Table of Contents

Welcome

Welcome to our **International Summer School 2014** on ”Methods and Microcompartments”. The summer school is organized by the PhD students of the ”Integrated Research Training Group” (IRTG), which is part of the University’s Collaborative Research Center (CRC) ”SFB 944”. Within the CRC, several research groups of the biology and physics departments from the Universities of Osnabrück and Münster work closely together with a common interest in studying microcompartments as basic functional units of a variety of cells.

The graduate training program on ”Membranes and Cellular Communication” was initiated in 2010 by research groups from the Physics, Chemistry and Biology Deparments at the Osnabrück University. The aim of the Summer School is to bring together distinguished scientists from different disciplines for intense scientific discussions on the topic of ”Methods and Microcompartments”.

The University of Osnabrück is a young university, founded in 1974, and is known for a large number of interdisciplinary degree programs, some of them rare or even unique among German universities, including European Studies, Applied Systems Science and Cognitive Science. Currently, about 10,000 students are enrolled for studies. The Science Institutes of the University (Biology, Chemistry, Physics, Cognitive Sciences) are located at the ”Westerberg Campus”, in close vicinity to the Botanical Garden of the University and the School for Applied Sciences (”Hochschule Osnabrück”).

We are delighted to welcome all of you in Osnabrück. We thank you for your contribution and look forward to an exciting meeting.

Schedule

Wednesday 23th July:

Methods & Microcompartments from Osnabrück

9.15 a.m Christian Ungermann
Opening of the Summer School
9.30 a.m Rainer Kurre
Beyond the Diffraction Limit - How Single Molecules Shed Light on Structure and Dynamics of Cellular Microcompartments
9.40 a.m Jürgen Klingauf
High-resolution microscopy for probing protein-protein interactions
9.50 a.m Johann Klare
Protein-Protein Interactions and Light-induced Conformational Changes in Archaeal Phototactic Signaling Arrays revealed by Electron Paramagnetic Resonance (EPR) Spectroscopy

10.00 a.m Coffee Break

Methods & Microcompartments International Guests

Session 1: Chair - Renate Scheibe

10.15 a.m Jörg Kudla
Integration of Calcium and ROS Signaling in Arabidopsis
10.50 a.m Thomas Surrey
Investigating the microtubule cytoskeleton by in vitro reconstruction
11.25 a.m Mary L. Kraft
Imaging the sphingolipid and cholesterol distributions in the plasma membrane with secondary ion mass spectrometry

12.00 a.m Lunch Break
1.00 p.m Poster Session A (even)

Wednesday 23th July:
Methods & Microcompartments International Guests

Session 2: Chair - Michael Hensel

2.30 p.m Christos Gatsogiannis
Syringe-like injection mechanism of bacterial ABC toxins revealed in molecular detail
3.05 p.m Jürgen M. Plitzko
Doing Structural Biology in situ – From cells to molecules

4.00 p.m Social event / Faculty Summer Party

Thursday 24th July:
Methods & Microcompartments International Guests

Session 3: Chair - Guy Hanke

9.15 a.m Carla Schmidt
The Chloroplast ATP Synthase – Insights from Mass Spectrometry
9.50 a.m Ulrich Rothbauer
Connecting Cell biology and Biochemistry with Chromobodies

10.25 a.m Coffee Break
Poster Session B (odd)

Session 4: Chair - Roland Brandt

11.55 a.m Franziska Fichtner
Precision gene targeting and genome editing using programmable DNA binding proteins
12.30 a.m Hans Ulrich Dodt
Visualization of neuronal networks in the mouse brain and mouse embryos by ultramicroscopy

1.05 p.m Lunch Break

2.00 p.m Career Coaching Workshop

Friday 25th July:
4th Annual Retreat of the IRTG

Session 1: Chair - Christian Kock

9.00 a.m Patrick Hörnschemeyer
Establishing ND technology for the characterization of a bacterial two-component system
9.20 a.m Simon Gohlke
Topology and protein interactions of Chs3 in Saccharomyces cerevisiae
9.40 a.m Dorthe Rippert
Regulation of cytokinesis in the milk yeast Kluyveromyces lactis
10.00 a.m Ariane C. Wilmes
Pericardin: A collagen IV like protein plays a major role in heart integrity of D. melanogaster

10.20 a.m Coffee Break

Session 2: Chair - Anna Lürick

10.45 a.m Amrita Jain
Manipulation of intracellular ceramide flows and its impact on cell fate and organization
11.05 a.m Erdal Yavavli
Toward understanding the function of AP-3 in fusion and membrane remodeling
11.25 a.m Manuel Twachtmann
Impact of FNR location on photosynthetic electron flow
11.45 a.m Bettina Rieger
Local pH differences in actively respiring mitochondria

12.05 p.m Lunch Break

Friday 25th July:
4th Annual Retreat of the IRTG

Session 3: Chair - Christoph Drees

1.00 p.m Agnes Borchers
Protein interaction dynamics of microtubule-associated proteins from Arabidopsis

1.20 p.m Dennis Janning
Tau's role in health and disease: What can we learn from single molecule imaging?

1.40 p.m Britta Barlag
Correlative super resolution/ atomic force microscopy unravels the localization of two protein secretion systems during invasion of polarized epithelial cells by Salmonella enterica

2.00 p.m Oliver Birkholz
Surface architecture for probing receptor interactions and conformations

2.20 p.m Coffee Break

Session 4: Chair - Daniel Klose

2.50 p.m Sarah Kopischke
Understanding the ancestral function of TCP transcription factors

3.10 p.m Markus Schneider
The cytosolic NAD-GAPDH as a hub for transmitting cellular redox changes

3.30 p.m Christian Rickert
Light- and ligand-induced structural changes detected by electron paramagnetic resonance

3.50 p.m Rajit Rajappa
Cleaning up the synaptic active zone: is synaptophysin a clearance factor post fusion?

Location

The meeting will take place in the newly built Bohnenkamp-Haus at the "Botanischer Garten Osnabrück" (botanical garden). The garden is maintained by the University of Osnabrück and was founded in 1984. It shows global plant biodiversity comprising the Mediterranean region, North America, Japan and China as well as protected regional plant populations. Plants are grown in two abandoned quarries of over eight hectares, giving the garden a special atmosphere with its setting surrounded by limestone, dating back over 200 million years. The collection consists of alpine plants, Swabian Alpine plants, medicinal plants, ferns, and heather as well as several greenhouses including a rainforest house with a focus on Amazonian vegetation. The newly built Bohnenkamp-Haus serves as the garden's environmental education facility for activities for all ages. In an attractive ambience, it provides the space for larger congresses, workshops as well as for small seminars and experimental practical courses.

Faculty Summer Party

The participants of the Summer School are cordially invited to attend our social event that will take place on 23rd of July from 4.00 p.m in the botanical garden. It is combined with annual summer party of the faculty, which is organized this year by the current dean Prof. Dr. Brandt and the department of Neurobiology. The program of the summer party includes as usual coffee, cake and ice-cream, a BBQ with a salad bar and different beverages, accompanied from 5.00 to 6.00 p.m by live music (Uni Big Band) and various outdoor games. Participants of the Summer School will find food vouchers in their bags, which can be redeemed for food and drinks at the summer party. Dessert enthusiasts have to pay the external ice-cream seller, who will be there in his traditional ice-cream truck from 5.00 p.m on, or buy self-made cakes and support the children foundation "Balu und Du" (http://www.balu-und-du.de).

Career Coaching

Thursday 2.00 p.m in the Bohnenkamp-Haus
In English and German

”From PhD to industry – Exchange of experciences with human resources managers”

”Mit dem Doktortitel in die Wirtschaft – Erfahrungsaustausch mit Personalmanagern”

Im Zuge der Promotion stellt sich vielen Promovierenden die Frage: in die Wissenschaft oder in die Wirtschaft. Und wenn Letzteres – wie? Diese Veranstaltung soll Ihnen die Chancen und Herausforderungen des Einstiegs mit Promotion deutlich machen, mögliche Fragen klären und Sie dabei unterstützen, Ideen für Ihr berufliches Fortkommen zu entwickeln sowie in die Praxis umzusetzen. Als Personalberater sind unsere Experten tagtäglich sowohl in den Bereichen Berufsorientierung und Bewerbung als auch Personalauswahl und -entwicklung in der Wirtschaft aktiv. Nutzen Sie die Gelegenheit zum Erfahrungsaustausch und lernen Sie, wie Sie zielorientiert vorgehen und Ihre Kompetenzen optimal einsetzen.

Es beraten Sie Bastian Thiebach und Philipp Ax von der B&B GmbH aus Georgsmarienhütte.

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Directions

By bus:

Take bus line 21 going to "Atterfeld" from the train station or "Neumarkt", which is the central bus station in Osnabrück. The bus stop "Westerberger Hochschulen" is located just outside the main entrance of the botanical garden. Alternatively, you can use bus lines 11 and 13, direction "Eversburg" from "Neumarkt" from the "Neumarkt" and exit at the bus stop "Sedanplatz" from where you just have to go up the hill following "Albrechtstraße".

On foot:

The botanical garden is also within walking distance (about 15 min uphill) from the Hotel Walhalla, if you prefer to walk. Leave the inner city through the "Heger-Tor" and cross the main road. Take the "Bergstraße" on your right hand and cross the "Bismarckplatz". Then take the "Edinghäuserweg" to your right and walk on straight until you reach the top of the Westerberg, where the botanical garden is located.

By car:

If you want to go by car and use a navigation system, direct it to "Albrechtstraße 29", zip code 49076, Osnabrück.

Speaker Portraits

Jörg Kudla

University of Münster, Germany

Jörg Kudla is Professor for „Molecular Genetics and Cell biology of Plants" at the University of Münster. He is also adjunct Professor for plant biology at the China Agricultural University in Beijing. He received his Ph.D. degree in genetics from the Martin-Luther University Halle in 1992. Dr. Kudla has held positions at the University of Freiburg (postdoctoral fellow), Berkeley (postdoctoral fellow) and Ulm (independent group leader). Dr. Kudlas research focuses on calcium signaling in plant development, calcium-regulated vesicle trafficking and membrane transport as well as agricultural appliances. Dr. Kudla received several research fellowships: The research fellowship of the Martin-Luther-University Halle (1988-1990), research fellowship of the German Academic Exchange Organization (DAAD, 1990), research fellowship of the Fund of the German Chemical Industry (1991), research fellowship of the state Baden-Württemberg (1992), research fellowship of the German Science Foundation (DFG, 1994-1995), research fellowship of the German Academic Exchange Organization (DAAD, 1996), visiting professorial fellowship of the German Academic Exchange Organization (DAAD, 2011) and Fellow of the San Diego Systems Biology Center (SDSBC, 2011) and won the research prize of the "Ulmer Universitätsgesellschaft" in 2000.

Integration of Calcium and ROS Signaling in Arabidopsis

Jörg Kudla, Universität Münster, Institut für Biologie und Biotechnologie der Pflanzen, Schlossplatz 4, 48149 Münster, Germany

Calcium (Ca2+) and reactive oxygen species (ROS) are important second messengers that function in many different biological processes in plants. Spatially and temporally defined changes in their cytoplasmic concentrations represent stimulus-specific signals. However, the modes of interaction and integration of these two important second messengers are largely unknown. Respiratory burst oxidase homologs (RBOHs) are membrane localized plant NADPH oxidases that produce ROS and form a family of 10 proteins in Arabidopsis. Cellular calcium signals are detected and transmitted by calcium-binding proteins that function as calcium sensors. In higher plants, calcineurin B-like (CBL) proteins and their CBL-interacting protein kinases (CIPKs) represent important relays in calcium signaling in many different response reactions. In Arabidopsis, 10 CBL-type calcium sensor proteins form a dynamic interaction network with 26 CIPKs. I will present mechanistic principles of the CBL/CIPK signaling network that on one hand enable specificity in signal-response-coupling and on the other hand allow for the required robustness and flexibility in signal output generation. Moreover, I will report in detail how distinct Ca2+-activated CBL/CIPK complexes regulate ROS production of specific RBOH proteins in Arabidopsis and how this contributes to generate specificity in ROS signaling. In this way I will detail mechanisms that integrate Ca2+ and ROS signaling, how activation of ROS signals occur and discuss a model how Ca2+ and ROS could function together in plant long distance signaling.

Thomas Surrey

London Research Institute, London, United Kingdom

Thomas Surrey is a senior group leader at the London Research Institute. He received his Ph.D. degree from the University of Tübingen and the Max-Planck Institute for Biology in Tübingen in 1995. Dr. Surrey has held positions at the European Molecular Biology Laboratory in the „Cell Biology and Biophysics Unit“ in Heidelberg (Group Leader (2006), Team Leader (2002), Staff Scientist (2011), Postdoc (1998).
Dr. Surreys research focuses on the question of how the microtubule cytoskeleton organizes itself within the boundary of the cellular membrane and how the mitotic spindle assembles and functions during cell division.

Investigating the microtubule cytoskeleton by in vitro reconstruction.

Thomas Surrey, London Research Institute, London, United Kingdom

The microtubule cytoskeleton is critical for a vast variety of essential processes in eukaryotic cells, including cell division and differentiation. How the microtubule cytoskeleton organises itself in space and how its dynamic properties are controlled are fascinating questions. The fact that the architecture and dynamic properties of this filament network can be observed by fluorescence microscopy make it an ideal object for the study of the design principles of a complex protein interaction network. Here we will present in vitro experiments aimed at dissecting microtubule cytoskeleton functioning by reconstructing it from purified components. Examples will be presented of how molecular mechanisms can be elucidated by reverse engineering of this major intracellular filament network.

Mary L. Kraft

University of Illinois, Illinois, USA

Mary L. Kraft is an assistant professor in the Department of Chemical and Biomolecular Engineering at the University of Illinois in Urbana-Champaign (2007 – present). She also is an affiliated member of the Department of Chemistry and the Center for Biophysics and Computational Biology. She received her Bachelor of Science degree in Biochemistry from the University of Illinois at Chicago, and her Ph.D. from the Department of Chemistry at the University of Illinois at Urbana-Champaign under the supervision of Prof. Jeffrey Moore. Dr. Kraft completed postdoctoral studies with Professor Steven Boxer at Stanford University. Dr. Kraft's research focuses on characterizing the lipid organization in cellular membranes with high-resolution secondary ion mass spectrometry. Other research interests include sphingolipid trafficking and signaling and influenza virus replication. Dr. Kraft's honors include a NIH NRSA Kirschstein Postdoctoral Fellowship (2004), and a Burroughs Wellcome Career Award at the Scientific Interface (2007). In 2014, she was awarded the Walter A. Shaw Young Investigator Award in Lipid Research from the American Society for Biochemistry and Molecular Biology (ASBMB) for her research in cell membrane organization.

Imaging the sphingolipid and cholesterol distributions in the plasma membrane with secondary ion mass spectrometry

Dr. Mary L. Kraft, Assistant Professor, Department of Chemical & Biomolecular Engineering, University of Illinois

Cholesterol and sphingolipids serve both as structural components in the plasma membranes of eukaryotic cells, and as bioactive signaling molecules that modulate fundamental cellular processes. Though the segregation of sphingolipids and cholesterol into distinct plasma membrane domains is widely hypothesized to be essential for cellular function, the sphingolipid and cholesterol distributions within the plasma membrane have not been established. To address this issue, we have used high-resolution secondary ion mass spectrometry (SIMS) to map the distributions of stable isotope-labeled lipids in the plasma membranes of intact cells with better than 100 nm lateral resolution. In this approach, we first metabolically incorporate the rare isotopes, 15N and 18O, into the sphingolipids and cholesterol, respectively in living fibroblast cells. Then we use a Cameca NanoSIMS 50 instrument to image the 15N- and 18O-enrichments that are characteristic of the 15N-sphingolipids and 18O-cholesterol, respectively, on the cell surface. Our experiments have revealed sphingolipid domains that are not enriched with cholesterol in the plasma membranes of fibroblast cells. By using this approach to visualize the lipid distributions after various drug treatments, we have determined that these sphingolipid domains are dependent on the cytoskeleton, and not favorable cholesterol-sphingolipid interactions.

Christos Gatsogiannis

FU Berlin, Germany

Christos Gatsogiannis is currently an expert scientist in the group of Prof. Raunser in the Institute Chemistry and Biochemistry at the Free University Berlin. He is a structural biologist with expertise in cryo electron microscopy. He completed a Diploma in Biology at the Johannes Gutenberg University of Mainz in 2006. He obtained his PhD in 2009 at the Institute of Zoology in Mainz where he worked with Prof. Jürgen Markl. While there, his research focused on the structure and function of oxygen transporters. This was followed by a four-year postdoctoral stint with Prof. Stefan Raunser at the Max Planck Institute of Molecular Physiology in Dortmund. His research focuses on the structural and functional analysis of macromolecular complexes that play important roles in biology and disease, in particular bacterial pore forming toxins and other virulence factors, using modern structural biology methods such as cryo-electron microscopy, 3D-single particle analysis, electron tomography and molecular modelling.

Syringe-like injection mechanism of bacterial ABC toxins revealed in molecular detail.

Christos Gatsogiannis, FU Berlin, Germany

Tc toxin complexes are dominant secreted virulence factors of many pathogenic bacteria such as the Far East scarlet-like fever pathogen *Yersinia pseudotuberculosis* and the plague pathogen *Yersinia pestis*. They are typically composed of TcA, TcB and TcC subunits that are only biologically active as tripartite complexes. TcB and TcC together form a closed cage, that encapsulates and sequesters the cytotoxic, C-terminal region of the C protein like the shell of an egg. However, little is known about the translocation of this toxic component into the cell by the TcA component. Here we show that TcA in *Photorabdus luminescens* (TcdA1) forms a transmembrane pore and report its structure in the prepore and pore state determined by cryoelectron microscopy and x-ray crystallography. We find that the TcdA1 prepore assembles as a pentamer forming an α-helical, vuvuzela-shaped channel less than 1,5 nanometres in diameter surrounded by a large outer shell. Membrane insertion is triggered not only at low pH as expected, but also at high pH, explaining Tc action directly through the midgut of insects. Comparisons with structures of the TcdA1 pore inserted into a membrane and in complex with TcdB2 and TccC3 reveal large conformational changes during membrane insertion, suggesting a novel syringe-like mechanism of protein translocation driven by an entropic spring. Our results allow us for the first time to understand key steps of infections involving ABC toxins at molecular level and shed new light on the interaction of bacterial pathogens, such as the plague pathogen *Yersinia pestis*, with their hosts. Furthermore, our data serve as a strong foundation for the development of protein shuttles targeting specific cell types in medical therapy.

References:

1. Meusch D, Gatsogiannis C, Efremov RG, Lang AE, Hofnagel O, Vetter IR, Aktories K, Raunser S.
Mechanism of Tc toxin action revealed in molecular detail.
Nature. 2014 Apr 3;508(7494):61-5. doi: 10.1038/nature13015.

2. Gatsogiannis C, Lang AE, Meusch D, Pfaumann V, Hofnagel O, Benz R, Aktories K, Raunser S.
A syringe-like injection mechanism in Photorhabdus luminescens toxins.
Nature. 2013 Mar 28;495(7442):520-3. doi: 10.1038/nature11987.

Jürgen Plitzko

University of Utrecht, Netherlands
Max-Planck Institute of Biochemistry, Martinsried, Germany

Jürgen Plitzko (45) has worked with the Electron Microscopy group at the leading Max-Planck-Institut für Biochemie in Martinsried (Germany) since 2002. The focus of his research is the development of new electron microscopy techniques to visualise biologically relevant macromolecular complexes.

Doing Structural Biology in situ – From cells to molecules

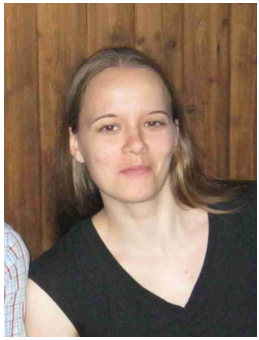
Juergen M. Plitzko Max Planck Institute of Biochemistry, Dept. Molecular Structural Biology, Martinsried, Germany and University Utrecht, Bijvoet Center of Biomolecular Research, Utrecht, The Netherlands

The application of cryo-electron tomography (cryo-ET) to complex cellular systems (i.e. large cells or tissues) is commonly referred to as “cellular tomography”, and allows the visualization of the supramolecular architecture of cells in a near-native state. However, only sufficiently small structures (< 500 nm) can be examined *in toto* by cryo-ET with a resolution that is high enough for the detection and identification of molecular structures. To advance the study of eukaryotic cells at molecular resolution it is therefore necessary to develop and deploy tools and techniques that provide controlled access to structural features buried deep inside cellular volumes. Moreover many structures of great interest are present only in low copy numbers or are so deeply rooted in their cellular environment that it is almost impossible to isolate them without violation of their structural integrity. Hence, it is highly desirable to study them *in situ* and the concept of doing structural biology *in situ* is an appealing one. Moreover, cryo-ET is challenged by the following particularity: Although the information content of a small volume increases (as one zooms further and further into the cell) sampling statistics decrease (as the volume imaged represents only a small proportion of the cell). Therefore approaches are needed that can be applied to a wide range of sample sizes (e.g. cells and tissue), that can bridge several orders of magnitude in spatial resolution and that provide a minimum level of statistical significance.

To study biological systems *in situ* and at different scales (e.g. from ‘cells to mo- lecules’), we need methods which are capable of navigating, targeting, examining and analyzing complex samples in their native environment. However, the combination of various methodologies into a robust and reliable workflow is challenging. Here we present our approach to combine three different methodologies for *in situ* structural biology with frozen hydrated biological specimens: (i) correlative cryo-fluorescence microscopy to localize features in all three dimensions, (ii) cryo-focused ion beam milling to thin previously identified subcellular structures in a targeted manner, and (iii) cryo-electron tomography to allow the structural analysis of the cellular environment at macromolecular resolution (approx. 2 nm). We aim to discuss approaches to improve the localization specificity and precision in 3D, the preparation reproducibility, yield and the overall throughput in cryo-FIB lamella preparation and furthermore advancements in cryo-ET data collection with direct detectors - all together key factors in advancing *in situ* imaging for structural biology.

Carla Schmidt

University of Oxford, United Kingdom



Carla is a senior post-doctoral researcher in the laboratory of Professor Dame Carol V. Robinson, University of Oxford, UK. Carla studied chemistry at the University of Leipzig and moved then to Göttingen for her PhD research at the Max Planck Institute of Biophysical Chemistry (Bioanalytical Mass Spectrometry Group). Her PhD work involved the quantification of functional intermediates of the human spliceosome. She obtained her PhD in 2010 from the University of Göttingen. After a short post-doctoral research stay she moved to Oxford to work with Carol Robinson. Carla combines mass spectrometry of intact protein assemblies with proteomic approaches to study the structure and function of soluble and membrane protein complexes. She is particularly interested in the regulation of F-type ATP synthases and protein-protein interactions within the eukaryotic translation initiation factors.

The Chloroplast ATP Synthase – Insights from Mass Spectrometry

Carla Schmidt, University of Oxford, Oxford, United Kingdom

Proteins undergo dynamic interactions with other proteins or ligands to form catalytic cores, fine-tuned for different cellular actions. The study of dynamic interactions between proteins and their ligands is therefore fundamental to the understanding of biological systems. Mass spectrometry is playing an increasing role in structural biology and, consequently, a multitude of techniques has evolved to address different questions in the structure elucidation of protein-protein and protein-ligand assemblies. We have applied and combined some of these techniques to study the ATP synthase purified from spinach chloroplasts. Mass spectra of the intact enzyme revealed stable subcomplexes and functional intermediates. To investigate subunit interactions at the molecular level we applied chemical crosslinking. Lipidomics allowed identification of associated lipids and arrangement of the 'lipid plug'. In addition, we have applied proteomic techniques to study the role of post-translational modifications. In this lecture I will provide an introduction to mass spectrometry to study protein (ligand) complexes. I will highlight the power of combining many MS methods by exemplifying this approach with our studies of the chloroplast ATP synthase.

Ulrich Rothbauer

University of Tübingen, Germany

Dr. Rothbauer has studied biology at the Ludwig-Maximilians University (LMU). He received his Ph.D in Biochemistry in 2003 revealing the pathomechanism of a mitochondrial disease. After his postdoctoral work on the regulation of the epigenetic keyfactors he became an independent group leader at the LMU-Biocenter in 2006.
As a junior group leader he directed R&D projects in a range of cellular diagnostic areas including cell cycle studies, apoptosis studies, proteomics and protein-protein interactions. 2008 he founded the Biotech spin-off company ChromoTek, which develops and commercializes new technologies to perform cellular diagnostics and proteomics. Since 2011 he is a full professor for Pharmaceutical Biotechnology at the University Tuebingen, Germany. 2008 and 2010 he was awarded with the GO-Bio Award of the BMBF for his innovative research on single domain antibodies and the development of new diagnostic probes based on this antibody format

Ulrich Rothbauer is working on new technologies to study proteins and protein interactions in living cells. His main focus lies on the development of disease relevant cell based assays and target specific nanoprobes for High Content Analysis based on the Chromobody-Technology.

Connecting Cell biology and Biochemistry with Chromobodies

Ulrich Rothbauer, University of Tübingen, Germany

One of the major challenges in early drug discovery is to understand cellular processes in response to external stimuli. To visualize dynamic processes of endogenous components in real time we have developed a new format of intracellular functional antibodies (chromobodies). In combination with high-throughput microscopy and automated image analysis we recently have demonstrated that chromobodies can be used to monitor cell cycle, induction of apoptosis and dynamic rearrangements of the cytoskeleton upon compound treatment. In addition we work on the development of new versatile nanotraps for biochemical analysis of cellular proteins and automated immuno diagnostics.

Bekir Ülker

University of Bonn, Germany

Change: Unfortunately, Dr. Ülker is unavailable but his former student Franziska Fichtner will act as a substitute for him.

Until recently, Dr. Ülker was a junior group leader at the University of Bonn heading the Plant Molecular Engineering Group. He received his Ph.D. degree in Crop Science from North Carolina State University, Raleigh U.S.A. in 1998. He held postdoctoral positions at Department of Genetics in North Carolina State University, Raleigh, U.S.A, at the Department of Plant Microbe Interactions in Max-Planck-Institute for Plant Breeding Research, Cologne, Germany, at the Department of Biological and Biomedical Sciences in University of Durham, Durham, U.K. and Senior Scientist position at CAMBIA (Center for Application of Molecular Biology to International Agriculture), Canberra, Australia.

His research interest is developing biosafe and environmental friendly enabling technologies for crop improvement against pathogenic plant microbes and abiotic stresses. His major research tool is Agrobacterium tumefaciens, a soil bacterium causing tumors (Crawn-gall disease) in plants. It is also the greatest tool for plant scientist in engineering plant genomes.

His major contribution to the science was the discovery of unintended transfer of Agrobacterium chromosomal DNA fragments to transgenic plants. His group has recently uncovered the mechanisms of these undesired DNA transfers and is currently developing engineered BioSAFE A. tumefaciens strains. Dr. Ülker is combining recent developments in precision gene targeting and genome editing with the Agrobacterium-mediated genetic engineering technologies to further improve the precision and efficiency of targeted genome editing.

Change: Unfortunately, Dr. Ülker is unavailable but his former student Franziska Fichtner will act as a substitute for him.

Precision gene targeting and genome editing using programmable DNA binding proteins

Bekir Ülker, University of Bonn, Germany

Programmable DNA-binding proteins such as ZFP/ZFNs, TALE/TALENs and CRISPR/Cas have produced unprecedented advances in gene targeting and genome editing in prokaryotes and eukaryotes. These advances allow researchers to specifically alter genes, reprogram epigenetic marks, generate site-specific deletions and potentially cure diseases. Unlike previous methods, these precision genetic modification techniques (PGMs) are specific, efficient, easy to use and economical. No doubt that PGMs are going change our lives, speed-up scientific leaps and lead to unprecedented applications all aspects of biology. In this seminar, the capabilities and pitfalls as well as future directions to how these technologies could be further improved will be discussed.

Relevant publications and patents

Fichtner, F, Castellanos, RU and **Ülker B.** Precision genetic modifications: a new era in molecular biology (2014). http://link.springer.com/article/10.1007/s00425-014-2029-y. *Planta*, 239 (4): 921-939.

Ülker B, Li Y, Rosso M G, Somssich I E and Weisshaar B. T-DNA-mediated transfer of *Agrobacterium* chromosomal DNA sequences into plants (2008). *Nature Biotechnology* 26:1015-1017.

Hommelsheim CM, Frantzeskakis FA, **Ülker B.** April 2013. Improved gene targeting and nucleic acid carrier molecule, in particular for use in plants.
The European Patent Office Application No. 13164966.7-1406.

Hans Ulrich Dodt

TU Vienna, Austria

Hans-Ulrich Dodt is Professor for Bioelectronics at the Technical University Vienna since 2007. He received his Ph.D. degree in medicin from the University of Heidelberg. Dr. Dodt has held positions at the MPI for psychiatry in Munich and the technical University of Munich. Dr. Dodts research is interdisciplinary, connecting Neurophysiology and Physics. It focuses on the neuronal network of the brain and its biological processing of information.

Visualisation of neuronal networks in the mouse brain and mouse embryos by ultramicroscopy

H.U. Dodt, N. Jährling, S. Saghafi, C. Hahn, K. Becker
Department of Bioelectronics, Institute of Solid State Electronics, TU Vienna, Vienna, Austria, and Section of Bioelectronics, Medical University Vienna. Vienna, Austria

It would be very helpful for the analysis of neuronal networks of the brain, if one could visualize these networks in 3 dimensions. Up to now this was only possible with limited resolution by sequential slicing and reconstruction of the brain. This time consuming attempt is easily hampered by artifacts as shrinkage and distortion induced by standard histological procedures. To overcome these problems we used a microscopy based on extreme darkfield illumination with a light sheet, once called ultramicroscopy. This microscopy allows optical sectioning of whole mouse brains and was combined with an approach to clear fixed neuronal tissue: Mouse brains were made completely transparent by immersion in oil of the same refractive index as protein. By illuminating the brains with blue light (λ = 488 nm), neurons labeled with GFP were visualized by fluorescence. This way we could detect single neurons in hippocampi inside whole brains. By surface rendering the shape and position of hippocampi relative to the brain surface could be depicted. In complete excised hippocampi subcellular resolution was obtained by 3D reconstruction from several hundred optical sections. The dendritic trees of CA1 hippocampal neurons with dendrites and dendritic spines could be visualized.
Many proteins can be labelled in transgenic mice with genetically encoded fluorescent markers. Using these markers our approach will represent a high-throughput screening method for protein expression in 3 D. This expression can be monitored with µm resolution and should allow the elucidation of complex neuronal networks in the brain.
In a genome-wide RNAi screen, flight defective Drosophila were isolated. A number of these lines show strong flight muscle defects, suggesting a muscle formation defect or extensive muscle degeneration. In our ongoing study we investigate the morphology of individual muscles in the intact fly. This enables us to assign the observed defects to particular muscle classes.
We show that ultramicroscopy allows also optical sectioning and detailed 3D reconstruction of whole mouse embryos by imaging autofluorescent structures. Especially the circulatory system in the body and brain became apparent as blood remaining in the preparation showed strong fluorescence. Also other applications like e.g. visualization of nerve bundles in whole embryos and visualization of plaques in brains of mice with Alzheimers disease will be shown. In general the method is well suited for high-throughput phenotype screening of

transgenic mice and thus will benefit the investigation of disease models.
We have started to apply ultramicroscopy to the investigation of specimens from human pathology like different cancers. In autofluorescence it is possible to visualize interesting structures in the pathological material which we hope will help the diagnosis and staging of malignant diseases.

Poster Abstracts

Understanding structural interactions and signal transduction within two component systems

Patrick Hörnschemeyer[1*], Sabine Hunke[1]

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Two component systems (TCS) are the major systems used by bacteria to communicate with their environment. Although studied for more than three decades, it is still poorly understood how a signal is recognized and transduced by a TCS. A TCS is comprised of a sensor kinase and a response regulator. The model studied by our group is the Cpx-TCS which consists of three major proteins: the sensor kinase CpxA, the response regulator CpxR, and the accessory protein CpxP. This signaling complex and the stimulus dependent dynamic interaction between its proteins can be defined as a “micro compartment”. CpxA activation is dependent on numerous stimuli that include, e.g. the lipid composition of the inner membrane and different factors from the periplasmic space. The target of this project is to investigate the structural interactions and the signal transduction between the core components CpxA and CpxR. To this end, the Cpx system was reconstituted using nanodiscs and subjected to functionality analyses and spectroscopic interaction studies. This approach avoids the problems of inside-out-orientation and spatial restrictions that are given in proteoliposomes [1]. Equal or improved kinase and phosphotransfer activities, respectively, could be shown using CpxA in nanodiscs compared to CpxA in proteoliposomes. Additionally, spectroscopic studies showed strong binding of CpxR to CpxA, which was controllable by adding Mg^{2+}-ATP.

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The role of the Cpx-system within the envelope stress systems in *Escherichia coli* analyzed by MRM and co-localization studies

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Bacteria rely on two-component systems (TCS) to acclimate in response to environmental changes and therefore to different stimuli [1].These systems use a phosphorylation cascade from a transmembrane sensor kinase (SK) to a cytoplasmic response regulator (RR). We investigate the Cpx-envelope stress TCS as a model for a microcompartment within and between bacterial membranes to understand signal recognition, signal transduction and signal coordination. The Cpx-TCS consists of the membrane-bound SK CpxA, the cytosolic RR CpxR and the periplasmic accessory protein CpxP, which inhibits the autophosphorylation activity of CpxA [2, 3].
To better understand the functional and the dynamic interactions within the Cpx-TCS, it is important to know the absolute amounts and the co-localization of CpxA, CpxR and CpxP under different growth conditions. Therefore, absolute quantification of the Cpx-TCS was performed under different Cpx-system affecting conditions by multiple reaction monitoring (MRM). Since a crosstalk between CpxA and the RR ArcA of the Arc-TCS is suggested [4], we additionally quantified the absolute amounts of ArcA and ArcB. By analyzing the relative abundance of other envelope stress systems as for example the Bae-TCS or the Rcs-TCS, we furthermore want to assess the role of the Cpx-TCS within the stress network in *E. coli*. These studies are currently accomplished by analyzing the co-localization of CpxA, CpxR and CpxP under various conditions. Together, our results give not only a deeper insight into the functional role of the Cpx-TCS within the stress network of *E. coli*, but also into the regulatory mechanisms within a TCS in general.

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The impact of Wsc1 microcompartmentation on cell wall integrity signaling in *S. cerevisiae*

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The yeast cell wall ensures cell shape and integrity. Five plasma membrane sensors detect surface stress and activate a conserved MAPK cascade through Rom2, Rho1 and Pkc1. Downstream of the cascade Mpk1 activates the transcription factors Rlm1 and SBF. These regulate the expression of genes whose products are involved in cell wall synthesis and cell cycle control.
Two small protein families with Wsc1, Wsc2 & Wsc3 on the one hand and Mid2 & Mtl1 on the other hand constitute the surface sensors. They contain a highly mannosylated extracellular serine/threonine-rich region, a single transmembrane domain and a cytoplasmic tail. The Wsc-family sensors carry an additional cysteine-rich domain (CRD) and the Mid-type sensors a glycosylated asparagine residue as headgroup [1].
Colocalization studies of the sensors with plasma membrane protein markers of the MCC (membrane compartment occupied by Can1), MCP (membrane compartment occupied by Pma1) and MCT (membrane compartment occupied by Tor2 complex) revealed that Wsc1 occupies a distinct plasma membrane domain, which is excluded from any of the three. The formation of this Wsc1 microcompartment (MCW) depends on the cysteine residues of the CRD headgroup and also affects the signalling process, shown by Mpk1 phosphorylation. Within this compartment, Wsc1 interacts with Wsc2 and Rom2 as shown by bimolecular fluorescence complementation (BiFC) analyses.

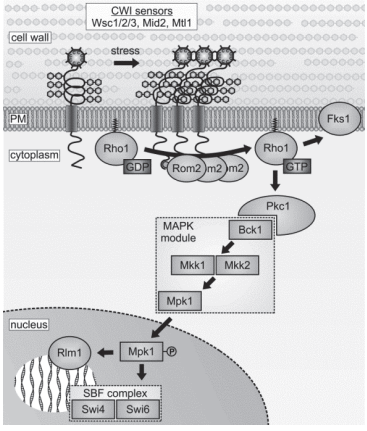

Figure 1. Simplified scheme of cell wall integrity (CWI) signaling in *Saccharomyces cerevisiae* showing Wsc1 clustering, activation of the MAPK signaling cascade and target proteins for the transcriptional response [1].

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Dynamic interactions within a bacterial two-component system

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Two-component systems (TCSs) are the main mechanisms by which bacteria sense and respond to environmental stimuli [1] and consist of a sensor kinase (SK) and a response regulator (RR). Some TCSs are additionally modulated by an accessory protein [2]. However, how these accessory proteins modulate the response is for most TCSs not known.

We use the Cpx-TCS as a model to investigate signal recognition and transduction in TCS signaling [3]. It consists of the SK CpxA, the RR CpxR and the periplasmic accessory protein CpxP. CpxP is a Cpx-TCS dependent factor that counteracts extracytoplasmic protein-mediated toxicities [4, 5]. Moreover, for misfolded proteins derived from the P pilus of uropathogenic E. coli CpxP appears to act as an adaptor protein for the periplasmic protease DegP [4]. On the other hand, cpxP overexpression results in reduced Cpx-response [6]. Thereby, CpxP inhibits autophosphorylation of reconstituted CpxA [7]. Physical interaction between CpxA and CpxP was predicted as a requirement for this regulatory interaction, but never shown. Now, we demonstrated physical interaction between CpxP and CpxA in unstressed cells using bacterial two hybrid assay (BACTH) and membrane-Strep-tagged protein interaction experiments (mSPINE) [8]. Moreover, mSPINE displayed that this interaction is detached by high NaCl concentration and misfolded pilus subunit PapE. Overall, our study emphasizes a model in which the inhibitory and supporting functions of CpxP for envelope stress response are linked: In unstressed cells, CpxP associates with CpxA to shut off the Cpx-TCS. Envelope-stress conditions induce the displacement of CpxP from CpxA resulting in Cpx-TCS activation. Together, our results suggest that CpxP modulates the activity of the Cpx-TCS by dynamic interaction with CpxA in response to specific stresses.

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Upconversion nanoparticles as interaction reporters and nanoscopic photoactuators in living cells

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Upconversion nanoparticles (UCNPs) are efficiently excited by multiphoton absorption of NIR light and emit photons in the UV/VIS regime and therefore can be detected with negligible background [1]. Moreover, luminescence resonance energy transfer (LRET) from the UCNP to molecules in the immediate proximity opens exciting possibilities as spectroscopic reporters or photoactuators with very high spatial resolution [2]. Accordingly, the interest in UCNPs grew drastically during the past years. However, even though strategies for improving the optical properties of UCNPs emerged [3], the characteristics of UCNP-based LRET as well as its application in a biological context are still poorly resolved. The aim of this collaborative project is to engineer UCNPs with suitable surface biofunctionalization for optimized LRET as novel reporters for spatially-resolved quantitative protein interaction analysis within living cells. For this purpose, core-shell UCNP with different photophysical properties were synthesized. To quantitatively analyze UCNP emission and energy transfer efficiency, we implemented microscopic and spectroscopic detection techniques. Remarkably, these phenomena strongly profited from laser powers far beyond commonly published values – in agreement with latest studies on this topic [3]. Moreover, successful manipulation of photoactivable fluorophores by LRET could be demonstrated. In order to utilize these unique optical properties in a biological context, biofunctional coatings for targeting to proteins in cells with high selectivity and efficiency were established. Accordingly, UCNPs equipped with an anti-GFP nanobody were specifically targeted to meGFP fusion proteins presented on the surface of living cells. Furthermore, employing the HaloTag system, rapid targeting of UCNPs inside living cells was achieved. By further optimization of the photophysical properties of UCNP in combination with an improved optical setup, successful development of novel nano-sized sensors and actuators for probing and manipulating signaling pathways in living cells can be envisaged.

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In vitro analysis of the Chitin synthase III complex in *Saccharomyces cerevisiae*

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The cell wall of fungi is important for survival in hostile environments. Though the fungal cell wall varies in composition and structure, one common component is chitin. The general pathway of chitin synthesis is highly conserved and involves a membrane-integral glycosyltransferase known as chitin synthase (Chs). In *S. cerevisiae*, there are 3 chitin synthases, of which one is part of the CSIII complex that produces more than 90 % of the cell wall chitin *in vivo*. The CSIII complex consists of the catalytic subunit Chs3 and the regulatory subunit Chs4 (Fig. 1). To obtain a better understanding of the role of Chs4 in chitin synthesis, we focussed on protein-protein-interactions between Chs4 and two different *N*- terminal domains of Chs3 (C1 and C3), which had been suggested in previous yeast-two-hybrid studies to be involved in binding of Chs4 [2].

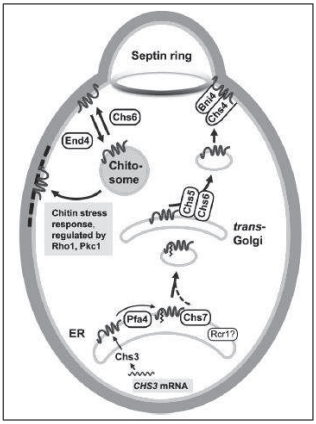

Figure 1. Chs3 trafficking [1].

For this purpose, his-tagged versions of Chs4 and the two Chs3 domains were expressed in *E. coli* as recombinant fusion proteins with the maltose binding protein and purified via affinity chromatography. Far-western blot analyses indicated a physical interaction between the Chs3 domains and Chs4, with C3 binding with higher affinities to Chs4 than the C1 domain.
While putative Chs3 phosphorylation sites at amino acid positions 26, 29, 32 and 40 could be confirmed by mass spectrometry, we tested the influence of Chs3 phosphorylation on Chs4 binding. Indeed, binding of Chs3-C1 to Chs4 was significantly increased upon *in vitro* phosphorylation by PKA, while binding of Chs3-C3 to Chs4 was not affected. As Chs4 exhibits a Ca^{2+}-binding EF hand motif, we further tested the influence of Ca^{2+} on Chs4 binding to Chs3. While Ca^{2+} treatment increases Chs4 stability, it did not affect binding to the two Chs3 domains.

Furthermore, we aimed to identify novel Chs4 interacting proteins. For this purpose, we performed three independent approaches based on affinity chromatography, co-immunoprecipitation and Bio-ID, an *in vivo* method based on the biotinylation of proximal proteins. We identified several candidate proteins interacting with Chs4, including the Glc7-interacting protein Gip4 and the protein kinases Vhs1 and Pkh3.

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Identification of Formiminotransferase Cyclodeaminase as a Binding Partner of Golgi-resident Sphingomyelin Synthase 1

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Sphingomyelin (SM) is an essential component of cellular membranes. Its synthesis involves enzymatic transfer of a phosphocholine head group from phosphatidylcholine to ceramide, yielding diacylglycerol (DAG) as a side product. This reaction is catalysed by sphingomyelin synthases (SMS) and occurs within the Golgi complex and at the plasma membrane [1].

In a GST-pull down experiment we recently identified formiminotransferase cyclodeaminase (FTCD) as a binding partner of the Golgi-resident sphingomyelin synthase, SMS1. FTCD is a microtubule-binding bifunctional enzyme involved in histidine degradation and folate metabolism [2,3]. Interestingly, both proteins co-localize at the Golgi complex. Therefore we assume that SMS1 might be required for recruitment of FTCD to the Golgi surface where it participates in the interaction of this organelle with the vimentin intermediate filament cytoskeleton.

To test this hypothesis, we investigate the impact of SMS1 removal on the Golgi localization of FTCD using human leukemia KBM7-SMS1 KO cells. To further dissect the interaction between SMS1 and FTCD, we perform pull down experiments using proteins produced in a liposome-based cell-free expression system.

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A chemical toolbox to explore the biological roles of lipids

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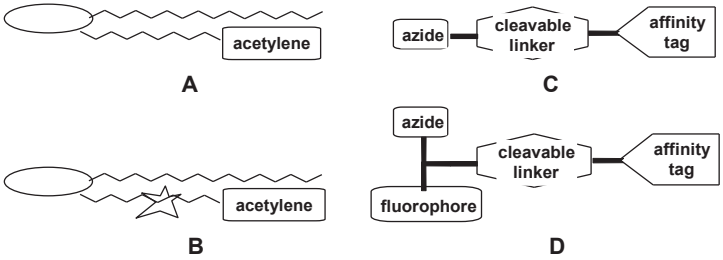
Discovery of the biocompatible conditions for cycloaddition of azides to acetylenes significantly expanded the possibilities to dissect biological processes at the molecular level. Application of this so-called 'click reaction' allows one to label pre-modified biomolecules with fluorophores, isotopes, radical species or other reporter molecules under mild conditions. [1-3]
Here, we used the click reaction philosophy to generate clickable lipids along with reagents for their detection and isolation as a chemical toolbox to investigate how these multipurpose molecules are used to execute vital cellular processes.
Clickable lipids **A** are analogues of natural lipids, armed with a triple bond. With this modification, the lipid can be labeled with a fluorphore, radical, isotope, affinity tag etc. to facilitate its detection.
Bifunctional lipids **B** are supplied with the photoactivatable function in addition to acetylenic bond. The photoactivated tag allows one to cross-link protein binding partners of the corresponding lipid.



Reagents **C** contain a cleavable affinity tag (e.g. biotin) that can be used in a click reaction with lipid **A** or **B**, allowing their isolation from a complex mixture (e.g. a cell extract) through capture and release.
Reagents **D** have an additional optical label to facilitate detection of the clickable lipids during their isolation from cell extracts.
We provide examples of how these chemical tools have been used to identify novel lipid transport machinery and effectors in cells.

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Exploring the biological role of ceramide phosphoethanolamine, an enigmatic sphingolipid spread throughout the animal kingdom

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Sphingolipids are vital components of cellular membranes that contribute to mechanical stability, signaling and molecular sorting. Sphingomyelin (SM), the most abundant sphingolipid in mammalian cells, is concentrated at the plasma membrane, where its high packing density and affinity for sterols contribute to the barrier function of the organelle. We previously identified a Golgi-resident SM synthase (SMS1) and uncovered a multiplicity of SMS genes in the human genome [1]. One of them, SMSr, produces low amounts of ceramide phosphoethanolamine (CPE) in the ER [2]. CPE levels in mammalian cells and tissues are very low and thus until recently went unnoticed. Unlike SM, CPE does not interact favorably with sterols and fails to form sterol-rich domains in model membranes [3]. This indicates that CPE unlikely serves a prominent structural role in membranes of mammalian cells.
Curiously, SMSr and its product CPE are conserved throughout the animal kingdom, occurring in a wide variety of invertebrate and marine organisms that lack SMS1 and SM [4]. Blocking SMSr-catalyzed CPE production in mammalian cells causes a dramatic rise in ER ceramides, triggering a mitochondrial pathway of apoptosis [5], suggesting that SMSr-derived CPE functions as a signaling molecule in sphingolipid homeostasis. To address the biological function(s) of CPE, we set out to manipulate local pools of CPE in mammalian cells using metabolic engineering. First, we identified key structural determinants of substrate specificity in SMS family members by domain swapping and site-directed mutagenesis. This allowed us to convert SM synthase SMS1 into a bulk producer of CPE. Next, $SMS1^{CPE}$ was transduced in human KBM7-SMS1KO cells, allowing a nearly complete substitution of SM for CPE. In future experiments, SMS1 and $SMS1^{CPE}$-expressing KBM7 cell lines will be used to investigate the impact of substituting SM for CPE on overall cell organization and function. In addition, the(phospho)proteomes of these cell lines will be compared to explore whether CPE is involved in any kinase signaling pathways. Finally, we will employ bifunctional lipid technology to search for CPE effectors.

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Redox-regulation of the multifunctional cytosolic enzyme GAPDH in redox signaling in *Arabidopsis*

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Apart from glycolysis the cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) participates in many other functions by its diverse activities as well as changes in its subcellular localization. The various localizations of GAPDH by redox post-translational modifications (RPTMs), subsequently lead to the transient microcompartmentation and transcriptional change in the nucleus which trigger cell death [1]. In plants, translocation and accumulation of GAPDH in the nucleus upon oxidative stress was found and its high redox sensitivity may play a role in oxidative stress signaling or protection [2,3]. However, the function in the nucleus and the translocation mechanism of GAPDH is unknown.

In this study, two cytosolic isoforms of phosphorylating GAPDH from *A. thaliana* (GapC1;At3g04120, GapC2;At1g13440) were localized in the nucleus upon oxidative stressed protoplasts. Individual regulation and function of the GapC isoforms under various redox-states, RPTMs and microcompartmentation of GapCs as well as their localization is currently being analyzed. In addition, structure predictions for *A. thaliana* GapCs were used to get more insight into the potential difference of the two isoforms.

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Endocytosis and the Drosophila heart: Pericardial cells as model for studies on endocytosis and cell functionality

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Drosophila pericardial cells represent a specific population of heart-associated cells. They locate along the heart tube and are hold in position by the cardiac extracellular matrix. It was recently shown that the pericardial cells act as nephrocytes (Weavers et al., 2008) but also contribute to the secretion and delivery of extracellular matrix proteins such as Pericardin, which is crucial for the formation of the cardiac ECM (Drechsler et al., 2013). Moreover, pericardial cells belong to the *Drosophila* cells with highest endocytic activity.

This prompted us to team up with the Biochemistry Department (AG Ungermann) to establish pericardial cells as a model to investigate (1) the composition and function of the endolysosomal CORVET and HOPS tethering complexes in flies and (2) to reveal the specific role of Vps8, a CORVET-specific subunit, in pericardial cell differentiation and functionality.

In yeast it could be shown that Rab5 binds to the CORVET complex at early endosomes, whereas Rab7 binds to the homologous HOPS complex along with the endosomal maturation process (Peplowska et al., 2007). Antibody stainings in *Drosophila* cells, here we used salivary gland cells, reveal a colocalization of Rab5 and Vps8, assuming that *Drosophila* Vps8 might also work as a Rab5-interacting subunit of CORVET.

Here, I will briefly outline which strategies and methods we will use to answer the questions noted above and what results I obtained so far.

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Molecular Dissection Of A Candidate Ceramide Sensor Controlling Mitochondrial Apoptosis

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Ceramides are essential but potentially lethal precursors of sphingolipids. Consequently, cells must monitor their ceramide levels to avoid killing themselves during sphingolipid biosynthesis. We recently identified SMSr, an ER-resident ceramide phosphoethanolamine (CPE) synthase, as suppressor of ceramide-mediated mitochondrial apoptosis [1, 2]. We show that SMSr-mediated ceramide homeostasis is critically dependent on both the enzyme's catalytic activity and its *N*-terminal sterile-alpha motif or SAM domain. BLAST searches revealed that SMSr-SAM is closely related to the SAM domain of diacylglycerol kinase DGKδ □□□ central regulator of lipid signalling at the plasma membrane. DGKδ activity is controlled by its SAM domain, which drives formation of helical polymers that sequester the enzyme in cytoplasmic puncta to prevent its mobilization at the plasma membrane until pathway activation. Native gel electrophoresis of recombinant SAM domains showed that SMSr-SAM, analogous to DGKδ□SAM, self-associates into oligomers. Co-immunoprecipitation analyses revealed that SMSr forms homo-oligomers in the ER and that SMSr oligomerization is critically dependent on the SAM domain. Moreover, we identified various SMSr mutants that are unable form oligomers. To further elucidate the mechanism by which SMSr controls ER ceramides, our ongoing work focuses on the functional analysis of oligomerization-defective SMSr mutants and on a proteome-wide search for SMSr binding partners.

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Arabidopsis Glutaredoxin S17 (GrxS17) as a possible redox-switch in several developmental processes

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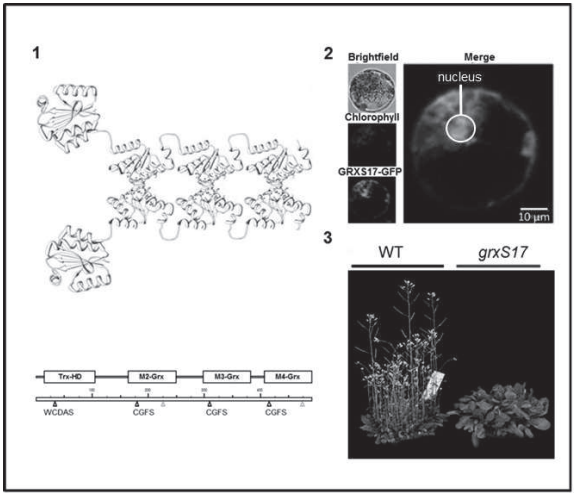
The atypical multi-domain glutaredoxin GrxS17 (At4g04950) belongs to the large family of thioredoxin-related proteins, consisting of an N-terminal Trx domain followed by three highly similar Grx domains. These Grx domains belong to the class of monothiol Grx with the typical CGFS motif [1]. Cellular redox homeostasis is balanced by thiol-disulfide exchange reactions mediated by the many “redoxins” in the plant cell. Redox imbalances serve as signals to adapt metabolism and to control development. The GrxS17 homologues in yeast (Grx3 & Grx4) are involved in iron-uptake regulation. Arabidopsis knockdown lines for GrxS17 respond to higher temperatures with an impaired phenotype [2]. The aim of the project is to investigate the redox properties of AtGrxS17 and its possible role as a redox switch in signal transduction. The interaction of GrxS17 with transcription factors and protein kinases suggests that GrxS17 controls gene expression. The *grxS17* mutant plants exhibit an impaired phenotype under defined environmental conditions indicating a central role in meristem growth and differentiation.



1. Hypothetical structure of GrxS17. The crystal structures of the single Grx and Trx domains are combined to an entire GrxS17 protein model.

2. Subcellular localization of GRXS17. Vectors encoding a C-terminal GFP fusion with GRXS17 were transformed into *A. thaliana* protoplasts.

3. Growth and development of *grxS17* KO plants. The *grxS17*-KO plants show a delayed flowering under long-day conditions in contrast to short-day where no phenotype is observed.

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Molecular Dissection of Ceramide-Induced Apoptosis Using Bi-functional Ceramide Analogues

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Ceramides are essential intermediates of sphingolipid biosynthesis as well as putative mediators of apoptosis. However, the mechanism whereby ceramides exert their apoptogenic activities remains to be established. Ceramides are synthesised on the cytosolic surface of the ER and then transported by ceramide transfer protein CERT to the Golgi for conversion into sphingomyelin (SM). We recently identified SM synthase-related protein SMSr as a candidate ceramide sensor in the ER. Disrupting sensor function caused an accumulation of ER ceramides and their mislocalisation to mitochondria, triggering a mitochondrial pathway of apoptosis. Mitochondrial apoptosis was blocked by targeting a bacterial ceramidase to mitochondria, indicating that translocation of ER ceramides to mitochondria is essential for committing cells to death.[1] How ER ceramides reach mitochondria and promote outer membrane permeabilisation to execute apoptosis remains to be established.

In this project, we use a bi-functional ceramide analogue (pacCer) to search for components of the ceramide transport machinery operating at ER-mitochondrial junctions as well as the down-stream ceramide effectors. Proteins in direct contact with pacCer can be tagged by photo-affinity labelling and then clicked with a reporter molecule (Alexa or biotin) to allow their visualization and identification.[2,3] This approach recently led to the identification of two mitochondria-associated lipid transfer proteins (LTPs). These LTPs are currently analysed for their potential involvement in the execution of ceramide-induced apoptosis.

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Functional characterization of yeast BLOC-1 complex, a new player in yeast endocytic pathway

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In metazoan cells, three BLOC complexes namely BLOC-1, 2, and 3 are involved in sorting of cargo to lysosomal related organelles (LRO) and mutations in subunits of any of these complexes lead to a hypopigmentation and bleeding disorder called Hermansky Pudlak Syndrome (HPS) (Ref.1). Recently, in a bioinformatic screen a heterohexameric putative BLOC-1 complex has also been identified in yeast (Ref. 2) that suggests a more general role of this complex in endosomal sorting and trafficking since yeast cells do not contain LROs. According to a recent finding of our lab, BLOC-1 is an effecter of endosomal Rab5 (Vps21) and acts as an adapter for Rab5 GAP (GTPase activating protein) Msb3, thus providing an intriguing connection between a Rab and its own GAP. We postulated that in absence of BLOC-1 subunits Rab5 is hyperactive (as in msb3Δ) and displaced to the vacuole (Ref. 3). In the present study, we address the possible role of BLOC-1 complex in regulating the Rab5-Rab7 switch (that is Vps21-Ypt7 in yeast) on endosomal membranes as early endosomes mature into late endosomes.

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Local pH differences in actively respiring mitochondria

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In mammals, the bulk of cellular ATP is produced at the crista membrane of mitochondria (Figure 1). Cristae are flat tube- or disk-like invaginations of the mitochondrial inner membrane (IM) extruding into the alkaline matrix space. A ΔpH and membrane potential ($\Delta\psi$), composing the protonmotive force (pmf), are build up across the IM/crista membrane. Proton pumps and the proton-driven ATP synthase can be segregated. Complexes I-IV are mainly found in the flat sheet membrane, while Immuno-EM and EM-tomography have revealed ribbons of F_oF_1 dimers lining the highly curved rim. These ribbons seem to be involved in folding the crista membrane [1]. It has been proposed that the concave side of the highly curved rim electrostatically up-concentrates protons at CV to augment the local pmf [2]. Realistically, a steady proton flow cycles between pumps and the distant ATP synthase in actively respiring mitochondria.

By attaching a fluorescent ratiometric pH-sensitive GFP variant to OXPHOS complex IV and the dimeric F_oF_1 ATP synthase, we determined the lateral pH profile along the p-side of cristae (intra-cristae space) in living HeLa cells [3]. To achieve an activated oxidative phosphorylation in glycolytic HeLa cells, glucose was replaced by galactose in the glutamine-containing growth medium.

Furthermore, we analysed the effect of inhibition of ATP hydrolysis under high glucose conditions by stably expressing IF1-H49K – the pH-independent (=active) mutant of inhibitory factor 1 (IF1) [4]. It prevents useless waste of energy that would excessively increase ΔpH.

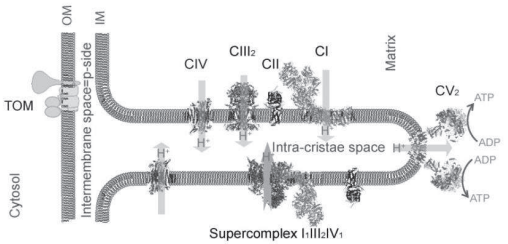

Figure 1. pH determination in different mitochondrial subcompartments

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Analysis and modification of the cardiac ECM in *Drosophila*

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The cardiac extracellular matrix (ECM), which represents a complex and highly dynamic structure, harbors tissue-specific structural proteins such as the Collagen IV like protein Pericardin (Prc) that are thought to determine the physical properties of the matrix. We established a technique in our lab that will allow the quantification of the physiological consequences of ECM modification in the *Drosophila* heart. To calculate heart parameters such as beating frequency, luminal distances and heart wall movement velocity, the beating heart is visualized by recording living animals with a high-speed video camera (200 frames per second). Data were analyzed with a matlab based software application (**Fig.1**) that was originally introduced to the scientific community by Rolf Bodmers lab (Burnham institute, La Jolla, US). We are able to analyse the heart beat in different developmental stages from embryo and larvae to adults.

We argue that the composition of the cardiac matrix determines its, e.g. elasticity and stiffness, which has a direct influence on parameters such as heart wall movement or systolic and diastolic interval. Therefore we want to change the composition of the cardiac matrix, e.g. by RNAi mediated knock down of Prc, and measure the effects on heartbeat parameters.

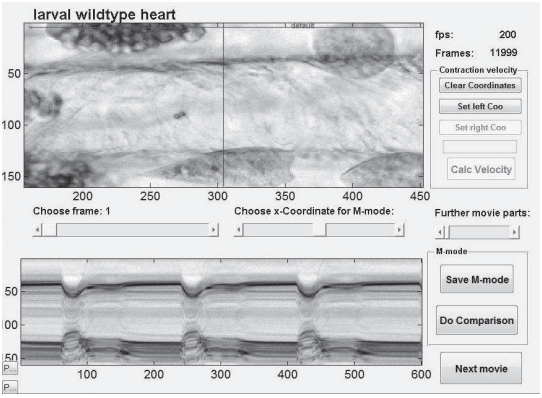

Figure 1. Overview of the matlab based software application for analyses of high-speed videos. Here beating of a semi-intact larval heart was recorded and analyzed. The red line indicates a digital vertical cut through the heart to visualize the movement of the heart tube edges over time.

Functional analysis of peritrophic matrix proteins in *Tribolium castaneum*

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The peritrophic matrix (PM) is an extracellular barrier that lines the midgut epithelium of most insects and protects the intestine from invasion by microorganisms and parasites. It consists of chitin fibrils that are embedded in a matrix of proteins and glycoproteins. Some of them have CBM14-type chitin-binding domains and are referred to as peritrophic matrix proteins (PMPs). To examine the function of individual PMPs in the midgut of the red flour beetle, *Tribolium castaneum*, we established a permeability assay based on fluorescein isothiocyanate (FITC)-dextrans. FITC-dextrans of defined sizes were added to a wheat flour-based diet and fed continuously to larvae. Next, cryosections were prepared to analyze the distribution of fluorescence signals in the larvae. While the PM of the anterior midgut has a high permeability for FITC-dextrans of even 2 MDa, the PM of the median midgut is almost impermeable for FITC-dextrans larger than 150 kDa.

We silenced the expression of three genes encoding *TcPMP3, TcPMP5-B* and *TcPMP9* by systemic RNA interference (RNAi). While injection of dsRNA specific for *TcPMP9* did not affect growth and development of the insects, those for *TcPMP3* and *TcPMP5-B* caused growth reduction, fat depletion due to starvation and lethality either at the larval-pupal molt or at the pupal-adult molt when dsRNA was injected into larvae or pre-pupae, respectively. The results indicate that the properties of the PM significantly vary in different midgut regions, and that some PMPs are required for PM integrity leading. Their loss leads to altered permeability, which in turn may compromise nutrition and fecundity. This study demonstrates for the first time the essential nature of PM proteins and sheds light on the mode of action of this class of proteins.

Make each photon count – Reconstructing dynamic microcompartments by Single-Molecule Localization Microscopy

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Cellular processes should be understood within the context of their specific micro-environment. Classical far-field light microscopy is limited to resolve such structural details below 200 nm. This limit is bypassed in Single-Molecule Localization Microscopy (SMLM), which achieves typically 20 nm lateral and 60 nm axial resolution theoretically only limited by the statistical localization precision of individual molecules and labelling-density according to the Nyquist-Shannon sampling theorem. However, the required optical isolation of individual emitters for subsequent localization leads to an inevitable trade-off between spatial and temporal resolution. The objective of this project is to develop experimental approaches and image analysis techniques with the long-term aim to reconstruct dynamic cellular nanostructures inside living cells. *In vivo* SMLM inflicts multiple non-trivial challenges in terms of sample preparation and evaluation. Labelling by a bright and photoswitchable dye compatible with cytosolic application is mandatory to retain adequate temporal resolution in a dynamically changing environment. We recently implemented life-cell direct Stochastic Optical Reconstruction Microscopy (dSTORM) for time-lapse superresolution imaging of dynamic cellular nanostructures.[1] For increasing the time resolution, decomposition of possibly overlapping point spread functions (PSF), especially in 3D-SMLM, must be handled in a robust manner. Therefore we are currently implementing state-of-the-art compressed sensing in combination with a phase-retrieved pupil function that fully describes the 3D-PSF and implicitly contains all of the aberrations introduced by the specific optical system in use. To fine-tune the experimental conditions and localization algorithm for astigmatism-based 3D-SMLM preliminary experiments are conducted *in vitro* on dye-functionalized micrometre silica beads and *in situ* on fixed giant endosomes.

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IRTG Retreat Talks

Establishing ND technology for the characterization of a bacterial two-component system

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Two component systems (TCS) are the major systems used by bacteria to communicate with their environment. Although studied for more than three decades, it is still poorly understood how a signal is recognized and transduced by a TCS. A TCS is comprised of a sensor kinase and a response regulator. The model studied by our group is the Cpx-TCS which consists of three major proteins: the sensor kinase CpxA, the response regulator CpxR, and the accessory protein CpxP. This signaling complex and the stimulus dependent dynamic interaction between its proteins can be defined as a “micro compartment”. CpxA activation is dependent on numerous stimuli that include, e.g. the lipid composition of the inner membrane and different factors from the periplasmic space. The target of this project is to investigate the structural interactions and the signal transduction between the core components CpxA and CpxR. To this end, the Cpx system was reconstituted using nanodiscs and subjected to functionality analyses and spectroscopic interaction studies. This approach avoids the problems of inside-out-orientation and spatial restrictions that are given in proteoliposomes [1]. Equal or improved kinase and phosphotransfer activities, respectively, could be shown using CpxA in nanodiscs compared to CpxA in proteoliposomes. Additionally, spectroscopic studies showed strong binding of CpxR to CpxA, which was controllable by adding Mg^{2+}-ATP.

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Topology and protein interactions of Chs3 in *Saccharomyces cerevisiae*

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The chitin synthase III complex (CSIII) in *S. cerevisiae* consists of the catalytic subunit Chs3 and the regulatory subunit Chs4, which is required for Chs3 activity. The topology of Chs3 is discussed controversial. Therefore, we examined Chs3 topology using protease accessibility assays. Based on these results, we provide a topological model for Chs3, which is in line with a homology model based on the crystal structure of the bacterial cellulose synthase complex, BcsA-BcsB [1]. An important characteristic of the cellulose synthase is a narrow channel formed by six conserved transmembrane helices (TMHs), which is involved in translocation of the sugar polymer across the membrane. Several TMHs of this channel are highly conserved also in insect chitin synthases and thus likely involved in the extrusion of chitin. However, there are only four TMHs in Chs3, a number which is presumably too low for pore formation. Therefore, we proposed that pore formation in the CSIII complex could involve di- or oligomerization of two or more individual Chs3 molecules. To test Chs3 oligomerization *in vivo*, we used bimolecular fluorescence complementation (BiFC) fusing N- and C-terminal halves of the YFP derivative Venus (VN, VC) to Chs3. We detected oligomeric complexes in the plasma membrane (PM) at the bud neck, in membranes of the endoplasmic reticulum (ER) and trans-Golgi network (TGN) vesicles. Oligomeric Chs3 complexes were not observed in *chs4*Δ cells, suggesting that Chs4 is involved in oligomerization. In contrast, depletion of Bni4, which links the CSIII complex to the septin ring, does not affect oligomerization. In this case, the Chs3 oligomers were exclusively found in the membranes of ER and TGN vesicles. Using BiFC, we further demonstrated that both subunits of the CSIII complex interact with the phosphatase Glc7 at the bud neck *in vivo*, supporting previous *in vitro* data [2]. Finally we analyzed the interaction between Chs3 and Chs4. We could localize the complex at the PM. In *chs7*Δ cells that are blocked in the exit of Chs3 from the ER, we could detect an interaction between Chs3 and Chs4 at the ER, supporting our previous hypothesis of Chs4 being tethered by Chs3 to the site of CaaX processing at the ER [3].

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Regulation of cytokinesis in the milk yeast *Kluyveromyces lactis*

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Cytokinesis in yeast and mammalian cells is a highly coordinated process mediated by the constriction of an actomyosin ring. It is accompanied by the formation of a chitinous primary septum in yeasts. We investigated the effects of various deletion mutants on cytokinesis in the milk yeast *Kluyveromyces lactis*. To determine the spatiotemporal parameters life imaging of fluorophor-tagged KlMyo1 and a new Lifeact probe for KlAct1 were employed. A *Klmyo1* deletion is temperature-sensitive but otherwise viable. Transmission- and scanning electron microscopy demonstrate a defect in the formation of a proper primary septum and in cell separation, which is also confirmed by FACS analyses.

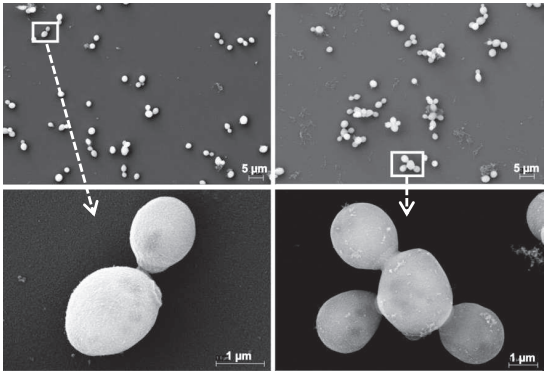

Figure 1: Morphological defect displayed by *Klmyo1* deletion. Scanning electron micrographs of cells from wild type (left) and a *Klmyo1* deletion (right) grown at 30°C in YEPD.

A lack of a conserved cytokinesis regulator, KlCyk3, is lethal in contrast to *Saccharomyces cerevisiae*, where mutants are only synthetically lethal with a *hof1* deletion. Growth of *Klhof1* mutants is osmoremedial at 25°C, like it is in *S. cerevisiae*. *CYK3* and *HOF1* genes cross-complement in both species and are thus functional homologs. Inn1, a common interactor for these two regulators, is essential in both yeasts and the encoding genes do not cross-complement. Species specificity is conferred by the C2 domain of the Inn1 homologs. This work establishes *K. lactis* as a model yeast with less genetic redundancy than *S. cerevisiae* to study cytokinesis. The viability of *Klmyo1* deletions provides an advantage over the latter. Lethality of *Klcyk3* null mutants indicates less functional redundancies with KlHof1 in *K. lactis*.

Pericardin: A collagen IV like protein plays a major role in heart integrity of *D. melanogaster*

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The Drosophila heart consists of a linear tube build by contractile cardiomyocytes and associated pericardial cells. Pericardial cells, together with adipocytes and hemocytes in the larvae, secrete large amounts of matrix proteins that are delivered to the heart tube and form an elastic cage in which the heart is embedded.
In a genetic screen searching for mutants that affect heart integrity, we identified two gene specific EMS-induced *pericardin* (*prc*) alleles, one of these turned out to be a protein null allele (PMM3-548), whereas the other one represents a hypomorphic allele where Prc is expressed but misassembled (PMM3-21). Prc is one of only four collagens in flies and specifically deposited to the heart (with a very few exception) and our newly identified mutants are the first discovered so far, beside a transposon insertion ($prc^{MB03017}$), which represents also a null allele. We could show that lack of Prc results in disintegration of the cardiac matrix, which leads to heart collapse upon aging (Drechsler et al., 2013). Furthermore we found that Prc interacts genetically with a prolyl-4-hydroxylase gene (P4H) cluster. Prolyl-hydroxlases convert proline into hydroxyproline in collagens, a prerequisite for collagen trimer formation (Myllyharju, 2003; Gorres and Raines, 2010) Our results indicate that Prc may form multimers, a notion confirmed by Western blot analysis. Furthermore we found that Pericardin, like Collagen IV and many other secreted matrix proteins, undergoes N- and O-glycosylation, which facilitates cross-linking within the heart matrix by interactions with other ECM proteins via carbohydrate chains (in preparation).
Our recent working hypothesis is that the cardiac matrix differs from other matrices in the required physical properties. The cardiac matrix has to be elastic to withstand the constant forces that act upon heart beating. We speculate that this is achieved by both a unique architecture and a specific chemical composition, build up by specific proteins and posttranslational modifications that define the properties of the matrix. We started to investigate the cardiac ECM architecture by superresolution microscopy (STORM) and could show that Prc co-assembles with the structural matrix proteins Collagen IV and Nidogen, but, e.g., not with Perlecan.

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Manipulation of intracellular ceramide flows and its impact on cell fate and organization

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Ceramides are central intermediates of sphingolipid metabolism with critical functions in cell organization and survival. They are synthesized on the cytosolic surface of the endoplasmic reticulum (ER) and then transported by ceramide transfer protein CERT to the Golgi for conversion into sphingomyelin (SM) by SM synthase SMS1. Our lab previously identified SMS-related protein SMSr (SAMD8), an ER-resident ceramide phosphoethanolamine (CPE) synthase, as suppressor of ceramide-mediated cell death. Disruption of SMSr catalytic activity causes a rise in ER ceramides and their mislocalization to mitochondria, triggering a mitochondrial pathway of apoptosis. Targeting a bacterial ceramidase to mitochondria rescued SMSr-deficient cells from apoptosis, arguing that ER ceramides exert their apoptogenic activity in mitochondria (Tafesse et al., 2014). To verify this concept, we set out to investigate the consequences of redirecting the biosynthetic ceramide flow from the Golgi to mitochondria on cell viability. To this end, the Golgi-directed PH domain of CERT was swapped for an outer mitochondrial membrane-targeting signal. Fluorescence microscopy revealed that the resulting protein, mito-CERT, accumulates at ER-mitochondrial junctions. Preliminary data indicate that mito-CERT is capable of binding ER ceramides and that its heterologous expression in HeLa cells triggers apoptosis. To rule out that the apoptotic response of mito-CERT-expressing cells is primarily due to the protein's ability to act as molecular bridge between the ER and mitochondria, we are currently analyzing the impact of expanding and/or tightening ER-mitochondrial junctions on cell viability using genetically encoded synthetic linkers. Moreover, we are using ceramide-binding defective mitoCERT mutants to address whether apoptosis induction relies on protein-catalyzed transfer of ER ceramides to mitochondria. Together, these studies should provide further insight into the mechanism by which ceramides commit cells to death.

Reference:

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Toward understanding the function of AP-3 in fusion and membrane remodelling

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The AP-3 complex is a heterotetrameric adaptor complex that mediates the cargo-selective transport from the trans-Golgi network (TGN) to the vacuole/lysosome[1]. This pathway bypasses the endosomal pathway. Whereas homotypic fusion of the yeast vacuole is well understood and needs the Rab7 GTPase Ypt7, SNAREs and the tethering complex HOPS (homotypic fusion and vacuole protein sorting)[2], less is known about fusion of AP-3 vesicles with vacuoles. During AP-3 vesicle biogenesis, the AP-3 complex is recruited to the TGN by the Arf1 GTPase and sorts cargo (e.g. Vam3, Nyv1, Yck3) to emerging vesicles. Importantly, AP-3 has a distinct binding site for the HOPS complex subunit Vps41, which is important for AP-3 vesicle fusion with vacuoles[3]. However, the molecular mechanism of fusion at the vacuole and the interplay of AP-3 and HOPS are not yet understood.
The aim of this study is to identify and purify all components involved to reconstitute this fusion event and to finally get new molecular insights into the AP-3 pathway.

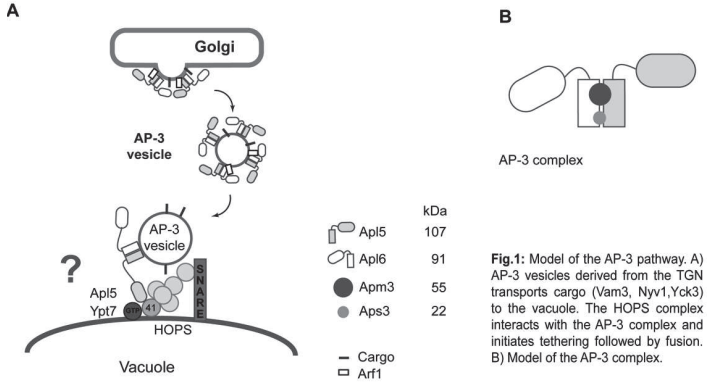

Fig.1: Model of the AP-3 pathway. A) AP-3 vesicles derived from the TGN transports cargo (Vam3, Nyv1,Yck3) to the vacuole. The HOPS complex interacts with the AP-3 complex and initiates tethering followed by fusion. B) Model of the AP-3 complex.

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Impact of FNR location on photosynthetic electron flow

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Photosynthesis demands dynamic changes in supercomplex composition in response to environmental and metabolic changes.
The enzyme ferredoxin:NADP+ reductase (FNR) catalyses the final reaction step in photosynthetic electron flow by receiving electrons from the electron carrier protein Ferredoxin to synthesize NADPH for metabolic processes. FNR is present as a soluble protein and bound to the interacting proteins Tic62 and TROL at the thylakoid membrane [1].
Some short term adaptations involve changes in micro-compartments at the thylakoid membrane to maximize photosynthetic efficiency while maintaining low stress levels. FNR can be found recruited into several protein complexes of these micro-compartments [1]. The direct function of FNR in these micro-compartments remains unclear but we have identified specific structural features that determine FNR recruitment [2].

Elucidating the principle of dynamic release and recruitment of FNR we have investigated the interaction of three maize FNR isoforms with small peptide sequences, containing the FNR-binding-motif of Tic62 and TROL, by analytical size exclusion chromatography. In addition, we have analysed how these structural differences between maize FNRs affect the composition of supercomplexes in the thylakoid membrane of transgenic Arabidopsis plants with blue native PAGE. Ultimately, we measured electron flow around the photosystems in these different transgenic plants to reveal the impact of FNR location on electron flow.

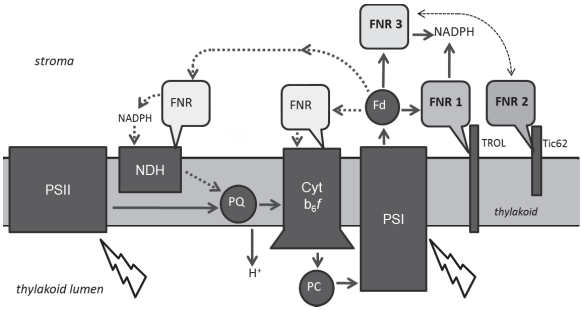

Figure 1: FNR is a soluble protein that synthesizes NADPH during photosynthesis. It can be bound to several interacting proteins on the thylakoid membrane. Recruitment into complexes with Tic62 and TROL seems to be isoform specific.

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Local pH differences in actively respiring mitochondria

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In mammals, the bulk of cellular ATP is produced at the crista membrane of mitochondria (Figure 1). Cristae are flat tube- or disk-like invaginations of the mitochondrial inner membrane (IM) extruding into the alkaline matrix space. A ΔpH and membrane potential ($\Delta\psi$), composing the protonmotive force (pmf), are build up across the IM/crista membrane. Proton pumps and the proton-driven ATP synthase can be segregated. Complexes I-IV are mainly found in the flat sheet membrane, while Immuno-EM and EM-tomography have revealed ribbons of F_oF_1 dimers lining the highly curved rim. These ribbons seem to be involved in folding the crista membrane [1]. It has been proposed that the concave side of the highly curved rim electrostatically up-concentrates protons at CV to augment the local pmf [2]. Realistically, a steady proton flow cycles between pumps and the distant ATP synthase in actively respiring mitochondria.

By attaching a fluorescent ratiometric pH-sensitive GFP variant to OXPHOS complex IV and the dimeric F_oF_1 ATP synthase, we determined the lateral pH profile along the p-side of cristae (intra-cristae space) in living HeLa cells [3]. To achieve an activated oxidative phosphorylation in glycolytic HeLa cells, glucose was replaced by galactose in the glutamine-containing growth medium.

Furthermore, we analysed the effect of inhibition of ATP hydrolysis under high glucose conditions by stably expressing IF1-H49K – the pH-independent (=active) mutant of inhibitory factor 1 (IF1) [4]. It prevents useless waste of energy that would excessively increase ΔpH.

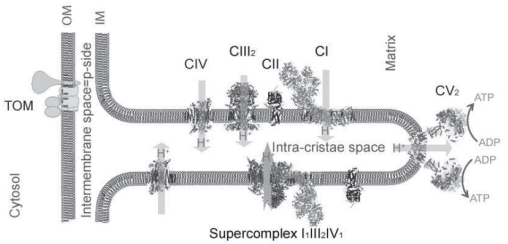

Figure 1. pH determination in different mitochondrial subcompartments

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Protein interaction dynamics of microtubule-associated proteins from *Arabidopsis*

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Microtubule-associated proteins (MAPs) regulate the polarity of cell division and the direction of cell elongation in plants. MAPs not only bind microtubules but they also tend to interact with each other. We investigate the dynamics of MAP protein interactions using a range of techniques. We employ proteomics and yeast-2-hybrid analyses to identify new protein interactions. The proteins are further analyzed by superresolution and single-molecule microscopy. This is performed in tobacco BY-2 cells and in intact seedlings of *Arabidopsis*. Interestingly, we identified signaling components that have the potential to regulate MAP microtubule binding and function.

Tau´s role in health and disease: What can we learn from single molecule imaging?

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The neuronal microtubule-associated phosphoprotein tau regulates microtubule (MT) dynamics and is involved in neurodegenerative diseases collectively called tauopathies. The general belief is that tau molecules decorate axonal MTs thereby organizing and stabilizing them. It is thought that increased phosphorylation of tau during disease (tau hyperphosphorylation) leads to the detachment of tau from MTs causing their breakdown. Since tau shares binding sites with kinesins on microtubules it is an apparent paradox that tau regulates microtubule dynamics but does not interfere with microtubule-dependent axonal transport.

To scrutinize the role of tau in regulating microtubule assembly and function single molecule imaging in living neurons was performed. Results and potential implications will be discussed.

Correlative super resolution/ atomic force microscopy unravels the localization of two protein secretion systems during invasion of polarized epithelial cells by *Salmonella enterica*

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Salmonella enterica is an important gastrointestinal pathogen of humans and animals with the ability to invade non-phagocytic cells and to persist and proliferate within mammalian cells. Invasion of polarized epithelial cells by *Salmonella* requires the cooperative activity of two protein secretion systems[1]. The type I secretion system (SPI4-T1SS) mediates close contact to the apical side of polarized epithelial cells via the giant non-fimbrial adhesin SiiE[3] binding an apical glycan structure[2,5] and the type III secretion system (SPI1-T3SS) translocates effector proteins inside the host cell leading to actin remodeling and uptake of *Salmonella*. Whereas synchronized activity of both SPI1-T3SS and SPI4-T1SS is known, the localization of these systems and the form of their interaction is ill-defined.
Direct Stochastic Optical Reconstruction Microscopy (dSTORM)[4] is a versatile tool to resolve structures in nanometer range and enables us for the first time to visualize both secretion systems in living bacteria. Local point pattern analysis according to Getis and Franklin[5] allows the calculation of clustering or co-clustering of both secretion systems. To distinguish between non-active and actively translocating SPI1-T3SS, we established a stably transfected MDCK cell line harboring the SPI1 effector SipA and its chaperone InvB fused to eGFP. This allows us to follow the recruitment of InvB to the site of translocation forming distinct foci[6,] while SipA is translocated. In combination with dual color dSTORM, we determined the co-clustering of active SPI1-T3SS with SPI4-T1SS. Combined with correlative atomic force microscopy (AFM) revealing the cellular positioning of the SPI1-T3SS and SPI4-T1SS in relation to the host cell surface, we are able to follow the formation of the microcompartment involving these two systems at the zone of contact between *Salmonella* and its host cells. Changes in the host cell surface structure like microvilli effacement or ruffle formation also indicate SPI1-T3SS activity and therefore reveal the state of invasion of the observed *Salmonella*. Our findings suggest that the cooperation of SPI1-T3SS and SPI4-T1SS is not only based on successive processes but also on the subcellular localization of these secretion systems.

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Surface architecture for probing receptor interactions and conformations

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The structural organization and the dynamics of membrane proteins in the context of a lipid bilayer play an important role for transport and communication across membranes. However, probing conformations of transmembrane proteins is obstructed as (i) expression and handling of these proteins take much effort and (ii) the properties and the composition of the lipid bilayer is a key determinant. The aim of this project is to develop surface architectures to study membrane protein conformation by metal-induced energy transfer (MIET) [1]. To this end, we developed several model systems using polymer-supported membranes (PSM) [2], which have the advantage to be accessible for surface-sensitive fluorescence-and spectroscopic techniques. Single transmembrane- as well as integral ß-barrel or GPCR proteins can be readily reconstituted in a synthetic or natural lipid environment and the diffusional behaviour of fluorescently tagged proteins can be investigated. Furthermore, regulation of the protein interactions and conformations by soluble interaction partners or the lipid environment can be systematically investigated under defined conditions. By incorporation of a gold layer under the polymer-support, fluorescence quenching by MIET is employed as a spectroscopic ruler for measuring the distance of a fluorescently labelled protein from the gold layer, which can be readily quantified from the fluorescence lifetime. In a first attempt, we tested this technique for varying the polymer length in polymer-supported membranes and confirmed the ability to control the thickness of the polymer cushion. Moreover, in a proof-of-concept experiment, we next investigated the extracellular domain of the epidermal growth factor receptor bound to a PSM. Upon addition of the soluble ligand EGF, a conformational change could be observed. As next step, we plan to explore this surface architecture for directly assessing the architecture of the type I interferon signalling complex in live cells by micropatterning of the respective receptors (Figure 1).

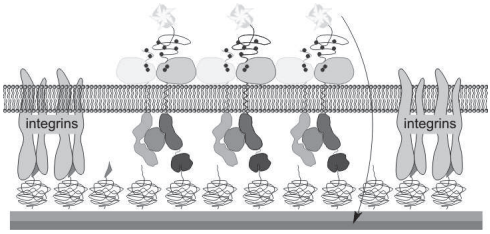

Figure 1. Metal-induced energy transfer (MIET) for the detection of the architecture of signalling complexes in real-time in live cells. Fluorescently labelled proteins of the signalling complex are captured to the support and remain biologically active. By determining the distance towards the support, distinct changes of the active complex can be dissected.

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Understanding the ancestral function of TCP transcription factors

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The land-plant specific TCP family (TEOSINTE BRANCHED 1 (TB1), CYCLOIDEA (CYC) and PROLIFERATING CELL FACTOR (PCF)) can, based on their amino acid sequence of the DNA-binding domain, be differentiated into two classes, the TCP-P (class I) and TCP-C (class II) class (1). Both play a pivotal role in the control of cell proliferation and in shaping the morphology of developing tissues in higher plants (2,3,4,5). Intriguingly, TCPs have been proven to control cell proliferation by exerting either positive or negative effects on this process. However, little is known about the ancestral function of this transcription factor family. As a member of the first land plant group that diverged from aquatic algae, the moss *Marchantia polymorpha* presents an excellent model organism to investigate the ancestral function of genes (6). *M. polymorpha* has not only the advantage of a dominant haploid gametophyte phase, it also comprises most major gene families of higher plants. However, due to not yet excessive occurring duplication events they consist of fewer members and redundancy effects as known for higher plants do most likely not appear. For example the *TCP* gene family has two members in the liverwort (one *TCP-P* (*MpTCP1*) and one *TCP-C* (*MpTCP2*)) and already 24 members in *Arabidopsis thaliana*. In our lab we try to elucidate the ancestral function of both classes. Hence we investigate the two *TCP* genes of *M. polymorpha* that are the first emerging members of this family yet known. In addition to expression studies, functional analysis by generating overexpression plants and knock-out transgenic lines via the TALEN technique will be conducted to reveal potential phenotypes, hinting towards the ancestral functions of those transcription factors. Here, we present our first results of the expression studies and functional analysis of *MpTCP1* and *MpTCP2* indicating intriguing activities for these key regulatory genes in the liverwort *Marchantia*

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The cytosolic NAD-GAPDH as a hub for transmitting cellular redox changes

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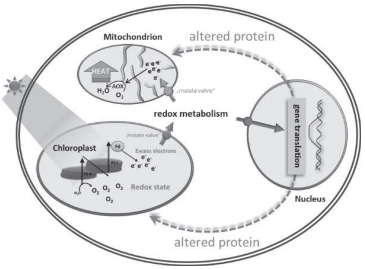
Plants maintain cellular homeostasis under changing environmental conditions, and adapt photosynthesis and CO_2 assimilation accordingly. The short-term adaptation response functions at the post-translational level. However, upon sustained stress (e.g., high light or drought), which can lead to over-reduction and radical formation in chloroplasts, nuclear gene expression is affected. It must be assumed that a system of retrograde signals from the chloroplast to the nucleus controls the synthesis of nuclear-encoded chloroplast proteins to alleviate the over-reduction. Candidate for a cytosolic component of such a signal transduction chain mediating the redox-signal to the nucleus is the NAD-dependent glyceraldehyde 3-P dehydrogenase (NAD-GAPDH, GapC) [1]. In *Arabidopsis thaliana* GapC can take over multiple functions dependent on the cellular redox-state where it is part of various microcompartments associated with the cytoskeleton, the mitochondria and in the nucleus. In the animal systems subcellular localizations associated with post-translational redox-modification of GAPDH, and these changes subsequently lead to the transient microcompartmentation and transcriptional changes in the nucleus which trigger cell death [2]. Plant GapC1 and GapC2 are oxidatively inactivated by *S*-glutathionylation and *S*-nitrosylation [3]. Under these conditions, GapC is located in nucleus. In addition to the role of GapC in glycolysis, we propose a moonlighting function of this enzyme in transferring a signal from the energy-imbalanced plastid through the cytosol into the nucleus where gene expression is changed according to the demand.



Retrograde signaling of chloroplast redox-state to nucleus.
Excess electron pressure in the chloroplasts leads to ROS/RNS formation, generating nitroso-glutathione (GSNO) that readily modifies sensitive thiols such as those of GapC. Changed properties of the modified cytosolic enzymes is supposed lead to altered microcompartmentation, and possibly to new functions, namely in gene expression, e.g. of NADP-MDH as part of the malate valve relieving electron pressure in the chloroplasts. Its increased expression is used as a read-out for the investigated redox-signaling cascade.

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Light- and ligand-induced structural changes detected by electron paramagnetic resonance

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In the past decades, X-ray crystallography, nuclear magnetic resonance, electron paramagnetic resonance (EPR) and cryo-electron microscopy have proven to be essential tools for the collection of structural information on ribonucleic acids and proteins in both aqueous solutions and in lipid-based environments.

Versatile methods making use of EPR spectroscopy can provide additional information on spin-labeled samples exceeding static structural information, for example conformational changes, structural dynamics as well as intra- and intermolecular distance distributions.

In this talk, *continuous wave* EPR and *pulsed* EPR data will document light-induced conformational changes in the phototactic receptor/transducer complex NpSRII/NpHtrII [1] and in the light-gated cation channel Channelrhodopsin-2 (ChR2) [2]. The transmembrane helix B (TMH B) movement observed in ChR2 is a new mode of rhodopsin conformational dynamics, which adds to the well-known displacement of TMH F observed in bacteriorhodopsin, sensory rhodopsin and halorhodopsin. Preliminary data for a protein-tRNA interaction will be shown for the CCA-adding enyzme [3].

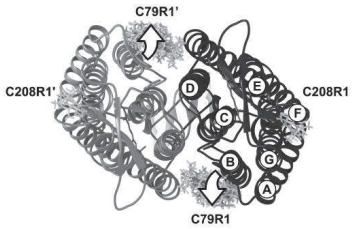

Figure 1:
Structural model of a spin-labeled ChR2 dimer.
The calculated spin-label rotamers displayed with ball-and-stick models cover 73 % of the total spin populations. The seven TMHs are labeled in one of the monomers. The light-induced TMH B movement following light activation is indicated by the arrows.

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Cleaning up the synaptic active zone: is synaptophysin a clearance factor post fusion?

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Fusion of synaptic vesicles (SVs) during synaptic transmission is mediated by SNARE complex assembly formed by coiled-coiling of the SNARE proteins synaptobrevin2 (Syb2), syntaxin-1A and SNAP-25. In order to maintain neurotransmission, exocytosed SV components need to be retrieved from the surface by compensatory endocytosis. Clathrin- mediated endocytosis (CME) is thought to be the predominant mechanism. However, it might be too slow for fast SV recycling.
Therefore, a pre-sorted and pre-assembled surface pool of SV proteins at the presynapse, the `readily retrievable pool´, might support a first wave of fast CME.
Using fluorescence nanoscopy of labeled SV proteins we show that the SV protein synaptophysin1 (Syp1) coordinates and sequesters Syb2 in larger nanodomains containing a few ten molecules of both Syb2 and Syp1. Using pHluorin-fusion constructs expressed in hippocampal neurons, we show that dimerization of Syb2 is necessary for proper sorting into endocytosing SVs, since Syb2 monomers stranded at the plasma membrane, resulting in a clearance defect from release sites.

This finding is corroborated in Syp1 knockout mice, where cultured hippocampal neurons show frequency-dependent short term depression as a result of compromised Syb2 clearance away from release sites. Our results hint at a functional role of the Syb2-Syp1 interaction in sequestering Syb2 into a RRetP for efficient release site clearance and prevention of cis-SNARE complex formation at the synapse.

Organizing Team

The organizing team was headed by Dennis Janning who is one of the speakers for the IRTG students and Dr. Nataliya Golovyashkina, the coordinator of the IRTG. We would like to express our gratitude to Inge Hasslöver and Sigrid Bröcker-Smidt from the CRC office and Dr. Wilfried Hamann for their help in organizing this event. Nevertheless, we thank all the technical staff who are helping us in the background. Members of the organizing team are the following graduate students from the CRC944 projects and can be easily identified by their yellow name-tags. They will help you with any concerns during the conference.

Agnes Borchers
Britta Barlag
Christian Kock
Christoph Drees
Dennis Janning
Henning Arlt
Lorene Penazzi
Manuel Twachtmann
Michael Holtmannspötter
Nataliya Golovyashkina
Oliver Birkholz
Sarah Kopischke
Severin Schweisthal

We hope you will enjoy the conference and would like to thank you for your input to this event.