a crucial step to initiate drug development programs, and also to develop probes for chemical biology studies. Target-based screening of DNA-encoded small molecule libraries (DELs) has emerged as a validated technology to interrogate vast chemical space.[1] DELs consist of chimeric molecules composed of drug-like low-molecular weight compounds that are covalently conjugated to individual DNA strands serving as identifier barcodes. DNA tagging of molecules allows for pooling of large compound libraries, and screening of these libraries by selection to identify low-molecular weight structures binding to the target protein. Screening of DELs has identified numerous bioactive compounds. Some of these molecules were instrumental in gaining a deeper understanding of biological systems, and a few compounds have even been progressed to clinical trials.

DELs are synthesized through combinatorial strategies with alternating organic preparative synthesis and (enzymatic) DNA-encoding steps giving access to large numbers. We have synthesized a DNA-recorded 27.000-membered library based on "privileged" scaffolds, and a DNA-encoded library using a newly developed DNA tagging scheme allowing for a large scope of chemical reactions for screening library synthesis.[2,3] We are currently offering these libraries for screening to any interested collaboration partners.

Session/Abstract ID: Other (free) topics / 16534 /OT-40

Classification of PAPS-synthase mutants and their stability in the cellular environment

Presenting author:

Oliver Brylski

Author(s):

Oliver Brylski, Jonathan Wolf Mueller, Simon Ebbinghaus

PAPS-synthase (PAPSS) is a bifunctional enzyme consisting of two well separated domains, ATP sulfurylase and APS kinase, generating 3'-phosphoadenosine-5'-phosphosulfate (PAPS). PAPS is the sole activated sulfate source in cells for the modification of biomolecules by sulfotransferases, which is why mutations affecting PAPSS activity result in disease states like bone or cartilage malformation and metabolic diseases. In vitro experiments comparing both PAPSS isoforms, PAPSS1 and PAPSS2, revealed that PAPSS is in general stabilized by its ligands but strikingly PAPSS2 is only marginally stable at physiological temperatures in a ligand unbound state. Buffered in vitro experiments cannot account for the heterogeneity of the cellular environment including varying metabolite concentrations, chaperoning systems and the physicochemical properties of the crowded environment like excluded-volume effects or quinary interactions.

Using Fast Relaxation Imaging we investigate the stability of PAPSS2 APS kinase domain and its disease relevant as well as catalysis inhibiting mutations inside the cell. First in cell thermodynamic data allowed a classification of the point mutants into either native-, diseaselike or destabilized states providing further insight into the disease mechanism due to PAPSS deficiency.

Session/Abstract ID: Cell signalling and membrane trafficking / 16158 /CS-08

The soluble adenylyl cyclase as a novel pivotal player in ISO/ICI-induced cardiac hypertrophy

Presenting author:

Heidi Budde

Author(s):

Heidi Budde, Nicole Klein, Ilona Schirmer, Diana Cimiotti, Philip Steinwascher, Andreas Mügge, Yury Ladilov, Kornelia Jaquet

Background: Soluble adenylyl cyclase (sAC) synthesizes cAMP in specific cell-compartments due to the localization in cytosol, nuclei and mitochondria and is also expressed in cardiomyocytes. sAC is involved in growth and proliferation acting via the B-Raf/Erk pathway and CREB. However, its physiological function in the heart is unclear.

Purpose: CREB and Erk pathways are involved in hypertrophic growth of cardiac tissue. Therefore, we investigated whether sAC participates in hypertrophy and acts via CREB or Erk.

Methods: In isolated adult rat cardiomyocytes hypertrophy was induced by 24h-treatment with Isoprenaline (ISO) in the presence of β 2-adrenoceptor antagonist (ICI). In cardiomyocytes sAC activity was inhibited either with the sAC specific inhibitor KH7 or by knockdown. Hypertrophic growth parameters i.e. cell size, RNA/DNA- and protein/DNA-ratio were examined. To identify the signaling pathway, expression levels of phosphorylated CREB, B-Raf, C-Raf and Erk were analyzed by Western Blot.

Results: All hypertrophic growth parameters were significantly elevated after ISO/ICI induced hypertrophy and clearly suppressed after inhibition of sAC. The results suggest the involvement of B-Raf, but not of CREB and Erk in sAC induced hypertrophy.

Conclusion: Our data suggest sAC as a novel pivotal player in ISO/ICI induced cardiac hypertrophy, though the pathway by which sAC promotes the hypertrophic response is still unclear.

Session/Abstract ID: Cell signalling and membrane trafficking / 16368 /CS-17

Intracellular signaling and dynamics of Alzheimer's disease relevant nuclear spheres

Presenting author:

Hassan Bukhari

Author(s):

Hassan Bukhari, Katharina Kolbe, Verian Bader, Konstanze F. Winklhofer, Thomas Günther-Pomorski, Thorsten Müller

The APP c-terminus corresponds to a docking site for a plenty of different proteins like FE65, which is capable of translocating to the nucleus generating so-called nuclear spheres. Over time, these structure fuse and grow to large spheres. These putatively toxic structures also include the histone acetyltransferase TIP60 and the DNA helicase BLM. Previously, we demonstrated the existence of nuclear spheres in the human brain using human frontal cortex brain samples from AD patients and age-matched control. Cell culture experiments demonstrated that APP phosphorylation at Thr668, which is known to play a crucial role in APP-FE65 binding, disrupts nuclear spheres generation and is significantly enhanced with a better membrane tethering of APP cleavage fragments like for C99 and AICD57. Now by using PALM imaging technique, we show that nuclear spheres are generated as tiny speckles at the nuclear envelope which later fuse in the nucleoplasm to form giant spheres. Expression of different canonical spheres interactors in the cells yields different phenotype which already points to the highly dynamic regulation of spheres generation. Taken together, our results show that Alzheimer's disease (AD) relevant nuclear spheres, which are highly dynamics structure, are enriched at the nuclear envelope and later fuse to generate larger spheres. Further experiments are needed to provide insights into the structure, dynamics and cellular localization of AD-relevant nuclear spheres.

Session/Abstract ID: Subcellular organisation / 16496 /SC-08

Hypercyclical patterns in the neuron cell: integration of information processing at cellular scale

Presenting author:

Victor Cavaller

Author(s):

Victor Cavaller

This article studies the connectivity patterns within the neuron resulting from both signalling and metabolic functions. It includes a detailed review and process analysis of the loops, feedbacks and feed-forward pathways that recurrent biophysical changes and biochemical reactions follow at different levels of sequencing in the internal organization of the neuron cell.

As baseline for a scale size analysis – from the micro to the manometer scale- of the neuronal integration of information, this paper considers different levels of specification, mainly corresponding to a progressive descent into the detail of the mechanisms that enable each phase of the signal transmission, such as the life cycle of neurotransmitters, the neurometabolic coupling' (NMC), etc.

From this multidimensional approach, the analysis unveils the tight hypercyclical coupling system of processes that lies behind the neuronal transmission of information and that is internally linked to a scale-organized collection of reactions and events. Based on this evidence, this article concludes that hypercyclicity explains not only the principle of natural self-organization of the cell in general (according to Eigen and Schuster, 1977-8), involving the neuro-metabolic or genetic mechanisms and related molecular pathways, but singularly, the process of neural signalling, coding and transmission.

Session/Abstract ID: Other (free) topics / 16586 /OT-46

The polymorphism V158M in myosin binding protein C affects its binding to cardiac troponin containing cardiac troponin I variants inducing infantile restrictive cardiomyopathy.

Presenting author:
Diana Cimiotti

Author(s):
Lisa Morgenstern, Natalia Smolina, Anna Kostareva, Hendrik Milting, Andreas Mügge, Kornelia Jaquet, Diana Cimiotti

Cardiac myosin binding protein C (MyBP-C) is a sarcomeric protein linking thick and thin filaments. Its N-terminal domain interacts with actin and modulates calcium sensitivity, thus probably acting in concert with cardiac troponin (cTn) in regulating muscle contraction. Here we show for the first time that the recombinant N-terminal MyBP-C fragment C0C2 also interacts with cTn in pull down assays. A $KD=336.8$ nM was determined by microscale thermophoresis.

The C0C2/cTn interaction was enhanced when the cTn contained variants of its inhibitory subunit (cTnI), namely cTnI-D127Y or -R170G, while KD was preserved for cTnI-R170W. These amino acid replacements result from missense mutations in the TNNI3 gene found in infants suffering from severe restrictive cardiomyopathy, who died within 1 year after diagnosis. Cosedimentation revealed a normal thin filament formation with cTnI-D127Y/R170G, but not with R170W.

Furthermore, we investigated the effects of a probably benign polymorphism (V158M) in the MyBP-C C0C2 fragment on C0C2/cTn interaction. The V158M replacement significantly strengthened binding of C0C2 to wildtype cTn ($KD=204.6$ nM). cTn containing cTnI-R170W interacted less strongly with C0C2-V158M than wildtype cTn. The polymorphism exhibited larger effects on the interaction with cTn than the TNNI3 mutation indicating that the MyBP-C/cTn interaction might not be as decisive for the development of restrictive cardiomyopathy as the interaction among thin filament proteins.

Session/Abstract ID: Cancer & Inflammation / 16439 /CA-11

The essential role of TAp73 in bortezomib-induced apoptosis in p53- deficient colorectal cancer cells

Presenting author:
Yasamin Dabiri

Author(s):
Yasamin Dabiri, Stefan Wölfl, Xinlai Cheng

Mutations in the tumor suppressor p53 are among the most highly occurring events in colorectal cancer (CRC). Such mutations have been shown to influence the sensitivity of cancer cells to chemotherapeutic agents. However their impact on the efficacy of the proteasomal inhibitor bortezomib remains controversial. We thus re-evaluated the toxicity of bortezomib in the CRC cell lines HCT116 wt (wild-type) and its p53^{-/-} clone. Transient resistance to bortezomib treatment was observed in p53-null cells that was later accompanied by an increase in levels and nuclear translocation of TAp73, an isoform of the p53-homologue p73, as well as induction of apoptosis. Knockdown of p73 in p53^{-/-} cells using CRISPR/Cas9 significantly prolonged the duration of resistance. Moreover, similar results were observed in HT-29 cells carrying mutated p53, but not human fibroblasts with expression of functional p53. Thus, our results clearly demonstrated that TAp73 served as a substitute for p53 in bortezomib-induced apoptosis in p53- deficient or mutated cells, implicating that TAp73 could be a potential therapeutic target for treatment of CRCs, in particular those lacking functional p53.

Session/Abstract ID: Other (free) topics / 15975 /OT-01

Follow-up of Healing of skin wounds by topical application of platelet-rich plasma in sheep.

Presenting author:
Badis Daikh

Author(s):
badis daikh

The use of surgical adjuvants such as platelet-rich plasma is the object of research in regenerative medicine. The purpose of this study was to evaluate the healing activity of PRP by its topical application to

experimentally injured skin in sheep. Ten adult 6-month-old sheep were used in this study. After sterile preparation of the skin, 06 wounds in full thickness (20 × 20 mm) were created on the back of each animal. Cutaneous biopsies were performed and interpreted by an experienced histopathologist. The morphometric data obtained were subjected to a statistical analysis (ANOVA: Tukey HSD test, P <0.05). The Chi-square test was used to analyze histopathological findings. In conclusion, it appears that the use of PRP promotes epithelialization and accelerates the process of wound healing in sheep during the first 14 days of healing.

Session/Abstract ID: Age-related pathologies and autophagy / 16590 /AP-13

Disruption of protein quality control has opposite effects on age-dependent protein aggregation in different tissues

Presenting author:
Della David

Author(s):
Della David, Marie Lechler, Raimund Jung

The formation of solid protein aggregates was thought to occur only in a disease context or exposure to extreme stress. This assumption was challenged by the finding that aging leads to the aggregation of several hundred proteins in *C. elegans* [David et al. PLoS Biol, 2010]. These results were confirmed by a number of studies and in different organisms. Significantly, age-dependent protein aggregation is likely to be a crucial player promoting aggregation in neurodegenerative diseases [Lechler et al. Cell Rep and Groh et al. Front Aging Neurosci, 2017]. Thus it is importance that we understand the cellular mechanisms controlling widespread protein aggregation with age.

We investigated the role of the main protein-quality-control (PQC) systems, namely the chaperone, proteasome and autophagy systems in two different tissues in *C. elegans*. As expected, PQC disruption caused increased age-dependent protein aggregation in body-wall muscles. Surprisingly however, disruption of these systems had a beneficial effect on protein aggregation in the pharyngeal muscles. To gain more insight, we analysed the aggregation kinetics of age-related protein aggregation. First we demonstrated that aggregation happens less than 24 hours after protein translation. Second, we show that PQC

disruption acts early-on in the aggregation process to prevent specifically the accumulation of newly synthesized aggregation-prone proteins. We are currently evaluating tissue-specific compensatory mechanisms.

Session/Abstract ID: Age-related pathologies and autophagy / 16564 /AP-11

Identification of autophagy-modulating natural products and derivatives

Presenting author:
Jana Deitersen

Author(s):
Jana Deitersen, Fabian Stuhldreier, David Schlütermann, Philip Böhler, Niklas Berleth, Nora Wallot-Hieke, Wenxian Wu, Björn Stork

Autophagy represents an intracellular degradation process that mediates the elimination of long-lived proteins and organelles. It is especially activated under stress conditions such as nutrient deprivation, growth factor withdrawal, hypoxia, or pathogen infection. Cancer cells can also activate autophagy in order to avoid cell death by nutrient and oxygen deprivation within the tumor environment. In addition, anticancer therapies, such as hormonal deprivation, radio- and chemotherapy, frequently induce autophagy as a pro-survival response that contributes to treatment resistance. Consequently, drugs that inhibit autophagy are applied in clinical trials in combination with several anticancer drugs to increase their cytotoxic potential. In contrast, it has also been postulated that autophagy is essential for the efficacy of certain anticancer therapies and that excessive autophagy might contribute to cell death of cancer cells. In these cases, the induction of autophagy might help to overcome resistance of cancer cells.

During one of our current PhD projects we have identified a series of novel natural products and their derivatives which specifically modulate autophagic responses both positively and negatively.

Session/Abstract ID: Age-related pathologies and autophagy / 16525 /AP-08

Function of SUMO in response to proteotoxic stress

Presenting author:

Fabian den Brave

Author(s):

Fabian den Brave, Stefan Jentsch

Posttranslational modification by the ubiquitin-related protein SUMO targets hundreds of substrates and is involved in a wide range of cellular processes. SUMOylation is widely implicated in the cellular response to stress, including hypoxic, osmotic or genotoxic stress. In particular, SUMOylation of a large number of substrates is strongly induced upon proteotoxic stress caused by acute heat shock, oxidative stress or proteasome inhibition. The substrates targeted under these conditions have been extensively mapped by mass-spectrometry based approaches. However, the functional significance of SUMOylation in response to stress remains largely unknown.

We chose to study the role of SUMO in response to acute heat shock, which is known to induce rapid and strong SUMOylation of diverse proteins. SUMO usually fosters protein-protein interactions via so-called SUMO-interacting-motifs (SIMs), thereby recruiting other factors to the modified protein. We can now demonstrate that the clearance of SUMO-conjugates after acute stress requires SIM-dependent interaction of SUMO with components of the proteins quality control machinery. We identified several of these factors and are studying how these proteins affect the fate of the SUMOylated proteins. Finally, this allows us to understand how SUMOylation of proteins enables the cell to deal with acute proteostasis impairment.

Session/Abstract ID: Other (free) topics / 16537 /OT-38

FUdR enables flexible adjustment of neuron/glia ratio in culture

Presenting author:

Irmgard D. Dietzel

Author(s):

Lars Klupal, Justus Wilke, Heiko Leßlich, Irmgard D. Dietzel

To study the role of glial cells on neuronal protein expression and function, cultures of defined neuron to glia ratio are desirable. Neuronal cultures are frequently treated with the cytostatic cytosine arabinoside (AraC) to inhibit overgrowth by glial cells. Due to its high neurotoxicity, the concentration of AraC is limited to 5 - 10 μ M for a few days (Wallace and Johnson, J. Neurosci. 9, 115-124, 1989), leading to a neuron to glia ratio of maximally 1:1. In the search for alternatives we incubated dissociated hippocampal cultures from p1-3 postnatal rats with 5-fluoro-2'-deoxyuridine (FUdR) (Oorschot, Exp. Brain Res 78: 132-138, 1989). Using ascending concentrations from 30 - 150 μ M in a defined medium composed of neurobasal medium (NB) supplemented with GS21 (both obtained from Sigma) this treatment resulted in an up to 8-fold decrease in astrocyte number and a 4-fold increase in neuron number compared with cultures incubated with 4 μ M AraC. Additionally, we observed an approximately 8-fold depletion of microglia. By changing the concentration of added FUdR, we are now able to flexibly adjust our neuron/glia ratio in culture to up to 8:1 in order to further elaborate effects of astrocytes and microglia (see e.g. Niederkinkhaus et al., Mol. Endocrinol 23:1494, 2009; Igelhorst et al., Phil. Trans. R. Soc. B 2015: 370, Klupal et al., Front. Neurol. doi: 10.3389/fneur.2016.00044, 2016) on the regulation of neuronal ion channel expression.

Session/Abstract ID: Other (free) topics / 16426 /OT-19

Atoh8 in reprogramming and maintenance of pluripotency

Presenting author:

Satya Srirama Karthik Divvela

Author(s):

Satya Srirama Karthik Divvela, Yamini Balachandran, Ajeesh Balakrishnan-Renuka, Markus Napirei, Holm Zaehres, Beate Brand-Saberi

Atoh8, an atonal bHLH transcription factor, have been implicated in multiple developmental events. We have previously reported its regulatory role in skeletal myogenesis, whereas recently we have identified its expression in the inner cell mass of the blastocyst. However, the role of Atoh8 in the context of pluripotency and early differentiation remains to be resolved. In this study, we plan to decipher the role of ATOH8 in the context of reprogramming, pluripotency and early differentiation. As the first step, we decided to study the role of Atoh8 in cellular reprogramming and maintenance of pluripotency by comparing wildtype and Atoh8 knockouts. The Atoh8 knockout fibroblasts cultured on feeders completely failed to reprogram, whereas the Atoh8 knockout fibroblasts cultured in feeder-free condition (Matrigel) were able to reprogram, but with very low efficiency. Corresponding to the above results, qRT-PCR performed at different time points representing different phases of reprogramming revealed significant alterations in the mRNA levels of genes involved in mesenchymal epithelial transition (MET) and maintenance of pluripotency. Furthermore, in our preliminary studies, we have also observed that the iPSCs derived from Atoh8 knockouts were undergoing differentiation earlier than wildtype suggesting the essential role of Atoh8 in maintaining the pluripotent state. Altogether, our data point towards a crucial role of Atoh8 in reprogramming and maintenance of pluripotency.

Session/Abstract ID: Cell signalling and membrane trafficking / 16217 /CS-11

Romol is a mitochondrial non-selective cation channel with viroporin-like characteristics

Presenting author:

Young Do Yoo

Author(s):

Young Do Yoo, Deok-gyun You, Hye-Ra Lee, Gi Young Lee

Reactive oxygen species modulator 1 (Romo1) is a nuclear-encoded small transmembrane protein located in mitochondrial inner membrane. It is known to induce mitochondrial reactive oxygen species (ROS) production in response to various cellular stresses. For a decade, Romo1 has been studied in the context of mitochondrial ROS production, cancer cell invasion, inflammation, replicative senescence, and mitochondrial dynamics. Although Romo1 is thought to be involved in mitochondrial superoxide production and functions as an essential redox sensor in mitochondrial dynamics, biophysical mechanisms that can explain Romo1 activity have not been proposed. Here, we report that Romo1 is a unique mitochondrial ion channel that differs from the currently identified eukaryotic ion channels. We found that Romo1 is a highly conserved protein with structural features of Class II viroporins, the virus-encoded non-selective cation channels. Indeed, Romo1 forms a non-selective cation channel with its amphipathic helical transmembrane domain necessary for pore-forming activity. Notably, channel activity was specifically inhibited by Fe²⁺ ions, an essential transition metal ion in ROS metabolism. Using structural bioinformatics, we designed an experimental data-guided structural model of Romo1 with a rational hexameric structure. We propose that Romo1 establishes a new category of viroporin-like non-selective cation channel in eukaryotes.

Session/Abstract ID: Other (free) topics / 16593 /OT-48

Red Color-Tuned variants of Channelrhodopsin-2 investigated by Time Resolved FTIR

Presenting author:
Max-Aymelt Dreier

Author(s):
Max-Aymelt Dreier, Jens Kuhne, Kirstin Eisenhauer, Stefan Tennigkeit, Klaus Gerwert

Generation of a red-shifted variant Channelrhodopsin-2 with a narrow action spectrum is a goal for optogenetics as blue light is more heavily scattered by the tissue and a narrow spectrum allows for two retinal proteins to be activated independently. For a rational design the detailed understanding of the functional mechanism of Channelrhodopsin-2 [1, 2] is a prerequisite. Structural comparison of Channelrhodopsin-2 with more redshifted microbial rhodopsins shows differences in the retinal binding pocket. Using this information we introduced several point mutants into the protein. Here we present a study on Channelrhodopsin-2 variants that exhibit a red-shifted absorption maximum as well as a high quantum efficiency and faster photocycle kinetics compared with the wildtype. Time resolved FTIR as well as UV/VIS spectroscopy were used for detailed studies.

[1]Eisenhauer, K., Kuhne, J., Ritter, E., Berndt, A., Wolf, S., Freier, E., Bartl, Franz Hegemann, Peter & Gerwert, K. (2012), JBC 287(9), 6904-6911

[2]Kuhne, J., Eisenhauer, K., Ritter, E., Hegemann, P., Gerwert, K., & Bartl, F. (2015), Angew. Chem. Int. Ed. 54(16), 4953-4957

Session/Abstract ID: Cancer & Inflammation / 16582/CA-23

Effect of different extraction techniques on anti-inflammatory activity of propolis

Presenting author:
Burak Durmaz

Author(s):
BURAK DURMAZ, BURAK DURMAZ

Propolis is a honeybee product and it has been used for its health benefits, which are strongly related to its phenolic content. The aim of this study was to investigate the effect of different extraction techniques on the phenolic content and the anti-inflammatory / antioxidant effects of solid propolis samples.

Antioxidant activity was determined by utilization of FRAP, TEAC and DPPH methods. Anti-inflammatory activity was evaluated through measuring the percent inhibition of hyaluronidase and xanthine oxidase activity by propolis extracts. The phenolic molecules were determined with LC MS/MS.

PEG-extracted propolis samples which have highest levels of Epicatechin (1.54 ng/mL), Vanilic acid (830 ng/mL), Quercetin (306 ng/mL) and Ellagic acid (348ng/mL), showed higher antioxidant activity compared to other propolis samples. Hyaluronidase activity was inhibited by propolis samples. Ultrasonic treated samples also returned results of significantly higher phenolic compounds mainly; caffeic acid, caempherol, ferulic acid, rutin, paracoumaric acid, ellagic acid, naringenin, pelargonidine, trans cinnamic acid in propolis samples compared to ethanol and PEG extracted propolis samples.

In conclusion, our data showed that the anti-inflammatory and antioxidant effect of propolis samples were closely correlated with its phenolic content which are released by extraction techniques. Ultrasonic treatment seems as an efficient and simple method to yield more phenolic compounds.

Session/Abstract ID: Cancer & Inflammation / 16343 /CA-08

Deciphering targets and function of microRNAs that play a role in aggressive B cell lymphomas

Presenting author:

Sonja Eberth

Author(s):

Natalie Freytag, Claudia Pommerenke, Yvonne Merkhoffer, Jéssica Arribas Arranz, Dieter Kube, Hans Drexler, Hilmar Quentmeier, Sonja Eberth

Malignant non-Hodgkin lymphomas (NHL) comprise a heterogeneous group of B and T cell lymphomas, the majority of which are of B cell origin (B-NHL). The microRNA cluster MIR23A~27A~24-2 (MIR23A cluster) encodes miR-23a, miR-27a and miR-24. It has been suggested that these microRNAs may act as oncomiRs in B-NHL because overexpression of miR-23a is correlated with negative outcome in B-NHL patients. However, the lymphoma-specific targets and functions of these microRNAs remain unknown. Accordingly, we have determined the direct and indirect targetomes of miR-23a and miR-27a by AGO2-RNA immunoprecipitation followed by RNA-Seq in a human B-NHL model cell line: comprising 26 direct target genes for miR-23a and 20 for miR-27a. GSEA and GO-term analyses of direct and indirect targets indicated that the MIR23A cluster might regulate processes in apoptosis. Moreover, BBC3 which encodes the pro-apoptotic protein PUMA was one of the identified direct targets of miR-27a. After etoposide induced apoptosis, miR-27a overexpressing B-NHL cells failed to induce PUMA at the protein level. Importantly, functional analyses confirmed that miR-23a overexpression reduces, and high levels of miR-27a significantly attenuate the ability of B-NHL cells to undergo apoptosis in response to DNA damage. Thus, high levels of miR-23a and miR-27a antagonize induction of apoptosis in the B-NHL model cell line, supporting an oncogenic role of the MIR23A cluster in aggressive B cell lymphomas.

Session/Abstract ID: Other (free) topics / 16142 /OT-08

Proteomic Characterization of Murine Muscle Fiber Types via Laser microdissection and LC MS/MS Mass Spectrometry

Presenting author:

Britta Eggers

Author(s):

Britta Eggers, Katalin Barkovits, Rolf Schröder, Christoph Clemen, Katrin Marcus

Skeletal muscles are composed of different fiber types known as type I and type II fibers. All can be distinguished via specific myosin isoforms. The fiber type composition of various muscles is differing and can change due to aging or training. These shifts can also be observed in neuromuscular diseases. Subclass specific analysis of fibers enables a more accurate analysis of aging, gender or disease related effects.

For the differentiation of fiber types and their isolation from muscle tissue a sophisticated method was established combining fiber type specific immunostaining, laser microdissection and mass spectrometry. Soleus muscle was stained with specific antibodies, fibers were dissected, lysed, digested and analysed via LC-MS/MS. We used the workflow in order to elucidate an influence of gender and age on the proteome level of wildtype mice.

First results led to the identification of over 1000 proteins in type I fibers of male and female mice. In total 24 proteins could be identified as interesting candidates for being differently expressed. E.g. proteins associated with cell stress could be identified as higher abundant in female mice. Studies with larger cohorts are still ongoing to strengthen the preliminary findings.

Further on we aim at the identification of differences in the protein pattern of a knock-in mouse strain with the most common mutation causing desminopathy in order to increase our knowledge about disease underlying molecular pathomechanisms.

Session/Abstract ID: Cell signalling and membrane trafficking / 16316 /CS-18

The F-BAR protein NOSTRIN regulates diastolic and systolic function of the heart

Presenting author:

Alexander Ehrke

Author(s):

Alexander Ehrke, Igor Kovacevic, Miriam Müller, Baktybek Kojonazarov, Tanja Hindemith, Ralph Theo Schermuly, Ingrid Fleming, Meike Hoffmeister, Stefanie Oess

Endothelial dysfunction is an early event in cardiovascular disease and characterized by reduced production of nitric oxide (NO). The F-BAR protein NOSTRIN interacts with the endothelial nitric oxide synthase (eNOS) and modulates its subcellular localization. We analyzed the consequences of deleting the NOSTRIN gene for NO production and cardiovascular function in both an endothelial specific and a global KO mouse model.

The levels of NO and cGMP were significantly reduced in NOSTRIN KO mice and cardiac dysfunction was identified. Furthermore, systemic blood pressure was increased and acetylcholine-induced relaxation of isolated aortic rings as well as resistance arteries was impaired. We identified that the muscarinic acetylcholine receptor M3R interacted directly with NOSTRIN and the latter was necessary for correct localization of the M3R at the plasma membrane in murine aorta.

Endothelial specific NOSTRIN KO mice show diastolic dysfunction of the heart whereas global NOSTRIN KO mice exhibit additionally systolic dysfunction. Alterations in number or size of cardiac myocytes, vascularization of these, as well as fibrosis or apoptosis within the tissue could be ruled out as possible causes of systolic dysfunction. Currently we focus on the role of NOSTRIN in the subcellular organization of cardiomyocytes. NOSTRIN expression peaks during t-tubular biogenesis but a possible role of NOSTRIN plays in this process still remains to be elucidated.

Session/Abstract ID: Cell signalling and membrane trafficking / 16110 /CS-03

Switching GPCR Signaling On and Off with Light: Melanopsin Variants for Transient versus Sustained Activation

Presenting author:

Dennis Eickelbeck

Author(s):

Dennis Eickelbeck

Our lab compared the biophysical properties of the orthologous melanopsins hOpn4L and mOpn4L (human/mouse melanopsin, long isoforms) and characterized their optogenetic applicability to control the Gi/o and the Gq/11 signaling pathway in vitro and in vivo. It could be shown that both opsins are not only able to activate and deactivate the Gi/o but also the Gq/11 signaling pathway with the requested high temporal and spatial precision using very low intensities of light. It could be confirmed that short light pulses are sufficient to induce sustained GPCR pathway activation via mOpn4L, which can be specifically deactivated with a second light pulse, whereas light stimulation of hOpn4L leads to temporary activation.

We could reveal that it is possible to control Gi/o dependent activation and deactivation of GIRK currents (via G protein-coupled inwardly rectifying potassium channels) and Gq/11 dependent increase in intracellular Ca²⁺ concentrations in HEK 293 cells. In addition, we could show that Gq/11 pathway activation leads to influx of cations and is sufficient to induce sustained/transient action potential firing in cerebellar Purkinje cells (cerebellar slices and in vivo) using mOpn4L/hOpn4L, which can be precisely switched on and off by light.

Thus, hOpn4L and mOpn4L now provide the opportunity to study and control differently shaped GPCR signals with high spatial and temporal precision in vitro and in vivo.

See: Current Biology 2016 DOI: 10.1016/j.cub.2016.03.007

Session/Abstract ID: Cancer & Inflammation / 16494 / CA-13

1,25(OH)2D3 REDUCES TXNIP EXPRESSION IN BREAST CANCER CELLS THROUGH A CROSS-TALK BETWEEN METABOLIC REWIRING AND PROTEIN DEGRADATION

Presenting author:

Mohamed Abu el Maaty

Author(s):

Mohamed Abu el Maaty, Yasamin Dabiri, Biljana Blagojevic, Fadi Almouhanna, Stefan Wölfel

1,25-dihydroxyvitamin D₃ (1,25(OH)2D₃), the hormonally active form of vitamin D, is now viewed as a promising chemotherapeutic. In this study, we investigated the influence of 1,25(OH)2D₃ on glucose metabolizing pathways in the breast cancer cell lines MCF-7 and MDA-MB-231. We performed real-time measurements of glycolytic/respiratory rates using a biosensor chip system, comprehensive mRNA expression analyses of metabolism-related genes and finally, assessment of intracellular energy charge. While 1,25(OH)2D₃ was found to modulate glucose metabolism in both cell lines, clear activation of AMPK signaling and reduction in TXNIP expression in response to treatment was only observed in MCF-7 cells. Detailed analysis of TXNIP regulation by 1,25(OH)2D₃ involved genetic/pharmacological modulation of CAMKK2/AMPK signaling, investigating the expression/activity of G6PD, and inhibition of proteasomal degradation as well as de novo protein synthesis. We observed rescuing of TXNIP expression in response to 1,25(OH)2D₃ in cells with knocked-down AMPK α levels, as well as delayed rescuing of expression by MG-132 and cycloheximide in 1,25(OH)2D₃-treated cells. Interestingly, while G6PD expression/activity were found to be significantly induced by 1,25(OH)2D₃, co-treatment with a G6PD inhibitor did not rescue TXNIP levels. Altogether, TXNIP regulation by 1,25(OH)2D₃ appears to be the result of cross talk between metabolic rewiring and protein degradation mechanisms.

Session/Abstract ID: Cancer & Inflammation / 16497 / CA-14

EXPRESSION OF TXNIP IN CANCER CELLS AND REGULATION BY 1,25(OH)2D3: IS IT REALLY THE VITAMIN D3 UPREGULATED PROTEIN?

Presenting author:

Mohamed Abu el Maaty

Author(s):

Mohamed Abu el Maaty, Fadi Almouhanna, Stefan Wölfel

Thioredoxin-interacting protein (TXNIP) was originally identified in HL-60 cells as the vitamin D₃ upregulated protein 1, and is now known to influence diverse cellular processes such as glucose homeostasis, redox balance and apoptosis. Besides the initial characterization, little is known about if and how 1,25-dihydroxyvitamin D₃ [1,25(OH)2D₃] induces TXNIP expression in other cancers. We previously observed non-canonical regulation of TXNIP by 1,25(OH)2D₃ in prostate cancer cells, which prompted us to evaluate the ability of 1,25(OH)2D₃ to induce TXNIP expression. Screening of multiple cancer cell lines of different tissue origins demonstrated induction, repression or no change in TXNIP expression in response to 1,25(OH)2D₃. In-depth analyses on HL-60 cells revealed a transient increase in TXNIP mRNA levels by 1,25(OH)2D₃, followed by a clear reduction at later time points. Furthermore, a strong induction in protein levels was observed only after 96h of treatment. Induction of TXNIP expression by 1,25(OH)2D₃ was found to be dependent on glucose availability as well as on the presence of a functional glucose transport system, highlighting the dependence of 1,25(OH)2D₃'s action on glucose-sensing mechanisms. Moreover, inhibition of de novo protein synthesis by cycloheximide reduced TXNIP half-life in 24h, but not in 96h 1,25(OH)2D₃-treated cells, demonstrating the possible influence of 1,25(OH)2D₃ on TXNIP stability rather than gene expression in long-term treatment.

Session/Abstract ID: Other (free) topics / 16433 /OT-26

Ca²⁺-dependent feedback mechanism of recoverin isoforms and G-protein-coupled receptor kinases in zebrafish phototransduction

Presenting author:

Dana Elbers

Author(s):

Dana Elbers, Karl-Wilhelm Koch

Vision is one of the most important sensory system of humans. Its primary steps are present in photoreceptor cells, the rods and cones. Humans are mainly using their cone vision, which is less sensitive but faster than rod vision. However the underlying molecular mechanism are less understood in cones than in rods. To study the cone phototransduction and therefore cone specific features of the photoresponse we use zebrafish (*Danio rerio*) as model organism. In zebrafish, a special feature is the expression of two paralogs of G-protein coupled receptor kinase 1 (GRK1a & 1b) and GRK7 (7a & 7b) and of four recoverin (zRec1a, zRec2a, zRec1b, zRec2b) isoforms. The mammalian GRK1 is under Ca²⁺-dependent control of recoverin and thereby plays a key role among different Ca²⁺-dependent feedback mechanisms. A similar operation mode of zebrafish GRKs can be hypothesized, but is not proven so far. Our aim is to investigate protein-protein interaction of recoverin and GRK isoforms by Surface Plasmon Resonance Spectroscopy as well as the specific Ca²⁺-sensing properties of the different recoverin isoforms by a ⁴⁵Ca²⁺-binding assay and fluorescence spectroscopy. The Ca²⁺-sensitivities of recoverin forms indicate a differential mode of target binding and regulation. Ongoing experiments are designed to identify which pair of recoverin and GRK is operating in which photoreceptor cell and whether different Ca²⁺-binding modes reflect a step-by-step activation/inhibition of the target GRKs.

Session/Abstract ID: Age-related pathologies and autophagy / 16377 /AP-04

Sarcopenia in Iraqi Subjects: A biochemical approach for diagnosis

Presenting author:

Hedef El-Yassin

Author(s):

Hedef El-Yassin, Walaa Jasim, Nazar Abdulatif

Older age is usually accompanied by functional decline due to loss of skeletal muscle mass and quality. Sarcopenia and muscle frailty are both highly relevant entities. However, no laboratory guidelines have yet been established for confirmatory tests of the diagnosis.

The aim of the present work is to identify specific biological markers that can quantify and serve in the qualitative assessment of the physical function impairment, to support the clinical diagnosis of sarcopenia.

The study included (100) sarcopenic subjects and (50) non sarcopenic subjects.

Methods:

1-clinical diagnostic measurements:

2- Biological markers (in serum): interleukin (IL)-6, C-reactive protein (CRP), and alpha1-antichymotrypsin (ACT), Procollagen type III N-terminal peptide (P3NP), C-terminal agrin fragment (CAF).

Results

Mean values of (ASM, LBM and α 1ACA) in control group > study group. Also there mean values were decrease with aging. While (P3NP, IL-6, hs-CRP, CAF and BMI) mean values in study group > control group.

Conclusions

Mean values of (ASM, LBM and α 1ACA) in control group were more than study group and in women less than men because sarcopenia defined as a reduction in ASM/height², and total lean body. Alpha1-antichymotrypsin has a direct relation with ASM, LBM. While (P3NP, IL-6, hs-CRP, CAF and BMI) have indirect relation with ASM, LBM and α 1ACA. So clinical variables values were increased:

1. with age,
 2. in study group more than control group
 3. in women more than men.
-

Session/Abstract ID: Other (free) topics / 16093 /OT-04

CARDIAC ACTIN MUTANTS CAUSING HYPERTROPHIC AND DILATED CARDIOMYOPATHIES

Presenting author:

Constanze Erdmann

Author(s):

Constanze Erdmann, Sebastian Schmitt, Matthias Geyer, Hans Georg Mannherz

Inherited cardiomyopathies are diseases of the cardiac sarcomere and caused by point mutations in genes encoding for contractile proteins. The most frequent forms are hypertrophic and dilated cardiomyopathy (HCM and DCM). One of the affected proteins causing these diseases is α -cardiac actin. We investigated the role of the α -cardiac actin mutations A295S, R312K and E361G which are correlated to HCM and DCM.

We isolated cardiomyocytes from neonatal rats and infected them with adenoviruses containing HA-tagged cardiac actin (wildtype and mutants). Subsequently we verified the incorporation of the transfected cardiac actins into microfilamentous or sarcomeric structures by immunostaining using confocal microscopy. In particular, we investigated the effects of these mutated cardiac actins on the integrity of the sarcomeric structures of the transfected cardiomyocytes.

One further aim is to analyze possible alterations in the biochemical properties of the mutated cardiac actins. Therefore, we expressed untagged cardiac actins in Sf21 insect cells by the baculovirus expression system. After affinity purification of the expressed cardiac actins using the C-terminal half of gelsolin (G4-6), we investigated possible changes in their polymerization behavior and activity like inhibition of DNase I and the stimulation of the myosin-subfragment 1 ATPase activity. In addition, we analyzed the formation of proper F-actin filaments of the wildtype and mutant actins by electron microscopy.

Session/Abstract ID: Cell signalling and membrane trafficking / 16066 /CS-02

Crosstalk of lipids and protein folding stress: Lipid fingerprints of a stressed ER

Presenting author:

Robert Ernst

Author(s):

Robert Ernst, Kristina Pesek, Kristina Halbleib, Roberto Covino, Harald Hofbauer, Dorith Wunnicke, Inga Hänelt, Gerhard Hummer

The unfolded protein response (UPR) is a conserved homeostatic program that is activated by unfolded proteins in the lumen of the endoplasmic reticulum (ER). Recently, it became evident that aberrant lipid compositions of the ER membrane, referred to as lipid bilayer stress, are equally potent in activating the UPR. The underlying molecular mechanism, however, remained unclear. We show that the most conserved transducer of ER stress, Ire1, uses an amphipathic helix (AH) to sense and respond to membrane aberrancies. In vivo and in vitro experiments, together with molecular dynamics (MD) simulations, identify the physicochemical properties of the membrane environment that control Ire1 oligomerization. Using mass spectrometry-based lipidomics, we follow the changes of the cellular lipidome in context of ER-stress in order to identify the lipid fingerprint of a stressed ER. This work establishes the molecular mechanism of UPR activation by lipid bilayer stress.

Session/Abstract ID: Cell signalling and membrane trafficking / 16127 /CS-07

Protein-membrane interactions as modulators in hormone signalling and protein aggregation

Presenting author:

Manuel Etzkorn

Author(s):

Manuel Etzkorn

While membrane proteins act as central interface in most signalling cascades, their surrounding lipid environment may modulate signalling by altering receptor and/or ligand structure as well as their accessibility. By means of NMR and fluorescence based approaches we investigated the role of lipid bilayers as potential modulators of two distinct

processes, i.e. hormone signalling and protein aggregation.

Using the melanocortin system consisting of the neuropeptide α -MSH, the hormone ACTH and the melanocortin-4 receptor, a class A GPCR, which is activated by both peptides, we identify a strong interaction of both peptides with lipid bilayers and determine their lipid dependent membrane binding modes. We demonstrate that membrane association can be weakened, leading to a required peptide release, by addition of Calcium ions at concentrations regularly found in neuronal Calcium gradients. We correlate our in vitro insights with cellular assays that reflect an ion dependence in MC4-receptor activation.

For the protein, α -synuclein (α S) which is linked to Parkinson's disease through its abnormal aggregation we structurally and kinetically characterize α S interaction with defined lipid bilayers in a quantitative and site-resolved way. We probe the role of membrane charge, plasticity and available surface area in modulating α S membrane binding modes and directly link these findings to their consequences for the nucleation and elongation process of α S amyloid fibril formation.

Session/Abstract ID: Pain / 16482 /PA-03

Inhibition of glycine transporter 1 as a novel approach for the treatment of chronic pain conditions.

Presenting author:
Volker Eulenburg

Author(s):
Anja Armbruster, Elena Naumann, Henning Hermanns, Robert Werdehausen, Volker Eulenburg

The perception of pain allows the appropriate reaction of organisms to potentially noxious stimuli. Chronic pain conditions caused by inflammation, systemic diseases or injuries can result in long-lasting neuronal maladaptation within the pain processing circuitries that contribute to spontaneous or facilitated pain responses. The treatment of chronic pain is difficult and the therapeutic results are in many cases not satisfactory. Thus, novel treatment approaches are urgently required. It has been postulated that altered spinal glycinergic neurotransmission contributes to the development and/or persistence of chronic pain. Glycine transporter 1 (GlyT1) plays an important role

in regulating extracellular glycine concentrations and might thereby constitute a useful target to facilitate glycinergic inhibition in pain signaling. Here, we show that the reversible GlyT1 inhibitor Bitopertin has beneficial effects in two different animal models of chronic pain. Here, Bitopertin ameliorated hyperalgesia and allodynia in the chronic constriction model of neuropathic pain and the carrageenan model of acute inflammatory pain, without affecting acute pain. During long term application, this beneficial effect was, maintained for more than 4 weeks. Neither single nor longterm application had effects on motor coordination and/or activity. Taken together, these findings suggest that modulation of the GlyT1 may be a useful therapeutic approach for the treatment of pathological pain states

Session/Abstract ID: RNA-Regulation und Transport / 16279 /RR-03

Mpp6 incorporation in the nuclear exosome contributes to RNA channeling through the Mtr4 helicase

Presenting author:
Sebastian Falk

Author(s):
Sebastian Falk, Fabien Bonneau, Judith Ebert, Alexander Kögel, Elena Conti

The main cellular machinery responsible for degrading RNAs in the 3'-to-5' direction is the RNA exosome complex. The exosome is a multisubunit macromolecular machine that associates with a distinct set of cofactors in the cytoplasm and the nucleus.

In the nucleus the exosome mediates the processing and decay of a large variety of transcripts, including defective pre-mRNAs and non-coding RNAs such as rRNAs, sn(o)RNAs and tRNAs. The exosome is formed by a 10-subunit core complex (Exo-10) that is present in both the nuclear and cytoplasmic compartments. In the nucleus, Exo-10 associates with the RNase Rrp6 and its interacting partner Rrp47, with the helicase Mtr4 and the small protein Mpp6. Biochemical and structural data have shed many insights on how the core complex and some of the cofactors function. The least understood cofactor is Mpp6.

Mpp6 is a 20 kDa protein predicted to be intrinsically disordered. How Mpp6 binds Exo-10 and how it

impacts on the activities of the nuclear complex is currently unclear. We have mapped the domain of Mpp6 that binds to the exosome core and determined the crystal structure of this complex at 3.2 Å resolution. The structure shows how the conserved central domain of Mpp6 binds onto conserved surface of Rrp40, which is supported by biochemical assays with structure-based mutations. Using RNase protection assays, we show that Mpp6 is required to effectively channel RNA through the Mtr4 helicase into the exosome core.

Session/Abstract ID: Cell signalling and membrane trafficking / 16518 /CS-35

A novel affinity-based method for the isolation of exosomes.

Presenting author:
Ivan Fedorov

Author(s):
Ivan Fedorov, Anna Tsimokha

Exosomes are cell-derived vesicles that mediate the exchange of cellular components between cells, and thereby play an important role in various physiological and disease processes. These features make exosomes potential diagnostic markers and targets for therapies. To obtain precise and complete data about protein composition of exosomes it is important to remove contaminating particles of the same size during exosome extraction. Existing methods based on ultracentrifugation mostly fail to address this problem.

One way to address this problem is to use purification methods based on exosome marker expression. To this end, we have added an auto-biotinylation sequence to CD63, a membrane exosome marker. However, because N- and C-termini of CD63 protein are hidden inside the vesicles, we have added an extra trans-membrane domain between CD63 protein and auto-biotinylation domain. We anticipated that the latter would be presented at the surface of exosomes, allowing their affinity purification. A TEV protease cleavage site was added between the CD63 and auto-biotinylation parts of the chimeric protein to allow the elution of exosomes in mild conditions. To validate the developed strategy, we have infected the HeLa cells with a retroviral construct expressing the chimeric protein. Exosomes isolated by our affinity purification method were significantly more enriched and uniform

in size than those isolated using conventional method based on ultracentrifugation.

Session/Abstract ID: Other (free) topics / 16265 /OT-15

Features of the two isoforms of the soluble receptor sTACI

Presenting author:
Miriam Fichtner

Author(s):
Miriam Fichtner, Heike Rübsamen, Michaela Smolle, Reinhard Hohlfeld, Franziska S. Thaler, Edgar Meinel

Background and Objective: B-cells contribute to the immunopathogenesis of Multiple Sclerosis (MS); their survival is regulated by BAFF and APRIL. One of their receptors is TACI. While B-cell depleting Abs are beneficial, surprisingly ataccept, consisting of the extracellular part of TACI unexpectedly worsened MS. Our lab has recently identified endogenous soluble TACI (sTACI) and found that it shares essential features with the pharmacological ataccept. In humans, TACI exists in two isoforms; the long form has two cysteine rich domains, the short form only one. The aim of this project is to elucidate properties of the two isoforms of TACI.

Results and Methodology: We produced both isoforms of sTACI recombinantly in HEK cells. Static light scatter analysis showed spontaneous dimerization of sTACI-long independent of a ligand, while sTACI-short appeared largely as a monomer. BAFF showed a similar affinity for both isoforms of TACI in a binding ELISA, while APRIL bound stronger to sTACI-long than to sTACI-short. In an NFκB reporter assay, similar results were obtained. We found that both TACI-long and TACI-short were expressed on the membrane and spontaneously shed from transiently transfected Hek293T cells. Both membrane-bound isoforms of TACI bound BAFF and APRIL.

Conclusion: We observed differences in binding affinity and decoy function between both soluble isoforms of TACI. These differences could be relevant for B-cell biology and drugs targeting the BAFF/APRIL-system.

Session/Abstract ID: Cancer & Inflammation / 16283 /CA-06

Synthesis and Biological Evaluation of Cyclic Chemerin-9 Analogs

Presenting author:

Tobias Fischer

Author(s):

Tobias Fischer, Tristan Zellmann, Annette Beck-Sickinge

Chemerin is an immunomodulating protein secreted mainly by adipose tissue and skin. It is released in its inactive form, namely the 143 residue prochemerin. Processing by different proteases removes the C-terminal residues of prochemerin and leads to various active isoforms. Multiple studies have shown that active chemerin induces migration in leukocytes and seems to have pro- as well as anti-inflammatory properties. These functions are mediated by the G-protein coupled receptor chemokine-like receptor 1 (CMKLR1), which is therefore an attractive drug target.

In 2004, Wittamer et al. discovered that a small nonapeptide derived from the C-terminus of chemerin, dubbed chemerin-9, is able to activate the CMKLR1 with nanomolar potency similar to the full-length protein. Previous work in our group showed that this peptide binds to its receptor in a hairpin-like conformation. Based on these results, we have synthesized cyclic chemerin-9 analogs that have the same potency and increased stability compared to the native peptide. Running molecular dynamics (MD) simulations of these novel cyclic peptides in combination with signal transduction studies helped to gain a more detailed insight into the conformational requirements necessary for receptor activation, which might be beneficial for the development of even more potent analogs.

Session/Abstract ID: Electron transport chains / 16133 /ET-01

Re-modelling plant mitochondrial respiration during drought

Presenting author:

Philippe Fuchs

Author(s):

Philippe Fuchs, Stephan Wagner, Thomas Nietzel, Marlene Elsässer, Markus Schwarzländer

Drought can severely limit growth and productivity of plants. To save water, plant leaves can close their stomata, which comes at the price of impaired gas exchange. Continuing photosynthesis can then deplete the available carbon-dioxide in the leaf and risk over-reduction of the photosynthetic and metabolic redox systems with detrimental effects for the cell. In that situation, mitochondrial electron transport can counteract over-reduction by acting as a cellular dump site for excess electrons. Mechanistically, this is thought to be mediated by flexible uncoupling of electron flux to oxygen from the phosphorylation of ADP. Yet, our understanding of the significance, regulation and integration of different uncoupling strategies in plant mitochondria is limited. To investigate how (un-)coupling impacts in drought acclimation, we have been manipulating and engineering mitochondrial uncoupling capacity. We have started mapping subcellular energy dynamics in response to drought and (un-)coupling using fluorescent protein sensors. We will discuss our insights into the flexibility of subcellular energy regulation in plant cells along with recent methodological innovations.

Session/Abstract ID: Other (free) topics / 16583 /OT-45

Time course analysis of lung proteome modulations in an ovalbumin mouse model of asthma bronchiale

Presenting author:

Lukas Funke

Author(s):

Lukas Funke, Maike Ahrens, Martin Eisenacher, Marcus Peters, Barbara Sitek, Stefanie Gnipp, Thilo Bracht

Asthma bronchiale is a chronic inflammatory disease of the respiratory airways, known as one of the most widespread diseases worldwide and considered a major factor of increasing health care costs in industrialized countries. Factors leading to asthma development require further investigation, especially on proteome level. This study aims to monitor dynamic proteome modulations during asthma development and discover novel actors in this process. An asthma mimicking phenotype, verified by e.g. immune cell counting, was established in an ovalbumin mouse model to monitor the lung proteome at four timepoints during a 12-week period. Three types of sample preparations were applied and a multifraction, mass spectrometry-based, label-free proteome analysis performed. Out of 3325 identified proteins, 435 were significantly differential abundant between the four groups (ANOVA p-Value ≤ 0.05 , Fold Change ≥ 1.5 , quantified with minimum 2 unique peptides). Nine clusters were formed using agglomerative hierarchical clustering and clusters were linked through further analyses to asthma associated pathophysiological events. Proteins previously not known to be related to asthmatic disease, like the Mpv17-protein and Charged multivesicular body protein 4b, were discovered. This study provides new insights into asthma development and associated modulations of the lung proteome.

Session/Abstract ID: Cell signalling and membrane trafficking / 16120 /CS-05

Investigating nanoparticle-cell interactions with scanning ion conductance microscopy

Presenting author:

Astrid Gesper

Author(s):

Astrid Gesper, Philipp Hagemann, Patrick Happel

Nanoparticles (NPs) are promising carriers for targeting drugs to specific cell types and thus possess a high potential to be used in medical applications. However, successfully developing NP-based drug carriers requires knowledge about NP-cell interactions and uptake mechanisms, which depend on the physico-chemical properties of the NP. Endocytotic uptake into vesicles would result in no direct access of the drug to the cytoplasm; however, it is also conceivable that, depending on size and surface functionalization, NPs can be taken up via transporters, channels or by diffusing through the membrane.

Since the interaction of NPs with the cell membrane and their active uptake, in contrast to diffusion through the membrane, require the formation of pits or protrusions, we investigated whether a change in roughness of the plasma membrane can be observed in HeLa cells after exposure to NPs.

Here, we show the development of an improved scanning ion conductance microscope using low-cost hardware, that allows short scanning times and lateral resolutions in the range of 100 nm. We found that carboxylate-modified latex-particles are taken up by HeLa-cells and that after 30 min of exposure the membrane roughness was significantly increased in a sub-set of cells compared to control cells. After 120 min, the increased roughness was not observed.

We thus conclude that NPs are taken up by HeLa-cells within two hours, while their interaction already is observable after 30 minutes of exposure.

Session/Abstract ID: Other (free) topics / 16475 /OT-36

Pitfalls of using total protein content for normalization of membrane proteins

Presenting author:

Astrid Gesper

Author(s):

Astrid Gesper, Heiko Leßlich, Birte Igelhorst, Denis Thatenhorst, Patrick Happel, Irmgard Dietzel

Protein expression in tissue is frequently normalized to the total protein content of the sample. Here we investigated how changes in the surface-to-volume ratio of cells can result in misinterpretations of the density of membrane proteins.

We studied the effect of basic fibroblast growth factor (FGF-2) on the density of Na⁺/K⁺-ATPases in the plasma membranes of cultured rat astrocytes using a ³[H]-ouabain binding assay and determined the protein content of the samples with a Lowry-based assay. Treatment with 6 nM FGF-2 for 4 days resulted in a reduced ³[H]-ouabain-binding by to about 35% of the control value after normalization to the total protein content. In addition, we quantified changes of membrane surface size and cell volume with a scanning ion conductance microscope (SICM) (Happel et al., J. Microsc. 212, 144-51, 2003) in response to treatment with 6 nM FGF-2. SICM-recordings showed a reduced cell surface as well as a decreased surface-to-volume ratio compared with untreated cells. The decline in ³[H]-ouabain binding sites was reduced by about 50% to only approximately 60% when normalized to the changes in the membrane surface. This suggests that changes in membrane proteins when normalized to the total protein content may reflect both, changes in the protein density in the membrane as well as changes in the ratio of the cell membrane to the cytoplasm volume (due to changes in the number of cells or their shapes) contained in the tissue under investigation.

Session/Abstract ID: Cell signalling and membrane trafficking / 16356 /CS-19

Molecular mechanism determining the interaction of K-Ras and Galectin-8

Presenting author:

Klaudia Giehl

Author(s):

Christopher Meinohl, Sarah-Jane Barnard, Johannes Klinke, Stefanie Wirth, Jens Bier, Marisa Heipel, Klaudia Giehl

The spatial organization of Ras isoforms in specific membrane microdomains is highly important for accurate and isoform-specific signal transduction, but the underlying mechanisms are poorly understood. Members of the Galectin family, which are β-galactoside-binding proteins comprising one or two carbohydrate recognition domains (CRDs) and a farnesyl-binding pocket, have been shown to regulate Ras nanoclustering and Ras-induced signal transduction. Our group identified Galectin-8 (Gal-8) as an interacting partner of K-Ras4B. Downregulation of Gal-8 protein expression by siRNAs activates Akt and ERK signaling and decelerates migration of Panc-1 pancreatic carcinoma cells. Gal-8 consists of two CRDs linked by a hinge region. The interaction interface of K-Ras4B and Gal-8 is not characterized, thus by co-immunoprecipitation and in vitro-interaction studies we demonstrate that the interaction is mediated via the N-terminal CRD regardless of the hinge region. Furthermore, Gal-8 and the N-CRD interact only with farnesylated K-Ras4B. Comparative binding studies with different Ras and Rho proteins as well as Ras mutants revealed that the lysine residues in the membrane anchor domain of K-Ras4B are crucial for the interaction with Gal-8. Since our in vitro studies show that Gal-8 can also bind other monomeric GTPases with a K-Ras4B comparable C-terminus, we suppose that Gal-8 might act as a GTPase-scaffold protein by interacting with a subset of farnesylated small GTPases.

Session/Abstract ID: Age-related pathologies and autophagy / 16108 /AP-01

Characterization of LRRK2

Presenting author:

Bernd Gilsbach

Author(s):

Bernd Gilsbach, Johannes Gloeckner, Arjan Kortholt, Giambattista Guaitoli, Katharina Rosenbusch, Franz Ho

Human leucine-rich-repeat kinase 2 (LRRK2) belongs to the Roco family of proteins and has been found to be thus far the most frequent cause of late-onset and idiopathic Parkinson's disease (PD). LRRK2 is a 2527 aa multi-domain protein that consists of three protein-protein interaction domains ARM, ANK, LRR and WD40 and two catalytic domains a GTPase and a kinase. Most of the PD mutations cluster around the catalytic core. To understand LRRK2 function and the effect of PD mutation we used an integrative approach to elucidate the LRRK2 structure. We combined negative stain EM, x-ray structures from orthologous protein, SAXS and XL-MS to obtain a LRRK2 model. Furthermore we analyzed GTPase and kinase activity in detail to understand the intra molecular regulation of LRRK2. The structural and biochemical analyses revealed that LRRK2 belongs to class of G-proteins activated by dimerization (GADs) and that COR is the dimerization domain of LRRK2. A deeper knowledge of the LRRK2 regulation will help to find new ways to target LRRK2 induced PD.

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16324 /GE-03

Recombinant production and mutagenesis of the 23-subunit dynactin complex

Presenting author:

Mathias Girbig

Author(s):

Mathias Girbig, Andrew Carter

The 23-subunit dynactin complex is an essential co-activator of cytoplasmic dynein-1 that transports numerous cargos along microtubules. Dynactin binding in the presence of cargo adaptors activates dynein into a highly processive motor. A key role in dynein binding and activation has been assigned to

the largest subunit, p150Glued, which binds both microtubules and the dynein intermediate chain. However, the precise mechanism of how p150Glued functions in the regulation of dynein activation is poorly understood due to the complex architecture of dynactin. Here, we report the recombinant production of the complete human ~1.1 MDa dynactin complex by co-expressing all subunits in mammalian cells. Size-exclusion chromatography, mass spectrometry, and negative stain electron microscopy confirm the integrity of the complex. To study how dynein-binding- and activation is regulated, we have expressed soluble dynactin and dynein mutant constructs, in which key subunits such as p150Glued and the dynein intermediate chain are systematically N-terminally truncated. The successful generation of the intact dynein/dynactin wild type and mutant complexes will enable the precise investigation of the dynactin-mediated activation of the dynein motor. Furthermore, the recombinant production of dynactin will serve as a tool to study how dynactin-mutations are linked to neurodegenerative diseases such as Perry syndrome.

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16330 /GE-04

Structural basis of CRISPR RNA-guided DNA recognition by a minimal Type I-Fv Cascade surveillance complex

Presenting author:

Daniel Gleditzsch

Author(s):

Daniel Gleditzsch, Patrick Pausch, Hanna Müller-Esparza, Florian Altegoer, Lennart Randau, Gert Bange

Prokaryotes can contain CRISPR-Cas adaptive immune systems that provide resistance against viruses and mobile genetic elements. CRISPR loci are transcribed and processed into CRISPR RNAs (crRNAs) containing spacer sequences that recognize foreign DNA. In Type I CRISPR-Cas systems, crRNAs and Cas proteins form surveillance complexes, termed Cascade. Target DNA contains a protospacer adjacent motif (PAM) that is recognized and bound by a large Cascade subunit. Small subunits bind the displaced non-target DNA strand.

We have identified a minimal Type I CRISPR-Cas variant in *Shewanella putrefaciens* CN32 that lacks large and small subunits. This CRISPR-Cas system was

transferred into *Escherichia coli* and PAM-dependent interference activity against phages and plasmids was observed. Cas7fv was identified to bind the crRNA spacer sequence and modulation of the crRNA length resulted in varying numbers of Cas7fv Cascade subunits (Gleditzsch et al. 2016). Structures of Cascade were solved in the absence and presence of target DNA (Pausch et al. [Mol Cell in press]). The subtype-specific protein Cas5fv was shown to bind to the crRNA 5'-tag and to recognize a 'GG' PAM sequence via a specific alpha-helical domain. In addition, Cas5fv and Cas7fv proteins were found to contain lysine-rich wrist loops, which guide and stabilize the non-target strand. These features compensate for the missing large and small Cascade subunits and highlight a minimalistic solution for efficient DNA surveillance.

Session/Abstract ID: Subcellular organisation / 16328 /SC-05

YFR016c-Characterization of a novel interaction partner of the yeast polarisome with a potential role in actin organization

Presenting author:

Oliver Glomb

Author(s):

Oliver Glomb, Nils Johnsson

The budding yeast *Saccharomyces cerevisiae* grows through budding. As a prerequisite, protein-protein interaction networks establish, organize and maintain a polarity axis. Herein, the polarisome forms a central hub, which coordinates exocytosis with actin polymerization: The scaffold proteins Spa2 and Pea2 bind to Bud6 and Bni1, which initiate the formation of actin cables and to Msb3/Msb4, which regulate exocytosis by their GTPase activating function on Sec4. These six core components assemble a large network of other proteins at the sites of polarized growth.

Using Split-Ubiquitin analysis as a method to determine protein-protein interactions *in vivo*, we discovered that a protein encoded by YFR016c interacts with the polarisome components Bud6 and Spa2. Supported by genetic and fluorescence microscopical experiments, we postulate that YFR016c assists the polarisome mediated actin cable assembly.

Session/Abstract ID: Other (free) topics / 16533 /OT-41

Intracellular modulation of protein folding stability probed by a SOD1 based folding sensor

Presenting author:

David Gnutt

Author(s):

David Gnutt, Jonas Ahlers, Benedikt König, Simon Ebbinghaus

The cell is a heterogeneous and highly crowded environment in which biomolecules evolved to fold. Still, protein folding has almost exclusively been studied *in vitro*. Such conditions, however, cannot mimic the complexity of the cellular environment. Thus, its role in modulation of protein folding remains elusive. Utilizing a combination of subsequent fast laser-induced temperature jumps and fast fluorescence microscopy, we simultaneously study thermodynamics and kinetics of a novel folding reporter based on a truncated superoxide dismutase 1 (SOD1) variant, an important enzyme in amyotrophic lateral sclerosis. Our reporter is a monomeric two-state folder which was mutated at different sites to study the effect of site-specific mutations on the intracellular folding modulation. Our results reveal that most SOD1 mutations lead to a destabilization of the folded state both inside the cell and in protein crowding *in vitro*. Strikingly, a single point mutation can overcome this destabilization and change protein folding modulation in environments crowded by proteins. The results highlight the need to introduce new methods and models to understand protein folding under native cellular conditions.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16620 /SM-08

Targeting protein-protein interactions via supramolecular ligands – Mechanistic insight by molecular modeling

Presenting author:

Jean-Noël Grad

Author(s):

Jean-Noël Grad, Daniel Hoffmann

Modulation of protein-protein interactions (PPIs) by small molecules is an established field with clinical relevance in disorders involving deregulated signaling pathways[1]. Recently, large multi-armed peptidic dendrimer scaffolds derived from β -tryptase inhibitors[2] were shown to stabilize 14-3-3/C-Raf complexes in vitro, with EC₅₀ values in the range 8-50 μ M – comparable to the natural stabilizer cotylenin-A (EC₅₀ = 60 μ M[3]) – with a high susceptibility to the position of a specific cationic group in the ligand scaffold. We carried out all-atom Molecular Dynamics (MD) simulations on the ligand with highest stabilization potency to monitor salt-bridge formation with the 14-3-3/C-Raf complex. Two Glu residues in the 14-3-3 cleft near the C-Raf binding sites were identified as critical salt-bridge partners, supporting the idea of the ligand sealing the cleft to prevent 14-3-3/C-Raf dissociation and thus stabilize the PPI. The ligand was too flexible to adopt a well-defined binding pose at the microsecond timescale; we show that for such intrinsically disordered ligands, FFT-based shape and electrostatic correlation techniques offer similar information to MD at a fraction of the computational cost[4] while avoiding the MD sampling problem[5].

[1] Aeluri et al., Chem. Rev. 2014, 114, 4640–4694

[2] Jiang et al., Chem. Sci. 2015, 6, 1792–1800

[3] Molzan et al., ACS Chem. Biol. 2013, 8, 1869–1875

[4] Grad et al., arXiv:1707.02614v2

[5] Zuckerman, Annu. Rev. Biophys. 2011, 40, 41–62

Session/Abstract ID: Subcellular organisation / 16119 /SC-01

Knowledge of cell membrane topography allows fluorescence correlation spectroscopy to reveal the molecular organization of the cell membrane at a new level of detail

Presenting author:

Philipp Hagemann

Author(s):

Philipp Hagemann, Astrid Gesper, Patrick Happel

The molecular organization of the cell membrane is still a matter of debate, and the organization of the wide variety of proteins anchored in or spanning the cell membrane is largely unknown. While various models of the molecular organization of the cell membrane have been proposed, they have not been validated yet, due to the the lack of tools that allow probing the cell membrane at a molecular level in living cells under physiological conditions. Since the diffusion of a molecule within the cell membrane depends on the molecular organization of the cell membrane, fluorescence correlation spectroscopy (FCS) has been applied to study the molecular organization of the cell membrane. However, FCS is biased by the unknown local curvature and topography of the cell membrane, which severely limits the conclusions that can be drawn from FCS data to the molecular organization of the cell membrane.

Here we show that knowledge of the cell membrane topography allows one to distinguish hindered diffusion due to local cell membrane curvature from hindered diffusion due to a different molecular structure of the cell membrane.

Our theoretical considerations suggest that a combined instrument that records topography and diffusion simultaneously would allow to investigate the molecular cell membrane structure with a hitherto unavailable level of detail.

Session/Abstract ID: Cell signalling and membrane trafficking / 16383 /CS-25

Does PDK1 phosphorylate the tumor suppressor LKB1?

Presenting author:

Ina Hallstein

Author(s):

Ina Hallstein, Barbara Schwertner, Lars Kullmann, Gudrun Mendl, Michael P. Krahn

The 3-Phosphoinositide-dependent protein kinase 1 (PDK1) is a serine threonine kinase that plays a role in the phosphoinositol-3-kinase (PI3K) pathway. The PI3K signaling pathway regulates cell proliferation and survival and is frequently overactivated in cancer. The recruiting of PDK1 to the plasma membrane occurs by binding to phosphoinositol (3,4,5)-triphosphate (PIP3). Subsequently, PDK1 phosphorylates and thereby activates Akt (PKB), which in turn regulates further downstream targets which play crucial roles in cell growth, survival and energy uptake.

The tumor suppressor liver kinase B1 (LKB1, also known as serine threonine kinase 11, STK11) is involved in the regulation of cell polarity as well as cellular metabolism, cell proliferation and cell migration.

Here we report that PDK1 interacts with and directly phosphorylates LKB1. Specifically, we identified a conserved PDK1 binding site in LKB1 as well as a phosphorylation motif using *in vivo* stainings.

In order to address the function of PDK1-mediated LKB1 phosphorylation, we used *Drosophila melanogaster* as model system and established transgenic flies expressing phosphodeficient or –mimetic variants of LKB1, which we analyze regarding localization and function in an *lkb1*-deficient background.

Our results suggest a new crosstalk between LKB1 signaling and PI3K signaling, which might contribute to the pathogenesis of cancer.

Session/Abstract ID: Cell signalling and membrane trafficking / 16380 /CS-24

Allosteric control of the K(+) uptake system KtrAB

Presenting author:

Inga Hänel

Author(s):

Marina Diskowski, Dorith Wunnicke, Ahmad Reza Mehdipour, Deryck Mills, Gerhard Hummer, Janet Vonck, Inga Hänel

In most bacterial and some eukaryotic ligand-gated potassium channels, domains termed regulator of K(+) conductance (RCK) regulate ion fluxes. A single regulatory mechanism has been proposed for all RCK-regulated channels, involving signal transduction from the RCK domain to the gating area.

We will present an ADP-bound structure of the bacterial K(+) uptake system KtrAB, determined by cryo-EM, which combined with pulsed EPR measurements and MD simulations, uncovers a novel regulatory mechanism for ligand-induced action at a distance. Exchange of activating ATP to inactivating ADP triggers severe changes in both the RCK and the K(+)-translocating domains, which stabilize the closed, inactive conformation of the system.

Session/Abstract ID: Systems biology and autophagy signal control / 16420 /SB-04

Identification of Proteins involved in LD Biogenesis by High-Throughput Screen

Presenting author:

Tobias Hansen

Author(s):

Tobias Hansen, Maria Bohnert, Nofar Harpaz, Wolfgang Girzalsky, Maya Schuldiner, Ralf Erdmann

Lipid droplets (LDs) are ubiquitous organelles, which play a crucial role in lipid and energy metabolism. They consist of an organic core which is mainly built up of sterol esters (SEs) and triacylglycerols (TAG) and are surrounded by a phospholipid monolayer. Recently, a close proximity between LDs and peroxisomes was identified. Thereby peroxisomal tail-like structures can enwrap LDs and remarkably, these structures can even face the inner core of the LD. The tight peroxisomal-

LD contact could indicate a direct transfer of fatty acids between these two organelles. The biogenesis of LDs in general, which is assumed to take place at the ER, is poorly understood. Same is true for the identity of proteins involved in peroxisome-LD-association. We want to perform a high-throughput screen in the yeast *Saccharomyces cerevisiae* to identify proteins that are involved in the biogenesis of LDs. A strain was constructed harboring genomic fusions of the ER protein Sec66p with GFP as well as the LD protein Erg6p with CFP. This strain will be crossed with a strain library of over 5000 deletion strains. The morphology of LDs and the localization of Erg6p will be analyzed via fluorescence microscopy. The outcome of this screen will reveal proteins that are involved in the biogenesis of LDs. In future this screen shall be expanded by the additional labeling of a peroxisomal protein to search for contact sites between LDs and peroxisomes.

Session/Abstract ID: Other (free) topics / 16196 /OT-11

Molecular consequences of a novel and spontaneous infantile mutation in TNNC1 leading to the expression of cTnC G34S

Presenting author:
Roua Hassoun

Author(s):
roua hassoun, Diana Cimiotti, Setsuko Fujita-Becker, Hans-Georg Mannherz, Constanze Erdmann, Andreas Mügge, Rasmus R. Schröder, Hendrik Milting, Kornelia Jaquet

A novel, spontaneous mutation in TNNC1 encoding the Ca²⁺ binding subunit cTnC of cardiac troponin (cTn), which regulates muscle contraction, was detected in a neonate with a fibrotic and sponge-like structured heart. It led to a G34S- exchange in the non-functional Ca²⁺ binding site I. Thus Ca²⁺ binding to site II might be impaired. We report consequences of cTnC-G34S on Ca²⁺ binding using fluorescence spectroscopy and IAANS-labeled TnC and on thin filament structure using electron microscopy (EM).

For Ca²⁺-binding measurements we used cTnC-G34S alone or in cTn or in thin filaments in the absence and presence of myosin -S1 (+/- ATP, +/- Myosin binding fragment C0-C2). cTnC-wt was used as control.

Preliminary results showed no effect of the cTnC variant on Ca²⁺ -binding to neither cTnC-wt-IAANS and -G34S-IAANS nor appropriate cTn complexes. pCa50 were significant different, when using thin filaments (pCa50 = 6.82±0.14 with cTnC-wt; pCa50 = 7.49±0.27 with cTnC-G34S; p<0,050). An addition of myosin S1 to the reconstituted thin filaments had only marginal effects on Ca²⁺ -sensitivity before and after the activation by ATP. The Addition of MyBP-C-C0-C2 increased the Ca²⁺ sensitivity ($\Delta pCa50=0.11$). Thin filaments with cTnC-G34S revealed partly shorter fragmented filaments in EM compared to actin filaments alone or thin filaments with cTnC-wt.

The obtained data clearly reveal an impaired Ca²⁺ handling in thin filaments and thin filament organization due to the mutation.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16606 /SM-06

Novel luminophores with aggregation induced emission properties for protein recognition

Presenting author:
Matthias Hayduk

Author(s):
Matthias Hayduk, Jens Voskuhl

Since the discovery of the fluorescence phenomenon called aggregation induced emission (AIE)[1], it has become a versatile tool in different disciplines in modern bio-chemistry[2]. The main advantage of AIE luminophores is their ability to light up when their motion is restricted. Only few examples are known from literature using this fluorophore class for the binding to proteins, so that the influence of the binding event on the protein structure and the protein activity in this context is largely unknown. This fluorescence "on" behaviour enables the direct read-out of binding processes or morphological changes such as gelation, crystallisation, binding or polymerisation, which gives the AIE important applications for bio-chemistry in future. Therefore our aim is the development of novel luminophores with AIE properties as ligands for the recognition of protein surfaces and binding pockets.

References

[1] a) J. Luo, Z. Xie, J. W. Y. Lam, L. Cheng, H. Chen, C. Qiu, H. S. Kwok, X. Zhan, Y. Liu, D. Zhu, B. Z. Tang,

Chem. Commun. 2001, 1740-1741; b) B. Z. Tang, X. Zhan, G. Yu, P. P. Sze Lee, Y. Liu, D. Zhu, J. Mater. Chem. 2001, 11, 2974-2978; c) J. Chen, C. C. W. Law, J. W. Y. Lam, Y. Dong, S. M. F. Lo, I. D. Williams, D. Zhu, B. Z. Tang, Chem. Mater. 2003, 15, 1535-1546

[2] a) Y. Zhang, D. Li, Y. Li, J. Yu, Chem. Sci. 2014, 5, 2710-2716; b) E. Wang, E. Zhao, Y. Hong, J. W. Y. Lam, B. Z. Tang, J. Mater. Chem. B 2014, 2, 2013-2019.

Session/Abstract ID: Other (free) topics / 16622 /OT-57

Molecular Tweezers for Specific Protein Surface Recognition

Presenting author:

Christian Heid

Author(s):

Christian Heid, Thomas Schrader

Molecular tweezers are excellent binders for lysine and arginine by non-covalent interactions.(1) Their unique binding mode counteracts pathologic protein aggregation with potential applications in the treatment of Alzheimer's disease (2) and HIV infection.(3) However, molecular recognition of lysines and arginines on protein surfaces is not selective. We now developed a new method for direct esterification of monoaryl phosphates. This method allows the convenient introduction of one or two additional linker arms for subsequent click reactions with modified peptides. These peptides act as additional recognition sites and direct the tweezers to a selected lysine residue on the protein surface. Herein we present the synthesis of these new molecular tweezers, including binding experiments.

[1] F.-G. Klärner and B. Kahlert, Acc. Chem. Res., 2003, 36 (12), pp 919–932.

[2] A. Attar, C. Ripoli, E. Riccardi, P. Maiti, D. D. Li Puma, T. Liu, J. Hayes, M. R. Jones, K. Lichti-Kaiser, F. Yang, G. D. Gale, C. Tseng, M. Tan, C. Xie, J. L. Staudinger, F.-G. Klärner, T. Schrader, S. A. Frautschy, C. Grassi, G. Bitan, Brain 2012, 135, 3735-3748.

[3] Lump et al., eLife 2015;4:e05397.

Session/Abstract ID: Other (free) topics / 16432 /OT-17

Elucidating a mechanism of microtubule stabilization contributing to chromosomal instability in cancer cells

Presenting author:

Dominic Heinecke

Author(s):

Dominic Heinecke

Precise regulation of microtubule (MT) dynamics during mitosis plays an important role in correct chromosome alignment and segregation. Cells with an imbalance between MT polymerization/depolymerisation are known to show chromosomal instability (CIN), an increased frequency in chromosome missegregation and hallmark of cancer cells. More specific, many cancer cell lines have hyperstabilized MT-kinetochore attachments that lead to errors in chromosome segregation. However, the genetic or molecular defects in tumours that contribute to MT hyperstabilization and CIN have not been fully elucidated.

We have recently shown that GTSE1, a protein often overexpressed in cancer cells, stabilizes MTs by interacting with and inhibiting the MT depolymerizing kinesin MCAK (Kif2C). GTSE1 is required for chromosome alignment and induces CIN when overexpressed. MCAK activity has been linked to CIN, and is important for mitotic processes, which dependent on the precise regulation of MT dynamics. How MCAK's potent activity is regulated in the cellular context, however, remains unclear.

We are therefore investigating the mechanism by which GTSE1 inhibits MCAK activity and its spatiotemporal regulation in cells to adjust MT dynamics. To this end, we are identifying specific regions by which GTSE1 and MCAK interact and establish mutations to disrupt this interaction. Future work aims to identify how this interaction puts MCAK into an inactive state and how it is regulated by phosphorylation.

Session/Abstract ID: Cancer & Inflammation / 16535 /CA-16

Cleavage of the transcription factor USF2 by Taspase1 counteracts heterodimerization with USF1 and CDK4 expression

Presenting author:

Christina Heiselmayer

Author(s):

Christina Heiselmayer, Shirley Knauer, Laura Bornes, Jana Reich

Taspase1 is a 50 kDa endopeptidase that was found to be overexpressed in many solid and non-solid human cancers. However, Taspase1 is not regarded as a causal factor for cancer development, but rather plays a permissive role and is required for tumor maintenance. Such "non-oncogene addiction" proteins are considered important novel targets in cancer therapy.

In 2011, the bHLH-LZ transcription factor USF2 (Upstream Stimulatory Factor 2) was identified as potential substrate for Taspase1. USF2 is ubiquitously expressed and binds to E-box promoter motifs as homo- and heterodimer with USF1. USFs regulate the expression of several tumor suppressors and proto oncogenes. Thus, a deeper understanding of the downstream effects of proteolytic USF2 cleavage by Taspase1 is urgently required. Therefore, we analysed USF2/USF1 heterodimerization and expression of the cell cycle regulator CDK4 upon proteolytic processing. First, we verified that full-length USF2, but not a non-cleavable USF2 mutant was processed by Taspase1. Indeed, cleavage of USF2 by Taspase1 significantly inhibited its interaction with USF1. Furthermore, cleaved USF2 but not the mutated form of USF2 significantly reduced CDK4 protein levels which in turn is capable to directly modulate cell proliferation.

In summary, we discovered a novel mechanism how Taspase1 acts as a switch in CDK4 transcriptional control with likely alteration of cell cycle regulation, and thus might contribute to carcinogenesis.

Session/Abstract ID: Cancer & Inflammation / 16348 /CA-07

Taspase 1 cleavage regulates Myosin 1f-induced formation of cell protrusions

Presenting author:

Astrid Hensel

Author(s):

Astrid Hensel, Lena Kunst, Christina Heiselmayer, Elisabeth Schröder, Shirley Knauer

The cancer relevant human protease Taspase1 mediates cleavage of important intracellular substrates, including the mixed lineage leukemia protein MLL, leukemia-provoking MLL-fusions, and the general transcription factor TFIIA. Here, we now verify the unconventional Myosin 1f as a novel substrate of Taspase 1, and shed light on the physiological relevance of this proteolytic cleavage. We could for the first time demonstrate Taspase1-mediated proteolytic cleavage not only of the isolated cleavage site in the context of a cellular biosensor system, but also of full-length Myosin. Overexpression of Myosin 1f-GFP in various cancer and non-cancer cell lines induces cell protrusions showing characteristic features of filopodia. Interestingly, increasing expression levels of active Taspase1 interferes with Myosin 1f-induced filopodia formation in a concentration-dependent manner. Whereas the C-terminal Taspase 1 cleavage product of Myosin 1f is still confined to the cytoplasmic compartment keeping contact with the cell membrane, the N-terminus is able to translocate to the nucleus, thus enabling additional nuclear functions. Moreover, we could show that Myosin 1f-GFP colocalizes with the Rho-family members Rac und Cdc42 in focal adhesions, finally resulting in increased cell adhesion.

In sum, we here identified a novel mechanism how proteolysis and regulated subcellular localization cooperatively fine-tune the function of unconventional Myosins.

Session/Abstract ID: Cell signalling and membrane trafficking / 16449 / CS-26

Purification and characterisation of the yeast plasma membrane ATP binding cassette transporter Pdr11p

Presenting author:
Sara Abad Herrera

Author(s):
Katrine Rude Laub, Magdalena Marek, Lyubomir Dimitrov Stanchev, Sara Abad Herrera, Tamara Kanashova, Gunnar Dittmar, Thomas Günther Pomorski

The ATP binding cassette (ABC) transporters Pdr11p and its paralog Aus1p are expressed under anaerobic growth conditions at the plasma membrane of the yeast *Saccharomyces cerevisiae* and are required for sterol uptake. However, the precise mechanism by which these ABC transporters facilitate sterol movement is unknown. In this study, an overexpression and purification procedure was developed with the aim to characterise the Pdr11p transporter. Engineering of Pdr11p variants fused at the C terminus with green fluorescent protein (Pdr11p-GFP) and containing a FLAG tag at the N terminus facilitated expression analysis and one-step purification, respectively. The detergent-solubilised and purified protein displayed a stable ATPase activity with a broad pH optimum near 7.4. Remarkably, and in contrast to Aus1p, ATPase activity of Pdr11p was insensitive to orthovanadate and not specifically stimulated by phosphatidylserine

upon reconstitution into liposomes. Our results highlight distinct differences between Pdr11p and Aus1p and create an experimental basis for further biochemical studies of both ABC transporters to elucidate their function.

Session/Abstract ID: Other (free) topics / 16437 /OT-28

Impact of cGMP-PKG Pathway Modulation on Titin Phosphorylation and Titin-Based Myocardial Passive Stiffness

Presenting author:
Melissa Herwig

Author(s):
Melissa Herwig, Soraya Hölper, Marcus Krüger, Doris Koesling, Wolfgang A. Linke, Nazha Hamdani

BACKGROUND: Titin phosphorylation by cyclic guanosine monophosphate (cGMP)-dependent protein kinase G (PKG) lowers cardiomyocyte passive stiffness (F_{passive}), thus mediating a mechanical signaling process that is impaired in heart failure (HF).

METHODS: We used knockout (KO) mouse models with deletion of the guanylyl cyclase (GC)-A receptor, cGMP-dependent PKG (cGKI) and soluble GC (sGC), as well as wildtype (WT) controls. We assessed titin phosphorylation by immunoblotting and quantitative mass spectrometry (MS). We measured F_{passive} before and after PKG administration and explored oxidative stress effects on cGMP-PKG signaling in myocardial biopsies from HF patients.

RESULTS: In all KO models, the PKG-dependent phosphosite S4080 within the N2-Bus region of titin was hypophosphorylated. Unexpectedly, MS analysis showed that most phosphosites were hyperphosphorylated in the cGKI KO hearts, perhaps because of compensatory processes following PKG loss. This was associated with upregulation of CaMKII and a clear rise of F_{passive} in KO vs. WT cardiomyocytes. While PKG administration lowered F_{passive} of KO cardiomyocytes in all models, this effect was more pronounced in cGKI KO. Increased oxidative stress in HF biopsies and cGKI KO correlated with increased CaMKII and reduced PKG activities.

CONCLUSION: Our findings suggest that a network formed by cGMP/PKG/oxidative stress/CaMKII plays an important role in the regulation of cardiomyocyte and diastolic stiffness.

Session/Abstract ID: Age-related pathologies and autophagy / 16418 /AP-06

Biochemical and Structural analyses of the RocCOR domain.

Presenting author:

Franz Y.F. Ho

Author(s):

Franz Y.F. Ho, Susanne Terheyden, Bert Poolman, Alfred Wittinghofer, Arjan Kortholt

Mutations in the human Leucine-Rich Repeat Kinase 2 (LRRK2) are the most frequent cause of inheritable Parkinson's Disease (PD). LRRK2 belongs to the Roco family of proteins within the Ras superfamily, which is characterized by a conserved Ras-like G-domain (Roc) and succeeded by a COR domain. Five out of seven PD-linked mutations are located in this domain tandem, however, its biochemistry is yet to be characterized. Recently, we for the first time were able to successfully express and purify sufficient stable human LRRK2 RocCOR from *Escherichia coli*. Together with its prokaryotic homologues, we study both the structure and biochemistry of RocCOR domain. By revealing their kinetics and structural characteristics, we are aiming to further elucidate the activation mechanism of LRRK2 and the impact of PD mutations towards the function of LRRK2.

Session/Abstract ID: RNA-Regulation und Transport / 16524 /RR-06

NAD-capped RNA in bacteria: Discovery, Scope and Function

Presenting author:

Katharina Höfer

Author(s):

Katharina Höfer, Andres Jäschke

A hallmark in prokaryotic gene expression was the absence of 5'-capped RNA. In eukaryotes, the m7G-cap protects mRNA from degradation and modulates maturation, localisation or translation. Recently, the ubiquitous redox cofactor nicotinamide adenine dinucleotide (NAD) has been found to be covalently linked to bacterial RNA. Recent work from our laboratory discovered a subset of small regulatory RNAs (sRNAs) in the bacterium *Escherichia coli* to be specifically 5'-modified with NAD in a cap-like manner

(1,2). Biochemical studies revealed that analogous to a eukaryotic cap, 5'-NAD modification stabilise RNA against 5'-processing by RppH and cleavage by RNase E. Moreover, the Nudix hydrolase NudC was found to act as a NAD-RNA decapping enzyme in vitro and in vivo. Crystal structures of *E. coli* NudC in complex with NAD and with cleavage product NMN reveal the catalytic residues lining the binding pocket and principles underlying molecular recognition of substrate and product. In in vitro competition experiments, NudC preferred NAD-RNA over NAD(H), suggesting that NAD-RNA may be its primary biological substrate. (3)

Given the central role of NAD in redox-biochemistry, protein-modification, and signaling, its attachment to RNA points to unknown roles of RNA in these processes and to undiscovered pathways in RNA metabolism and regulation.

Session/Abstract ID: Cell signalling and membrane trafficking / 16198 /CS-10

NOSIP is essential for craniofacial development and developmental neurogenesis in mice

Presenting author:

Meike Hoffmeister

Author(s):

Meike Hoffmeister, Alexander Ehrke, Stefanie Oess

The loss of the E3 ligase NOSIP in mice causes holoprosencephaly and facial anomalies including cleft lip/palate, cyclopia and facial midline clefting. By a mass spectrometry based protein interaction screen we identified NOSIP as a novel interaction partner of protein phosphatase PP2A. NOSIP mediates the monoubiquitination of the PP2A catalytic subunit and the loss of NOSIP results in an increase in PP2A activity in craniofacial tissue in NOSIP knockout mice. To investigate a potential role of NOSIP in neurogenesis we employed the neurosphere assay system. Neurospheres derived from Nosip knockout embryos exhibited reduced growth and the capability to differentiate into neurons in vitro was almost completely abolished. In vivo, we found that the formation of postmitotic neurons was greatly diminished in NOSIP KO embryos, concomitant with a reduced number of apical and basal neural progenitor cells and hypoplasia of the cortical plate. Mass spectrometry analysis of the neurospheres proteome revealed a reduced expression of Rbp1, a regulator of

retinoic acid synthesis, when Nosip was absent. We conclude, that NOSIP is a critical factor for brain development and neural stem cell/progenitor self-renewal in mice and a candidate gene for holoprosencephaly and developmental neurogenesis in humans.

Session/Abstract ID: Other (free) topics / 16565 /OT-43

The C-terminus of the human guanylate-binding protein 1 regulates its unique GTPase activity

Presenting author:

Semra Ince

Author(s):

Semra Ince, Ping Zhang, Miriam Kutsch, Sergii Shydlovskiy, Christian Herrmann

Human guanylate-binding protein 1 (hGBP-1) is an interferon-inducible large GTPase which unites immune related activity on the cellular level with a unifying two-step GTP hydrolysis mechanism on molecular level. While ongoing studies aim to drive the link between observed cellular effects and molecular details, the nucleotide-dependent dimerization of hGBP-1 has been already identified as a central property not only controlling the subcellular localization but also its enzymatic activity. Accordingly, dimerization of hGBP-1 was revealed as an essential prerequisite to catalyze the two-step GTP hydrolysis by successive cleavage to a mixture of GDP and GMP in a defined ratio. However, it is still unknown how exactly the share of either product is regulated.

Here, we identified that nucleotide-driven intramolecular interactions between the C-terminal GTPase effector domain (GED) and the large GTPase domain (LG), and in particular the release of the GED do essentially influence the second hydrolysis step. Appropriate hGBP-1 mutants with tightened and weakened LG:GED contacts, respectively, yielded either no GMP or exclusively GMP as product. FRET data further suggested that the GED release correlates with dimerization of the protein. As the mutants with weaker LG:GED interactions showed both enhanced dimerization and favored GMP production, these data altogether suggest that the intramolecular interactions regulate the dimer lifetime and thereby coordinate the product share.

Session/Abstract ID: Emerging Analytical Methods / 16284 /AM-01

Quantitative mapping of the interaction surface of nucleosome with the chromatin remodeler, RSC, in vivo

Presenting author:

Neha Jain

Author(s):

Neha Jain, Bryan Wilkins, Petra Geue, Heinz Neumann

Chromatin remodelers govern nucleosome dynamics by fine-tuning the packaging and access to DNA, allowing adaptation to numerous cellular needs. They use energy derived from ATP hydrolysis to either slide, eject or replace nucleosomes. However, understanding of their mechanism of action is still rudimentary.

To investigate the interaction of remodelers with their substrate, we use genetically encoded UV-activable crosslinkers site-specifically installed on histone proteins in living yeast to trap the binding partners. By this, it is possible to follow protein interactions in a synchronized process and monitor their dependence on further factors. With this strategy we have recently been able to identify an inter-nucleosomal interaction between the H4 N-terminal tail and the acidic patch of a neighbouring nucleosome that drives condensin independent chromatin condensation in mitosis.

Using a library of amber mutants, we have surveyed the entire nucleosome for sites that produce crosslinks to the chromatin remodeler RSC and in turn revealed the footprint of its catalytic subunit, Sth1, by gel shift assays. Mutational studies show requirement for an acetylatable lysine residue in the H3 tail (H3 K14) for its binding to Sth1. Our results identify that Sth1 binding to nucleosomes is enhanced by sumoylation. We are currently developing a method to determine the crosslink products by mass spectrometry. This will provide unprecedented insights into mechanisms that shape mitotic chromatin structure.

Session/Abstract ID: Other (free) topics / 16385 /OT-24

A comparative analysis of Sec61- and SecY-mediated transport of intrinsically disordered proteins

Presenting author:
Sebastian Jung

Author(s):
Sebastian Jung, Anika Gonsberg, Sarah Ulbrich, Andrea Origi, Anke Ziska, Michael Baier, Hans-Georg Koch, Richard Zimmermann, Konstanze F. Winklhofer, Jörg Tatzelt

About one-quarter to nearly one-third of the proteins synthesized in the cytosol of eukaryotic cells are integrated into the plasma membrane or are secreted. Translocation of secretory proteins into the lumen of the endoplasmic reticulum (ER) of eukaryotes or the periplasm of bacteria is mediated by a highly conserved heterotrimeric membrane protein complex denoted Sec61 in eukaryotes and SecY in bacteria. Previous studies in neuronal cells revealed that authentic N-terminal ER signal peptides cannot promote efficient ER import of intrinsically disordered proteins. We now analyzed secretion of proteins with defined secondary structure in mammalian, yeast and *E. coli* cells and show that the impaired translocation of unstructured client proteins is an evolutionary conserved feature of the Sec61/SecY translocon.

The use of more efficient signal peptides or increasing substrate length did not significantly improve translocation. However, alpha-helical domains restored translocation, even if they are added more than 150 amino acids C-terminally to the signal peptide. Interestingly, beta-sheet domains neither promoted efficient translocation of unstructured domains, nor were efficiently translocated in the absence of alpha-helical domains.

Our findings indicate that alpha-helical domains are required in addition to the signal peptide to promote productive translocation of secretory proteins through the Sec61/SecY complex.

Session/Abstract ID: Subcellular organisation / 16580 /SC-11

Evolutionary divergent organelles provide novel anti-parasite drug targets

Presenting author:
Vishal Kalel

Author(s):
Vishal Kalel, Ralf Erdmann, Wolfgang Schliebs

Eukaryotic pathogens harbor unique or divergent organelles. Glycosomes are divergent peroxisomes in Trypanosomatid parasites which compartmentalize glycolytic enzymes inside organelle. As trypanosomal glycolytic enzymes lack feedback regulation, unique compartmentation of ATP-consuming steps inside glycosomes with limited ATP and net ATP-generating steps in the cytosol is necessary for the regulation of glycolysis. Since glycolysis is the sole source of ATP for trypanosomes in the host bloodstream, disruption of glycosome biogenesis is an attractive drug target. We are characterizing proteins required for glycosome biogenesis called peroxins (PEX). We identified trypanosomal PEX16 involved in glycosomal membrane protein import and show its essentiality for glycosome biogenesis and parasite survival, thereby providing a novel drug target. Using structure-based drug design, we developed high-affinity small molecule inhibitors of the glycosomal matrix protein import by targeting PEX5-PEX14 interaction. The inhibitors disrupt glycosomal matrix protein import leading to mislocalisation of glycosomal enzymes to the cytosol which makes glucose toxic to trypanosomes. Unregulated glucose phosphorylation in the cytosol depletes cellular ATP, accumulates glucose metabolites to toxic levels and kills the parasites. We show that better understanding of organelle biogenesis in pathogens enables identification of new drug targets and design of new drugs which are urgently needed.

Session/Abstract ID: Electron transport chains / 16578 /ET-02

The obligate respiratory supercomplex from the actinobacterium Corynebacterium glutamicum

Presenting author:

Wei-Chun Kao

Author(s):

Wei-Chun Kao, Syed H. Mir, Christophe Wirth, Carola Hunte

Actinobacteria are closely linked to human life as industrial producers of bioactive molecules and as pathogens. Respiratory cytochrome (cyt) bcc complex and cyt aa3 oxidase form a supramolecular association in *Corynebacterium glutamicum*. We define the cyt bcc-aa3 supercomplex as an obligate supercomplex and phylogenetic analysis showed that it is characteristic for Actinobacteria [1]. The prototype bcc-aa3 supercomplex from *C. glutamicum* was purified and its biophysical and structural properties were characterized [1]. The bcc complex is fine-tuned for oxidation of menaquinol, as predicted on the basis of the PDWY sequence for the Qo motif of actinobacterial cyt b [2]. The aa3 oxidase mediates the low potential cofactors of the bcc complex and the high potential binuclear centre to accomplish dioxygen reduction. The molecular model of the supercomplex is in line with distance constraints for biological electron transfer. Recombinant antibodies were generated for structural characterization. We will discuss why a defined solid-state architecture of the supercomplex is beneficial for energetically efficient coupling of menaquinol oxidation and dioxygen reduction in one supramolecular entity. The actinobacterial supercomplex is of interest for improving efficiency of industrial production strains and for development of drugs against diphtheria and tuberculosis causing pathogens.

[1] *Biochim. Biophys. Acta* 1857, 1705-1714 (2016).

[2] *Genome Biol. Evol.* 6, 1894-1910 (2014).

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16538 /GE-07

Towards identifying therapy resistance-inducing "driver" mutations in the human non-coding genome.

Presenting author:

Manuel Kaulich

Author(s):

Manuel Kaulich

Drug resistance is defined as the failure of transformed cells to respond to a drug that used to kill or weaken them and can be present at the beginning of treatment, or develop during the course of drug exposure.

Advancements in gene perturbation approaches enable robust and comprehensive identification of genetically encoded drug resistance markers (DRMs). However, results are incomplete and fail to mimic "in-patient"-occurring resistance mechanisms.

Although protein-coding regions have received most attention, the majority of mutations occur in non-coding genomic regions. While the role of non-coding driver mutations in cellular transformation is widely accepted, their role in therapy resistance has remained elusive mainly due to the large number of existing variants and technological limitations.

Here we address the hypothesis of non-coding genomic variants acting as driver mutations therapy resistance development. We invented a novel method for the generation of CRISPR/Cas gene perturbation libraries that relies on the generation of Covalently Closed Circular synthesized (3Cs) gRNAs. Using our technique, we generated the first "truly-genome-wide" CRISPR/Cas gRNA library that targets 20 million SpCas9-target sites in the coding and non-coding human genome. We apply this reagent to patient-derived acute lymphoblastic leukemia (ALL) cells in the presence of first-line chemotherapeutic agents to identify non-coding genomic regions that act as drivers for therapy resistance.

Session/Abstract ID: Other (free) topics / 16307 /OT-09

genphen: A tool for quantification of genotype-phenotype associations using statistical learning techniques and Bayesian inference

Presenting author:

Simo Kitanovski

Author(s):

Simo Kitanovski, Daniel Hoffmann, Karl Lang, Vikas Duhan

Genome Wide Association Studies (GWAS) have become an essential tool for studying the relationship between genotypes and phenotypes. One important challenge which arises with conventional (frequentist) statistical approaches for GWAS is a massive multiple-testing problem, that is often tackled by rigorous corrections leading to huge amounts of false negatives, i.e. many small but real associations are likely to be discarded. Bayesian inference and statistical learning techniques provide a more consistent approach for GWAS and alleviate the multiple-testing problem.

Here we present *genphen*, an R package for statistical inference in GWAS. With *genphen* we can quantify the association between genotypes and phenotypes using statistical learning techniques such as random forests and support vector machines, as well as Bayesian inference using hierarchical models. To evaluate *genphen*, we studied the association between 4 million SNPs made publicly available by the mouse HapMap project, and experimentally derived phenotypes from a study of viral replication of vesicular stomatitis virus (VSV) and lymphocytic choriomeningitis virus (LCMV) in 17 strains of laboratory mice.

We identified multiple SNPs having significant association with VSV replication, including coding SNPs found in genes associated with the immune system.

The version 1.4 of *genphen* is available as part of Bioconductor.

Session/Abstract ID: Subcellular organisation / 16334 /SC-06

Functional characterization of a newly identified interaction site between the peroxisomal pore components PEX5 and PEX14.

Presenting author:

Jessica Klümper

Author(s):

Jessica Klümper, Leonidas Emmanouilidis, Wolfgang Schliebs, Michael Sattler, Ralf Erdmann

Peroxisomes are ubiquitous organelles in all eukaryotic cells. They form subcellular compartments for many reactions that require oxidative conditions like β -oxidation of long chain fatty acids. The essential meaning of peroxisome metabolism for human life is indicated by peroxisomal biogenesis disorders (PDBs). Most of these patients are characterized by defects in the posttranslational import of peroxisomal matrix enzymes. Two peroxisomal targeting signals (PTS1, PTS2) on completely folded, even oligomerized peroxisomal proteins have been characterized so far. Cargo proteins carrying these signals are transported by the cytosolic receptor PEX5 to the peroxisomal membrane. PTS1 proteins are interacting directly with the C-terminal half of PEX5, whereas PTS2 protein interaction is bridged by the PTS2 receptor PEX7. At the peroxisomal membrane PEX5 interacts with the so-called docking complex, consisting of the integral membrane proteins PEX13 and PEX14. The multivalent interaction between the N-terminal region of PEX14 and the N-terminal region of PEX5 has been extensively documented. Recently we identified an additional PEX5-PEX14 interface formed between the C-terminal region of human PEX14 and the C-terminal cargo-binding region of human PEX5. Here, we analyzed by structural and biochemical means the in vitro effects of various mutations within this interface and demonstrate its functional importance in mammalian cells.

Session/Abstract ID: Emerging Analytical Methods / 16384 /AM-04

Advanced fluorescence spectroscopy and stopped-flow experiments, a perfect couple for studying protein association dynamics

Presenting author:

Julian Koch

Author(s):

Julian Koch, Jan-Hendrik Budde, Thomas-Otavio Peulen, Christian Herrmann, Claus A. M. Seidel

The self-association (oligomerization) of proteins often depends on structural changes of the involved domains. To simultaneously track oligomerization kinetics and domain movements, we combined time-resolved Förster-Resonance-Energy-Transfer (FRET) experiments with a stopped-flow setup. Fluorescence intensities recorded with picosecond time-resolution and the polarized fluorescence detection achieve Ångström precision. Domain movements, structural heterogeneities and oligomerization kinetics are resolved by multiple measurements of intra- and intermolecular distance measurements via FRET. We apply this approach to the human guanylate binding protein 1 (hGBP1), a member of the dynamin-superfamily of large GTPases. In a nucleotide dependent manner hGBP1 binds to lipid membranes leading to tethering of vesicles and it forms oligomers leading to ring like structures. hGBP1 is a prime example to study the relevance of structural dynamics as a prerequisite for the self-association of proteins. It shows internal dynamics from microseconds to milliseconds. Furthermore, it requires a rearrangement of its super-tertiary structure for its dimerization. We use hGBP1 as a system to establish our approach which may open new ways to understand the relevance of conformational heterogeneity and domain movements for oligomerization processes.

Session/Abstract ID: Cell signalling and membrane trafficking / 16353 /CS-21

Nuclear spheres are highly enriched in the human AD brain and are regulated by APP T668 phosphorylation

Presenting author:

Katharina Kolbe

Author(s):

Katharina Kolbe, Hassan Bukhari, Verian Bader, Konstanze F. Winklhofer, Carsten Theiss, Thomas Günther-Pomorski, Thorsten Müller

The role of the amyloid precursor protein (APP) in Alzheimer's disease (AD) has been studied since decades. A putative function of this protein or its intracellular domain (AICD) is the generation of protein aggregates within the cellular nucleus termed "nuclear spheres" consisting of FE65, TIP60, BLM, and other yet unknown proteins. So far, the complexes were solely studied in cell culture model systems. However, immunohistochemical studies of our group using human frontal cortex brain samples from AD patients and age-matched controls revealed a highly significant enrichment of these spherical structures in the AD brain. Co-staining of neuronal or astrocytic marker protein with the nuclear spheres showed that neurons are distinctly affected by nuclear spheres. The formation of the spheres seems to be affected by the phosphorylation of the APP Thr668 residue as the aggregates were predominantly found in neurons negative for the Thr668 phosphorylation. Cell culture experiments further revealed that the number of nuclear spheres positive cells is reduced by JNK3-mediated APP phosphorylation. The results support the early, cell culture based assumption that "nuclear spheres" play a relevant role in the neurodegenerative molecular mechanism of AD. In future experiments, we plan the investigation of nuclear sphere distribution in hippocampal areas as well as the search for additional interaction proteins to gain further information about the relevance of nuclear spheres AD.

Session/Abstract ID: Other (free) topics / 16592 /OT-47

A fluorescence-based calcium phosphate nanoparticle probe to elucidate lysosomal entrapment in living cells

Presenting author:
Sebastian Kollenda

Author(s):
Sebastian Kollenda, Matthias Epple

Calcium phosphate naturally occurs in bone tissue and teeth and is generally considered to have a good biocompatibility and a high biodegradability in biological applications.[1] Due to these characteristics of calcium phosphate, nanoparticles based on this material have become a potent delivery system of a vast range of molecules for in vitro and in vivo experiments. Our aim is to create a tool to visualize the pathway and fate of nanoparticles inside the cell.

Calcium phosphate nanoparticles (CaP-NPs) are synthesized by a fast precipitation from aqueous solutions of calcium lactate and diammonium hydrogen phosphate. The nanoparticles are colloiddally stabilized with poly(ethylene imine) and loaded with a tandem fusion protein consisting of DsRed-eGFP (red and green fluorescence).[2] The nanoparticles are taken up by the cell together with the protein via endocytic pathways and then directed to lysosomes.[3] At a physiological pH of 7.4 the fluorescence of both proteins (red and green) is detectable. Under the conditions of an acidic pH of about 4.5-5 inside the lysosomes, the green fluorescence will disappear[4] due to the protonation of the chromophore.

As control, unloaded nanoparticles were synthesized and applied to the cells as well as the soluble protein. An fluorescence assay is used to confirm the pH-dependent fluorescence decrease of eGFP alone and its fusion variant to DsRed. Particles were characterized by dynamic light scattering and scanning electron microscopy.

Session/Abstract ID: Other (free) topics / 16472 /OT-35

HOBbing proteins with DNA

Presenting author:
Katja Koßmann

Author(s):
Katja Koßmann, Cornelia Ziegler, Alessandro Angelin, Rebecca Meyer, Marc Skoupi, Kersten Rabe, Christof Niemeyer

HOB, the Halo-based oligonucleotide binder protein, is an improved version of the self-ligating HaloTag® fusion protein, which enables efficient coupling of proteins with DNA.

DNA nanostructures allow for precise arrangement of other molecules (e.g. proteins) at nanometer scale. Those molecular pegboards are essential tools for the investigation of distance dependent processes, e.g. enzyme cascades or receptor signaling mechanisms.

To perform such studies, efficient (biorthogonal) immobilization techniques are required. By rational engineering of the HaloTag® binding interface an optimized linker for DNA nanostructures was obtained. Five amino acids positioned around the active site entry channel of the HaloTag® protein were exchanged against positively charged lysine amino acids to produce the HOB (Halo-based oligonucleotide binder) protein.

HOB was genetically fused with the enzymes Cytochrome P450 BM3 as well as with BMR, the separated reductase domain of BM3. The resulting HOB-fusion proteins revealed significantly improved rates in the ligation with CH-modified oligonucleotides and DNA origami nanostructures at equimolar reactant ratios. These results suggest that the efficient self-assembly of protein-decorated DNA structures can be largely improved by fine tuning of the electrostatic interactions between proteins and the negatively charged nucleic acid nanostructures.

Session/Abstract ID: Subcellular organisation / 16558 /SC-10

VMC1: Mitochondrial Targeting of a Virus-encoded Protein

Presenting author:

Sebastian Kreimendahl

Author(s):

Sebastian Kreimendahl, Jan Schwichtenberg, Kathrin Günnewig, Dina Sträter, Joachim Rassow

The Mimiviridae mimivirus was first identified as a possible pathogen in *Acanthamoeba polyphaga*. Its viral 1,2 Mbp genome contains 911 genes, of which approximately 10% encode proteins of known function, including a functional homologue of the mitochondrial carrier family (MCF), the viral mitochondrial carrier 1 (VMC1). Members of the MCF enable the transport of metabolites and cofactors across the inner membrane of mitochondria. Given the ability of VMC1 to transport dATP and dTTP in artificial membrane studies, we raised the question if and how VMC1 interacts with mitochondria. We found that the VMC1 targets mitochondria in intact mammalian cells. Using a cell-free system to investigate protein import into isolated mitochondria, we investigated the import pathway of the VMC1, from its initial recognition to its final location in the mitochondrial inner membrane. The aim of our project is to investigate a possible role of the VMC1 in the reproduction of the mimivirus.

Session/Abstract ID: Subcellular organisation / 16306 /SC-04

The tip localization of the polarisome depends on its interaction with the type V myosin Myo2

Presenting author:

Jan-Michael Kromer

Author(s):

Jan-Michael Kromer

The yeast *Saccharomyces cerevisiae* is a preferred model organism for studying polar growth and asymmetric cell division of eukaryotic cells. The growth of the yeast occurs through budding. Focussed at the tip of the bud is a network of proteins, the so-called polarisome, that coordinates the incorporation of new cell wall material and plasma membrane at this site. The polarisome consists of four core components.

Spa2 und Pea2 are central scaffold proteins that bind to each other as well as to Bud6 and the formin Bni1. The latter two proteins initiate actin cable formation at the bud tip. The type V myosin Myo2 walks on these filaments and pulls post-Golgi vesicles, organelles and other cargo along to the tip.

Using a systematic high-through-put Split Ubiquitin protein interaction screen we could identify a direct link between Pea2p and the Cargo-Binding-Domain (CBD) of Myo2.

We exchanged surface residues of the CBD and used these mutants to map the binding site for Pea2p on the CBD. We are in the process to identify and characterize mutants of Myo2p that have lost the ability to specifically interact with Pea2. Once characterized these mutants will help to understand how the polarisome assumes its focussed distribution at the tip and how its connection to the polarisome influences Myo2p to carry specific cargo to the bud.

Session/Abstract ID: RNA-Regulation und Transport / 16587 /RR-07

The role of piRNAs in planarian regeneration

Presenting author:

Claus Kuhn

Author(s):

Claus Kuhn

Planarian flatworms possess fantastic regenerative abilities that can be attributed to the presence of stem cells all across their bodies. Intriguingly, piRNAs are essential for proper stem cell function and hence regeneration. However, how piRNAs contribute to regeneration is not understood.

This is why we study the role of planarian piRNAs during regeneration. We employ biochemical techniques paired with next-generation sequencing, immunostaining and in vivo RNAi.

We can demonstrate that planarian piRNA keep transposable elements in check in a transcriptional, as well as post-transcriptional manner. Thereby piRNAs clearly contribute to stem cell integrity. Beyond that, planarian piRNAs also seem to regulate mRNA levels. To understand how mRNAs are regulated by piRNAs we combine RNA-seq and small RNA-seq with ChIP-seq data of heterochromatin marks.

Our results help to establish how small RNAs contribute to the pluripotency of stem cells. In addition, understanding the role of piRNAs during planarian regeneration will facilitate attempts to grow human organs from pluripotent stem cells.

Session/Abstract ID: Cancer & Inflammation / 16595/CA-24

Colon cancer tissue sections investigated by IR - Imaging

Presenting author:

Claus Küpper

Author(s):

Claus Küpper, Frederik Großerüschkamp, Angela Kallenbach-Thieltges, Axel Mosig, Klaus Gerwert

The field of IR Imaging has shown many promising results in the last decade. The capability of this label-free method to distinguish many different tissue types and disease patterns has been proven in numerous studies. We extended the approach of spectral annotation by several layers of analysis regarding colorectal cancer. Our studies show that differential diagnostics for distinguishing cancerous regions with following differential diagnosis of the dedifferentiation grade of the tumor cells is possible for FFPE and fresh frozen tissue samples.

Therefore, we established supervised learning algorithms providing label free annotation. This allowed us to couple this technique with laser capture microdissection (LCM) for subsequent -omics-studies of label-free annotated tissue samples. A first proof of principle of this workflow was performed in our group on mesothelioma samples. A colorectal cancer FTIR-guided -omics study of cancerous lesions and healthy crypts is in progress.

Utilizing a Spero QT IR-microscope of Daylight Solutions (San Diego, CA, USA) allows us to push the frontiers of the time scale to a reasonably short time frame. First preliminary results show that these QCL based instruments offer new possibilities in rapid and reliable data acquisition and may pave the way to translate this technique into clinics.

Session/Abstract ID: Cell signalling and membrane trafficking / 16552 /CS-40

Lipid-conjugated pH-sensors for advanced biological approaches

Presenting author:

Ronja Marie Kühnel

Author(s):

Ronja Marie Kühnel

Advanced molecular sensors is an area of research in rapid development, and proton nanosensors attract special interest because of their applicability in monitoring proton concentrations in biological microcompartments. Proton concentration gradients play pivotal roles by providing the driving forces for the uptake of nutrients and other cell constituents through an array of secondary transport systems. Previously, we reported on two lipid-linked pH sensors employing amine-reactive forms of the probes seminaphthorhodafluors (SNARF®-1 dye) and rhodamine (pHrodo™ Red dye), respectively (Kemmer et al., Analyst, 2015, 140, 6131). Here, we present an improved synthesis protocol for lipid-linked pH sensors, and demonstrate their application to monitor pH changes in reconstituted liposomal systems. The lipid-conjugated derivatives were characterized regarding pH dependence of their fluorescence emission and their applicability for monitoring pH changes in reconstituted liposomal systems and in cellular systems.

Session/Abstract ID: Other (free) topics / 16529 /OT-39

Molecular insights into the networking of human guanylate binding proteins

Presenting author:

Miriam Kutsch

Author(s):

Miriam Kutsch, Semra Ince, Sergii Shydlovskiy, Christian Herrmann

The human guanylate-binding proteins (hGBPs) are key players in the cellular response against viral and bacterial pathogens. The family of hGBPs consists of seven isoforms and each isoform homo dimerises and localises to a specific cellular compartment. Upon hetero dimerisation hGBPs are able to recruit each

other to their specific compartment. Thus, homo and hetero dimerisation allow the hGBPs to build a network within the cell which seems crucial for their anti-pathogenic functions. In this network, hGBP-1 recruits hGBP-5 from the Golgi apparatus to the cytosol. Here, we investigated the homo and hetero dimerisation of the two isoforms hGBP-1 and hGBP-5 in vitro with FRET-based experiments. This allowed us to obtain the affinities and the dynamics of the homo and hetero dimers which build the molecular bases for understanding the hGBP network and therefore the hGBPs' anti-pathogenic activities.

Britzen-Laurent, N., Bauer, M., Berton, V., Fischer, N., Syguda, A., Reipschläger, S., Naschberger, E., Herrmann, C., and Stürzl, M. (2010). Intracellular trafficking of guanylate-binding proteins is regulated by heterodimerization in a hierarchical manner. *PLoS ONE*, 5:1–11.

Ince S., Kutsch M., Shydlovskiy S., and Herrmann C. (2017). The human guanylate-binding proteins hGBP-1 and hGBP-5 cycle between monomers and dimers only. *FEBS J.*, doi: 10.1111/febs.14126.

Session/Abstract ID: Age-related pathologies and autophagy / 16005 /AP-02

Lysine-acetylation in cellular regulation, ageing and disease.

Presenting author:
Michael Lammers

Author(s):
Michael Lammers, Nora Kuhlmann, Magdalena Kremer, Linda Baldus

Today, more than 15.000 acetylation sites have been identified in proteins covering all essential cellular functions, in all cellular compartments from bacteria to man. The model that dietary restriction extends lifespan in several organisms such as *Drosophila*, *C. elegans* and mice is generally accepted, while the molecular mechanisms underlying this are poorly understood.

Due to the tight connection of metabolism and lysine deacetylase (KDAC)/lysine acetyltransferase activities, lysine acetylation might play an important role in the ageing process. The sirtuins (Sir; silent information regulator) are NAD⁺-dependent deacetylases play

protective roles in the development of cardiovascular and neurodegenerative diseases as well as cancer.

Despite the huge gain-of-knowledge by mass spectrometry-based approaches, the functional and structural consequences of lysine acetylation on protein function are only poorly investigated so far. Indeed, for less than 1% of all identified acetylation sites, a thorough functional characterisation has been performed.

The genetic-code expansion concept (GCEC) enables to incorporate acetyl-L-lysine co-translationally into proteins using a synthetically evolved, orthogonal acetyl-lysyl-tRNA-synthetase/tRNACUA-pair from *Methanosarcina barkeri*. We use a combined synthetic biological, biophysical and biochemical approach to identify acetylation sites with physiological relevance and to judge the consequences of a dysregulation at this site.

Session/Abstract ID: Cancer & Inflammation / 16144 /CA-03

Epigenetic control of Hematopoiesis by Histone Arginine Methylation

Presenting author:
Jörn Lausen

Author(s):
Jörn Lausen, Olga Kuvardina, Stefanie Herkt

Hematopoietic differentiation is driven by transcription factors, which orchestrate a fine tuned transcriptional network. At bipotential branching points lineage decisions are made, where key transcription factors initiate cell type specific gene expression programs. These programs are stabilized by the epigenetic activity of recruited chromatin modifying cofactors. An example gives the association of the transcription factor RUNX1 with the protein arginine methyltransferase 6 (PRMT6). We could show that PRMT6 is differentially recruited to RUNX1 target genes during differentiation. Here, PRMT6 mediates a repressive chromatin environment by establishment of a histone modification pattern with high H3R2me2a and low H3K4me3. Interestingly, the repressive RUNX1/PRMT6 complex is formed cell-type and promoter dependent. This way RUNX1 is able to initiate a specific cell type dependent gene expression program, while actively repressing the competing program. Importantly, inhibition of PRMT6 by shRNA

or small molecule inhibitor leads to growth inhibition and a promotion of erythropoiesis. Our data reveal that the RUNX1/PRMT6 axis could be a molecular target to facilitate enhanced erythropoiesis for regenerative medicine and may suppress cell growth in a therapeutic setting for the treatment of leukemia.

Session/Abstract ID: Cancer & Inflammation / 16614 /CA-25

Non-invasive diagnosis of bladder cancer in urine by label-free Raman micro-spectroscopy

Presenting author:

Tatjana Lehtonen

Author(s):

Tatjana Lehtonen, Hesham Yosef, Sascha Krauß, Hendrik Jütte, Andrea Tannapfel, Heiko Käfferlein, Thomas Brüning, Florian Roghmann, Joachim Noldus, Axel Mosig, Samir El-Mashtoly, Klaus Gerwert

The current gold standard for diagnosis of bladder cancer is cystoscopy, which is an invasive and painful for patients. Non-invasive diagnosis of urothelial carcinoma (UC) remains challenging. The only non-invasive diagnostic method used in clinical practice is urine cytology, which is used as an adjunct for cystoscopy. However, urine cytology has been criticized for its low sensitivity (~80% for high-grade), especially in cases of low-grade (20-53%). Here, a novel non-invasive, label-free method applied on urine is presented with high sensitivity. Coherent anti-Stokes Raman scattering (CARS) imaging of urine sediments was used first for fast preselection of urothelial cells. In the second step, Raman spectral imaging of urothelial cells was performed. A supervised classifier, random forest, was then used to differentiate between normal and cancerous urothelial cells with 100% accuracy. In addition, the Raman spectra do not only provide morphological changes but also specific marker bands provide molecular resolution. For instance, Raman marker bands show a decrease in the glycogen level and an increase of fatty acids levels in cancer cells in comparing with the normal cells. These results pave the way for "spectral" cytology of urine using Raman micro-spectroscopy. Furthermore, we demonstrate that CARS/SHG microscopy has a prospective use as a fast label-free imaging method for prescreening large amounts of cells in cytopathological samples.

Session/Abstract ID: Other (free) topics / 16604 /OT-51

Structural insights into different Tc toxins of human and insect pathogenic bacteria

Presenting author:

Franziska Leidreiter

Author(s):

Franziska Leidreiter, Daniel Roderer, Christos Gatsogiannis, Stefan Raunser

Tc toxin complexes are α -helical pore-forming toxins that use a syringe-like mechanism to penetrate the membrane of specific target cells and deliver a toxic enzyme into the cytosol. Tc toxins are composed of three components: TcA, TcB and TcC; and only the tripartite complex (A5BC) is biologically active. TcA forms a translocation pore, whereas TcB and TcC assemble into a cocoon that harbors the toxic cargo. While the structure of TcdA1 from *P. luminescens* has been studied in detail, it is not yet known whether the structure of TcAs is conserved and consequently shared by other members of the family. Most importantly, the basis for the host specificity, which has been described for Tc toxins, remains elusive. We therefore determined atomic resolution structures of different TcAs: XptA1 (*X. nematophila*), TcdA4 (*M. morgani*) and TcdA1 (*P. luminescens*). The structures were obtained using cryo-EM at a resolution of 2.7, 3.0 and 2.6 Å, respectively. The overall composition and domain organization of the TcAs is identical, they assemble as a pentameric bottle-shaped structure with a central α -helical channel surrounded by an outer shell composed of conserved α -helical domains and more variable β -sheet domains. Our results suggest that TcAs from different organisms share a common mechanism of action, while the variability in β -sheet regions where potential receptor binding domains are located enable targeting of different hosts.

Session/Abstract ID: Cancer & Inflammation / 16566 /CA-21

The enzymatic activity of glycerophosphodiesterase EDI3

Presenting author:
Gregor Leonhardt

Author(s):
Gregor Leonhardt

The importance of choline and phospholipid metabolism in physiological and pathological conditions, such as cancer is increasingly recognized. We previously described the glycerophosphodiesterase (GDE) Endometrial Differential carcinoma 3 (EDI3, GPCPD1; GDE5) as a key enzyme in both metabolic pathways due to its hydrolysis of glycerophosphocholine (GPC) to choline and glycerol 3-phosphate (G3P). The enzyme has two functional domains, the GDE domain which is the enzymatic active centre, and a carbohydrate binding domain (CBM), the function of which is unknown. EDI3 is a member of the evolutionary conserved GDE protein family whose members have diverse functions. Early work showed an association between high EDI3 expression in the primary tumours of endometrial cancer patients with metastasis and worse survival. Subsequent in vitro analyses showed EDI3 to be relevant for specific cellular processes, such as migration. However, EDI3's function is not fully elucidated. We developed a simple kinetic assay with which we are able to measure the enzymatic choline turnover. This can be mirrored as the activity of EDI3. Here, we show how specific point mutations of EDI3 lead to decreased enzymatic activity, and a deletion of the different EDI3 domains completely deactivate the enzymatic function. Further analysis will investigate the mechanism underlying the migration phenotype, and whether the decreased enzymatic activity diminishes the migration and other EDI3-related phenotypes.

Session/Abstract ID: Cancer & Inflammation / 16090 /CA-01

p21-activated kinases (PAKs) regulate invadopodia formation in cancer cells

Presenting author:
Michaela Lesjak

Author(s):
Michaela Lesjak, Nicole Nicholas, Aikaterini Pipili, Claire Wells

PAKs, Ser/Thr kinases best known as effectors of the small GTPases Cdc42 and Rac1, have been implicated in the regulation of various cellular processes. Overexpression of PAKs found in cancer correlates with poor prognosis and metastasis. During invasion cancer cells form protease-secreting protrusions, termed invadopodia, which enable them to invade the extracellular matrix.

Using patient-derived cell strains, we demonstrated that PAK1 and PAK4 expression are significantly increased in invasive melanoma compared to melanocytes. Isoform-specific depletion in two invasive melanoma cell lines (WM-115 and A-375M2) showed that PAK1 and PAK4 are both required for in vitro invadopodia formation. In a zebrafish embryo in vivo model, depletion of both PAK isoforms reduced cell invasion from the yolk sac to the tail. Our detailed actin analysis in PAK-depleted cells revealed that the two isoforms have distinct functions during the invadopodia life cycle; PAK1 is needed for nascent invadopodia formation, while PAK4 drives maturation.

Since PAK4 expression was also increased in high-grade breast cancer patient samples, we wanted to confirm the importance of PAKs for invadopodia formation in this context. Indeed, both siRNA-mediated knockdown and isoform-specific inhibition in invasive MDA-MB-231 breast cancer cells resulted in similar findings, supporting a crucial role for PAKs in cancer dissemination across tissue types and identifying these proteins as a potential therapeutic target.

Session/Abstract ID: Cell signalling and membrane trafficking / 16488 /CS-32

Proteolytic Control of Interleukin-11 Signalling

Presenting author:

Juliane Lokau

Author(s):

Juliane Lokau, Christoph Garbers

Proteolytic processing of cytokine receptors is an important regulatory element for their signalling capacity, which not only regulates the amount of the receptors on the cell surface, but also creates soluble receptors with distinct biological functions.

Interleukin (IL)-11 is a member of the IL-6 cytokine family with several biological functions, including bone formation, fertility and haematopoiesis. Additionally, recent studies revealed a role for IL-11 in the development of gastric cancer. IL-11 signalling is initiated by binding of the cytokine to the IL-11 receptor (IL-11R), which induces the recruitment of two molecules of the signal transducing receptor glycoprotein (gp)130 and the activation of intracellular signalling cascades, mainly the Jak/STAT pathway.

Here, we show the IL-11R is a substrate for the protease ADAM10 and that the IL-11R stalk region is the main determinant of ectodomain shedding. Activation of ADAM10 results in loss of cell-surface IL-11R and the release of a soluble (s)IL-11R. The sIL-11R binds IL-11, and this complex is able to activate cells that do not express the IL-11R. We have termed this pathway IL-11 trans-signalling, which can be specifically blocked with the anti-inflammatory designer molecule sgp130Fc.

The initial cleavage induces the degradation of the remaining IL-11R membrane stub via the γ -secretase complex and the proteasome. In conclusion, we uncovered a novel IL-11 signalling pathway that is controlled by proteolysis.

Session/Abstract ID: Molecular machines in Bioenergetics / 16618 /MM-02

Zn-ATPase: Observation of Hydrolysis Reaction by Vibrational Spectroscopy

Presenting author:

Mathias Lübben

Author(s):

Benjamin Blask, Daniel Mann, Carsten Kötting, Klaus Gerwert, Mathias Lübben

ZntA is a detoxifying P-type ATPase which drives the transport of Zn out of the cytoplasm of *Escherichia coli*, using metabolic energy in form of ATP. We started to investigate the reaction mechanism of this integral membrane protein using FTIR spectroscopy. To this purpose we overexpressed ZntA and reconstituted the purified enzyme in phospholipid vesicles. The concentrated samples were depleted of water and applied on CaF₂ windows. Using the caged substrate precursor NPE-ATP at substoichiometric concentrations, we triggered the reaction by liberation of ATP via laser-induced photolysis. Although the protein has been incubated at low temperatures, we observed a two-phase process with a time constant of about 1 s of the first partial reaction. Comparison of the recorded absorbance changes with reference spectra of other ATPases yielded band assignments and corresponding time courses of ATP, ADP, free phosphate. Moreover, many absorbance changes in the amide-II region and in the range of amino acid side chains suggest the presence of a protein intermediate. Due to the complexity of the P-type ATPase reaction mechanism, it is expected that additional partial reaction steps are either superposed in the observed absorbance time courses or may be too fast to be kinetically resolved. However, these extra steps might be resolved by application of another caged ATP compound allowing much faster substrate release.

Session/Abstract ID: Other (free) topics / 16607 /OT-52

Local Mode Analysis: Decoding IR Spectra by Visualizing Molecular Details

Presenting author:

Matthias Massarczyk

Author(s):

Matthias Massarczyk, Till Rudack, Jürgen Schlitter, Carsten Kötting, Klaus Gerwert

Integration of experimental and computational approaches to investigate chemical reactions in proteins has proven to be very successful. Experimentally, time-resolved FTIR difference-spectroscopy monitors chemical reactions at atomic detail. To decode detailed structural information encoded in IR spectra, QM/MM calculations are performed. Here, we present a novel method which we call local mode analysis (LMA) for calculating IR spectra and assigning spectral IR-bands on the basis of movements of nuclei and partial charges from just a single QM/MM trajectory [1]. Through LMA the decoding of IR spectra no longer requires several simulations or optimizations. The novel approach correlates the motions of atoms of a single simulation with the corresponding IR bands and provides direct access to the structural information encoded in IR spectra. Either the contributions of a particular atom or atom group to the complete IR spectrum of the molecule are visualized, or an IR-band is selected to visualize the corresponding structural motions. Thus, LMA decodes the detailed information contained in IR spectra and provides an intuitive approach for structural biologists and biochemists. The unique feature of LMA is the bidirectional analysis connecting structural details to spectral features and vice versa spectral features to molecular motions.

REFERENCES

[1] M. Massarczyk*, T. Rudack*, J. Schlitter, J. Kuhne, C. Kötting, and K. Gerwert,

J. Phys. Chem. B, 121, 3483–3492 (2017).

Session/Abstract ID: Lunch Sessions / 16370 /OT-59

Illuminating the serotonergic system - Modulation of GPCR signaling by light

Presenting author:

Olivia Masseck

Author(s):

Olivia Masseck, Katharina Spoida, Stefan Herlitze

G-Protein coupled receptors (GPCRs) integrate extracellular cues into intracellular signals to modulate the cellular state. Because of their diverse modulatory function GPCRs represent one of the major drug targets of the pharmaceutical industry. Until now, the characterization and control of GPCRs and their intracellular signaling cascades has mainly relied on chemical compounds, which either activate or inhibit GPCR pathways albeit with limited receptor and cell-type specificity and low temporal and spatial resolution. Recently, serotonergic chimeric GPCRs, which can be activated by light, have been developed by our laboratory to control signaling cascades in a cell- and receptor type specific way. Serotonin (5-HT) has been shown to modulate emotional behavior and alterations in 5-HT levels have been related to different disease states including anxiety disorders and depression. We now use light activated serotonergic receptors to investigate neuronal circuitries, which are thought to be involved in the development and manifestation of anxiety and depression. Additionally, our research focuses on the development of genetically encoded sensors to illuminate the role of the 5-HT system in general.

Session/Abstract ID: Other (free) topics / 16314 /OT-16

FRET monitoring of conformational changes in a nonribosomal peptide synthetase

Presenting author:

Florian Mayerthaler

Author(s):

Florian Mayerthaler, Jonas Alfermann, Henning D. Mootz

Nonribosomal peptide synthetases (NRPSs) are large modularly organized enzymes that synthesize a plethora of bioactive and pharmaceutically interesting peptides. One set of discrete domains acts as a module and incorporates specifically one of over 530 different monomers (1). During the enzymatic cycle, the domains adopt multiple structural configurations and undergo large conformational changes. However, the regulation and the dynamics of the domain interactions are poorly understood (2). Here, we apply Förster Resonance Energy Transfer (FRET) spectroscopy to monitor in solution and in real-time crucial interactions between NRPS domains (3). The required fluorophores were either introduced genetically or using site-specific Michael-like addition with maleimides. Even though FRET is often applied to elucidate protein dynamics (4), so far it has not been applied to NRPS because of their size and complexity. In this study, we report first insights from solution measurements into the conformational dynamics of the catalytic cycle of NRPS. These investigations will foster our understanding of the biosynthetic NRPS machinery and, consequently, will facilitate their bioengineering.

(1)S. Caboche et al., J. Bacteriol., 2010

(2)J. Zettler et al., FEBS J., 2010

(3)J. Alfermann et al., 2017 (accepted)

(4)T. Heyduk, Curr. Opin. Chem. Biol., 2002

Session/Abstract ID: RNA-Regulation und Transport / 16135 /RR-01

Drosophila Sister of Sex-lethal antagonizes Sxl-lethal-dependent splicing to maintain a male-specific gene expression pattern

Presenting author:

Jan Medenbach

Author(s):

Rebecca Moschall, Oliver Rossbach, Gerhard Lehmann, Lars Kullmann, Norbert Eichner, Daniela Strauss, Nicholas Strieder, Julia Engelmann, Gunter Meister, Michael Krahn, Jan Medenbach

In *Drosophila* female development is governed by a single RNA-binding protein, Sex lethal (Sxl), that controls the expression of key factors involved in dosage compensation, germline homeostasis and the establishment of female morphology and behaviour. Sxl expression in female flies is maintained by an auto-regulatory, positive feedback loop with Sxl controlling splicing of its own mRNA. Until now, it remained unclear how males completely shut down the Sxl expression cascade and protect themselves against runaway protein production.

We have identified the protein Sister of sex lethal (Ssx) as an antagonist of Sxl auto-regulatory splicing. Sxl and Ssx have a comparable RNA-binding specificity and compete for RNA regulatory elements present in the Sxl transcript. Ablation of the *ssx* gene results in a low level of productive Sxl mRNA splicing in male flies; and in cultured *Drosophila* cells, Sxl-induced changes to alternative splicing can be reverted by the expression of Ssx. In sum, this demonstrates that Ssx safeguards male animals against Sxl protein production to establish a stable, male-specific gene expression pattern.

Session/Abstract ID: Cancer & Inflammation / 16376 /CA-09

Bifunctional Immunoactive siRNAs as an Approach to Personalized AML Therapy

Presenting author:

Hanna Meinl

Author(s):

Hanna Meinl, Marcus Zeithöfler, Samet Kocabey, Tim Liedl, Wolfgang Hiddemann, Marion Subklewe, Stefan Endres, Simon Rothenußer, Felix Lichtenegger

We strive to combine two different concepts to develop a new specific and personalized treatment for acute myeloid leukemia (AML). siRNAs are used to knock down a gene that is essential for the survival of the leukemic cells. By adding a triphosphate modification to the 5' end, the siRNA molecules additionally become ligands for the cytosolic pattern recognition receptor RIG-I (retinoic acid inducible gene I). Its activation mimics viral infection and leads to the production of inflammatory cytokines and induction of apoptosis in the target cell.

This concept was successfully tested in vitro for several target genes in AML cell lines. We could demonstrate that the specific gene knockdown inhibited proliferation and increased apoptosis. Activation of RIG-I by triphosphate-modification of the identified siRNA additionally stimulated an inflammatory response by the leukemic cells and increased their apoptosis rate.

A major hurdle for all siRNA-based anti-cancer strategies is the specific delivery of the RNA into tumor cells. We are testing DNA-based nanoparticles coupled with folic acid that target the folic acid receptor which is overexpressed in several tumor cells. These nanoparticles delivered the siRNA largely to the endosomes. Mechanisms regulating the delivery from endosomes to the cytosol need to be worked out.

Our in vitro studies showing the bifunctional activity of modified siRNAs in AML cells is the basis for further analysis of this concept in vivo in mouse models.

Session/Abstract ID: Other (free) topics / 16469 /OT-34

TGFβ1-induced cell migration of NSCLC lung cancer cells by Smad-dependent and -independent mechanisms

Presenting author:

Andre Menke

Author(s):

Julia Seiz, Laura Scharlibbe, Dirk Lohfink, Klaudia Giehl, Andre Menke

TGFβ1 concentration is enhanced in many epithelial tumors but its role during development of carcinomas remains contradictory. TGFβ1 causes diverse and sometimes opposing effects during carcinogenesis even in the same type of tissue. In many tumor types TGFβ1 promotes tumor progression and metastasis in later tumor stages. Here, we compared TGFβ1-induced cell migration with Smad4-dependent and -independent signaling in human NSCLC cell lines.

Most of the examined cell lines express TGFβ receptor proteins and exhibit Smad-mediated gene expression. 6 of 8 NSCLC cell lines responded to TGFβ1 with Smad2/3 phosphorylation and activation of Smad-responsive luciferase reporter constructs. 5 of 8 NSCLC cell lines react to TGFβ1 also with activation of further signaling pathway not related to Smad-signaling such as the MAPK p38 and the phosphoinositol-3-kinase/AKT pathway.

In contrast to other carcinoma types, in NSCLC cell lines TGFβ1 maintain its capability to inhibit cell proliferation and gain the property to induce cell migration and invasion.

The small GTPase Rac1b has been reported to be a potent regulator of TGFβ-driven cell migration. In our study we detected Rac1b expression in many NSCLC cell lines, but a major influence on spontaneous or TGFβ-induced cell migration was not observed.

In summary, our data support an important role of TGFβ1 in invasive growth of NSCLC cells, which is not strictly correlated with inactivation of the TGFβ-Smad-mediated proliferation control.

Session/Abstract ID: Cell signalling and membrane trafficking / 16271 /CS-13

The Parkin-coregulated gene PACRG rescues defective NF- κ B signaling in SHARPIN-deficient cells by stabilizing the linear ubiquitin assembly complex

Presenting author:

Jens Meschede

Author(s):

Jens Meschede, Maria Sadic, Kathrin Müller-Rischart, Jörg Tatzelt, Konstanze F. Winklhofer

The E3 ubiquitin ligase Parkin shares a bi-directional promoter with PACRG, the Parkin-coregulated gene. As the expression patterns of Parkin and PACRG widely overlap we explored a possible role of PACRG in pathways linked to the function of Parkin. We observed that PACRG has no impact on mitophagy but influences NF- κ B signaling. In PACRG-deficient cells, TNF α -induced NF- κ B-dependent transcription and nuclear translocation of p65 are markedly decreased. This defect in canonical NF- κ B activation in cells silenced for PACRG expression is accompanied by a decrease in ubiquitination mediated by the linear ubiquitin assembly complex (LUBAC), which is composed of the two RBR (RING-between-RING) E3 ubiquitin ligases HOIP and HOIL-1L and the adaptor protein SHARPIN. PACRG is recruited to LUBAC and interacts with HOIP similarly to SHARPIN and HOIL-1L. In SHARPIN-deficient cells, PACRG restores HOIP-dependent linear ubiquitination and prevents TNF-induced cell death. Our study identified the scaffold protein PACRG as a regulator of linear ubiquitination by stabilizing LUBAC.

Session/Abstract ID: Cell signalling and membrane trafficking / 16490 /CS-33

APEX-based Proximity Labeling reveals Hedgehog-induced Remodeling of the Ciliary Proteome

Presenting author:

David Mick

Author(s):

David Mick, Marian Kalocsay, Steven Gygi, Maxence Nachury

Primary cilia are central signaling hubs of mammalian cells that form specialized compartments by dynamically concentrating signaling molecules to transduce external stimuli. To fulfill this function, primary cilia must rapidly alter their protein content in response to various signals. Yet, the extent of the proteomic changes in cilia during signaling remains unknown because of the limited means to assess ciliary protein abundances in an unbiased and highly sensitive manner.

To overcome our inability to purify primary cilia with classic biochemical methods, we established a proximity labeling-based approach using cilia-targeted ascorbate peroxidase (cilia-APEX) to specifically biotinylate ciliary proteins in living cells, which allows effective isolation and identification by mass-spectrometric methods. We have expanded this technology to resolve the temporal protein distribution in cilia by state-of-the-art quantitative mass-spectrometry approaches. Here, we describe the proteomic changes in cilia during Sonic Hedgehog signaling, a central signaling pathway in embryonic development and tissue patterning. Our comprehensive analyses provide further insights into the mechanistic involvement of cAMP-dependent protein kinase (PKA) in Sonic Hedgehog signaling. Moreover, our work demonstrates that proximity labeling can be applied to time-resolved proteomics of cellular subdomains and provides novel clues on how the primary cilium generates its unique signaling environment.

Session/Abstract ID: Cancer & Inflammation / 16556 /CA-17

A novel role for the anti-apoptotic protein Survivin in DNA repair and replication

Presenting author:

Stefanie Mosel

Author(s):

Stefanie Mosel, Elisabeth Schröder, Shirley Knauer

Survivin is a member of the inhibitor of apoptosis protein (IAP) family and a fundamental protein for genomic stability during mitosis. In cancer, a markedly increased Survivin expression is associated with tumor resistance against chemo- and radiotherapy, thus designating it an attractive therapeutic target. In contrast to its well-studied role during mitosis, little is known about the functional role during interphase, especially during replication.

We could detect Survivin expression already during S phase, and thus significantly before its previously described stringently regulated increase during G2M transition. Functionally, knockdown of Survivin leads to a reduction in replication fork speed as investigated by DNA fiber assay analyses. During S phase, the chromosomal passenger complex (CPC) proteins Survivin, Aurora B kinase, Borealin and INCENP accumulate in distinct nuclear foci co-localizing with the replication associated protein PCNA. An in situ proximity ligation assay revealed a direct interaction of PCNA and the CPC. Also co-immunoprecipitation analyses suggest a biochemical interaction of PCNA with the members of the CPC during S phase. Indeed, a potential PCNA binding motif could be identified within INCENP.

In sum, our data indicate a novel, previously unknown function for Survivin and the other CPC members apart from mitosis, once more underlining the pivotal role of Survivin in the context of genomic instability and tumorigenesis.

Session/Abstract ID: Emerging Analytical Methods / 16554 /AM-06

Optogenetic AKT and RAF for the study of oncogenic pathways and processes

Presenting author:

Wignand Mühlhäuser

Author(s):

Wignand Mühlhäuser, Lena Werstein, Wilfried Weber, Gerald Radziwill

Optogenetics, compared to other techniques, grants unmatched spatiotemporal control over chimeric signaling proteins used in the study of signaling. Using light as activator, it is straight forward to address and mimic dynamic aspects of cell signaling by alternating duration and/or intensity of irradiation. We focus on the *A. thaliana* originating cryptochrome 2 (CRY2) / cryptochrome interacting basic helix-loop-helix protein 1 (CIB1) interaction pair. CRY2 interacts with CIB1 upon irradiation with blue light (~ 450 nm) in the range of seconds. The interaction disassembles in the dark with a half-life of 5 min. Cryptochrome 2 is a versatile tool that has been used in the generation of various optogenetic signaling proteins. We are interested in the PI3K/AKT and RAF/MEK/ERK pathways and the cross-talk between those. Our repertoire of optoSignaling molecules is composed of optoRAF, optoAKT and optoCNK1. Additionally, an optogenetic toolbox with CIB1 variants anchored at endomembranes of subcellular compartments is available. This toolbox can be used to recruit a protein, fused to CRY2, easily towards a specific compartment. Such a system allows characterization of the dynamic signaling properties and the interactome of the protein of interest at the specific compartment and is independent from upstream activation. This independence is suited for the investigation of isoform specific signaling. Here we provide examples of how optogenetic can be used to study oncogenic signaling.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16078 /OT-33

Biosensing of Extracellular Complexes of Complete Glycosylphosphatidylinositol-Anchored Proteins and Phospholipids as Biomarkers for Stress-Induced Diseases

Presenting author:

Günter Müller

Author(s):

Günter Müller, Andreas Herling, Matthias Tschöp

A novel "phenomenological" approach may lead to biomarkers for the prediction and stratification of stress-induced disorders, such as type 2 diabetes (T2D) with higher informative value than traditional phenotypic, peptidic and genotypic ones. It relies on extracellular complexes constituted by glycosylphosphatidylinositol-anchored proteins (GPI-AP), equipped with the complete GPI anchor, and phospholipids (GAPEC): GPI-AP have been shown to exhibit high susceptibility for release in GAPEC from the surface of mammalian cells in response to metabolic stress, such as high glucose, fatty acids and reactive oxygen species [1,2] prevalent during T2D. However, the presence of GAPEC in body fluids of T2D patients has not been studied so far.

To overcome these hurdles, a novel chip-based biosensor was used for the detection and biophysical characterization of GAPEC. Its principle relies on the generation of horizontal surface acoustic waves (SAW) within the gold surface of a microfluidic four-channel chip. Any interaction of (macro)molecules with the gold surface will result in corresponding changes in frequency and amplitude of the SAW reflecting binding of GAPEC to and their biophysical properties (size, shape, viscoelasticity and rigidity). Preliminary data indicate that SAW signatures will be characteristic for the overall contents and biophysical characteristics of all GAPEC, either as summation signals or as 1D-/2D-signatures of high informative value.

Session/Abstract ID: Other (free) topics / 16462 /SM-01

Biosensing of Complete Glycosylphosphatidylinositol-Anchored Cell Surface Proteins (GPI-AP) in Serum as Biomarkers for Metabolic Diseases

Presenting author:

Günter Müller

Author(s):

Günter Müller, Andreas Herling, Matthias Tschöp

GPI-AP with complete glycolipid anchor which become released from the plasma membrane of a donor cell in response to certain nutritional or hormonal stimuli upon incorporation into phospholipid micelles, lipoprotein-like particles or small vesicles, such as exosomes and microvesicles (EMVs), routinely escape detection by standard proteomic procedures. To study the putative transfer of the GPI-AP from donor to acceptor cells and its physiological roles for the (dys)regulation of glucose and lipid metabolism in the normal and diabetic/obese state, a novel dynamically operating biosensor technology was introduced for the detection of complete GPI in its various assembly states, i.e. in mixed phospholipid micelles as multimers, in lipoprotein-like particles and in EMVs. Biosensing of serum from normal, diet-induced obese and diabetic rats revealed the presence of GPI-AP in the various assembly states in differential quantitative and qualitative fashion depending on body weight and insulin-resistant state (i.e. blood glucose and insulin levels) as monitored by differential signatures of amplitude and phase of the surface acoustic waves. In future, the relationship between the differential release of GPI-AP, the putative systemic availability of the released GPI-AP and the pathogenesis of diabetes will be tested in diabetic mice. Causality would suggest the usefulness of the complete GPI-AP released as functional biomarkers and of the releasing mechanism as drug target.

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16375 /GE-05

Structure and function of the chromatin remodeling enzyme ISWI

Presenting author:

Felix Müller-Planitz

Author(s):

Felix Müller-Planitz

Chromatin remodeling enzymes assume important roles in all nuclear processes by regulating access to nucleosomal DNA. To determine the architecture of the ISWI remodeling enzyme, we have developed an integrative structural approach that combines protein cross-linking coupled to mass spectrometry and small-angle X-ray scattering with computational modeling. Our results show the ATPase module in a resting state with both ATPase lobes twisted against each other, providing support for a conformation that was recently trapped by crystallography. The NTR, a conserved but functionally poorly characterized domain, docks against the ATPase lobes in configuration that is reminiscent of the remodeling enzyme Chd1, despite the NTRs of ISWI and Chd1 being unrelated. Motifs in the NTR, including AutoN and AcidicN, help recognize the histone H4 N-terminal tail, an epitope ISWI critically depends on. The H4 tail binds to the ATPase domain but its binding site does not overlap with the docking site of AutoN as hypothesized before. Finally, we show that the full-length enzyme adopts a compact structure in solution with the C-terminal HSS domain packing against the ATPase module. Our data imply a series of conformational changes to reach previously characterized structural and functional states. In summary, our data shed light on the intricate structural and functional regulation of ISWI by peripheral domains and uncover surprising parallels with remodelers of other families.

Session/Abstract ID: Other (free) topics / 16027 /OT-03

Expression of genes involved in Taxol biosynthetic pathway in Taxus baccata L. and application of Magnetic- and carbon-based nano-adsorbents for pre-purification of Taxol

Presenting author:

Mohammad Reza Naghavi

Author(s):

Mohammad Reza Naghavi, Jaber Nasiri

This work was undertaken to elucidate the consequences of some environmental cues (i.e. day length, temperature, hours of sunlight and relative humidity) on the expression patterns of TXS, DBAT, BAPT and DBTNBT genes contributed to the taxol biosynthetic pathway. Our results indicated that environmental cues have synergistic or antagonistic regulatory roles of on transcription activity and taxanes accumulation in yew, though DBAT activity is less influenced, could be accordingly a rate-limiting enzyme. Furthermore, a modified analytic hierarchy process (AHP) approach was employed to monitor the most reliable callus maintenance media of *T. baccata* callus cultures in terms of five criteria. Our results connoted that L-glutamine-based feeding appears to generate more significant results either for calli growth continuously or taxanes production, while, for stems, both amino acid supplies had fairly equal worth. Meanwhile, considering decolorization efficiency, purity of taxol, recovery and reusability of adsorbents, Fe₃O₄NPs@GO (50 g/L) in dichloromethane was selected as the best medium for pre-purification of paclitaxel. Finally, based on RSM data, the optimum conditions to simultaneously acquire the maximum EPPR (94.0 %) and ETP (11.4 %) were recorded as adsorbent dosage of 37.7 g L⁻¹, sorption temperature of 30.7 °C and agitation power of 153.1 rpm; and the predictive results were confirmed using experimental rechecking survey.

Session/Abstract ID: Cell signalling and membrane trafficking / 16473 /CS-28

Ha-Ras mediated neuroprotection - involvement of plasma membrane localized Voltage-dependent anion channel 1 (VDAC-1)?

Presenting author:

Sebastian Neumann

Author(s):

Sebastian Neumann, Konstantin Kuteykin-Teplyakov, Rolf Heumann

The proto-oncogene GTPase Ras regulates neuronal neurite growth, differentiation and survival. For the investigation of Ras in the nervous system, we established the synRas mouse model in which constitutively activated V12-Ha-Ras is selectively expressed in postmitotic neurons. Besides other changes, quantitative proteome analysis uncovered a decrease of 70% for the voltage-dependent anion channel-1 (VDAC-1) in cortex and hippocampus of adult synRas mice. The outer mitochondrial membrane contains VDAC-1 (mt-VDAC-1) and in addition the plasma membrane (pl-VDAC-1). In mouse, VDAC-1 mRNA is alternatively spliced leading to mt-VDAC-1 and pl-VDAC-1 mRNA. Here we investigated if possible changes of VDAC-1 expression participates to the neuroprotection in the synRas mouse model.

In adult synRas mice, the mRNA level of pl-VDAC-1 is selectively decreased whereas the mt-VDAC-1 mRNA is unchanged in hippocampus and cortex. Co-expression of activated Ras prevented cell death, which was mediated by the overexpression of either mt-VDAC-1 or pl-VDAC-1 in combination with a neurotoxic insult in PC12 cells. Further experiments in synRas mice derived primary cortical cultures confirmed the selective decreased expression of pl-VDAC-1 level. The splicing of VDAC-1 mRNA might be altered by activated Ras resulting in a reduced expression of pl-VDAC-1, which contributes to the mechanism of neuroprotection in synRas mice.

Session/Abstract ID: Cancer & Inflammation / 16410 /CA-05

FAK-Inhibitor-PF-573228 can reduce the Ionizing Radiation-induced Adhesion of Cancer Cells to Endothelial Cells in Vitro.

Presenting author:

Pascaline Nguemgo Kouam

Author(s):

Pascaline Nguemgo Kouam, Helmut Bühler, Anja Kochannek, Bettina Priesch, Thomas Hero, Irenäis A Adamietz

Radiotherapy is a most widely used treatment to manage cancer diseases. While about 30% of irradiation-treated patients will be cured, the remaining 70% will relapse locally or develop metastases. Here we investigated whether ionizing radiation influences the metastatic potential of cancer cells by affecting the interaction of tumor cells (TC) and endothelial cells (EC) during the dissemination process. We analysed changes in the adhesion of glioblastoma (U-87 MG, U-373 MG) and breast cancer cell lines (MDA-MB-231, MCF7) to a layer of EC. The cells were irradiated with 0, 0.5, 2, 4, or 8 Gy or treated with FAK-Inhibitor-PF-573228. The adhesion of TC to EC, both irradiated or not, was determined with 2 different methods: the VybrantTM cell adhesion assay based on fluorescent labeling of TC and the Ibidi pump system that allows to mimic the physiological blood stream in the vasculature. Our results show an increasing adhesion of TC to EC after irradiation. FAK-Inhibitor-PF-573228 was able to reduce the adhesion of non-irradiated cells but also the irradiation-induced increase in adhesion of both cells types. Furthermore, qRT-PCR and western blot analyses have revealed that the expression of adhesion-related proteins was increased in all cell types 4h after irradiation with 4 Gy. We assume that the irradiation of TC as well as of EC with photons might enhance adhesive interactions of these cells and thereby may affect important steps of metastasis.

Session/Abstract ID: Cancer & Inflammation / 16412 /CA-10

Extravasation of Tumor Cells might be promoted by Photon Irradiation

Presenting author:

Pascaline Nguemgo Kouam

Author(s):

Pascaline Nguemgo Kouam, Helmut Bühler, Irenäus A. Adamietz

Metastases are formed by cancer cells that have left the primary tumor mass and travelled by the body's highways-blood and lymphatic vessels to seek out new sites throughout the body where they may found new colonies (Weinberg R, 2014). This late step of tumor progression represents the major cause of death of cancer patients and remains not well understood until yet. In the present project, we questioned how photon irradiation affects extravasation, a crucial step in the metastatic cascade, using tumour cells of breast cancer and glioblastoma. To do so, we first analysed changes in the permeability of endothelium when it was exposed to irradiation, with a particular focus on metalloproteases (ADAM10 and ADAM17) and VE-cadherin (CD144). Our results indicate that photon irradiation increased the permeability of endothelial monolayers which was effectively blocked by a potent and selective ADAM10 metalloproteinase inhibitor (GI254023X). This effect seems to be related to the increased expression and activity of this protease under irradiation which leads to an increasing degradation of VE-cadherin in endothelial cells. ADAM10 was overexpressed in breast cancer and glioblastoma cells lines and was modulated under irradiation. Our results suggest a mechanism of increased activation of ADAM10-mediated VE-cadherin permeability in endothelial monolayers under irradiation. This effect might be influenced by VEGF. Since its expression is enhanced in tumor cells after irradiation.

Session/Abstract ID: Molecular machines in Bioenergetics / 16515 /MM-01

*Application of the TwinStrep-tag/Streptactin system for purification of photosynthetic membrane protein complexes from *T. elongatus**

Presenting author:

Marc Nowaczyk

Author(s):

Pasqual Liauw, Marc Nowaczyk

Membrane protein complexes, especially the photosystems, play a vital role in photosynthetic electron transfer. Photosystem II (PSII), a large multisubunit membrane protein complex found in the thylakoid membranes of cyanobacteria, algae and plants, catalyzes light-driven oxygen evolution from water and reduction of plastoquinone. Biogenesis of PSII requires coordinated assembly of at least 20 protein subunits, as well as incorporation of various organic and inorganic cofactors. The stepwise assembly process is facilitated by numerous protein factors that are transiently bound to distinct assembly intermediates. However, the characterization of these intermediate PSII species is hampered by their instability and low abundance. One way to elucidate the role of such transiently binding PSII factors is to enrich PSII complexes using a tagged-version of the PSII factor. Here we present the purification of PSII assembly intermediates using the TwinStrep-Tag – Streptactin System. The PSII species obtained are characterized by very high purity thus reducing samples complexity in their downstream analyses. This allows identification and unambiguous assignment of novel protein components.

Session/Abstract ID: Molecular machines in Bioenergetics / 16635 /MM-04

THE ROLE OF SMALL SINGLE TRANSMEMBRANE PROTEINS FOR NDH-1 ASSEMBLY AND STABILITY IN CYANOBACTERIA

Presenting author:

Marc Nowaczyk

Author(s):

Marc Nowaczyk

The cyanobacterial NADPH:plastoquinone oxidoreductase complex (NDH-1), that is related to Complex I of eubacteria and mitochondria, plays a pivotal role in respiration as well as in cyclic electron transfer (CET) and is involved in unique carbon concentration mechanisms (CCMs). Despite many achievements in the past, the complex protein composition and the specific function of many subunits of the different NDH-1 species remain elusive. We have discovered two novel small single transmembrane proteins (NdhP, NdhQ) with molecular weights below 5 kDa in a NDH-1 preparation from *Thermosynechococcus elongatus*. NdhP is a unique component of the ~450 kDa NDH-1L complex, that is involved in respiration and CET at high CO₂ concentration, and not detectable in the NDH-1MS and NDH-1MS' complexes that play a role in carbon concentration. C-terminal fusion of NdhP with his-tagged superfolder GFP and the subsequent analysis of the purified complex by electron microscopy and single particle averaging revealed its localization in the NDH-1L specific distal unit of the NDH-1 complex, that is formed by the subunits NdhD1 and NdhF1. Moreover, we could show that this small single transmembrane subunit, which was previously overlooked in genome and proteome analysis, is essential for function of the 450 kDa NDH-1L complex.

Session/Abstract ID: Other (free) topics / 16584 /OT-44

Nonstructural proteins 7 / 8 of Middle-East respiratory syndrome coronavirus: Primase or RNA-dependent RNA polymerase?

Presenting author:

Wasifa Nuriev

Author(s):

Wasifa Nuriev, Heike Laschin, Raffaele Ciriello, Rolf Hilgenfeld

Emerging coronaviruses such as SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus) and MERS-CoV (Middle-East Respiratory Syndrome Coronavirus) caused alarming outbreaks in recent years. While the SARS outbreak of 2003 was contained within 6 months, the number of MERS cases have been gradually accumulating since 2012. To this day, more than 2000 laboratory-confirmed cases have been reported, mainly on the Arab peninsula, with a very high fatality rate of about 35%. No vaccines or antiviral drugs are available for prevention or treatment.

In order to contribute to a better understanding of the viral replication mechanism of MERS-CoV, we focus on nonstructural proteins Nsp7 and Nsp8, which form a complex and play an important role in the replication machinery. The SARS-CoV Nsp8 is regarded a primase, which synthesizes a short primer for Nsp12, the canonical RNA-dependant-RNA-polymerase (RdRp) (Subissi et al., 2014). Furthermore, it has been observed that Nsp8 of feline coronavirus as well as of SARS-CoV has a primer-independant RdRp-activity by itself, which is enhanced by Nsp7 (Xiao et al., 2012; te Velthuis et al., 2012).

We investigate the molecular properties of MERS-CoV Nsp7+8 complexes by dynamic light-scattering (DLS) and RdRp assays. Furthermore, we aim at generating high-quality crystals to determine the structure by X-ray crystallography.

Session/Abstract ID: Cell signalling and membrane trafficking / 16548 /CS-38

Investigation of possibly new glutamate receptor auxiliary proteins - claudins

Presenting author:

Sebastian Obst

Author(s):

Sebastian Obst, Michael Hollmann

Ionotropic glutamate receptors (iGluRs) are the main excitatory receptors in the vertebrate central nervous system. They are further subdivided regarding their pharmacology and sequence identity into non-NMDA receptors that consist of AMPA and kainate receptors, NMDA receptors, and the elusive delta receptors. A functional receptor is formed as a homo- or heterotetrameric complex of receptor subunits and opens a cation-conducting channel pore upon release and binding of glutamate from the presynaptic site.

The transport and anchoring of AMPA receptors in the postsynaptic membrane is affected by TARPs (transmembrane AMPA receptor regulatory proteins). Most of them also alter the current response of iGluRs in different manners. TARPs are four-transmembrane domain proteins that belong to the tetraspanin superfamily.

The closely related claudins form tight junctions that are crucial for cell-cell adhesion of different tissues. Although it has been shown that TARPs can have similar tight junction-like properties, an investigation whether claudins can also show TARP-like effects on the currents of iGluRs has not been published.

We thus investigated 18 rat claudins and found claudin-20 and -24 that potentiate AMPA receptor currents and are currently looking into the remaining rat claudins. This project mainly focusses on electrophysiological identification and characterization of new claudin AMPA-receptor interactions and investigating general modulatory mechanisms by domain exchange.

Session/Abstract ID: Subcellular organisation / 16374 /SC-07

Linking microtubule and actin-based transport systems

Presenting author:

Zeynep Ökten

Author(s):

Zeynep Ökten

Inner organization of eukaryotic cells intimately depends on the active transport of diverse intracellular cargo on the ubiquitous actin and microtubule networks. The underlying mechanisms of such directional transport processes have thus been of outstanding interest. Despite decades of work, however, underlying mechanisms that link these two ubiquitous transport systems largely remained mysterious. We studied a physiologically relevant motor complex composed of Rab27a, melanophilin and myosin Va and found that it is surprisingly the adaptor protein melanophilin that toggled the binding preference towards actin or microtubules. Our results unmasked an unexpected regulatory dominance of the melanophilin adaptor protein over its associated motor and offer an unexpected mechanism for how filaments of the cytoskeletal network compete for the moving organelles to accomplish directional transport on the cytoskeleton in vivo.

Session/Abstract ID: Other (free) topics / 15996 /OT-02

BIOCHEMICAL IMPLICATIONS OF ADMINISTRATION OF HALOFANTRINE HYDROCHLORIDE (HALFAN) ON ESTRADIOL LEVELS OF FEMALE WISTAR RATS

Presenting author:

UCHENNA ONOCHIE

Author(s):

UCHENNA ONOCHIE

This study determines the effects of doses of halofantrine hydrochloride, a phenanthrene methanol

drug used in the therapeutic treatment of malaria on the estradiol levels of female wistar rats. A suspension of drugs at a dose of 0.2ml/kg body weight three times at six hourly intervals were administered orally to different groups of mature female rats for 2 weeks, 4 weeks and 6 weeks duration, control groups received similar treatment doses of normal saline. The animals were sacrificed on the 14th day, 28th day and 42nd day respectively after drug administration by cervical dislocation. Whole blood sample were collected for full blood count: (WBC, RBC, PCV, Hg and platelets counts). From the plasma, hormonal level was determined by radio-immunoassay, the activities of AST, ALT, ALP, TB and CB, lipid profile test: (TC, TG, HDL-C, LDL-C) and Kidney profile test: (urea, creatinine, BCO₃, Na, K and Cl) were also determined. The level of estradiol level following 2 weeks, 4 weeks and 6 weeks treatment was higher significant ($p < 0.05$) in all the groups compared to the control. The activities of AST, ALT, ALP, TB, CB, TC, TG, HDL-C, and LDL-C increased significantly ($p < 0.05$). The full blood counts and kidney profile tests increased in a dose dependent manner. These findings could signify toxicity of the drugs on the bone marrow of the rats, the increase in the full blood count indicates a pathological condition and renal dysfunction for the kidney profile tests.

Session/Abstract ID: Genome-editing proteins and other macromolecular structures / 16514 /GE-06

Characterization of the Type IV CRISPR-Cas system of *Aromatoleum aromaticum* EbN1

Presenting author:

Ahsen Özcan

Author(s):

Ahsen Özcan, Thomas Heimerl, Lennart Randau

CRISPR-Cas are adaptive and heritable immune systems found in many bacteria and most archaea. Cas proteins and processed CRISPR array transcripts, termed crRNAs, form a ribonucleoprotein complex targeting foreign DNA. Diversified CRISPR-Cas systems are found in nature and six types (I-VI) and sixteen subtypes have been classified. Type IV CRISPR-Cas systems have not been characterized experimentally. Therefore, we analyzed the Type IV CRISPR-Cas system of *Aromatoleum aromaticum* EbN1, which contains a megaplasmid-encoded CRISPR array and four cas genes (*csf1*, *csf2*, *csf3* and *csf5*).

Reconstitution of a Type IV CRISPR-Cas complex from *A. aromaticum* EbN1 was established in *E. coli* BL21 AI with a single crRNA sequence. The Csf2 protein was shown to form extended filaments suggesting that it acts as the backbone protein binding the mature crRNA spacer. This role resembles Cas7 protein functionality in Type I CRISPR-Cas systems. In addition, we observed processed repeat regions of crRNAs yielding a unique 7 nt-5'-terminal tag and a stable 3'-terminal stem loop structure. The Csf5 protein was identified to be responsible for the maturation of precursor-crRNAs. Recombinant ribonucleoproteins were isolated, purified and visualized by electron microscopy, providing first experimental evidence for the existence of Type IV surveillance complexes.

Session/Abstract ID: Cell signalling and membrane trafficking / 16486 /CS-31

Toxin mediated delivery of functional Nurr1 fusion protein as promising tool for programming of dopaminergic cells

Presenting author:

Dennis Paliga

Author(s):

Dennis Paliga, Sebastian Neumann, Fabian Raudzus, Rolf Heumann

The transcription factor nuclear receptor-related 1 (Nurr1) plays a key role in embryonic development and maintenance of mesencephalic dopaminergic neurons. These neurons degenerate in Parkinson's disease (PD) and are therefore of interest for cell replacement therapies. A prime downstream target of Nurr1 is the rate-limiting enzyme in dopamine synthesis, tyrosine hydroxylase. We bacterially expressed and purified a novel Nurr1 fusion protein which is able to enter the outer cell membrane via a cell-penetrating domain derived from bacterial toxin. After cytoplasmic cleavage of the fusion protein, Nurr1 is transported into the nucleus where it develops its transcriptional effects.

After protein transduction in dopaminergic SHSY-5Y cells, immunoblot analysis allowed the detection of the cleaved Nurr1 fusion protein fragment and showed an increased amount of tyrosine hydroxylase protein. Promoter activity assays revealed that the Nurr1 fusion protein transactivates the mouse and human tyrosine hydroxylase promoter. Neurotoxicity assays with 6-hydroxydopamine demonstrated a concentration-

dependent enhancement of cell viability compared to untreated controls. This finding suggests a protective effect of the Nurr1 fusion protein which may contribute to a novel concept in the field of future PD treatments.

Session/Abstract ID: Electron transport chains / 16546 /ET-03

Structural and functional characterization of the interaction of yeast cytochrome bcl with cytochrome c

Presenting author:

Vishnupriya Pandey

Author(s):

Vishnupriya Pandey, Carola Hunte

Biological energy conversion relies on cofactor-mediated transfer of electrons, which requires precise spatial and temporal control of the distance, orientation and environment of cofactors. A snapshot of a transient electron-transfer complex of the respiratory chain was captured with X-ray structures of *Saccharomyces cerevisiae* cyt bc1 complex with the mobile carrier cytochrome c bound. The interaction is mainly stabilized by non-polar forces provided by a small contact area which surrounds the respective heme clefts. The peripheral electrostatic interactions are in agreement with a steered binding which enforces the distinct orientation of the electron donating and accepting heme cofactors. Yet, it is not known whether physiological electron transfer is accomplished by this single conformation or by multiple productive complexes and why only a single cytochrome c is bound to the dimeric cyt bc1 in the X-ray structures. In order to address the role of single interface residues for structure and function of the electron transfer complex, we produced recombinant yeast cyt c. Production conditions were optimized and the protein quality was assessed. The interaction of recombinant cyt c with yeast cyt bc1 was structurally and functionally characterized. The complex was successfully crystallized and its structure determined at 2.0-Å resolution. This opens the way to systematically study structure-function relationships in the electron transfer complex.

Session/Abstract ID: Cell signalling and membrane trafficking / 16118 /CS-04

Phosphoinositide-dependent regulation of GABAergic neurotransmission at inhibitory postsynapses

Presenting author:

Theofilos Papadopoulos

Author(s):

Theofilos Papadopoulos

The formation of neuronal synapses and the dynamic regulation of their efficacy depend on the proper assembly of the postsynaptic neurotransmitter receptor apparatus. Receptor recruitment to inhibitory GABAergic postsynapses requires the scaffold protein gephyrin and the guanine nucleotide exchange factor collybistin (Cb). In vitro, the pleckstrin homology domain of Cb binds phosphoinositides, particularly phosphatidylinositol 3-phosphate (PI3P). In this work, we used a membrane-permeant PI3P derivative, time-lapse confocal imaging, electrophysiology, as well as knockdown and overexpression of PI3P-metabolizing enzymes in neurons. We provide the first in cellula evidence that PI3P located at early/ sorting endosomes regulates the postsynaptic clustering of gephyrin and GABAA receptors and the strength of inhibitory postsynapses. Our results show that an endosomal pool of PI3P, generated by the class III phosphatidylinositol 3-kinase, is important for the Cb-mediated recruitment of gephyrin and GABAA receptors to developing inhibitory postsynapses and thus the formation of postsynaptic membrane specializations. Furthermore, we provide first evidence that small Rho-like GTPases, particularly TC10, modulate the phosphoinositide-specificity of Cb and suggest a new model for the role of the TC10/Cb-interaction in the assembly of the gephyrin scaffold at postsynaptic membranes.

Session/Abstract ID: RNA-Regulation und Transport / 16652 /RR-08

Are symplasmic tissues appropriate routes for signaling based on RNA and other stuff? Not necessarily.

Presenting author:

Winfried Peters

Author(s):

Winfried Peters, Michael Knoblauch

All multicellular organisms maintain a degree of cytoplasmic continuity between at least some of their cells. Cytoplasmic connections (plasmodesmata) are ubiquitous and large in plants, allowing transcellular cytoplasmic bulk flow in extreme cases. The long-distance transport of photoassimilates is such a cytoplasmic bulk flow. It occurs in the sieve tubes of the phloem, a specialized network of symplasmic micro-pipes that traverses the entire organism. The discovery of RNA molecules in sieve tubes in the 1990ies fostered the idea that the tubes provide an "information superhighway" through which the plant's physiology and development is integrated and controlled. Consequently, the notion that symplasmic tissues are particularly well suited for the translocation of signaling molecules became popular among plant cell biologists.

Developmental and ultrastructural studies lead us to question the plausibility of the idea. As autonomously functional precursor cells differentiate into anucleate sieve tube members that lack the machinery for transcription and translation, the molecular elements of this machinery are released into the sieve tubes. This happens thousands of times per hour even in a tiny plant like *Arabidopsis*, which explains the presence of a confusing multitude of molecules in mature sieve tubes that have no apparent function in these cells – such as mRNAs. What looks like an information superhighway might in reality be a trash can.

Session/Abstract ID: Other (free) topics / 16615 /OT-55

Hierarchical assembly of DNA filaments with designer elastic properties

Presenting author:

Wolfgang Pfeifer

Author(s):

Wolfgang Pfeifer, Pascal Lill, Christos Gatsogiannis, Barbara Saccà

The elastic features of protein filaments are encoded in their component units and in the way they are connected, thus defining a biunivocal relationship between the monomer and the result of its self-assembly. Using DNA origami approaches, we constructed a reconfigurable module, composed of two quasi-independent domains and four possible interfaces, capable of facial and lateral growing through specific recognition patterns. Whereas the flexibility of the intra-domains region can be regulated by switchable DNA motifs, the inter-domain interfaces feature mutually and self-complementary shapes, whose pairwise association leads to filaments of programmable periodicity and variable persistence length. Thus, we show here that the assembly pathway leading to oligomeric chains can be finely tuned and fully controlled, enabling to emulate the formation of protein filaments using a single construction principle. This results into artificial materials with a large variety of ultrastructures and mechanical strengths comparable, or even superior, to their natural counterparts.

Session/Abstract ID: Cell signalling and membrane trafficking / 16501 /CS-34

Study of ionotropic glutamate receptors using photoswitchable ligands

Presenting author:

Stefan Pollok

Author(s):

Stefan Pollok, Leonie Inderwiedenstrasse, Andreas Reiner

Ionotropic glutamate receptors (iGluRs) mediate fast excitatory synaptic neurotransmission in the central nervous system of vertebrates. iGluRs are ligand gated ion channels assembled from four subunits. Glutamate

binding, channel opening and activation occur in the sub millisecond range and are followed by rapid desensitization of the receptor.

A key aspect for understanding these gating mechanisms is to study how receptor occupancy of the four ligand binding domains (LBDs) contributes to activation, desensitization and reactivation. We study homo- and heteromeric kainate receptors by expressing different subunits, e.g. GluK1, GluK2 and GluK5 in HEK293T cells and record their channel activity with patch clamp recordings. Fast receptor kinetics can be measured using outside-out patches and an ultra-fast perfusion system. To directly control receptor occupancy of specific subunits like GluK1 and GluK2 we use a family of photoswitchable tethered ligands (PTLs): the maleimide-azobenzene-glutamate (MAG). This photoswitch allows covalent coupling at the LBD via a cysteine substitution and cis/trans isomerization of the azobenzene group to bind/unbind the glutamate head group to the receptor with light pulses in the microsecond range. Combining these PTLs with electrophysiological recordings and pharmacological manipulations provides detailed insight into the gating processes of heteromeric kainate receptor complexes and their role in the nervous system.

Session/Abstract ID: Emerging Analytical Methods / 16272 /AM-02

Establishment of an in vivo and cell-specific method for the identification of palmitoyltransferase-interacting proteins in Drosophila using BioID

Presenting author:
Elena Porcellato

Author(s):
Elena Porcellato, Mahmoud Elsakka, Felix Wieland, Christoph Metzendorf

We are interested to identify palmitoylated transmembrane proteins (PTMPs) that cause branching of membrane protrusions. One strategy to obtain candidates is to identify PTMPs in highly branched cells, such as neurons. However, no methods exist to extract PTMPs cell-specifically. To overcome this shortcoming, we are establishing the proximity biotinylation system (BioID) in the fruit fly to then identify neuron specific PTMPs in vivo, by expressing

palmitoyltransferase-BioID fusion proteins cell-specifically.

Here we present the current state of establishing this method. Of the 22 different palmitoyltransferases (DHHC protein family) in *Drosophila*, we focused on a subset of 10 DHHCs that show neuronal or ubiquitous expression. As proof of principle in cell culture we find that DHHC-BioID is able to identify the specific interaction between the DHHC Hip14 and its known ligand SNAP25, and the highly similar SNAP24 in a co-overexpression system. Furthermore, by mass-spectrometric identification of biotinylated proteins we also found an interaction of endogenous SNAP24 in Hip14-BioID expressing cells. Applying Bioinformatics we are presently analyzing mass-spectrometric data generated in DHHC-BioID cell culture for the subset of 10 DHHCs mentioned above. First experiments in transgenic Hip14-BioID flies are promising: Hip14-BioID can be expressed and results in a moderately strong biotinylation pattern.

Session/Abstract ID: Other (free) topics / 16600 /OT-49

Near-atomic structure of jasplakinolide-stabilized malaria parasite F-actin reveals the structural basis of filament instability

Presenting author:
Sabrina Pospich

Author(s):
Sabrina Pospich, Esa-Pekka Kumpula, Julian von der Ecken, Juha Vahokoski, Inari Kursula, Stefan Raunser

The protozoan parasite *Plasmodium* is the causing agent of malaria, which is widespread in tropical and subtropical regions and results in more than half a million deaths every year.

In contrast to other apicomplexan parasites, the human pathogen *Plasmodium falciparum* (Pf) expresses two actin isoforms. With a sequence identity of less than 80% relative to each other and canonical actins, the Pfactins are among the most divergent ones.

Of particular interest is Pfactin 1, as it only forms short, unstable filaments in vitro. Moreover, the helical symmetry parameters of filaments that have been stabilized by Jasplakinolide (JAS), a toxin originally

isolated from the marine sponge *Jaspis johnstoni*, differ slightly from those of canonical actins.

Up to now only a low resolution structure of filamentous Pfactin 1 has been published¹, hampering a detailed comparison of Pfactin 1 to other actin isoforms.

We used transmission electron cryo microscopy to determine the three-dimensional structure of Pfactin 1 at a resolution of 3.8 Å. Based on the electron density map we were able to build a complete atomic model including JAS. By comparing the filamentous structure of Pfactin 1 and α -actin from *Oryctolagus cuniculus*, we identified several residues that have an impact on major interaction interfaces and possibly reduce the filament stability. Moreover, we characterized the binding site of JAS and the underlying hydrophobic interactions which stabilize the filament for the first time.

Session/Abstract ID: Other (free) topics / 16605 /OT-50

Structural studies of a toxin loaded Type VI secretion (T6S) effector complex using electron microscopy

Presenting author:
Dennis Quentin

Author(s):
Dennis Quentin, John C. Whitney, Joseph Mougous, Stefan Raunser

Participating in interbacterial competition and mediating virulence are two major tasks of almost every bacterium in order to survive in their respective environmental niche. To do so Gram-negative bacteria developed sophisticated protein secretion machineries for translocating a variety of effector proteins across their two membranes into the cytoplasm/periplasm of the host cell.

One of the latest discovered secretion systems is the T6S apparatus, contributing to the virulence of several human pathogens like *V. cholera* and *P. aeruginosa*. It uses a unique translocation mechanism, which is functionally and structurally related to the effector delivery of bacteriophages. Upon contraction of an outer sheath, an inner tube, consisting of stacked Hcp hexameric rings, is propelled outwards and finally pierces the target cell. Located at the tip of the inner

tube, is a tapering VgrG trimer attached, building the basis for the secreted effector complex.

In this study, we show for the first time the architecture of the Tse6 effector-loaded VrgG complex using negative stain EM. Surprisingly, Tse6-mediated toxicity requires the binding to an essential housekeeping protein, translation elongation factor Tu (EF-Tu). Furthermore we show, that a putative chaperoning protein, EagT6, is part of the complex and observe the adoption of different conformations in the presence/absence of detergent hinting to a conformational change upon membrane contact.

Session/Abstract ID: Other (free) topics / 16633 /OT-58

Directed protein evolution for thermophile biotechnology

Presenting author:
Kersten Rabe

Author(s):
Kersten Rabe

Recently there has been a growing interest in the use of non-standard organisms in biotechnology. Screening and prediction methods for directed protein evolution taking into account non-standard reaction conditions have to be developed, amongst them selection of a promising starting point for the protein evolution and the ability to screen for activity at non-standard conditions. In my group we are using a combination of several bioinformatics approaches enabling us to generate a small library of protein variants with improved thermostability as well as activity at an increased reaction temperature. As a first example, we have used a ketoacid decarboxylase from *Lactococcus lactis*, since its homo-dimeric architecture, with an active site shared between the monomers, renders stabilization challenging. Once improved variants have been identified these can in turn be utilized to test screening procedures which enable the evaluation of random mutant libraries. We have successfully used this procedure to evolve variants which have an increase activity at 60°C as well as drastically increased stability at lower temperatures.

In combination with new molecular tools for extremophile organisms enabling a quantitative, in vivo analysis of molecular events (e.g. protein expression) down to the single cell level, these

advances enable a continued expansion of the use of non-standard host systems for biotechnological application, especially those envisioned for whole cells biocatalysis.

Session/Abstract ID: Systems biology and autophagy signal control / 16547 /SB-07

Screening for novel factors involved in the peroxisomal de novo synthesis in S. cerevisiae

Presenting author:

Juliane Lara Radke

Author(s):

Juliane Lara Radke, Wolfgang Schliebs, Einat Zalckvar, Maya Schuldiner, Ralf Erdmann

Peroxisomes are ubiquitous organelles involved in many cellular pathways, such as detoxification of reactive oxygen species or β -oxidation of fatty acids. Peroxisomes originate either by growth and division from pre-existing organelles or de novo by budding from the ER. One of the proteins which is essential for the ER-derived formation of peroxisomes is the peroxisomal biogenesis factor PEX19.

The aim of the study described here was the identification of novel factors involved in de novo formation of yeast peroxisomes. To this end, we generated a conditional peroxisomal mutant of *Saccharomyces cerevisiae*, in which PEX19 expression is under control of an inducible galactose promoter. Under repressing growth conditions this strain is characterized by complete depletion of peroxisomes. Upon induction of PEX19 expression, these cells exhibit de novo formation of peroxisomes. For systematic screening of genes involved in this process, we crossed this strain with a library of over 6000 deletion strains and screened for mutants, which are impaired in peroxisomal import of a fluorescent marker protein. As expected, we could identify all known genes involved in peroxisome protein import, proliferation and de novo formation. In addition, we selected several mutants displaying delayed formation of peroxisomes. The corresponding genes with uncharacterized function have been chosen for further analysis.

Session/Abstract ID: Cell signalling and membrane trafficking / 16551 /CS-39

The scaffold protein CNK1 controls RAF and AKT signaling and cell fate decisions

Presenting author:

Gerald Radziwill

Author(s):

Adrian Fischer, Wignand Mühlhäuser, Kerstin Leberecht, Mandy Neumann, Wilfried Weber, Bettina Warscheid, Gerald Radziwill

CNK1 is a multidomain protein without enzymatic function. Recent studies from our group demonstrated that growth factor-induced phosphorylation of the N-terminal SAM domain induces clustering of CNK1 correlating with CNK1-triggered signaling. Using an optogenetic approach based on light-induced clustering of cryptochrome 2 (CRY2) we generated optoCNK1 to control CNK1 signaling uncoupled from physiological stimuli. We identified selective clusters of CNK1 that either stimulate RAF-MEK-ERK or AKT signaling depending on the light intensity applied. CNK1 clustering decides on proliferation and differentiation in a cell type- and cell stage-dependent manner by orchestrating AKT and RAF signaling. In addition, we identified acetylation of CNK1 in its PH domain as late step in activation of CNK1 signaling. Growth factor-stimulated ERK signaling induces CNK1 acetylation and acetylated, membrane-associated CNK1 promotes ERK signaling correlating with increased cell proliferation and migration. Searching the COSMIC database listing mutants found in human tumors, we identified CNK1 mutants that activate oncogenic signaling. Mutants in the SAM domain constitutively form clusters and stimulate AKT signaling but not ERK signaling. CNK1 mutants in the PH domain show constitutive acetylation and membrane localization accompanied with ERK but not AKT signaling. Thus, CNK1 cluster size and the spatiotemporal control of CNK1 regulate RAF/ERK and AKT signaling in a mutually exclusive manner.

Session/Abstract ID: Cell signalling and membrane trafficking / 16526 /CS-37

Remote Control of Cell Signaling Pathways with Magnetic Nanoparticles inside Living Cells

Presenting author:

Fabian Raudzus , Hendrik Schöneborn

Author(s):

Fabian Raudzus, Hendrik Schöneborn, Rolf Heumann

Neurodegenerative disorders are a major public health issue affecting the worldwide aging population. Especially, Parkinson's disease (PD) caused by loss and dysfunction of dopaminergic neurons is not curable by pharmacological treatment. To this date, progressing PD can be retarded mainly by deep brain stimulation (DBS) or antiparkinson medication like levodopa (L-DOPA). In efforts to treat Morbus Parkinson, cell replacement strategies are considered very promising. However, controlling the cell behavior after transplantation, in particular the directed fibre outgrowth for functional integration in the neuronal network will be a fundamental challenge.

Therefore, we aim to remote control cellular functions by means of magnetic manipulation. Our method is based on magnetic nanoparticles to stimulate cellular signaling cascades. After modification, the nanoparticles are delivered into target cells thereby aiming to act as intracellular signaling structures. Particularly, we are focusing on tools to remote control neuronal fibre outgrowth and survival of iPS-derived dopaminergic neuron precursor cells.

Session/Abstract ID: Cell signalling and membrane trafficking / 16560 /CS-42

optogenetic control of eyeblink conditioning in mice

Presenting author:

Pia Reiterer

Author(s):

Pia Reiterer, Stefan Herlitze

Eyeblink conditioning (EBC) is a form of associative motor learning which depends on cerebellar circuits. An unconditioned stimulus (US), which leads to the

closure of the eyelid is associated with a conditioned stimulus (CS). The neuronal circuits underlying EBC have been described in detail and involve two main pathways projecting onto Purkinje cells (PC). The information of the CS is conveyed via a mossy fiber – parallel fiber input, while climbing fiber projections are carrying the US information from the inferior olive. These circuits seem to be localized in the C3 or D0 zones of the cerebellar cortex. To date plasticity mechanisms underlying EBC remain largely unknown. To investigate the role of synaptic plasticity and underlying signaling cascades we will control network activity and G-protein signals during EBC using optogenetic techniques. Specific expression of optogenetic probes in neurons of the eyeblink region will be achieved by injection of floxed viruses into different cre-mouse lines. Based on electrophysiological responses of PCs, following air puff application onto the ipsilateral eye electrophysiological in vivo recordings in awake mice will be performed to monitor activity during learning and during light-control. Thus, the study should lead to a precise understanding of how G-protein signals and neuronal activity shapes motor learning. My preliminary results indicate that light (ChR2)-controlled depolarization of PCs impairs learning during EBC.

Session/Abstract ID: Subcellular organisation / 16498 /SC-09

Towards the mechanism of peroxisomal matrix protein import in mammalian cells

Presenting author:

Maren Reuter

Author(s):

Maren Reuter, Jessica Klümper, Eva Hambruch, Daniel Blum, Wolfgang Schliebs, Richard Wagner, Ralf Erdmann

Pex5, the cycling import receptor for folded peroxisomal matrix proteins, can switch between a soluble and a membrane bound state. Thus, PEX5 becomes an integral part of a transient import channel in the peroxisomal membrane. It is generally believed that cargo protein-receptor interactions in the cytosol lead to conformational changes of the receptors enabling docking of the receptor-cargo complex at the membrane. It has been shown that PEX5 interacts with peroxisomal phospholipids and membrane proteins

like PEX14, thereby resulting in the assembly of the translocon in the peroxisomal membrane.

To understand the mechanism of matrix protein import in more detail, we analyse the optical and electrophysiological characteristics of various forms of purified human PEX5 bound to artificial membranes. To this end, we are using liposome flotation analysis as well as horizontal lipid bilayers coupled with opto-electrical recording. Upon incubation with fluorophore-labelled receptor we observed its spontaneous insertion into membranes and formation of an ion-transducing channel. Ongoing experiments using horizontal lipid bilayer technology include purified cargo-proteins and reconstituted peroxisomal membrane proteins like PEX14 to study the influence of these protein interactions on pore formation and characteristics. Furthermore, we are mapping lipid-binding sites and protein-protein interacting sites, to study *in vivo* their contribution on the assembly of the import machinery.

Session/Abstract ID: Cell signalling and membrane trafficking / 16602 /CS-43

Transfection Agents with Aggregation Induced Emission Properties

Presenting author:
Steffen Riebe

Author(s):
Steffen Riebe, Jens Voskuhl

In 2001 Tang et al. discovered a phenomenon called aggregation induced emission which describes the emission of a luminophore in the aggregated state but not when dissolved.[1] This effect was used for different applications in the biomedical chemistry.[2] In the past we investigated the formation of fluorescent micelles using hydrogen bonding[3] as well as the recognition of lectins and carbohydrates using a novel class of AIE active compounds.[4] Careful design of our luminophores enables the use as transfection agents with unique AIE properties. Suprastructures (micelles, vesicles, rods) formed by amphiphilic AIE luminophores in aqueous media can undergo efficient transfection (lipofection). These structures can bind negatively charged DNA or RNA on their positively charged surface and transport it into living cells.

References

- [1] J. Luo, Z. Xie, J. W. Y. Lam, L. Cheng, H. Chen, C. Qiu, H. S. Kwok, X. Zhan, Y. Liu, D. Zhu and B. Z. Tang, *Chem. Commun.*, 2001, 1740-1741.
- [2] J.-X. Wang, Q. Chen, N. Bian, F. Yang, J. Sun, A.-D. Qi, C.-G. Yan and B.-H. Han, *Org. Biomol. Chem.*, 2011, 9, 2219-2226.
- [3] H. Frisch, D. Spitzer, J. Voskuhl, P. Besenius, *Org. Biomol. Chem.*, 2016, 14, 5574-5579.
- [4] B. Schmidt, S. Sankaran, L. Stegemann, C. A. Strassert, P. Jonkheijm, J. Voskuhl, *J. Mater. Chem. B*, 2016, 4, 4732-4738

Session/Abstract ID: Cell signalling and membrane trafficking / 16124 /CS-06

An intramolecular pathway for the voltage dependence of muscarinic GPCRs

Presenting author:
Andreas Rinne

Author(s):
Anika Hoppe, Maria Marti-Solano, Peter Kolb, Moritz Bünemann, Andreas Rinne

Muscarinic receptors (MRs) are activated by binding of specific agonists to an orthosteric site, which is formed by the seven trans-membrane (TM) helices of these receptors. This site is exposed to the electrical field across the membrane, resulting in voltage-dependent MR signaling. The function of MRs can be also regulated by allosteric modulators (AMs) binding to an extracellular allosteric site involving extracellular loops (EL) and TM 7. Both types of modulation require an orthosteric agonist and thus, the active receptor conformation. To address, if the membrane potential can be regarded as AM, we compared voltage-dependent signaling changes of M1R, M3R and "allosteric" M1R/M3R chimeras to signaling changes induced by AMs in single HEK 293 cells with a FRET biosensor for Gq protein activation. In terms of kinetics and amplitudes, changes in signaling were similar for wild type receptors, but some chimeras were insensitive to voltage, indicating that the allosteric site may affect voltage dependence. This was confirmed by a mutation that caused functional uncoupling of the allosteric site and removed the voltage sensitivity of the M3R. Molecular dynamics simulations of active MR

structures revealed a specific interaction of EL 2 with tyrosine residues of the orthosteric site, which controls agonist binding and receptor signaling. This intramolecular pathway is specific for each MR subtype and defines the voltage dependence of MRs in a receptor subtype-specific manner.

Session/Abstract ID: Cell signalling and membrane trafficking / 16425 /CS-23

Crosslinking the Neuropeptide Y5 Receptor by Photoreactive Biotin Analogues

Presenting author:

Sarina Rudolf

Author(s):

Sarina Rudolf, Irene Coin, Annette G. Beck-Sickinger

Based on the influence on fundamental physiological processes such as food intake, regulation of blood pressure and memory retention, the neuropeptide Y (NPY) family is also associated with pathological processes as obesity, cancer, mood disorders and epilepsy. The neuropeptide Y family is a multiligand/multireceptor system consisting of four G-protein-coupled Y receptors in humans - Y1R, Y2R, Y4R, and Y5R - and three native ligands - neuropeptide Y (NPY), pancreatic polypeptide (PP), and peptide YY (PYY) - that bind and activate the Y receptors with different affinity and potency. While the Y1R, Y2R and Y4R show preferences towards those ligands, the Y5R displays high affinity to all endogenous ligands.

We selectively introduced the photoreactive unnatural amino acid p-benzoyl-L-phenylalanine (Bpa) at seven positions into the ligand NPY by solid phase peptide synthesis. Those NPY analogues were probed for their potential to activate the Y5R and to crosslink to the Y5R. All analogues retained their ability to activate the receptor but exhibited varying potencies. Notably, three of the analogues crosslinked efficiently to Y5R, indicating the proximity of those positions to the ligand binding pocket and moreover demonstrating that only central positions can be modified with the photocrosslinker without affecting the binding.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16555 /SM-03

Ultrasmall Gold Nanoparticles for Protein-Specific Targeting: Investigation by NMR Spectroscopy

Presenting author:

Tatjana Ruks

Author(s):

Tatjana Ruks, Christine Beuck, Matthias Epple, Peter Bayer

Ultrasmall gold nanoparticles with a diameter below 2 nm were prepared to target epitopes on the surface of proteins. They are small enough (smaller than most proteins) to be able to interact with just one protein molecule or one epitope on the protein surface.

Functionalized gold nanoparticles were prepared by reduction of HAuCl₄ with NaBH₄ and subsequent functionalization with peptides. Peptides form a strong Au-S-bond via cysteine. In this case, the hexapeptide CGGpTPA was attached that is known to address the WW domain of the protein hPin1.

The attachment of the peptide to the gold nanoparticle was shown by NMR spectroscopy, i.e. 1D-1H NMR spectroscopy and 2D-1H,1H-TOCSY NMR spectroscopy. 1H-DOSY and 15N-DOSY NMR spectroscopy of the synthesized gold nanoparticles gave the hydrodynamic radius of the functionalized gold nanoparticles.

Specific protein binding was demonstrated by 2D-1H,15N-HSQC NMR. The 15N-isotope-labeled protein hPin1 was titrated with CGGpTPA-functionalized gold nanoparticles to show the interaction with the WW domain.

We thank the Deutsche Forschungsgemeinschaft (DFG) for funding in the framework of SFB 1093: Supramolecular Chemistry on Proteins.

Session/Abstract ID: Cell signalling and membrane trafficking / 16467 /CS-29

Palmitoylation - Emerging roles in TNF-R1 endocytic trafficking and signaling

Presenting author:

Vinzenz Särchen

Author(s):

Vinzenz Särchen, Timo Glatter, Philipp Zingler, Stefan Schütze, Jürgen Fritsch

TNF-R1 as well as other members of the TNF-Receptor superfamily and their ligands have been described to be involved in various physiological processes ranging from regulating immune response to organ formation. On the other hand they have pathological function i.e. in cancer formation, chronic inflammatory and neural diseases. It is known that TNF-R1 can induce diametrically opposed biological effects upon ligand binding: inflammation/proliferation versus cell death. The differences in signal quality depend on the localization of the receptors. Plasma membrane resident receptors activate pro-inflammatory/survival signals, while endocytosed receptors can induce cell death.

We recently showed that TNF-R1 ubiquitination is mandatory for signaling bifurcation and that signaling cascades are regulated by protein complexes, which are assembled in distinct membrane enclosed compartments upon ligand binding. One mechanism to induce or modulate membrane localization of proteins is their post translational modification by palmitoylation. We here present unpublished data showing that TNF-R1 itself is palmitoylated, it induces biological effects which can be modulated by the application of (de-)palmitoylation inhibitors and that it interacts with palmitoyl transferases (PAT) and thioesterases (PTE). Thereby, activation of TNF-R1 induces dynamic changes in the palmitoylation status of various trafficking associated proteins, highlighting their role in endocytic TNF-R1 signaling.

Session/Abstract ID: Systems biology and autophagy signal control / 16456 /SB-05

isiKnock: a tool for in silico knockouts of signaling pathways

Presenting author:

Jennifer Scheidel

Author(s):

Jennifer Scheidel, Heiko Giese, Leonie Amstein, Jörg Ackermann, Ina Koch

Signaling pathways are complex and intertwined processes. The perturbation of biological systems can reveal the complicated interplay and dependencies of the pathway components. In silico knockout is a valuable method to detect inconsistencies within the current knowledge of a signaling pathway. Furthermore, in silico knockouts can predict unknown knockout behavior to support experimental design.

We developed the tool isiKnock to automatically conduct in silico knockouts for signaling pathways. isiKnock predicts the knockout behavior based on the calculation of signal flows at steady state [1] and visualizes the results appropriately using a matrix representation. We applied the method to study the autophagic capturing of the pathogen Salmonella Typhimurium. The antibacterial autophagy pathway, also known as xenophagy, has been discovered recently. We developed a mathematical model of this key defense mechanism [1]. The model combines the functional information derived from literature data and represents the current understanding of the pathway. To verify the topology of the model, we compared the results of the in silico knockouts with those of experimental gene knockouts or knockdowns. Additionally, each non verified matrix entry represents a hypothesis of unknown knockout behavior for future experimental studies towards a better understanding of xenophagy.

[1] Scheidel et al. PLOS Computational Biology, 2016, 12(12), e1005200

Session/Abstract ID: Age-related pathologies and autophagy / 16588 /AP-12

The variability of the CSF proteome: An important aspect for protein biomarker studies

Presenting author:

Lukas Schilde

Author(s):

Lukas Schilde, Simone Steinbach, Steffen Kösters, Michael Turewicz, Maike Ahrens, Martin Eisenacher, Sara Galozzi, Katalin Barkovits, Brit Mollenhauer, Caroline May, Katrin Marcus

Many diseases of the central nervous system are difficult to diagnose as a result of overlapping clinical symptoms and inaccessibility of the brain. For many of them, like Parkinson's disease, there is no reliable disease biomarker. Due to Cerebrospinal fluids proximity to the brain, it is used for diagnostic routine tests as well in discovery studies for biomarkers in neurodegenerative diseases. Many possible biomarkers were identified but many could not be validated. High variation of CSF protein composition and protein abundance between and within individuals might be one reason for this. In our study, CSF samples from human individuals with no neurological disease were taken at three time points over 4 years. Proteins were analysed using a mass spectrometry based approach and evaluated. 791 proteins were identified, for further analysis, 217 of them were chosen for further analysis and it may help to assess the obstacles that we face in protein biomarker studies in CSF. Representatively, we investigated intra- and interindividual variability of Haptoglobin (P00738) as a validation obstacle.

Session/Abstract ID: Other (free) topics / 16616 /OT-56

The Collective Behavior of Spring-like Motifs Tethered to a DNA Origami Nanostructure

Presenting author:

Elisa-Charlott Schöneweiß

Author(s):

Elisa-Charlott Schöneweiß, Barbara Saccà

Dynamic DNA nanotechnology relies on the integration of small switchable motifs at suitable positions of DNA nanostructures, thus enabling to

manipulate matter with nanometer spatial accuracy in a trigger-dependent fashion. Typical examples of such motifs are hairpins, whose elongation into duplexes can be used to perform long-range, translational movements. In this work, we used temperature-dependent FRET spectroscopy to extract the thermal stabilities of distinct sets of hairpins integrated into the central seam of a DNA origami structure. We then developed a hybrid spring model to describe the energy landscape of the tethered hairpins, combining the thermodynamic nearest-neighbor energy of duplex DNA with the entropic free energy of single-stranded DNA estimated using a worm-like chain approximation. We show that the organized scaffolding of multiple hairpins enhances the thermal stability of the device and that the coordinated action of the tethered motors can be used to mechanically unfold a G-quadruplex motif bound into the inner cavity of the origami structure, thus surpassing the operational capabilities of freely diffusing motors. Finally, we increased the complexity of device functionality through the insertion of two sets of parallel hairpins, resulting in four distinct states and in the reversible localization of desired molecules within the reconfigurable regions of the origami architecture.

Session/Abstract ID: RNA-Regulation und Transport / 16493 /RR-05

TFIIA transcriptional activity is controlled by a "cleave-and-run" Exportin-1/Taspase 1-switch

Presenting author:

Elisabeth Schröder

Author(s):

Christian Schrenk, Verena Fetz, Cecilia Vallet, Christina Heiselmayer, Elisabeth Schröder, Astrid Hensel, Angelina Hahlbrock, Désirée Wunsch, Dorothee Goesswein, Carolin Bier, Negusse Habtemichael, Günter Schneider, Roland Stauber, Shirley Knauer

Transcription factor TFIIA is controlled by complex regulatory networks including proteolysis by the protease Taspase 1, though the full impact of cleavage remains elusive.

Here, we demonstrate that in contrast to the general assumption de novo produced TFIIA is rapidly confined to the cytoplasm via an evolutionary conserved nuclear export signal (NES, aa²¹VINDVRDIFL³⁰), interacting with the nuclear export receptor Exportin-1/Crm1. Chemical export inhibition

or genetic inactivation of the NES not only promotes TFIIA's nuclear localization but also affects its transcriptional activity. Notably, Taspase 1 processing promotes TFIIA's nuclear accumulation by NES masking, and modulates its transcriptional activity. Moreover, TFIIA complex formation with the TATA box binding protein, TBP, is cooperatively enhanced by inhibition of proteolysis and nuclear export, leading to an increase of the cell cycle inhibitor p16^{INK}, which is counteracted by prevention of TBP binding. We here identified a novel mechanism how proteolysis and nuclear transport cooperatively fine-tune transcriptional programs.

Session/Abstract ID: Subcellular organisation / 16301 /SC-03

An unexpected Liaison of Lipid Droplets and Peroxisomes - PEX19-mediated Protein Targeting to Lipid Droplets

Presenting author:

Bianca Schrul

Author(s):

Bianca Schrul, Ron Kopito

Lipid droplets (LDs) are endoplasmic reticulum (ER)-derived neutral lipid storage organelles uniquely encapsulated by phospholipid monolayers. Integral LD proteins are embedded into this monolayer in a unique monotopic hairpin topology. UBXD8 belongs to a subfamily of hairpin proteins that localize to both the ER and LDs, and that are initially inserted into the cytoplasmic leaflet of the ER bilayer before partitioning to the LD monolayer. We investigated the molecular machinery responsible for inserting hairpin proteins into membranes and show that newly synthesized UBXD8 is post-translationally inserted into discrete ER subdomains by a mechanism independent of the canonical ER-targeting pathways. Instead UBXD8 insertion requires cytosolic PEX19 and membrane-integrated PEX3; both proteins hitherto exclusively implicated in peroxisome biogenesis. Furthermore, we report that farnesylation of PEX19 uncouples ER/LD and peroxisome targeting as it is dispensable for peroxisome biogenesis but essential for correct ER/LD localization of UBXD8. Quantitative comparative proteomic analyses revealed that PEX19-dependent LD targeting is not unique to UBXD8. We are currently exploring lipid metabolic changes in cells lacking PEX19-dependent LD proteins.

Together, our findings expand the function of PEX19/PEX3 to a novel ER/LD-targeting pathway and suggest a coordinated biogenesis of LDs and peroxisomes, both ER-derived organelles with complementary roles in lipid metabolism.

Session/Abstract ID: Cell signalling and membrane trafficking / 16266 /CS-12

Insight into receptor targeting by the peroxisomal AAA+ complex Pex1p/Pex6p of the yeast Saccharomyces cerevisiae

Presenting author:

Daniel Schwerter

Author(s):

Daniel Schwerter, Immanuel Grimm, Wolfgang Girzalsky, Ralf Erdmann

The receptor-cycle of type I peroxisomal matrix protein import is completed by ubiquitination of the membrane-bound receptor Pex5p and its subsequent export back to the cytosol. The receptor export is the only ATP-dependent step of the whole process and is facilitated by two members of the AAA+ family of proteins (ATPases associated with various cellular activities), namely Pex1p and Pex6p.

Electron-microscopic analyses of the hetero-hexameric Pex1p/Pex6p complex gave a first impression of a possible mechanism for the export of Pex5p [1]. Conformational changes of the AAA+ complex, observed at different arrested states during ATP hydrolysis, suggest an involvement of the conserved pore loop motifs of Pex1p and Pex6p in threading the receptor through the central pore of the complex. To gain further insight into substrate recognition by the AAA+ complex, we generated an artificial Ubiquitin-Pex5p fusion protein. This construct is able to functionally complement a PEX5-deletion in *S. cerevisiae*. Furthermore, it interacts with key components of the import machinery in vitro and is deubiquitinated by the deubiquitinating enzyme Ubp15p. Through in vitro pull-down assays as well as crosslinking studies we are able to show, that Pex5p recognition by the AAA+ complex depends on the presence of the Ubiquitin moiety and is mediated by Pex1p.

1. Ciniawsky, S., et al., Molecular snapshots of the

Pex1/6 AAA+ complex in action. Nat Commun, 2015. 6: p. 7331.

Session/Abstract ID: Other (free) topics / 16122 /OT-07

Optogenetic control of cerebellar output abolishes generalized seizures

Presenting author:

Jan Claudius Schwitalla

Author(s):

Jan Claudius Schwitalla

Absence epilepsy (AE) is a form of generalized epilepsy, characterized by a brief loss of consciousness and behavioral arrest. The electrophysiological hallmarks, bilaterally synchronized 4-12 Hz spike-and-wave discharges, arise from an abnormal thalamocortical network. There are several different drugs available for the treatment of generalized seizures. However, for patients where these drugs lack efficiency, excisions and deep brain stimulation are the only available treatment options. Typical rodent models for AE have a knockout (KO) or point mutation of a specific gene, which disables it for the analysis of the contribution of specific areas to AE. Therefore, our laboratory created a conditional KO model to test the area specific contribution and generated new animal models for the study of AE. Since the common dogma excludes the cerebellum from the development of AE, we tested if Cre-dependent KO of the $\alpha 1$ subunit of P/Q type calcium channels restricted to the Purkinje or granule cells of the cerebellum show AE. We found that both lines show AE similar to the widely used mouse models with a typical frequency between 4-9 Hz. Treatment with the antiepileptic drug ethosuximide reduces the occurrence of AE in freely moving animals, while valproic acid lacks efficiency. By applying closed-loop optogenetic stimulation to the cerebellar nuclei, we were able to stop ongoing seizures and proof that the cerebellar network is involved in AE and could be a new therapeutic target.

Session/Abstract ID: Lunch Sessions / 16492 /OT-60

Design and bio-functionalization of magnetic nanoparticles for intra-cellular remote control of signaling pathways

Presenting author:

Emilie Secret

Author(s):

Emilie Secret

Cell replacement therapy is among the most promising approach to treat neurodegenerative disorder such as Parkinson's, Alzheimer's or Huntington's diseases. In the work presented here, part of the MAGNEURON European project, we aim at having magnetic nanoparticles (MNPs) that are stable inside the cytoplasm of cells and bio-functionalized to trigger neurons' differentiation and growth along the direction of use of the external magnet gradient.

To this goal, $\gamma\text{Fe}_2\text{O}_3@SiO_2$ core-shell nanoparticles and $\gamma\text{Fe}_2\text{O}_3$ nanoparticles coated with polymers such as poly(ethylene glycol) or poly(acrylic acid) were synthesized. The particles were optimized to have size, charge and magnetization to obtain a good colloidal stability, render them injectable in cells, and facilitate intracellular motion. These MNPs were then functionalized with a HaloTag ligand in order to interact specifically with proteins able to trigger different pathways in the cell. MNPs were microinjected in the cell and showed intra-cellular biased diffusion toward the micro-magnet. The magnet can then be used to displace target proteins attached to the MNPs inside the cell, and trigger signaling events such as actin polymerization at particular subcellular localizations. Finally the synthesis of Fe_3O_4 nanorods, coated with silica or polymers will also be presented. Nanorods are of interest for their potentiality to be internalized without endocytosis and for their higher magnetic hyperthermia capacities.

Session/Abstract ID: Cell signalling and membrane trafficking / 16276 /CS-15

Linear ubiquitination is essential for the neuroprotective activity of Parkin

Presenting author:

Dominik A. Sehr

Author(s):

Dominik A. Sehr, Lena A. Berlemann, Lena Angersbach, Pia Mittweg, A. Kathrin Müller-Rischart, Jörg Tatzelt, Konstanze F. Winklhofer

Mutations in the gene encoding the E3 ubiquitin ligase Parkin are responsible for the majority of autosomal recessive Parkinsonism. Parkin has a wide neuroprotective activity, preventing cell death in various stress paradigms. Parkin acts together with the mitochondrial kinase PINK1 in a mitochondrial quality control pathway called mitophagy. We recently observed that Parkin prevents stress-induced cell death in a mitophagy-independent manner and identified NF- κ B signaling as an essential pathway mediating the pro-survival activity of Parkin. In response to cellular stress, Parkin is recruited to LUBAC, the linear ubiquitin assembly complex which is composed of the two E3 ubiquitin ligases HOIP and HOIL-1L and the adaptor protein SHARPIN. Thus, linear ubiquitination of NEMO (NF- κ B essential modulator) is increased. In support of a crucial role of this pathway, Parkin cannot prevent stress-induced cell death in the absence of HOIP or its substrate NEMO.

In this study we searched for deubiquitinating enzymes that counteract the pro-survival activity of Parkin. Interestingly, we identified OTULIN, a protease that specifically hydrolyzes linear polyubiquitin in a substrate-assisted manner. OTULIN abolishes the stress-protective activity of Parkin without affecting PINK1/Parkin-mediated mitophagy. These data reveal that linear ubiquitination is essential for the pro-survival activity of Parkin and that this pathway is independent from the role of Parkin in mitophagy.

Session/Abstract ID: Other (free) topics / 16440 /OT-12

Inhibition and knockout of subunits of immunoproteasome decrease the efficiency of reprogramming murine fibroblasts into iPSCs

Presenting author:

Anastasiia Selenina

Author(s):

Anastasiia Selenina, Sergey Sinenko, Ulrike Seifert, Anna Tsimokha, Alexey Tomilin

Embryonic stem cells and induced pluripotent stem cells (iPSC) are not only interesting objects of fundamental research, but also extremely promising in various fields of applied medicine, regenerative medicine. Despite the great interest to pluripotent cells, the mechanisms of maintaining protein homeostasis in these cells by the ubiquitin-proteasome system have been studied rather poorly. The proteasome consists of a 20S core and regulatory 19S particles. Also the constitutive catalytic subunits β 1, β 2 and β 5, can be replaced by alternative subunits - β 1i, β 2i and β 5i and form immunoproteasome(IP). There is also an additional regulator - PA28 α/β . The role of IP in the process of reprogramming of somatic cells into iPSC has been little studied. We induced reprogramming of mouse embryonic fibroblasts(MEFs) into iPSC and we observed the increase expression of β 1i and β 5i subunits. Furthermore, during the treatment with selective inhibitor of the β 5i - PR-957 and inhibitor of β 5 and β 5i - MG-132, alkaline phosphatase staining showed a significant decrease in the formation of iPSC clones, which indicates the important functions of both proteasomes and IP in the reprogramming. Analysis of reprogramming of MEFs derived from β 2i- and PA28 α/β -deficient embryos also showed a strong decrease in the ability to give rise to iPSC clones, again, implying important functions of proteasomes, IP and PA28 in the process of reprogramming.

This work was supported by RSF 16-04-10343

Session/Abstract ID: Systems biology and autophagy signal control / 16507 /SB-06

The tail of human guanylate-binding protein 1 is switched by GTP and triggers polymerization and membrane binding

Presenting author:
Sergii Shydlovskyi

Author(s):
Sergii Shydlovskyi, Anke Y. Zienert, Semra Ince, Annika Hohendahl, Ailisa Blum, Miriam Kutsch, Aurélien Roux, Gerrit J.K. Praefcke, Christian Herrmann

Dynamain-like proteins (DLPs) play an important role in fusion and fission of cellular membranes. Some of DLPs are involved in the immune responses within the cell, in particular the guanylate-binding proteins (GBPs). Human guanylate-binding protein 1 (hGBP1) is the founding member of human GBPs. Besides its participation in antimicrobial and antiviral responses, hGBP1 regulates also cell adhesion and migration. Within the cell, hGBP1 undergoes isoprenylation via attachment of a farnesyl anchor to its C-terminus, which is required for membrane association. Here, we show that farnesylated hGBP1 exposes this lipid anchor triggered by GTP-binding and we term this a nucleotide-dependent "farnesyl switch". Thus, GTP-dependent membrane binding of farnesylated hGBP1 is revealed, and furthermore, tethering of lipid vesicles, mediated by GTP-bound hGBP1, is observed. Moreover, we report nucleotide-dependent polymerization of hGBP1 once it carries the farnesyl tail. Intriguingly, membrane binding of farnesylated hGBP1 competes with protein polymerization, suggesting two different pathways in the course of its GTPase cycle. Finally, we show data which suggest the involvement of hGBP1 in the autophagy pathway.

Session/Abstract ID: Other (free) topics / 16415 /OT-25

Application of calcium phosphate nanoparticles for transfection and gene silencing in 2D and 3D cell culture models

Presenting author:
Viktoriya Sokolova

Author(s):
Viktoriya Sokolova, Leonardo Rojas, Matthias Epple

The transfer of genetic information into living cells to manipulate their internal processes is of high interest in cell biology and in the treatment of genetically caused diseases ("gene therapy"). Calcium phosphate has a high biocompatibility because it is the inorganic component of human bone and teeth. Therefore, its application in biomedical materials science is widespread [1-2]. Here we report on the preparation of calcium phosphate nanoparticles and their further application in 2D and 3D cell culture models for transfection (expression of the corresponding protein) and gene silencing (selective inhibition of protein synthesis). 3D culture systems exploit more realistic spatial, biochemical and cellular parameters compared to 2D monolayer cell culture and serve as a bridge between in vitro and in vivo studies. By using appropriate precipitation methods, multi-shell nanoparticles, loaded with nucleic acids (plasmid DNA or siRNA), were prepared and characterized. Inside the particle, the nucleic acid is protected from intracellular degradation by nucleases in the cells. The uptake of these functionalized nanoparticles was studied in 2D and 3D cell culture models.

- [1]S. Chernousova, M. Epple, Gene Ther. 24 (2017) 282.
[2]M. Kopp, O. Rotan, C. Papadopoulos, N. Schulze, H. Meyer, M. Epple, PLoS ONE 12 (2017) e0178260.
[3]B. Neuhaus, B. Tosun, O. Rotan, A. Frede, A.M. Westendorf, M. Epple, RSC Adv. 6 (2016) 18102.
[4]M. Zanoni, F. Piccinini et al., Sci. Rep. 6 (2016) 19103.

Session/Abstract ID: Other (free) topics / 16443 /OT-30

Crystallographic and Biochemical Studies Reveal Insight into Phycoerythrobilin Biosynthesis

Presenting author:

Johannes Sommerkamp

Author(s):

Johannes Sommerkamp, Marco Aras, Nicole Frankenberg-Dinkel, Eckhard Hofmann

The pink-coloured open-chain tetrapyrrole phycoerythrobilin (PEB) is one of the major chromophores in phycobiliproteins of cyanobacteria and cryptophytes. For the biosynthesis of PEB, biliverdin IX α (BV) serves as the substrate for ferredoxin-dependent bilin reductases (FDBRs). BV is reduced by 15, 16-dihydrobiliverdin (15, 16-DHBV):ferredoxin oxidoreductase (PebA) to the intermediate 15, 16-DHBV. A second consecutive two-electron reduction by PEB:ferredoxin oxidoreductase (PebB) generates the final chromophore PEB. In contrast, phycoerythrobilin synthase (PebS) from cyanophages combines the activities of PebA and PebB in one single enzyme.

Several crystal structures of PebA and PebS were published by our laboratories, but structural evidence for PebB was missing. Here, we present crystal structures of PebB from the cryptophyte *Guillardia theta* in the substrate free form and with bound substrate DHBV. While the sequence identity of PebA, PebB and PebS is rather low, all reductases show the typical α - β - α sandwich fold with two conserved aspartate residues in the substrate binding pocket, which are essential for substrate protonation. We identified a strictly conserved arginine residue, specific to PebB, which varies the shape of the active site and therefore binding of the substrate. Utilizing UV/Vis spectroscopy and HPLC analysis, we investigated PebB wildtype and variants and verified the arginine residue as an additional critical residue for the conversion of DHBV to PEB.

Session/Abstract ID: Other (free) topics / 16297 OT-18

Artificial cobalt-sulfur proteins as redox biocatalysts

Presenting author:

Ingrid Span

Author(s):

Lisa Galle, Ingrid Span

Metalloenzymes combine the reactivity of a transition metal centre with the potential to modulate the reactivity and substrate selectivity of a protein environment. However, the scope of reactions catalyzed by native metalloenzymes is limited. One approach to broaden the scope of chemical transformations is based on exploring substrate promiscuity and protein engineering.

To overcome this limitation, we are aiming at generating artificial metalloproteins by incorporating metal complexes within proteins lacking their native metal-containing cofactor. Our focus lies on replacing the native metal in iron-sulfur proteins by cobalt. We have established methods for the incorporation of cobalt within the active sites of *Pseudomonas oleovorans* Rubredoxin 1 and 2 by using chemical reconstitution. The binding of the metal to the cysteine residues in the active sites has been determined by electronic absorption spectroscopy and mass spectrometry. In addition to the reconstitution *in vitro*, we have developed a protocol for the incorporation of cobalt within the protein *in vivo*.

The presented method to generate artificial cobalt-sulfur proteins can be extended to clusters with higher nuclearities and various transition metals, enabling the assembly of a wide range of novel redox biocatalysts.

Session/Abstract ID: Emerging Analytical Methods / 16362 /AM-05

Directed Evolution of Lysine Deacetylases

Presenting author:

Martin Spinck

Author(s):

Martin Spinck, Heinz Neumann, Petra Geue

Posttranslational modifications of lysine residues are involved in a wide array of regulatory biological functions like gene expression, protein degradation or protein interaction. Due to the vast amount, widespread nature, redundancy and dynamic interplay of different modifications their function is not fully understood yet. [1]

To address this problem and add to the biological toolkit we developed a selection system to alter deacetylase activity. This allowed us to create Sirtuin variants with altered or new substrate selectivity for natural or artificial lysine modifications.

These selective deacetylase variants will enable us to disentangle the physiological roles of diverse lysine modifications.

1.Choudhary, C.; Weinert, B. T.; Nishida, Y.; Verdin, E.; Mann, M. *Nat Rev Mol Cell Biol* 2014, 15, (8), 536-550.

Session/Abstract ID: Cell signalling and membrane trafficking / 16522 /CS-36

Chemical Synthesis of Switchable Protein-Based Nanopores

Presenting author:

Lena Karin Steinacker

Author(s):

Lena Karin Steinacker, Andreas Baumrueck, Alesia A. Tietze

The basic principle of a modified nanopore is to detect chemical or physical properties transducing them through a microelectronic device into electrical signals. Therefore such pores are highly suitable to be incorporated into miniaturized electronic components for biomedical or analytical applications. In living organisms, the transport of ions is performed by ion channels and transporters. Basic principles of the

working mechanism and the reason for their efficiency/high selectivity are well understood to date. However, their functionality is only provided in mechanical instable lipid membranes. Synthetic nanopores are mainly based on silica materials or organic polymers and were demonstrated to be useful as sensory devices for analytical applications. Unfortunately, their sensitivity and selectivity is much lower than for biological pores. Thus, the combination of biological and synthetic nanopores as a hybrid pore system should overcome current limitations of conventional pore systems.

For this reason we focused our studies on the development of switchable protein-based nanopores mimicking biological ion channels that can be integrated into solid state material. We here present first results towards the synthesis of the viral potassium channel KcvNTS [1] using Solid Phase Peptide Synthesis (SPPS) and Native Chemical Ligation (NCL).

References

[1] C. J. Braun, et al., *BBA - Biomembranes*, 1838, 1096-1103 (2014)

Session/Abstract ID: Age-related pathologies and autophagy / 16541 /AP-09

Effects of evaluation of possible agents in the context of Parkinson's disease

Presenting author:

Simone Steinbach

Author(s):

Simone Steinbach, Katrin Marcus, Gisa Ellrichmann, Ralf Gold, Dirk Voitalla, Caroline May

Parkinson's disease is a neurodegenerative disorder that affects the central and the enteric nervous system. It is characterized by the loss of dopaminergic neurons in the substantia nigra and the spread of Lewy bodies from the brainstem to the cortical parts of the brain. The main component of Lewy bodies is α -synuclein. In the last decades, several studies reported the presence of α -synuclein aggregates in the enteric nervous system of Parkinson's disease patients. Based on these observations the intestine is hypothesized as the starting point of the disease, moving along the vagus nerve in an upstream direction towards the brain. The aim of this study is to analyse possible agents based on their effect on α -synuclein

aggregates in the intestine and in the brain in rotenone treated mice.

Low doses of rotenone treatment induce α -synuclein aggregates in the brain and in the intestine in C57BL/6 mice. In this study, male mice were treated with rotenone in combination with various neuroprotective drugs to investigate the effect on α -synuclein aggregates in the intestine. Followed by treatment, the intestine was excised longitudinally to access the muscle layers containing enteric nerves and used for immunohistochemistry. First results revealed the presence of α -synuclein aggregates in the intestine of rotenone treated mice.

Our outlook is to investigate the possible effects of neuroprotective drugs on α -synuclein in the gastrointestinal tract and in the brain.

Session/Abstract ID: Age-related pathologies and autophagy / 16543 /AP-10

Which proteins are responsible for the selective neuronal vulnerability in Parkinson's disease?

Presenting author:
Simone Steinbach

Author(s):
Simone Steinbach, Marianna Molina, Helmut Heinsen, Lea T. Grinberg, Renata Leite, Katrin Marcus, Caroline May

Within neurodegenerative disorders specific neuron populations are degenerating, e.g. dopaminergic neurons in the substantia nigra during Parkinson's disease [1, 2]. Furthermore, not all dopaminergic neurons within the substantia nigra are equally affected. Studies revealed that ventral located neurons within the substantia nigra have a higher prevalence to degenerate during Parkinson's disease compared to neurons in the dorsal tier of the substantia nigra [3]. Reasons for this selective neuronal vulnerability are still an open issue [4]. To gain a better understanding of molecular differences between these types of neurons, they were isolated specifically with laser microdissection [5]. While methods in the past had the disadvantage that it was not possible to isolate specific cell populations of a complex tissue, laser microdissection overcomes this problem. Using this approach in combination with data independent acquisition mass spectrometry dorsal and ventral located neuron populations within the substantia nigra

were analyzed. The results of this study revealed proteomic differences between these two types of neurons. With that hints a deeper understanding of the protective mechanisms against neurodegeneration in specific neuron populations can be gained.

Session/Abstract ID: Cancer & Inflammation / 16557 /Ca-18

Quantification strategies in phosphoproteomics: A Comparison of SILAC, TMT and label-free techniques to study EGFR signal transduction networks and phosphorylation dynamics in the colorectal cancer cell line DiFi upon Cetuximab treatment

Presenting author:
Markus Stepath

Author(s):
Markus Stepath, Abdelouahid Maghnouj, Birgit Korte, Kristin Rosowski, Stephanie Tautges, Dominik A. Megger, Stephan Hahn, Barbara Sitek, Thilo Bracht

Proteomic techniques have been shown to be a valuable tool for discovering disease-related molecular mechanisms in cellular signal transduction networks in an unbiased way. In particular, post-translational modifications (PTM) such as phosphorylation can be assessed by this technology. A prominent disease related signaling network is the Epithelial growth factor receptor (EGFR) and its downstream signal components, which play an essential role in abnormal cell growth and survival of cancer cells. The foci of this study are the systematic comparison of different phosphoproteome quantification strategies and to gain insights into cellular signaling networks.

Therefore, the EGFR expressing colorectal cancer cell line DiFi was selected. Downregulation and dynamics of the EGFR signalling network upon treatment with anti-EGFR antibody Cetuximab have been confirmed and characterized by immunoblotting. State-of-the-art mass spectrometry-based proteomics were applied primarily. In detail, relevant experimental conditions have been optimized including the in-house TiO₂ phosphoprotein enrichment protocol as well as the liquid chromatography-based peptide separation and the mass spectrometer parameters. Furthermore, different labeling techniques on metabolic level via SILAC and chemical level by TMT were tested and

compared to the established label-free approach. The current workflow allows the detection of more than 4000 class 1 phosphosites and proteins including EGFR, Akt and Erk.

Session/Abstract ID: Age-related pathologies and autophagy / 16609 /AP-14

An Immuno-Infrared Sensor Enables the Preselection of Drug Candidates for Alzheimer's Disease

Presenting author:
Julia Stockmann

Author(s):
Julia Stockmann, Klaus Gerwert

Alzheimer's disease (AD) affects millions of people worldwide with a continuously increasing number of new cases. The aggregation of amyloid-beta (A β) and Tau proteins lead to the forming of plaques and neurofibrillary tangles in the brain. These aggregates are discussed as possible causes of Alzheimer's Disease (AD) progression.

An immuno-infrared-sensor was developed, which immobilizes A β or Tau proteins out of body fluids. The recorded conformation sensitive amide I band represents the secondary structure distribution of the extracted target protein fraction. For AD patients a spectral downshift of the amide I band –indicating an increased content of β -sheet enriched isoforms of A β or Tau- was detected. Thus, AD patients could be distinguished from healthy people using a simple threshold classifier.

The immuno-infrared-sensor is also able to preselect drug candidates regarding their effectiveness on A β and Tau aggregates. Drug candidates change the secondary structure from β -sheet enriched to monomeric states of the target proteins due to re-folding or digesting of the aggregated target proteins. A successful treatment is measured, when the amide I band shifts during the treatment from a diseased state to a healthy one.

Session/Abstract ID: Cell signalling and membrane trafficking / 16058 /CS-01

Claudins: An Unexpected Source For More Tetraspanning Proteins Acting As Transmembrane AMPA Receptor Modulatory Proteins

Presenting author:
Tobias Strasdeit

Author(s):
Tobias Strasdeit, Simon C. Haering, Subhrajit Bhattacharya, Muhammad Aslam, Jakob von Engelhardt, Stephen F. Traynelis, Michael Hollmann

Although ionotropic glutamate receptors (iGluRs) are able to function on their own, many interacting and modulating auxiliary subunits have been found that either influence biophysical properties of iGluRs or modulate their trafficking to the plasma membrane. The most commonly known proteins are the transmembrane AMPA receptor regulatory proteins (TARPs), cornichon homologs (CNIH), and neuropilin- and tolloid-like proteins (NETOs).

We show that certain subunits of the claudin family, which have high structure and sequence homology with the TARP proteins, act as AMPAR modulators. Claudins are generally known as tight junction proteins that seal passageways within and in between plasma membranes. Of the 29 different claudin genes identified in rat, few have been functionally characterized. We show that claudin-20 and claudin-24 potentiate the current amplitude and modulate the desensitization of certain AMPA receptors.

Amplitude potentiations were seen with GluA1 and GluA2 receptors, with effects being more pronounced with flip than with flop splice variants and with R than with Q editing variants. We further show that both claudin-20 and claudin-24 are expressed in the brain, particularly in the granule cell layers of the cerebellum and the olfactory bulb, and claudin-24 in addition at low levels in principal cells of the hippocampus. Finally, we used FRET after acceptor bleaching to demonstrate that claudin-20 and claudin-24 interact directly with both GluA1 and GluA2.

Session/Abstract ID: Other (free) topics / 16651 /OT-10

Cerebellar Involvement in Spatial Information Processing

Presenting author:

Tatjana Surdin

Author(s):

Tatjana Surdin

Spatial navigation (SN) relies on a multitude of parallel systems which integrate sensory input about environmental landmarks as well as selfmotion-based information in order to generate a functional cognitive map. It comprises declarative and procedural memory components. The declarative component provides semantic information about the environment, whereas the procedural component provides knowledge about the egocentric position and movements. A continuously growing body of evidence supports the notion that the cerebellar circuit serves to foster the procedural optimization of goal-directed navigation through synaptic longterm depression (LTD) at the parallel fiber-Purkinje cell synapse. The role of other cerebellar celltypes for SN is not known.

In order to determine the function of cerebellar granule cells (GCs) and molecular layer interneurons (MLIs) for SN, we used optogenetic online-stimulation of respective celltypes in mice during two different SN paradigms: Morris watermaze (MWM) and starmaze. The MWM requires both components – procedural and declarative. The starmaze, however, relies mainly on declarative capacities. Optogenetic activation of respective celltypes resulted in different patterns of performance decrease in the two paradigms indicating their distinctive roles in SN. Whereas optogenetic activation of GCs suppressed efficient learning in both mazes, MLIs became more important in the late phase of the starmaze, i.e. in the declarative component of SN.

Session/Abstract ID: Other (free) topics / 16613/OT-54

Biophysical Investigations on microbial rhodopsins using theoretical methods

Presenting author:

Stefan Tennigkeit

Author(s):

Stefan Tennigkeit, Max Dreier, Jens Kuhne, Klaus Gerwert

Channelrhodopsin 2 (ChR2) is a light-gated cation channel from the unicellular algae *Chlamydomonas reinhardtii*. ChR2 is a seven transmembrane protein with retinal as a chromophore. After blue light excitation the retinal isomerizes and the protein undergoes a photocycle with different structural intermediates. It is widely used as an optogenetic tool. A detailed mechanistic understanding of these optogenetic tool is necessary to improve their properties depending on the respective application.

We used a combination of FTIR, theoretical techniques (e.g. Molecular dynamic (MD) simulations) and electrophysiological measurements, to identify molecular mechanisms of several microbial rhodopsins. With these methods we could describe the early channel opening of channelrhodopsin 2 recently. MD simulations are very helpful to interpret spectroscopic results on small biological systems like proteins in order to study their activation mechanism in atomic detail. With several theoretical methods such as water density calculations and absorption spectra calculations we will identify molecular mechanisms of microbial rhodopsins and optimize these proteins for optogenetic applications (e.g. color tuning, ion selectivity).

Session/Abstract ID: Age-related pathologies and autophagy / 16479 /AP-07

Biochemical characterization of prokaryotic Roco protein family members

Presenting author:

Susanne Terheyden

Author(s):

Susanne Terheyden, Lina Wauters, Wim Versées, Arjan Kortholt

The Roco protein family has come into the focus of research when the human Roco protein LRRK2 (Leucine-rich repeat kinase 2) was discovered to be the most frequent cause of late onset Parkinson's disease (PD). LRRK2 is a large multi-domain protein with a complex regulatory mechanism and numerous functions. PD-linked mutations in LRRK2 are found primarily in the catalytic core of the protein consisting of a Ras like G-domain (Ras of complex proteins, short Roc), a COR domain (C-terminal of Roc) and a kinase domain. It is known that the G-domain of LRRK2 functions as a GTP-binding protein, and that GTP binding is essential for LRRK2 kinase activity. Moreover there is accumulating evidence that the Roc domain is not a classical G-domain but follows an unconventional activation mechanism involving dimerization. However, little is known about the regulation of LRRK2 activity and especially how the RocCOR domain tandem is regulated. The understanding of the G-proteins activation mechanism is essential in order to understand the activation mechanism of LRRK2 and its malfunction in PD.

Here we employed prokaryotic LRRK2 homologues to biochemically characterize the Roc domains' activity of several members of the Roco protein family. Michaelis-Menten kinetics and nucleotide binding affinities point towards a complex GTP hydrolysis mechanism involving more than one step. Moreover we found that the dimerization state of the protein plays a role in its activity.

Session/Abstract ID: Cell signalling and membrane trafficking / 16634 /CS-44

The role of receptor CXCR4 in the developing peripheral nervous system

Presenting author:

Daniel Terheyden-Keighley

Author(s):

Daniel Terheyden-Keighley, Carsten Theiss

The development of the peripheral nervous system (PNS) is an intricate process that is mediated, to a large degree, by intercellular signalling. Here we investigate one of these signalling systems, that of the receptor CXCR4 and its exclusive ligand, SDF1. This includes studying their roles in axon guidance, both in vitro and in vivo, differentiation and cellular migration. In the case of axon guidance, we developed multiple models to measure the effect of diffusible signalling molecules in vitro based on chick primary sensory neurons in 3D culture. Microfluidics chambers or micro-bead implantation were used to establish gradients of specific signalling proteins to allow the study of their chemoattractive/repulsive properties. For studying CXCR4/SDF1's role in differentiation, we directed the in vitro differentiation of human pluripotent stem cells into sensory neurons using a timed regime of signalling molecules. In addition, in situ hybridisation was used to map the spacio-temporal expression of CXCR4 and SDF1 mRNA during early chick development. Here we show preliminary in vitro data indicating CXCR4's role in dorsal root ganglia condensation. Through these experiments, we hope to enable the development of novel neuroregenerative therapies by helping to elucidate the mechanisms governing axon outgrowth initiation and guidance in the PNS.

Session/Abstract ID: Other (free) topics / 16612 /OT-53

Investigation of the G α s Modification by Cholera toxin with time resolved FTIR spectroscopy

Presenting author:
Christian Teuber

Author(s):
Christian Teuber, Nefeli Lewer, Klaus Gerwert, Carsten Kötting

Heterotrimeric G-Proteins are important switches in many signaling pathways of the cell. The G α subunit of this protein family acts as a GTPase and is associated with cholera, an infectious disease caused by the microorganism *Vibrio cholerae*. The bacteria produce an enterotoxin, the so called cholera toxin with its active subunit A1 (CTA1). This subunit acts as an ADP-ribosyl-transferase and transfers an ADP-ribose group from its substrate NAD onto the catalytically important Arginine 201 of the α -subunit of the stimulatory G-Protein Gs, resulting in an inactive GTPase.

In this work the subunit CTA1 of the cholera toxin was produced in *E.coli* and isolated via affinity- and size-exclusion chromatography. Its NADase activity was monitored using high performance liquid chromatography and the ADP-ribosyl-transferase activity with G α s was elucidated via Western-Blot analysis using 1,N⁶-Etheno-NAD and a monoclonal antibody (1G4) against etheno-adenosine. To get information on the mechanistic effect of the ADP-ribosylation for the GTPase reaction of G α s we performed time resolved Fourier Transform Infrared Spectroscopy using caged GTP on ADP-ribosylated G α s. Compared to unmodified G α s the spectrum of the GTP state is severely changed and almost no GTPase activity is observed. In the next step, these changes will be assigned by isotopic labelling and site-specific mutants. Further, we want to elucidate the reaction mechanism of the ADP-ribose transfer reaction by means of caged-NAD.

Session/Abstract ID: Cell signalling and membrane trafficking / 16429 /CS-27

Interactions and conformational changes of Bcl-2 proteins regulating cell death

Presenting author:
Markus Teucher

Author(s):
Markus Teucher, Stephanie Bleicken, Enrica Bordignon

Proteins of the Bcl-2 family are essential regulators in the mitochondrial pathway of apoptosis. They form a complex regulatory network whose understanding is a central objective in cancer research. Members of the Bcl-2 family share Bcl-2 homology (BH) domains, forming three distinct groups: Bax and Bak constitute the group of pro-apoptotic effectors whose activity is mediated by anti-apoptotic regulator proteins like Bcl-2 and Bcl-xL. The group of proapoptotic BH3-only proteins (e.g. Bid, Bim, Bad) can inhibit the activity of the anti-apoptotic regulators and/ or activate the pro-apoptotic effectors Bax and Bak [1].

Upon activation, cytosolic monomeric Bax undergoes major conformational changes, oligomerizes and enters the mitochondrial outer membrane [2]. Pore formation and the subsequent release of cytochrome c lead irreversibly to cell death. Here, we show how it is possible to monitor interactions in a minimal regulatory Bcl-2 network comprising of Bax, cBid and Bcl-xL via site-directed spin labeling EPR. To this end, we performed continuous wave EPR kinetic experiments to follow the mobility of spin labeled Bax, cBid or Bcl-xL in the presence of the other Bcl-2 regulators and liposomes. Furthermore, we performed DEER experiments to unveil how the interactions modify the structure of the proteins at the membrane and ODNP experiments giving insight in the membrane-water interfaces of the proteins.

References:
[1] doi:10.1038/nrm3722
[2] doi:10.1016/j.molcel.2014.09.022

Session/Abstract ID: Other (free) topics / 16341 /OT-21

RD3 and dysregulation of photoreceptor guanylate cyclase E (GC-E)

Presenting author:

Jonas Trautvetter

Author(s):

Jonas Trautvetter, Hanna Wimberg, Karl-Wilhelm Koch

Retinal guanylate cyclases and their regulation through proteins are involved in phototransduction. This process is crucial for cGMP and Ca²⁺ homeostasis in rod and cone photoreceptor cells. Mutations that impair GC-E regulation frequently lead to phenotypes associated with retinal diseases. GC-E activity is controlled by a set of guanylate-cyclase-activating-proteins (GCAPs), which regulate its activity in a Ca²⁺-dependent manner. The 23 kDa protein retinal degeneration 3 (RD3) mediates transport of GC-E and GCAP1 from the photoreceptor inner segments to the outer segments. RD3 is reported to inhibit GC-E at low Ca²⁺ by competing with GCAP1 for binding sites on GC-E. It is unknown whether RD3 affects GC-E activity in presence of dysfunctional GCAP1 mutants. Therefore, regulatory capabilities of RD3 in presence of GCAP1 mutants were tested. Human RD3 was heterologously expressed in *E. coli* and purified using fast protein liquid chromatography (FPLC). Enzyme assays were used to investigate RD3- and GCAP1-dependent GC-E activity. Cyclic nucleotide levels were determined as a measure of GC-E activity using high performance liquid chromatography (HPLC). Parameters of enzyme kinetics like IC₅₀ values were obtained and compared to wildtype data. Results indicate that competitive binding of RD3 to GC-E is impaired in presence of GCAP1 mutants. Impaired RD3 interaction may contribute to the retinal disease phenotype observed with GCAP1 mutations.

Session/Abstract ID: Cancer & Inflammation / 16460 /CA-12

Oocyte DNA damage quality control requires consecutive interplay of CHK2 and CK1 to activate p63.

Presenting author:

Marcel Tuppi

Author(s):

Marcel Tuppi, Volker Dötsch

The survival rate of cancer patients is steadily increasing due to more efficient therapies. These advances in cancer therapy, however, create a new and increasing problem since treatment with chemotherapeutic drugs and radiation increases the risk of premature ovarian insufficiency (POI) for cancer patients. Assisted reproductive technologies can address the problem of infertility but these measures cannot restore the hormonal functions important for women's health. Recent advances in whole organ replacement that could eventually restore long-term hormone function and fertility are promising, a more detailed understanding of the molecular mechanisms of therapy-induced POI could identify targets for pharmacological prevention of POI during gonadotoxic therapies. Loss of the primordial follicle reserve is the most important cause of POI, with the p53 family member p63 being identified to be responsible for DNA damage induced apoptosis of resting oocytes. Here, we provide the first detailed mechanistic insight into the activation of p63, a process that requires phosphorylation by both the priming kinase CHK2 as well as by the executioner kinase CK1. We further describe the structural changes induced by these phosphorylation events that enable p63 to adopt its active tetrameric conformation. Inhibition of CK1 rescues oocytes of the primordial follicles from doxorubicin and cisplatin induced cell death, thus uncovering a new target for the development of fertoprotective therapies

Session/Abstract ID: Cancer & Inflammation / 16247 /CA-04

Transcriptional expression of the proliferation marker Ki-67

Presenting author:

Sigrid Uxa

Author(s):

Sigrid Uxa, Paola Castillo-Binder, Gerd A. Müller, Kurt Engeland Ki-67, the protein encoded by the human gene MKI67, is the most frequently used proliferation marker in pathology for cancer diagnostics. Although Ki-67 has been widely employed as a diagnostic tool for more than two decades, the cellular function and the regulation of the gene expression remained largely unknown. Only recently, it was discovered that Ki-67 serves as a chromosome separator during mitosis and an organizer of heterochromatin. Ki-67 protein levels vary throughout the cell cycle with an expression maximum in G2 phase. In G0 Ki-67 is not expressed. Our experiments demonstrate that MKI67 mRNA levels also fluctuate in a cell cycle-dependent manner preceding expression of the protein. This observation suggests that Ki-67 expression is controlled on the transcriptional level. Indeed, we show that MKI67 expression is mainly regulated via two CHR transcriptional elements together with a CDE promoter site. Remarkably, all three sites are involved in transcriptional repression as well as activation of MKI67 through binding of the DREAM and MMB transcription factor complexes, respectively. This promoter structure represents a novel type of MuvB target genes. Therefore, we identify the mechanism of MKI67 cell cycle-dependent transcriptional regulation and explain the long observed expression pattern of the Ki-67 proliferation marker.

Session/Abstract ID: Supramolecular chemistry on disease-relevant targets / 16563 /SM-04

Molecular characterization and modulation of Survivin's cellular functions with supramolecular ligands

Presenting author:

Cecilia Vallet

Author(s):

Cecilia Vallet, Marcel Mertel, Dennis Aschmann, Martin Ehlers, Sandra Bäcker, Jana Reich, Christine Beuck, Peter Bayer, Carsten Schmuck, Shirley Knauer

Survivin was found to be upregulated in virtually all types of human cancers. It is associated with resistance against chemo- and radiotherapy, an increased tumor recurrence and an abbreviated patient survival, making it a promising target for cancer therapy. Survivin is involved in two key processes of carcinogenesis. As a member of the IAP family, it exhibits anti-apoptotic functions, but is also necessary for proper chromosome segregation during mitosis.

Our project aims to interfere with Survivin's (patho)biological role during cell death and proliferation using novel supramolecular ligands to target surface-accessible glutamate/aspartate residues either on Survivin's Histone H3 binding site or nuclear export signal (NES), which mediates interaction with the export receptor Crm1.

We were able to identify several promising candidate ligands affecting Survivin/Crm1 interaction and Survivin dimer formation in cell-based FRET- and proximity ligation assays. With NMR titration experiments, we could map binding of one peptide ligand to acidic amino acid residues on Survivin's surface spanning across the Histone H3 binding site and reaching out to the vicinity of the NES and the dimerization interface. Currently, we are setting up additional cellular assays to investigate an effect on Survivin's anti-apoptotic and mitotic functions.

Session/Abstract ID: Lunch Sessions / 16532 /OT-61

Attachment of protein-targeting molecules to the surface of ultrasmall gold nanoparticles (2 nm) by click chemistry

Presenting author:

Selina van der Meer

Author(s):

Selina van der Meer, Matthias Epple

Ultrasmall nanoparticles with a diameter of 2 nm or smaller were prepared in a one-pot synthesis by reducing tetrachloroauric acid in the presence of functional ligands. These nanoparticles are autofluorescent and permit a label-free tracking in biological setups like cells.

The ultrasmall particles were functionalized with alkyne groups either in situ or after a reduction step with azido groups carrying ligands. By click chemistry, molecules with an azido or an alkyne function were covalently bound to the particles under mild reaction conditions.

These surface-functionalized ultrasmall particles permit a specific targeting of epitopes on the surface of a protein as they are smaller than most proteins. This is of special interest to influence the function or conformation of a protein, e.g. to inhibit its function. The protein-binding affinity is increased by the multiavidity (more than one ligand on each nanoparticle) or heteroavidity (different ligands on one nanoparticle) of nanoparticles. The heteroavidity can be established by clicking several molecules simultaneously to the particle surface.

We thank the Deutsche Forschungsgemeinschaft (DFG) for funding in the framework of SFB 1093: Supramolecular Chemistry on Proteins.

Session/Abstract ID: Other (free) topics / 16465 OT-14

Recombinant expression of murine DNase1-like 2 in Pichia pastoris

Presenting author:

Lukas Verhuelsdonk

Author(s):

Lukas Verhuelsdonk, Katja Jakubowski, Beate Brand-Saberi, Hans Georg Mannherz, Markus Napirei

Deoxyribonuclease1 is an extensively investigated mammalian secretory deoxyribonuclease, however, less information exists about the biochemical and structural features of the further nucleases belonging to the DNase1-protein family.

To investigate these aspects, we started recombinant expression of murine DNase1-like 2 (DNase1I2) using the eucaryotic Pichia pastoris secretory expression system. This yeast expression system guarantees protein processing similar to the mammalian situation.

By varying several culture conditions like pH-value, aminoacid source, temperature or incubation time during growth and expression phase we established a reproducible expression protocol. In addition, we tried to enhance expression levels by co-transformation and co-expression of the yeast signal peptidase KEX2 or by increasing the copy number of the DNase1I2 expression cassette.

First results concerning protein purification from the yeast culture medium as well as concentration of the purified recombinant DNase1I2 protein were achieved.

Session/Abstract ID: Other (free) topics / 16444 OT-31

Towards time resolved kinetics of Biliverdin reduction by FDBRs

Presenting author:

Kai Vocke

Author(s):

Kai Vocke, Daniel Mann, Johannes Sommerkamp, Nicole Frankenberg-Dinkel, Klaus Gerwert, Carsten Kötting, Eckhard Hofmann

Phycocerythrin (PEB), an open chain tetrapyrrole, belongs to the class of Phycobiliproteins and serves as a photopigment in the light harvesting structure of cyanobacteria. Biosynthesis of PEB starts with the cleavage of Heme by heme oxygenases to produce Biliverdin (BV). BV is reduced in two consecutive two-electron-reductions by Ferredoxin dependent Bilin reductases (FDBRs). In cyanobacteria, BV reduction to the intermediate 15,16-Dihydrobiliverdin (DHBV) is catalysed by 15,16 dihydrobiliverdin: ferredoxin oxidoreductase (PebA). The subsequent reduction of DHBV to PEB is catalysed by Phycocerythrin: ferredoxin oxidoreductase (PebB). While structural and biochemical data for several FDBRs with and without bound substrate or product are known, the catalytic mechanism is still not fully understood. To measure the protonation states in the active site of PebA, we developed a photoactivated FDBR Assay. Using Proflavine or 5' Carboxyeosin as photosensitizer and cyanobacterial ferredoxin as electron mediator the catalysis is investigated by ongoing time resolved FTIR spectroscopy experiments.

Session/Abstract ID: Other (free) topics / 16381 /OT-23

Real-time imaging of the bacillithiol redox potential in the human pathogen Staphylococcus aureus using a genetically encoded bacilliredoxin-fused redox biosensor

Presenting author:

Van Loi Vu

Author(s):

Van Loi Vu, Manuela Harms, Thi Thu Huyen Nguyen, Chris J. Hamilton, Marret Müller, Falko Hochgräfe, Jan Pané-Farré, Haike Antelmann

Bacillithiol (BSH) is the major low molecular weight thiol of *Staphylococcus aureus*. Under oxidative stress, BSH forms mixed disulfides with proteins, termed as S-bacillithiolation which can be reduced by glutaredoxin-like bacilliredoxins (Brx). Here we have constructed the first genetically encoded bacilliredoxin-fused roGFP2 biosensor for dynamic live-imaging of the BSH redox potential in *S. aureus*. The Brx-roGFP2 biosensor showed a specific and rapid response to low levels bacillithiol disulphide (BSSB) in vitro which required the active-site Cys of Brx, but was unresponsive to cystine, GSSG or MSSM. Dynamic live-imaging revealed fast and dynamic responses of the Brx-roGFP2 biosensor inside *S. aureus* USA300 and

COL strains under hypochlorite and H₂O₂ stress and constitutive oxidation of the probe in isogenic BSH-deficient mutants. Using confocal laser scanning microscopy, the changes in the BSH redox potential in *S. aureus* are confirmed in single cells. In phagocytosis assays with THP-1 macrophages, the biosensor was 87 % oxidized in *S. aureus* COL. However, no changes in the BSH redox potential were measured after treatment with different antibiotics classes indicating that antibiotics do not cause oxidative stress in *S. aureus*. This novel Brx-roGFP2 biosensor can be used for live imaging of the BSH redox potential in *S. aureus* under oxidative stress, infection-like conditions and drug-treatments

Session/Abstract ID: Cell signalling and membrane trafficking / 16483 /CS-30

Assembly of peroxisomal pre-import complexes

Presenting author:

Thomas Walter

Author(s):

Thomas Walter, Wolfgang Girzalsky, Ralf Erdmann

The import of natively folded proteins into the peroxisomal matrix is facilitated by two specific peroxisomal targeting signals, PTS1 and PTS2. PTS1-proteins are imported via binding to the cytosolic PTS1-receptor Pex5p, whereas PTS2-proteins are recognized and bound by Pex7p. In contrast to Pex5p, Pex7p requires a co-receptor for association with the peroxisomal membrane. In *Saccharomyces cerevisiae* these co-receptors are Pex18p and Pex21p. The most abundant PTS2-protein is the thiolase Fox3p. Upon formation of the Fox3p/Pex7p complex in the cytosol, Pex18p associates with the complex and mediates binding to the peroxisomal membrane by association with the docking complex consisting of Pex13p, Pex14p and Pex17p. Finally, Pex18p becomes part of a distinct PTS2-import pore, which mediates the translocation of the cargo protein into the peroxisomal matrix. In this work, we analyzed the localization of Fox3p in wild-type and mutant cells more closely by biochemical means. A cell-free homogenate of oleic-acid grown cells was applied to a sucrose density gradient and obtained fractions were analyzed for presence of organelle-marker proteins. To gain more insight into the nature of specific Fox3p-subpopulations, we performed flotation analysis, size exclusion chromatography and Blue Native PAGE. The goal of this work is to investigate the pre-peroxisomal

complex formation between different cargo proteins and the organization of matrix proteins within the peroxisomal matrix.

Session/Abstract ID: Other (free) topics / 16450 /OT-32

Interaction of RD3 with enzymes of the nucleotide cycle in photoreceptors

Presenting author:
Hanna Wimberg

Author(s):
Hanna Wimberg, Ulrike Janssen-Bienhold, Karl-Wilhelm Koch

RD3 (retinal degeneration 3) is necessary for photoreceptor cell survival. Mutations in rd3 lead to an unstable, nonfunctional protein. These mutations are linked to an early onset of photoreceptor degeneration in patients with Leber congenital amaurosis (LCA12). The molecular mechanisms leading to LCA12 caused by rd3 mutations are not understood. So far two different functions for RD3 in photoreceptor cells are proposed. RD3 can inhibit the retinal guanylate cyclase (GC-E), a key enzyme in the phototransduction cascade. Further RD3 supports the GC-E transport in rods and cones. The aim of this project is to gain a better understanding of RD3 function in photoreceptors. Here we show that RD3 interacts at different steps within the nucleotide cycle in photoreceptor cells. Enzyme assays were used to analyze the regulatory function of RD3. Key enzymes of phototransduction (GC-E, phosphodiesterase (PDE6) and guanylate kinase (GUK)) were tested. Nucleotide levels upon RD3 interaction with these enzymes and their catalytic activity were analyzed by an HPLC assay. A spectrophotometric assay was used to define the effect of RD3 on GUK activity. RD3 revealed in addition to its inhibitory function on GC-E an activating effect towards GUK. A direct interaction between GUK and RD3 was tested by BSI (Backscattering Interferometry). In addition colocalization of RD3 and GUK was detected in the inner segments of rods. Our findings point towards a more complex role for RD3 in photoreceptors.

Session/Abstract ID: Cell signalling and membrane trafficking / 16354 /CS-20

Post-translational Labeling of Cardiovascular Receptors to Investigate Receptor Dimerization

Presenting author:
Philipp Wolf

Author(s):
Philipp Wolf, Katharina Gröger, Oliver Seitz, Annette Beck-Sickinger

Cardiovascular homeostasis is maintained by tight regulation of the endothelin receptors (ETR) ETAR and ETBR – two G protein-coupled receptors (GPCR). However, the formation of ETR multimers influences signaling and internalization. Therefore, dimerization studies are required for functional receptor characterization. C-terminally fluorescent fusion proteins achieve receptor visualization so far, although these modifications can block docking interfaces and prevent protein-protein interaction. Novel labeling techniques based on N-terminal tags are required to mark membrane-bound receptors extracellularly without disturbance of the intracellular docking interfaces. Recently, we displayed such an on-surface labeling method for GPCRs by short peptide tags [Reinhardt and Lotze et al., Bioconjugate Chem., 2015]. Here, we report on the expanded combination of two tags with complementary, fluorophore-bearing probes that interact by specific coiled-coil motifs. This allows the site-specific transfer of the fluorophore to the receptor. We demonstrate for N-terminally modified ETRs that they are able to bind their endogenous ligand without loss of receptor activation potency and that receptor internalization is maintained after ligand binding. This novel on-surface technique to label both ETRs differently is a promising tool to investigate the interplay between ETAR and ETBR, as well as their regulation, internalization and trafficking.

Session/Abstract ID: Systems biology and autophagy signal control / 16091 /SB-01

Anti-autophagy mechanism of the Legionella pneumophila

Presenting author:

Yaowen Wu

Author(s):

Aimin Yang, Supansa Pantoom, Yaowen Wu

Autophagy is a conserved cellular process involved in the elimination of proteins and organelles. It is also used to combat infection with pathogenic microbes. The intracellular pathogen *Legionella pneumophila* manipulates autophagy by delivering the effector protein RavZ to deconjugate Atg8/LC3 proteins coupled to phosphatidylethanolamine (PE) on autophagosomal membranes. To understand how RavZ recognizes and deconjugates LC3-PE, we prepared semisynthetic LC3 proteins and elucidated the structures of the RavZ:LC3 interaction. Semisynthetic LC3 proteins allowed the analysis of structure-function relationships. RavZ extracts LC3-PE from the membrane before deconjugation. RavZ initially recognizes the LC3 molecule on membranes via its N-terminal LC3-interacting region (LIR) motif. The RavZ $\alpha 3$ helix is involved in extraction of the PE moiety and docking of the acyl chains into the lipid-binding site of RavZ that is related in structure to that of the phospholipid transfer protein Sec14. Thus, *Legionella* has evolved a novel mechanism to specifically evade host autophagy.

Session/Abstract ID: Age-related pathologies and autophagy / 16378 /AP-05

Sirtuin 6 Activation by Synthetic Small Molecules

Presenting author:

Weijie You

Author(s):

Weijie You, Dante Rotili, Christian Kambach, Antonello Mai, Clemens Steegborn

Sirtuins are NAD⁺-dependent protein lysine deacylases, they regulate pathways in metabolism, stress responses, and aging processes. There are seven mammalian sirtuin isoforms (Sirt 1–7) at different sub cellular compartments, and they catalyze different

forms of deacylation. Increased activity of most Sirtuins has been implicated as beneficial for lifespan extension and protection against aging-associated disease, thus small molecule activators for sirtuins are considered for the treatment of cancers and neurodegeneration diseases. Resveratrol and synthetic STACs, which activate Sirt1 through interactions with an N terminal domain of Sirt1, are currently evaluated in clinical trials. However, no potent activators have been found for other sirtuin isoforms, which lack Sirt1's activator binding domain. Sirt6 plays crucial functions in metabolic adaptations and DNA homeostasis, it increases the life span of male mice and promotes resistance to aging phenotypes and cancer growth. We identified pyrrolo[1,2-a]quinoxaline-based compounds as first pharmacological activators for Sirt6. The compounds improve Sirt6-dependent deacetylation of acetylated peptide, histone octamers and nucleosomes, and activate Sirt5's dominant desuccinylation reaction, but have no effect on Sirt1, 2 and 3. Crystal structures of Sirt6/activator complexes reveal a novel target pocket within the acyl binding channel as activator binding site and provide a structural basis for drug development.

Session/Abstract ID: Cancer & Inflammation / 16544 /CA-15

Application of a highly miniaturized microarray for target-oriented search strategy for new (Hsp) inhibitors

Presenting author:

Qing Yue, Sabine Helmsen Carsten Zeilinger

Author(s):

Qing Yue, Sabine Helmsen, Sona Mohammadi Ostad-Kalayeh, Frank Stahl, Thomas Scheper, Andreas Kirschning, Carsten Zeilinger

Since heat shock proteins (Hsps) are targets for several natural or chemical drugs to hinder pathogenic developments like cancer or pathogenic bacteria, different purified Hsps were spotted on nitrocellulose membrane chip surfaces. These Hsp samples are capture proteins for ligands (e.g. ATP), isolated or synthesized inhibitors or labelled proteins. With the miniaturized target-oriented microarray assay an efficient screening was developed to assign targets susceptibility with synthesized specific small molecules or isolated natural products. Using fluorescence-labelled ATP, competitive binding of ligands can be measured by detecting changes in fluorescence

intensity as a function of inhibition of Hsp. Our data revealed that the purified proteins are stable after transfer and incubation time. Minimal amounts of the Hsps and inhibitors are sufficient. Only 50 pmol of purified Hsps are required per array, which can be stored for up to several weeks at 4 °C or -20 °C. It is possible to monitor simultaneously several proteins in a single test field. Some drugs showed different binding properties to human and bacterial Hsps. Furthermore, we can show that dye-labelled Hsp90 interacts with several clients and cochaperones as predicted before. Therefore the method can be used to screen for selective binders for Hsp proteins and enables multidimensional use for the detection of diverse interactions, e.g. protein-ligand/inhibitor and protein-protein/inhibitor.

Session/Abstract ID: Other (free) topics / 16106 /OT-06

A Proteomics approach to childhood maltreatment

Presenting author:
Johannes Zang

Author(s):
Johannes Zang, Caroline May, Katrin Marcus, Robert Kumsta

Early trauma experience has been linked to a wide range of psychological and physical health problems in adulthood. To identify the impact of childhood maltreatment on the functional level of cellular action we investigated the proteome of 118 monocyte samples derived from 30 participants with a history of traumatic experience and a matching control group before and after exposure to social stress. Utilizing LS-MS/MS so far we were able to identify 3519 protein groups by a minimum of 2 unique peptides of which 1162 protein groups were present in at least 80% of the trauma and/or the control group. Preliminary analyses indicate trauma induced differences in the monocyte proteome and highlight the feasibility of studying protein expression following stress exposure in the context of bio-psychological trauma research.

Session/Abstract ID: Pain / 16336 /PA-02

Landscape of pain in Parkinson's disease

Presenting author:
M.A. Samis Zella

Author(s):
M.A. Samis Zella, Thomas Müller, Caroline May, Maïke Ahrens, Lars Tönges, Ralf Gold, Katrin Marcus, Dirk Woitalla

Pain is a non-motor symptom in Parkinson's disease (PD). Various forms of pain in PD are discussed in the recent literature. However, only few systematic studies on pain features have been carried out. Aim of our study was to draw a topography of pain of a large PD study group including 1204 women and 1610 men. 469 of the PD patients were classified as tremor-dominant phenotype, 1178 as akinetic-rigid and 1167 as equivalence phenotype. A structured questionnaire was sent to PD patients to evaluate the relationship between pain and various clinical as well as demographic characteristics of PD. Each of the following items was correlated to gender and to phenotype: pain as 1st symptom, pain localization, mean pain intensity and pain as impairment to quality of life. Most of the patients reported spinal-paravertebral pain, followed by joint pain, musculoskeletal pain and headache. Furthermore, a significant correlation was demonstrated between gender and pain as 1st symptom (p -value=1.0e-03), pain topography (p -v.<7.0e-05), mean pain intensity (p -v.=1.0e-10) and pain as impairment to quality of life (p -v.=5.2e-14). Additionally, a significant correlation was shown between phenotype and pain as impairment to quality of life (p -v.=7.0e-04). Thus, gender and phenotype strongly influence pain perception in PD. Therefore, this study represents a premise for better understanding the pathophysiology of pain and for drawing appropriate therapeutic strategies in PD.

Session/Abstract ID: Molecular machines in Bioenergetics / 16631 /MM-03

Accessory subunits of mitochondrial complex I

Presenting author:

Volker Zickermann

Author(s):

Heike Angerer, Kristian Parey, Flora Kahlhöfer, Katarzyna Kmita, Stefan Schönborn, Jan Hoffmann, Klaus Zwicker, Ilka Wittig, Volker Zickermann

Complex I is the largest enzyme complex of the respiratory chain. Fourteen central subunits are conserved from bacteria to humans. Mitochondrial complex I comprises some 30 accessory subunits with largely unknown function. We have studied selected accessory subunits in complex I of the aerobic yeast *Yarrowia lipolytica*. Deletion of the NUMM gene or mutation of residues forming its zinc binding site blocked a late step in complex I assembly. NDUF54 is a hot spot for pathogenic mutations in humans. Deletion of the gene for the corresponding NUYM subunit caused distortion of iron sulfur clusters of the electron input domain leading to decreased complex I activity and increased release of reactive oxygen species. Complex I from *Y. lipolytica* comprises two acyl carrier proteins (ACPM1 and ACPM2) that are bound to the complex by LYRM proteins NB4M (NDUFA6) and NI2M (NDUFB9). ACPM1 was also detected as a subunit of the NFS1/LYRM4 complex of FeS cluster biogenesis, and as a free matrix protein. We have studied modification of both ACPMs by acyl groups and N-terminal acetylation. Deletion of the NB4M gene caused complete loss of ubiquinone reductase activity despite assembly of all central subunits. Site-directed mutagenesis identified critical interactions of NB4M residues with the long TMH1-2 loop of central subunit ND3 and with the N-terminal beta sheet of the 49-kDa subunit.

Session/Abstract ID: Cell signalling and membrane trafficking / 16172 /CS-09

Internalization and Resensitization of Neuropeptide Y1 and Y2 Receptors

Presenting author:

Isabelle Ziffert

Author(s):

Isabelle Ziffert, Stefanie Babilon, Karin Mörl, Annette Beck-Sickinger

Human neuropeptide Y receptors belong to the Rhodopsin-like G protein-coupled receptors and are involved in the regulation of various physiological and pathophysiological processes. Together with their three native ligands neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) the four receptor subtypes hY1R, hY2R, hY4R and hY5R build up a multi-ligand/multi-receptor system. Since this system is a promising target for pharmaceutical research, comprehensive knowledge about receptor signaling, as well as desensitization and downregulation is crucial to generate subtype-selective compounds and to reduce undesired side effects or drug resistance. In this study, we demonstrate a ligand dependent activation and internalization of the hY1R and hY2R in intracellular compartments. After the recycling period, both receptors relocate back to the membrane. However, in contrast to the Y1 receptor, the Y2 receptor does not recover with respect to G-protein activation, internalization and arrestin recruitment in a following restimulation with ligand. Furthermore, we could verify reduced ligand binding after recycling. Therefore, we hypothesize a new mechanism, which additionally controls receptor activation in terms of sensibility and selectivity within this multi-ligand/multi-receptor system.

Session/Abstract ID: Other (free) topics / 16435 /OT-27

Zebrafish GCAP5 - a redox-sensitive regulator of the guanylate cyclase?

Presenting author:

Sarah-Karina Zlomke-Sell

Author(s):

Sarah-Karina Zlomke-Sell, Nele Griepenstroh,
Alexander Scholten, Karl-Wilhelm Koch

The zebrafish guanylate cyclase activating protein 5 (zGCAP5) is one out of six GCAPs expressed in photoreceptors of the zebrafish (*Danio rerio*). GCAPs are neuronal Ca^{2+} -sensor proteins with a key function in photoreceptor physiology. The onset of zGCAP5 expression has not been proven before 12 to 15 dpf, which is later than other zGCAPs start expression. It might be involved into the phototransduction cascade by regulating the target enzyme of the known GCAPs, sensory membrane bound guanylate cyclases (GCs). However, initial experiments had shown that zGCAP5 activates sensory GCs much less in comparison to the other zGCAPs. Surprisingly, we have preliminary results showing that zGCAP5 regulates sensory GCs in a redox-sensitive manner, which might be related to the binding of Fe^{2+} . This unique property among all known GCAPs probably originates from an unusual arrangement of cysteine residues with a cysteine cluster at its N-terminal. Replacement of cysteines in zGCAP5 by site-directed mutagenesis revealed critical involvement in target regulation and Ca^{2+} -triggered conformational changes.
