2nd NETWORK MEETING

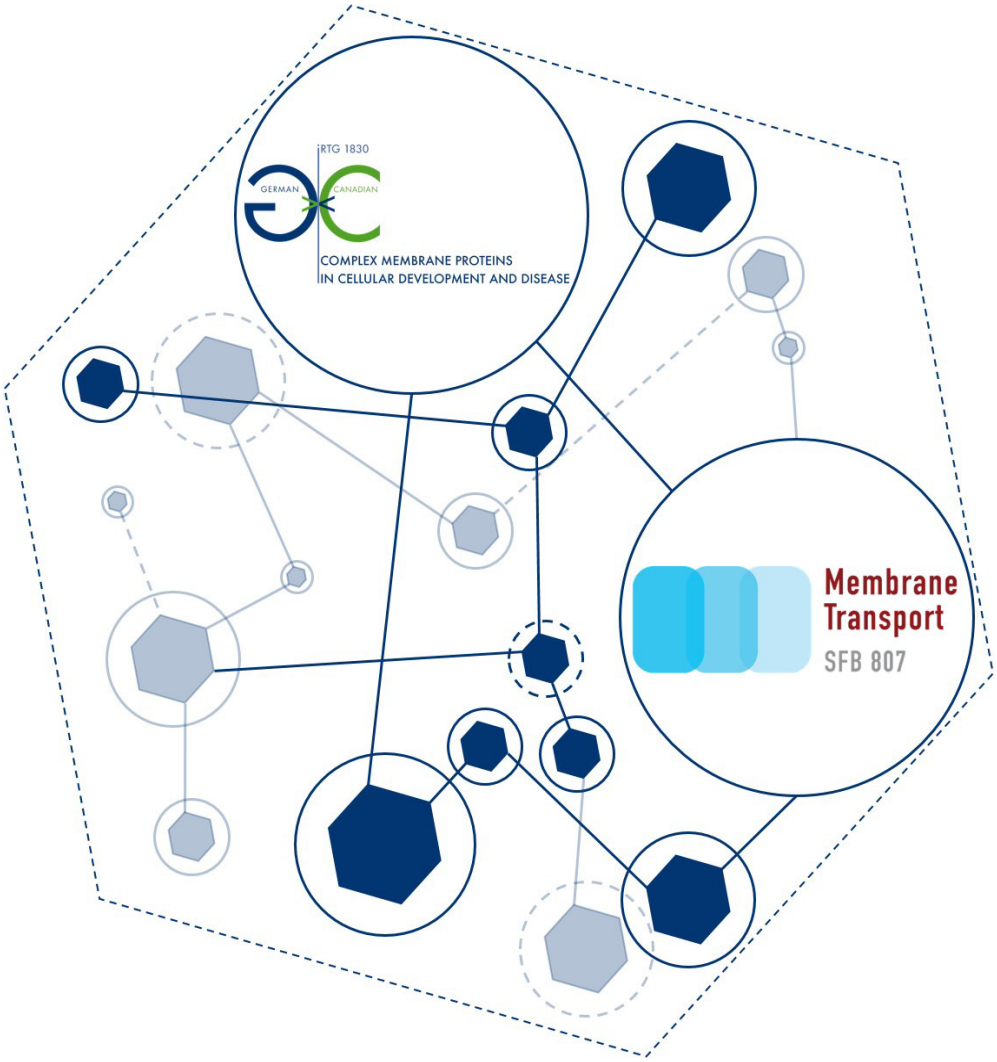
IRTG 1830

"Complex Membrane Proteins in Cellular Development and Disease"

&

TRAM-MGK

"Transport and Communication across Biological Membranes"



March 16/17, 2015
University of Kaiserslautern

DFG

Compendium

	Monday 03/16/15		**Tuesday 03/17/15**
		8.30	***Lab visits***
		10.00	Introduction IRTG1830 (10 + 5 min)
		10.15	Introduction TRAM (10 + 5 min)
		10.30	***Short break***
		10.40	Lightning Talks (10 x 3+2 min)
		11.30	***Lunch***
12.30	***Arrival of trainees*** ***Check-in to the hotel*** ***Walk to meeting location*** ***Welcome / Snacks***	12.30	Lightning Talks (10 x 3+2 min)
		13.20	***Short break***
		13.30	Lightning Talks (10 x 3+2 min)
		14.20	***Short break***
14.30	Workshop	14.30	Poster Session TRAM-MGK
16.30	***Coffee break***	16.00	Poster Session IRTG 1830
17.00	Alumni talk & discussion		
18.00	Walk to the restaurant	17.30	***Concluding remarks*** ***Dinner*** ***Departure***
19.00	***Dinner***		

Day One (Monday, March 16, 2015)

10.00 Departure of TRAM-MGK Trainees in Frankfurt

12.30 Arrival of TRAM-MGK Trainees in Kaiserslautern and check-in to the hotel

13.00 Walk to the University Campus

13.30 – 14.30 Registration of attendees and welcome snacks (42-foyer)

Session 1 (42-110 & foyer; Chair: Gabriele Amoroso)

14:30 – 16:30 **Workshop "Self-presentation and networking"**
(Silke Oehrlein-Karpi & Heike Schubert)

16:30 – 17:00 Coffee break

Session 2 (42-110; Chair: Katharina Dolata)

17:00 – 17:30	**Dr. Thomas Sattig**	Alumni TRAM-MGK
17.30 – 18:00	**Dr. Sebastian Longen**	Alumni GRK 845

18:00 Walk to restaurant (Sommerhaus)

19:00 Dinner

Day Two (Tuesday, March 17, 2015)

08:30 – 09:45 Lab visits (Details in the appendix)

09:45 Arrival of PIs

Session 3 (42-110)

10:00 – 10:15	**Ekkehard Neuhaus**	Introduction IRTG 1830
10:15 – 10:30	**Klaas Martinus Pos**	Introduction TRAM-MGK

10:30 – 10:40 Short break

Session 4 (42-110; Chair: Christopher Girke)

10:40 – 10:45	**Christian Backes**	A technique to distinguish two modes of immune cell killing on single cell level
10:45 – 10:50	**Hélène Lyrmann**	Quantitative analysis of calcium dependent migration in human killer cells
10:50 – 10:55	**Anna-Maria Miederer**	A novel STIM2 splice variant functions as a break for STIM mediated activation of Orai calcium channels
10:55 – 11:00	**Isabelle Lang**	Are members of the LRRC protein family expressed in mammalian inner hair cells?
11:00 – 11:05	**Hawraa Bzeih**	Maturation of endocytosed synaptobrevin2-containing vesicles
11:05 – 11:10	**Christoph Bock**	Structure determination by solution NMR of the interaction and targeting domain of the lysosomal peptide transporter TAPL
11:10 – 11:15	**Jenifer Cuesta Bernal**	Identification of stable candidates for crystallization studies of RND transporters from gram-negative bacteria
11:15 – 11:20	**Marina Diskowski**	Exploring the function of the K(+) uptake system KtrAB”
11:20 – 11:25	**Carl Elias Eckert**	Spectroscopic studies of blue light quenching effect on proteorhodopsin mutant E108Q
11:25 – 11:30	**Bianca Eisel**	Biochemical and structural characterization of MgtC, a novel bacterial virulence factor

11:30 – 12:30 Lunch (42-foyer)

Session 5 (42-110; Chair: Anastasia Salisowski)

12:30 – 10:35	**Valentina Peleh**	Protein oxidation in the intermembrane space of mitochondria is substrate-specific rather than general
12:35 – 12:40	**Alican Erdogan**	Redox processes in complex I assembly
12:40 – 12:45	**Armin Melnyk**	Maintenance of cellular calcium homeostasis by sealing of the SEC61 translocation channel
12:45 – 12:50	**Sara Schwartz**	Structural and functional analysis of yeast killer toxin K28
12:50 – 12:55	**Martin Textor**	In-plane topology of a membrane protein involved in bacterial biofilm formation
12:55 – 13:00	**Erik Henrich**	Lipid dependent activities of cell-free expressed MraY translocase homologues
13:00 – 13:05	**Beate Hoffmann**	Functional analysis of cell-free expressed voltage-sensing domains of proton channels
13:05 – 13:10	**Anna Klinger**	Targeting of Beta-Barrel proteins in plants
13:10 – 13:15	**Miyer Patiño-Ruiz**	Electrophysiological study of eukaryotic CPA2 Na^+/H^+ antiporters"
13:15 – 13:20	**Judith Warnau**	Applying MDFF to drive a transformation from an inward-open to an outward-open state of Na^+/H^+ antiporter MjNhaP1

13:20 – 13:30 Short break (42-foyer)

Session 6 (42-110; Chair: Ralf-Bernhard Rues)

13:30 – 13:35	**Zinnia Naoshin**	Intracellular sodium changes mediated by the electrogenic sodium-bicarbonate co-transporter NBCe1 in mouse cortical astrocytes
13:35 – 13:40	**Linda Forero-Quintero**	The role of the monocarboxylate transporters (MCTs) in neurons and astrocytes in the uptake of ketone bodies
13:40 – 13:45	**Kerstin Duscha**	Salt stress signaling: Characterization of an Arabidopsis mutant overexpressing the C-terminus of the sodium transporter SOS1
13:45 – 13:50	**Manuel Daumann**	Structure-function-relationships of the *Arabidopsis thaliana* equilibrative nucleoside transporter 1
13:50 – 13:55	**Teqiyya, Bentrcia**	Identification and characterization of the TRPV6/TRPC6 proteins
13:55 – 14:00	**Kristina Pesek**	Activation mechanism of the UPR by lipid bilayer stress
14:00 – 14:05	**Katrin Reichel**	*De novo* prediction of membrane protein structures using CS-Rosetta with sparse NMR data
14:05 – 14:10	**Michael Urban**	Membrane transport processes analyzed by a highly parallel nanopore chip device
14:10 – 14:15	**Katharina Wiesemann**	Leucine zipper system to stabilize low affine dimerization
14:15 – 14:20	**David Wöhlert**	Structure and ion binding of electroneutral Na^+/H^+ antiporters

14:20 – 14:30 Short break

14:30 – 16:00 Poster session TRAM-MGK (42-foyer)

16:00 – 17:30 Poster session IRTG 1830 (42-foyer)

17:30 Concluding remarks (Richard Zimmermann) & dinner (42-foyer)

End of Meeting

IRTG 1830

Poster abstracts in alphabetical order

No	Referee	Title
1	Backes, Christian (AG Hoth)	A technique to distinguish two modes of immune cell killing on single cell level
2	Bentrcia, Teqiyya (AG Flockerzi)	Identification and characterization of the TRPV6/TRPC6 proteins
3	Bzeih, Hawraa (AG Rettig)	Maturation of endocytosed synaptobrevin2-containing vesicles
4	Daumann Manuel (AG Möhlmann)	Structure-function-relationships of the *Arabidopsis thaliana* equilibrative nucleoside transporter 1
5	Duscha, Kerstin (AG Neuhaus)	Salt stress signaling: characterization of an Arabidopsis mutant overexpressing the C-terminus of the sodium transporter SOS1
6	Erdogan, Alican (AG Riemer)	Redox processes in complex I assembly
7	Linda, Forero Quintero (AG Deitmer/AG Becker)	The role of the monocarboxylate transporters (MCTs) in neurons and astrocytes in the uptake of ketone bodies
8	Lang, Isabelle (AG Engel)	Are members of the LRRC protein family expressed in mammalian inner hair cells?
9	Lyrmann, Hélène (AG Hoth)	Quantitative analysis of calcium dependent migration in human killer cells
10	Melnyk, Armin (AG Zimmermann)	Maintenance of cellular calcium homeostasis by sealing of the SEC61 translocation channel
11	Miederer, Anna-Maria (AG Niemeyer)	A novel STIM2 splice variant functions as a break for STIM mediated activation of Orai calcium channels
12	Naoshin, Zinnia (AG Deitmer)	Intracellular sodium changes mediated by the electrogenic sodium-bicarbonate co-transporter NBCe1 in mouse cortical astrocytes
13	Valentina Peleh (AG Herrmann)	Molecular dissection of Mia40 functions in *Saccharomyces cerevisiae*
14	Sara Schwartz (AG Schmitt)	Structural and functional analysis of yeast killer toxin K28
15	Martin Textor (AG Keller)	In-plane topology of a membrane protein involved in bacterial biofilm formation

1 A technique to distinguish two modes of immune cell killing on single cell level

C. Backes, R. Schoppmeyer, G. Schwär, C. Stephan, M. Hoth & C. Kummerow
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Death of cells in the body is involved in many diseases or injuries, but also occurs during development under physiological conditions. The killer cells of the immune system like cytotoxic T lymphocytes (CTL) or natural killer (NK) cells can eliminate malignant cancer cells or virus-infected cells by inducing cell death in their targets. Cell death can occur as a highly organized process during apoptosis or by plasma membrane disruption during necrosis. We have generated cell lines expressing a genetically-encoded sensor to quantify both necrosis and apoptosis in single living target cells by time-lapse fluorescent microscopy. We have observed that NK cells induce both types of cell death in a clonal population of target cells. Interestingly, individual NK cells can switch from necrosis to apoptosis during serial target cell killing. We postulate that the relative contribution of apoptosis and necrosis is important in regulating the immune response towards cancer and infection. We have also established a high-content protocol for this assay on an automated microscope and an analysis in a three-dimensional collagen matrix using light sheet fluorescence microscopy. This will enable us to use the assay for screening purposes and under conditions as physiological as possible.

2 Identification and characterization of TRPV6 and TRPC6 proteins

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Experimentelle und Klinische Pharmakologie und Toxikologie, Universität des Saarlandes, Homburg

The TRPV6 and TRPC6 proteins are members of the transient receptor potential (TRP) protein family. These proteins form cation-permeable channels which allow organisms to detect changes in temperature, chemicals and light, and which are essential for calcium and magnesium homeostasis. Both TRPC6 and TRPV6 are membrane proteins and share 18% amino acid sequence identity. Expression of the TRPV6 or TRPC6 cDNAs in HEK293 cells or other cell culture cells is sufficient to yield ion conducting TRPV6 or TRPC6 channels. These results indicate that the TRPV6 and TRPC6 proteins are the ion conducting pores or alpha subunits of these channels. The TRPV6 channels are highly selective for calcium ions, the TRPC6 channels are non-selective. They predominantly carry calcium and sodium ions. Whether additional non-TRP proteins contribute to the TRPV6 and TRPC6 channel complexes *in vivo* as beta or gamma subunits is not known. We have generated antibodies for TRPV6 and for TRPC6 which allow detecting the respective proteins in tissue homogenates and in Western blots, with TRPV6 being predominantly present in placenta, TRPC6 in platelets. Initial attempts have been made to establish an antibody-based affinity purification scheme to enrich both proteins solubilized from placenta and platelets, respectively, in the presence of 1% digitonin. So far, bound TRPV6 could only be eluted from the immobilized antibodies under denaturing conditions but not by the antigenic peptide which was originally used to generate the anti-TRPV6 antibody. In contrast to TRPV6 the antibody-bound TRPC6 protein was efficiently eluted by the antigenic TRPC6 peptide under non-denaturing conditions and run on blue native gels followed by denaturing SDS-polyacrylamide gel electrophoresis and Western blot. By pursuing these procedures we try to get hold of additional proteins associated with TRPC6 which can then be identified by mass spectrometry. For applying a similar protocol on TRPV6 we are generating a tumor cell line stably expressing the FLAG-tagged TRPV6 cDNA. The tumor cell line T47D was selected because these cells endogenously express the TRPV6 gene. Using

these cells we want to enrich both the endogenous TRPV6 (and associated proteins) attached to the FLAG-tagged TRPV6 using immobilized anti-FLAG antibodies. Progress also of this part of the project will be presented.

3 Maturation of endocytosed synaptobrevin2-containing vesicles

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Cytotoxic T Lymphocytes (CTLs) are specialized cells of the immune system that function in killing viral infected cells and tumor cells. The main mechanism by which these cells kill their targets is by releasing the content of their lytic granules at the interface formed between these two cells, the Immunological Synapse (IS). After being exocytosed at the IS, lytic granules need to be recycled to be reused in another cycle of killing by the T cell. This process is termed endocytosis. We aim to study the pathway followed by a recycling lytic granule, to better understand the effects of these processes on the function of CTLs.

To study endocytosis in primary mouse CTLs, we took advantage of the synabtobrevin2-mRFP knock-in (SybKI) mouse line we have generated in our lab. Synaptobrevin2 (syb2) is the v-SNARE required for the final fusion of lytic granules at the IS. In our SybKI mice the mRFP is exposed to the lumen of the lytic granules, thus upon exocytosis it will exposed to the external medium. Therefore we used fluorescently labeled anti-RFP antibodies to track the pathway of internalized (endocytosed) syb2-mRFP molecules.

We were interested to see at which time point endocytosed vesicles containing syb2-mRFP were acidified; for that we coupled both alexa-405 and pHrodo dyes to anti-RFP antibody. Alexa-405 dye is a pH-insensitive dye, however pHrodo dye is only fluorescent in acidic pH. Using LSM780 Zeiss microscope, we can perform live cell confocal imaging to visualize endocytosis in real time. T cells are allowed to form conjugates with P815 target cells, fluorescently labeled anti-RFP antibodies are then added to the medium. Having both dyes we can visualize the signal from the alexa-405 dye once lytic granules are exocytosed, and track it until we see pHrodo fluorescence and measure the time required for this to happen.

Another point we are addressing is to check how these endocytic vesicles are colocalized with different compartments of the endocytic pathway. For this experiment we are using anti-RFP antibody coupled only to alexa-405 dye. SybKI CTLs are transfected with different rab-EGFP constructs (rab5, early endosomes; rab7, late endosomes; rab11, recycling endosomes). Then transfected SybKI CTLs are allowed to form conjugates with target cells with anti-RFP antibody present in the medium, and are then fixed at different time points. Live imaging was not feasible because high resolution is needed to perform more reliable colocalization analysis. Samples were then imaged using SIM microscope. Preliminary data show that syb2-containg endocytic vesicles are partially colocalized with rab5-EGFP starting after 10 min of conjugation with target cells. Colocalization with rab11-EGFP starts about 10 min later. These results however still need to be confirmed.

4 Structure-function-relationships of the *Arabidopsis thaliana* equilibrative nucleoside transporter 1

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Nucleoside and nucleobase transporters possess crucial importance in order to facilitate uptake of nucleotide precursors in a wide range of eukaryotic organisms. On the one hand these precursors can be used to regenerate the nucleotide pool via salvage processes, on the other hand, nucleosides and nucleobases can liberate free nitrogen after total degradation, thus distribution of these valuable compounds is of major importance. Nucleoside transport across membranes in *A. thaliana* is exclusively mediated by 8 isoforms of equilibrative nucleoside transporters (AtENT1-8). Structural characteristics (11 TMD´s, cytosolic N-terminus, extracellular loop between TMD6 & 7) are shared by all AtENT´s. Interestingly, only AtENT1 exhibits a prolonged N-terminal cytosolic region with high homologies to ENT´s known from mammals (human ENT3, mouse ENT3). All of these proteins contain an acidic dileucin motif which targets these proteins to endomembranes, in case of AtENT1 tonoplastic localization will be shown. In contrast to mammalian ENT´s plant members don´t possess sensitivity to common inhibitors used for viral disease or cancer treatment (Dilazep, NBMPR, Dipyridamol). Results of selective mutagenesis of 2 residues will be shown, indicating sensitivity to these inhibitors for the former insensitive AtENT1. In addition, inhibitor analysis revealed a competitive inhibition. Finally, first results of the substituted cysteine accessibility method will be shown to gain further insides into protein topology.

5 Salt stress signaling: characterization of an Arabidopsis mutant overexpressing the C-terminus of the sodium transporter SOS1

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[2]Department of Biochemistry, University of Alberta, Edmonton

Soil salinity is a major environmental factor impairing plant growth and world-wide crop yield. The predominant overabundance of sodium imposes both, an osmotic and an ionic stress, thereby limiting cell metabolism and photosynthesis. In order to maintain sodium homeostasis, plants have developed three different mechanisms: (i) restriction of sodium influx, (ii) active extrusion of sodium from the cell across the plasma membrane or (iii) sodium sequestration in the vacuole. In *Arabidopsis thaliana*, the salt overly sensitive (SOS) signaling pathway is crucial for ion homeostasis and salt tolerance. It consists of three key components: SOS3, a calcium-sensor protein; SOS2, a serine/threonine protein kinase; and SOS1, a highly specific Na^+/H^+ exchanger localized to the plasma membrane.
SOS1 exhibits 12 transmembrane domains followed by a remarkably long (about 700 aa) hydrophilic C-terminal extension facing the cytosol. The necessity and function of this C-terminal tail unique to the SOS1 antiporter is still poorly understood. Potential novel binding partners interacting with the C-terminal region have been identified by us in a pull-down assay. These physical interactions will be confirmed using Bimolecular Fluorescence Complementation. In addition, physiological analyses revealed that the overexpression of the C-terminus results in a multitude of metabolic changes in the mutant plants during salt stress. The most stunning finding is that these mutants accumulate far less sodium in leaves when exposed to salt stress, even though they only overexpress the soluble, cytosolic part of the transporter. The cellular trigger for these fascinating changes still has to be elucidated.

6 Redox processes in complex I assembly

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Complex I of the respiratory chain of mitochondria is crucial for cellular energy production. It is also a major source of reactive oxygen species, and dysfunctions of the complex have been implicated in the pathogenesis of a variety of neurodegenerative disorders. Dysfunctions often occur as a result of an impaired assembly, but so far only little is known about the biogenesis and maintenance of Complex I in mammalian cells. Seven of its subunits are encoded in the mitochondrial genome, while the remaining 38 subunits have to be imported from the cytosol. Subunits are then sequentially assembled to give rise to the holoenzyme. We aim to characterize the function of four proteins in the assembly/maintenance of Complex I. The four uncharacterized nuclear-encoded Complex I subunits NDUFS5, NDUFB7, NDUFB10 and NDUFA8 lack typical mitochondrial import signals but instead contain conserved cysteine residues. The proteins are likely imported and trapped in the intermembrane space in a redox-dependent manner (twin-Cx_9C proteins). This process of oxidative folding is facilitated by the oxidoreductase Mia40 and is coupled to the activity of the respiratory chain. It is unknown how this redox pathway affects the assembly and maintenance of Complex I and whether the coupling to the respiratory chain provides a feedback control for respiratory chain assembly. Here, we will present our findings on the import and the function of these proteins as well as the role of Mia40 in Complex I biogenesis and maintenance on the molecular level.

7 The role of the monocarboxylate transporters (MCTs) in neurons and astrocytes in the uptake of ketone bodies

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The SLC16 gene family of monocarboxylate transporters (MCTs) comprises 14 isoforms, the first four of which (MCT1-4) mediate electroneutral co-transport of one high energy metabolite like lactate and ketone bodies with one proton. In the brain, MCT1 is expressed ubiquitously, MCT2 predominantly in neurons and MCT4 exclusively in astrocytes (Pierre, K. *et al.*, J. Neurochem., 2005). It has been suggested that MCTs play an important role in the treatment of refractory epilepsy in patients that follow a ketogenic diet (Lauritzen, F. *et al.*, Brain Struct Funct., 2013); some studies on animal models and humans have shown that ketone bodies are able to diminish the occurrence of seizures (Greene, A. *et al.,* J. Neurochem., 2003; Nehlig, A.*,* PLEFA., 2003; McNally M. *et al.*, J.Neurochem., 2012). However, the molecular mechanisms by which ketone bodies reduce the incidence of seizures under ketogenic diet still remain poorly understood. In this study, we have characterized the transport of ketone bodies in primary neuron-astrocyte co-culture from mouse cerebral cortex using H^+ imaging. The intracellular proton concentration ($[H^+]_i$) was measured using live fluorescence imaging with confocal laser microscopy and SNARF-5F as proton sensitive probe. Determination of the K_m value for lactate and ketone bodies as measured in both cell types indicated activity of MCT1 and MCT2 in neurons and MCT1 and MCT4 in astrocytes. On the other hand, we mimicked ketogenic conditions in the co-culture through the incubation of the cultures with 2 mM β-Hydroxybutyrate (BHB) over three days. Astrocytes incubated with BHB showed an increase in the uptake of lactate and acetoacetate (ACA) in comparison with untreated astrocytes. However, no

significant changes in the uptake of lactate, BHB and ACA in neurons were observed. From these results we hypothesize that the MCTs expressed in cortical astrocytes are up-regulated in the presence of high concentration of BHB in the extracellular environment, and that a similar scenario might be expected under ketogenic conditions.

Supported by the Deutsche Forschungsgemeinschaft (IRTG 1830/1 and DE 231/25-1).

8 Are members of the LRRC protein family expressed in mammalian inner hair cells?

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The mammalian inner hair cell (IHC) transduces sound into depolarization, followed by transmitter release resulting in neuronal activation. The big conductance, voltage and Ca^{2+}-activated K^+ (BK) channels of the mature IHC are responsible for fast repolarization of the receptor potential and for the small time constant of the IHC, enabling it to respond very quickly to changes in membrane potential. BK channels are widely expressed in the body and serve many functions, e.g. controlling smooth muscle tone and neuronal excitability. To date, the exact function of IHC BK channels is not known: Although BK currents of the IHCs are largely insensitive to Ca^{2+} influx they show a dependence on intracellular Ca^{2+} under certain experimental conditions. Furthermore, IHC BK channels are activated around -60 mV, whereas in heterologous expressions systems, they do not activate below +100 mV. The underlying mechanism for the very negative activation of BK channels in IHCs is not known at present.

As has been discovered recently, members of the leucin-rich-repeat-containing protein family (LRRC proteins) can function as a regulatory subunit of BK channels (Yan & Aldrich, 2010, 2012) transforming them from a Ca^{2+}- and voltage-dependent into a purely voltage-gated K^+ channel. This may explain the negative voltage range of BK channel activation in IHCs. It also has been shown that LRRC26 is an auxiliary subunit of BK channels in arterial myocytes and is colocalized with BK channels (Evanson et al., 2014). To elucidate this hypothesis we performed transcript analysis of those members of the LRRC protein family that substantially shift the activation of BK channels to more negative potentials, LRRC26 (140 mV shift) and LRRC52 (100 mV shift).
To this end, inner and outer hair cells from organs of Corti were selectively harvested using micropipettes under the microscope with micromanipulator control. Nested RT-PCR for the two LRRC proteins was performed with cDNA reverse-transcribed from reference tissues (salivary glands, testis), organ of Corti and hair cells. We found transcripts for LRRC26 and LRRC52 in the organ of Corti and for LRRC52 in IHCs and OHCs. For LRRC26 we have derived new primers and work is in progress. We also studied the expression and localization of LRRC26 and LRRC52 proteins with immunohistochemistry. LRRC26 largely colocalized with BK channels. Immunofluorescence experiments for LRRC52 are in progress. Taken together, evidence is accumulating that members of the LRRC protein family are expressed in IHCs and are colocalized with BK channels, which could be the reason for the very negative voltage range of activation of BK channels.

Supported by IRTG1830

9 Quantitative analysis of calcium dependent migration in human killer cells

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*,# Equal contribution
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The killer cells of the immune system, such as cytotoxic T lymphocytes (CTL) and natural killer cells (NK cells) search the body for virus-infected and cancerous cells. After recognition of a target cell, the killer cell releases lytic granules to kill the target. To optimize the immune function, the migration machinery of the killer cells has to be tuned to maximize the probability of target cell encounter.

We studied the calcium dependence of NK and CTL migration by time-lapse microscopy on a flat substrate (2D). Our analysis revealed that the cells alternate between a mobile and a stationary state, where the probability of each state depends on the external calcium concentration. On a short time scale (seconds/minutes), the killer cells show directional persistence, whereas migration on a longer time scale (hours) is random. This mode of migration can be modelled as an intermittent, persistent random walk. Our model predicts that the direction as well as the speed of the cell is determined by the number of active motors within the cell. Through shape analysis during migration we want to test if the number of active motors predicted by the model coincides with the number of lamellipodia formed by the cell membrane. Furthermore simulations with this model reveal that, depending on the persistence time, a search time optimum can be reached in a given space for certain boundary conditions. We are currently investigating the behaviour of migrating killer cells upon contact with boundaries in 2D. We believe that modelling killer cell migration and search behaviour can help us modify and optimize the immune response in health and disease.

10 Maintenance of cellular calcium homeostasis by sealing of the SEC61 translocation channel

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The endoplasmic reticulum (ER) of human cells is a major storage compartment for calcium ions (Ca^{2+}) and an important site for protein synthesis. The translocation of newly synthesized proteins into the ER lumen by the membrane resident Sec61 complex represents an occasion when calcium ions can leak from the ER into the cytosol due to the ion concentration gradient between ER and cytosol. Important regulators of the Ca^{2+} leakage control at the level of Sec61 have been identified by live cell Ca^{2+} imaging in HeLa cells using the ratiometric Ca^{2+} indicator FURA-2 in combination with siRNA mediated cellular depletion of proteins of interest. In previous work, calmodulin was shown to bind to the cytosol located IQ motif of Sec61α in a calcium dependent manner, thus leading to channel closure after calcium has started to leak out. Furthermore, the luminal Hsp70 chaperone BiP, an essential component of the translocation machinery, has been shown to counteract the loss of Ca^{2+} during translocation by binding to the ER luminal loop7 of Sec61α and closing of the channel. To investigate and characterize this interaction of BiP and Sec61α we performed several binding studies such as surface plasmon resonance (SPR) spectroscopy, peptide arrays and photo-cross-linking experiments. Based on the findings that Kar2p, the yeast orthologue of BiP as well as a BiP mutant which does not bind to J-domain proteins are both unable to seal the channel, we performed further studies,

addressing the binding of Hsp40 co-chaperones to BiP and Kar2p, to gain knowledge about the mechanism of sealing of the translocation channel. Furthermore, the membrane bound ERj1, Sec63 and ERj7 as well as the ER resident J-domain proteins ERj3, ERj4, ERj5 and ERj6 were analyzed by live cell Ca2+ imaging after their depletion from HeLa cells. Only depletion of ERj3 and ERj6 lead to an increased cytosolic calcium influx which was even more pronounced after simultaneous depletion of BiP, which led us to the model that these two ERj-proteins are co-chaperones of BiP in limiting ER Ca^{2+} leakage at the level of the Sec61 complex.

11 A novel STIM2 splice variant functions as a break for STIM mediated activation of Orai calcium channels

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[2]Department of Biochemistry, Bld. 44, Saarland University, 66421 Homburg, Germany

Cell homeostasis and Ca^{2+} signaling depend on a tight regulation of the intracellular Ca^{2+} concentration. Alterations in basal [Ca^{2+}] can lead to various diseases and likely contribute to development of abnormal growth. Different regulators such as calmodulin and Ca^{2+} pumps limit cytosolic [Ca^{2+}] and their down-regulation by siRNA lead to an increased basal [Ca^{2+}]. Another important regulator is the stromal interaction molecule 2 (STIM2) that shows a reduction in basal [Ca^{2+}] following knock down. STIM2 (as the second isoform STIM1) is an ER resident membrane protein which senses the Ca^{2+} content of the ER via its luminal EF-hand. After partial or complete store depletion STIM-proteins multimerize and trigger store-operated calcium entry (SOCE) by directly gating Orai channels localized at the plasma membrane.

Here, we characterize a novel STIM2 splice variant, STIM2.1, differing in a single exon inserted within the channel activating domain (CAD). Expression of STIM2.1 is ubiquitous but highest in naïve T-cells with expression relative to conventional STIM2 (=STIM2.2) changing upon activation or cell type. STIM2.1 knockdown increases SOCE in naïve CD4 + T cells, contrasting knockdown of STIM2.2 decreasing Ca^{2+} and SOCE. Vice versa, overexpression of STIM2.1 but not of STIM2.2 decreases SOCE, indicating its inhibitory role.

12 Intracellular sodium changes mediated by the electrogenic sodium-bicarbonate co-transporter NBCe1 in mouse cortical astrocytes

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The electrogenic sodium bicarbonate co-transporter isoform 1 (NBCe1, *SLC4A4*) is one of the major transporters for Na^+ and HCO_3^- in many epithelial cells. In the brain, NBCe1 is predominantly expressed in astrocytes operating with 1 Na^+: 2 HCO_3^- stoichiometry and has been reported to be an important regulator of intracellular pH (Deitmer and Chesler, 2009). In this study, NBCe1-mediated changes in intracellular sodium, $[Na^+]_i$, in primary cortical astrocyte cultures of wild type (WT, C57BL/6) and of NBCe1 gene-deficient mice (NBCe1-KO) were studied by calibrated live-cell imaging by confocal microscopy with the Na^+-sensitive fluorescent indicator Asante Sodium Green 2–AM (ANG-2 AM). Changing from HEPES-buffered, nominally CO_2/ HCO_3^- free, to CO_2/ HCO_3^- -buffered saline resulted in an increase in the $[Na^+]_i$ by ~7 mM in WT astrocytes. This increase was reduced or even reversed in NBCe1-KO astrocytes. Imposing an intracellular acid load by a weak acid (40 mM butyrate) to challenge the NBCe1 activity also led to significant increase in $[Na^+]_i$ and rate of sodium rise in CO_2/HCO_3^- saline in WT as com-

pared to NBCe1-KO astrocytes. Removal of extracellular Na^+ abolished the $[Na^+]_i$ transient observed during application of CO_2/ HCO_3^-, but removal of extracellular Cl^- did not affect CO_2/ HCO_3^- -mediated sodium transient. These observations imply that NBCe1 plays an essential role for the rise in $[Na^+]_i$ when challenged by intracellular acidification in mouse cortical astrocytes. We conclude that the changes in $[Na^+]_i$ recorded are largely dependent on the Na^+/K^+ pump and on NBCe1.

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13 Molecular dissection of Mia40 functions in *Saccharomyces cerevisiae*

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The majority of mitochondrial proteins are encoded in the nucleus, synthesized in cytosol and imported into the mitochondria. Proteins targeted to the mitochondrial matrix carry an N-terminal targeting sequence which allows the translocation across the outer and the inner mitochondrial membranes. Many proteins of the mitochondrial intermembrane space (IMS) lack the presequence but contain conserved cysteine residues that are organized in so-called Cx_3C or Cx_9C motifs. In addition, IMS proteins contain special hydrophobic binding sequences named MISS regions. The import of these proteins into the IMS is mediated by the mitochondrial oxidoreductase Mia40. Mia40 possesses two characteristics which are combined within a single protein – the ability to oxidize incoming substrates via a redox-active CPC motif and a chaperone-like activity via a hydrophobic binding cleft. In this study, we addressed the possibility to separate both Mia40 functions on molecular level. Our preliminary results show that both functions have to be present within a single protein. However, different Mia40 substrates differ considerably in their dependence on the chaperone and oxidase activities of Mia40.

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14 Structural and functional analysis of yeast killer toxin K28

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Controlled disulfide bond reduction is a key step in the intracellular trafficking of A/B protein toxins such as cholera toxin, Shiga toxin, ricin and yeast killer toxin K28. To investigate and molecularly dissect this process, we use K28 as model A/B toxin. Killer toxin K28 is produced and secreted by virus-infected *S. cerevisiae* strains and consists of two subunits (α and β) connected by a single disulfide bond. It is taken up by sensitive host cells by receptor-mediated endocytosis and travels the secretory pathway backwards via early endosomes, Golgi and ER to finally translocate into the cytosol. The molecular mechanism of its ERAD- and ubiquitin-independent retrotranslocation and the nature of the translocation channel in the ER membrane are still largely unknown. After reductive cleavage of the connecting disulfide bond in the cytosol, cytotoxic K28α is released and enters the nucleus to kill the host cell by blocking DNA synthesis. While K28 producing cells likewise take up K28, the retrotranslocated α/β hetero-dimer is rapidly degraded after being complexed by the cytosolic preprotoxin (pptox) precursor. To achieve this self-protection, S-S bond reduction within the K28/pptox immunity complex should be tightly controlled. In the present project we therefore investigate the properties of

cysteine residues (Cys) and thiol redox control in K28 and try to solve its 3D crystal structure. Mature K28 possesses four cysteines (C56 in the α-subunit; C292, C307, C333 and C340 in the β-subunit). Mutational analysis and subsequent phenotypic and biochemical analyses of mutant K28 variants indicated that C56 and C333 are the most likely candidates which form the single inter-chain disulfide bond connecting both toxin subunits. We recently discovered that the three remaining Cys residues (C292, 307, and 340) are actively involved in the dissociation of the α/β heterodimer, while C292 seems not essential for K28 *in vivo* toxicity. In addition, we found that K28 is irreversibly inactivated at pH higher than 6 through the formation of S-S-linked toxin oligomers. Since such pH conditions are likely to occur *in vivo* during toxin passage through the Golgi (pH 6) and the ER (pH 7.2), we assume the existence of cellular factors in the ER (e.g. PDI) that control these reactions and prevent the undesired formation of toxin oligomers and α-subunit release.

15 In-plane topology of a membrane protein involved in bacterial biofilm formation

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Investigations on membrane protein topology are usually limited to determining the sequences and lengths of alternating transmembrane regions and extramembranous loops. However, membrane-protein function depends on the particular translational and rotational arrangement of the entire protein or individual transmembrane helices within the membrane [1]. With the aid of fluorescence quenching experiments using acrylamide and brominated lipids, we show here that the four-helix bundle membrane protein Mistic from *Bacillus subtilis* [2] indeed resides within the lipid bilayer despite its uncommonly hydrophilic surface. However, Mistic appears to be the first exception from the commonly observed transmembrane orientation of α-helical membrane proteins, since it exhibits a highly unusual in-plane topology, with each of its two pairs of helices inserted into one of the two bilayer leaflets. This novel topology is confirmed by oriented circular dichroism spectroscopy and goes in line with coarse-grained molecular dynamics simulations of Mistic in a self-assembled DPPC bilayer [3]. This topology raises the question on how it is related to Mistic's function as an essential regulator of biofilm formation in *Bacillus subtilis* and, in particular, how it affects Mistic's putative interaction with the potassium ion channel YugO [4].

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TRAM-MGK

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16 Structure determination by solution NMR of the interaction and targeting domain of the lysosomal peptide transporter TAPL

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All ABC-transporter share a similar fold of two NBDs and TMDs, but also accessory domains are often found: cytoplasmic, cytosolic regulatory, accessory catalytic or membrane-embedded domains. Several molecular structures of ABC transporters are available; however, the structure of an additional membrane-embedded domain is not solved yet. One of these membrane-embedded domains is TMD0 (transmembrane domain 0), which is found as an additional N-terminal domain in several ABC transporters of the ABCB and ABCC subfamilies and consists of three to five TMHs. We are interested in the membrane-embedded TMD0 of the homodimeric polypeptide transporter TAPL (transporter associated with antigen processing like, ABCB9). TAPL translocates cytosolic peptides into the lumen of the lysosomes driven by ATP hydrolysis. Functionally, this transporter can be divided into coreTAPL, comprising the transport function, and the mentioned additional N-terminal TMD0. TMD0 is not essential for peptide transport or dimerization of TAPL, but includes the lysosomal targeting signal for cellular trafficking to lysosomes and interacts with the lysosomal associated membrane proteins (LAMP).

To elucidate the structure of this unique domain, we developed protocols for the production of high quantities of cell-free expressed TMD0, by screening different N-terminal expression tags. High expression was detected for AT-rich sequences in the first seven codons independently of the amino acid sequence, decreasing the free energy of RNA secondary structure formation at translation initiation. Furthermore, conditions were optimized for liquid NMR studies in respect of high solubilization efficiency, long-term stability and high quality spectra. A critical step was the detergent exchange to 6-DHPC after solubilization in LMPG. Several constructs, different in size, were tested in order to stabilize folding of TMD0 as well as to reduce the conformation exchange. TROSY NMR spectra with sufficient resolution and homogeneity were finally obtained with a TMD0 derivate, modified by a C-terminal His10 -tag and a codon optimized AT-rich sequence. With the help of differential labeling schemes the full backbone chemical shift assignment was performed and the secondary structure derived. To obtain spatial distance restraints due to the paramagnetic relaxation enhancement (PRE) for 3D structure calculations, we established spin probe labeling procedure and collected PREs.

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17 Identification of stable candidates for crystallization studies of RND transporters from gramnegative bacteria

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Antibiotic resistance has become a global public health concern due to the appearance of resistant strains (especially from pathogen Gram-negative bacteria), against many clinically used chemotherapeutic agents[1]. Among the resistance mechanisms, drug efflux pumps play a key role due to active export of antibiotics from the cell, preventing that noxious compounds reach their intracellular targets and exert their lethal actions. Efflux pumps belonging to the **Resistance- Nodulation cell Division (RND)** superfamily have been shown to often act as

proton/substrate antiporters. In Gram-negative bacteria, RND proteins are located in the inner membrane and assemble in a tripartite system with a membrane fusion protein (MFP) and an outer membrane protein (OMP)[2]. Most studied systems are the pumps **AcrAB-TolC** of ***Escherichia coli*** and **MexABOprM** of ***Pseudomonas aeruginosa*** from which the tertiary structure of each single component has been solved by X- ray crystallography and allowed to propose a transport mechanism[3].

Recently, an increased concern has been shown for the antibiotic resistance of food-bornepathogens, in the case of Gram-negative bacteria: ***Campylobacter jejuni*** and ***Salmonella enterica serovar Typhimurium***. Both are the leading cause of gastroenteritis in developed countries due to the consumption of contaminated products. In order to compare structurally and functionally the RND proteins from *C. jejuni* and *S. typhimurium* (CmeB, CmeF, AcrB, AcrD, AcrF and MdsB), a strategy based on Membrane Protein - Green Fluorescent Protein (MP-GFP) fusions was employed[4]. Initially 12 RND-GFP constructs were generated by FX cloning[5]; screening and optimization of expression conditions in *E. coli* was followed by whole cell fluorescence measurements. All 6 RND transporters were expressed and inserted into the plasma membrane of *E. coli*, and 4 of them were solubilized by common detergents as determined by Fluorescence Size Exclusion Chromatography (FSEC)[6]. After further purification steps, it was possible to produce 4 RND proteins that exhibited monodisperse peaks by Size Exclusion Chromatography (SEC) as evidence of stability, and are suitable material for crystallization studies.

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18 Exploring the function of the K(+) uptake system KtrAB

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In nature, many intermediates between species exist. At the molecular level, the KtrAB K^+ uptake system may represent an intermediate between a channel and a transporter. KtrAB is a Na^+- and ATP-dependent bacterial K^+ uptake complex. The complex is composed of the K^+-translocating subunit KtrB and the regulatory subunit KtrA. While KtrB forms a functional dimer within the membrane, the cytoplasmic protein KtrA constitutes an octameric ring that controls the uptake system by binding of ATP. The most fascinating feature of this translocating system is the fact that it may represent an evolutionary intermediate between a channel and a transporter. While the B-subunit probably harbors channel-like activity, the complex as a whole may acts as a secondary active transporter. The A-subunit confers velocity, Na^+-dependency and ion selectivity to the complex. It is possible that in the course of evolution a former channel was converted into a transporter by the addition of a non-covalently linked regulatory subunit.

This poster summarizes the thermodynamic parameters of K^+-binding to the B-subunit at different salt concentrations as determent by isothermal titration calorimetry (ITC). Furthermore, ITC was employed to determine the affinity of KtrAB for (I) ATP in the presence of potassium and (II) potassium in the presence of ATP. The results shed new light on the mechanism of K^+-translocation via KtrAB. Furthermore, we have elucidated the stoichiometry and connectivity of the KtrAB complex using the novel laserinduced liquid bead ion desorption mass spectrometry (LILBID-MS). In summary our current view of the physiology of the KtrAB system will be presented.

19 Spectroscopic studies of the blue light quenching effect on proteorhodopsin mutant E108Q

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Proteorhodopsin (PR) belongs to a newly identified family of retinal proteins from marine bacteria. It is a homologue of archaeal bacteriorhodopsin (BR), which plays an important role in the energy balance of the biosphere [1]. On absorption of green light, PR undergoes a series of conformational shifts (a photocycle), causing a proton to be transported across the membrane, thereby increasing the proton motive force, which can be utilized by the cell for different purposes, e.g. driving ATP synthesis or secondary active transporters.

As well-known from electrophysiological experiments on PR [1] (and BR as well [2]), absorption of blue light by the so called M-state (an intermediate occurring during the photocycle) interrupts the proton transfer process. Neither the underlying photo kinetics of this so called “blue light quenching effect” nor the physiological relevance is clear.

Ultrafast transient absorption spectroscopy and laser flash photolysis will be used, to gather insight into the photo dynamics of the blue light quenching effect. Additionally, resonance Raman measurements will be performed to detect conformational changes of the retinal chromophore. Object of study is the E108Q-mutant, which is known to accumulate a sufficient amount of M-intermediate.

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20 Biochemical and structural characterization of MgtC, a novel bacterial virulence factor

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Intracellular pathogens such as *Mycobacterium tuberculosis* and *Salmonella enterica* harbour a specific mechanism to survive in macrophages of their hosts. The infection mechanism includes that the pathogen can survive within a membrane-derived acidic compartment inside the macrophage and finally cause a lethal infection, e.g. in mice. Recently, the membrane protein MgtC was identified to play an essential role in host infection. This pathogenic factor is able to regulate the activity of rotary F-type ATPases and control the acidification and ATP levels in the phagosomes during the early state of an infection. MgtC is also involved in adaption to Mg2+ deprivation in several pathogenic and non pathogenic bacteria. The structure and precise

function of MgtC proteins is unknown, hence we aim to characterize the molecular mechanisms underlying these membrane proteins by using a structural biology approach. In the first step, we purified MgtC proteins from different species (Pseudomonas, Salmonella and Mycobacteria). Next we investigated the MgtC stoichiometries of the different isolates using LILBID experiments and crosslinking approaches. We found out that the Pseudomonas MgtC protein oligomerized as a heptamer whereas the Salmonella und Mycobacteria MgtC formed dimers. The results obtained so far indicate that the stoichiometry of MgtC might be linked to its dual function either in macrophages or in Mg2+ transport. With respect to structural investigations, first crystals were obtained (3D), which showed already that they were capable of diffracting X-rays to low resolution.

21 Lipid dependent activities of cell-free expressed MraY translocase homologues

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Rapidly spreading multiple antibiotic resistances request new approaches for the screening of chemotherapeutic compounds. Although enzymes of bacterial cell wall biosynthesis have been among the first targets, drugs directed against the membrane integrated catalysts have been rarely found. The first membrane bound step of cell wall biosynthesis is catalysed by the integral membrane protein MraY. Expression of membrane proteins in cellular expression systems is still challenging and availability of the enzymes for biochemical characterization was limited. We have developed efficient cell-free expression protocols for the production of MraY homologues. The expression efficiency was dependent on the design of the mRNA and on the translation initiation. The quality of the synthesized enzymes was modulated by modifying the hydrophobic environment, by selecting conditions for the post-translational solubilization and by screening of the lipid composition in preformed nanodiscs. The specific activity of the produced MraY samples was determined by *in vitro* lipid-I formation. We demonstrate that cell-free expression strategies as well as the solubilization conditions are specific to the individual MraY homologues. The *B.subtilis* MraY can be synthesized as a stable enzyme with high activity in a variety of different conditions, whereas enterobacterial MraY homologues were inhibited by detergents and high quality protein samples could only be produced in presence of specific lipids.

22 Functional analysis of cell-free expressed voltage-sensing domains of proton channels

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Voltage-gated proton channels are highly selective for protons as transported ions. The channel opening is caused by a depolarization in the membrane, but their voltage dependence is strongly regulated by the pH gradient across the lipid bilayer (ΔpH), with the result that in most species they normally conduct only outward current[1]. A more detailed investigation of the

function and mechanisms of the proton-gating proteins is needed e.g. for the identification of those as effective targets for anticancer drugs.

The main aim of the project is to obtain a better understanding of the gating mechanism of voltage-gated proton channels by comparing the structural dynamics of Hv1 from different organisms. We have adapted the cell-free expression technique for the production of these channels. This offers the advantage of controlling the environment of our membrane proteins directly at the expression level by using artificial membrane systems like detergents, liposomes or nanodiscs. We could show for the first time that cell-free expressed voltage-gated proton channels are functional. We obtained first hints that these functionality is dependent on the lipid composition of liposomes. Future work is aimed at obtaining chemical shift changes in Hv1 NMR spectra at varying temperatures and pH-environments and comparing these with supposed mechanistic models.

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23 Targeting of Beta-Barrel proteins in plants

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Plant cells contain two types of endosymbiotically derived organelles: mitochondria and plastids, which both transferred most of their genes to the host genome and contain a variable number of organelle specific β-barrel proteins in their outer envelopes. These get synthesized in the cytosol and so the plant cell had to develop dedicated mechanisms to guaranty targeting to their correct destination membrane. Except of Toc75III, all other beta-barrel proteins are devoid of a classical cleavable topogenic signal.[1] So there must be other targeting mechanisms like a still unidentified non-cleavable targeting signal either on protein or mRNA basis, structural information or the involvement of so far unknown cytosolic targeting factors.

We could show, that the exchange of the C-terminal eight to two beta-strands of the plastidic betabarrel protein OEP24 with the corresponding beta-strands of the mitochondrial beta-barrel protein VDAC1 led to retargeting of these chimeric proteins to mitochondria in transformed *Arabidopsis thaliana* protoplasts (Groß, L., unpublished). The exchange of only the last C-terminal beta-strand had no effect on targeting, while the switch of the second-to-last beta-strand led partially to a mitochondrial localization as examined via the self-assembling GFP-system and confocal laser scanning microscopy. So the last beta-hairpin or the second-to-last beta-strand of VDAC1 seems to contain a signal sufficient for mitochondrial targeting. Whether this signal lies in the peptide or the mRNA sequence or is based on structural information is so far not clear.

In order to validate the localization of the chimeric beta-barrel proteins cellular subfractionation and proteolytic digestion assays will be performed. Alkaline extractions will show, if the proteins integrate into the organellar outer membrane or accumulate in the intermembrane space. Since it was shown, that plastidic beta-barrel proteins can integrate *in vitro* into both outer membranes, mitochondrial and plastidic ones[2], dual import experiments with the chimeric proteins will show if the putative targeting signal of VDAC1 is sufficient to guaranty organelle specificity.

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24 Electrophysiological study of eukaryotic CPA2 Na^+/H^+ antiporters

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Maintaining the intracellular pH and sodium content are vital processes for all the living cells. Indeed organisms have evolved mechanisms that regulate strictly Na^+ and H^+ across biological membranes. Within those mechanisms Na+/H+ antiporters play a crucial role. These integral membrane proteins belong to the superfamily of monovalent Cation Proton Antiporters (CPA). This superfamily is subdivided into CPA1, CPA2 and NaT-DC where CPA1 and CPA2 are the largest clades. The most characterized members of CPA1 are the nine mammalian isoforms NHE1-NHE9, also known as $Na^+(K^+)/H^+$ exchangers. NHE's are electroneutral and extrude H^+ using a Na+ inward gradient as a driving force, which is established by the Na^+/K^+ ATPase. On the other hand, EcNhaA (*Escherichia coli* NhaA) is the CPA2 family prototype. NhaA utilizes the inwardly-directed electrochemical H^+ gradient generated by the inner membrane H^+ ATPase to export Na^+ (or Li^+) electrogenically with a stoichiometry of $2H^+$:$1Na^+$. Two recently discovered eukaryotic Na^+/H^+ antiporters (NHA1 ad NHA2) have higher sequence similarity to the pro-karyotic NhaA than to NHE's, leading to their classification as CPA2 members. Although some experimental studies carried out on yeast suggested NHA2 as mediating a H^+-driven Na^+ efflux in the same way as NhaA does, a functional characterization that strongly supports this transport mechanism is still missing.

In order to characterize electrophysiologically the transport activity of human HsNHA2, cDNA coding this membrane protein was cloned as YFP-fusion into a vector containing the flanking 5' and 3' UTR *Xenopus* β-globin regions. The linearized construct was used as template for the RNA production via *in vitro* transcription. NHA2-YFP cRNA was injected into *Xenopus laevis* oocytes and protein expression was checked through fluorescence microscopy after 3 to 4 days of incubation. NHA2-YFP expressing oocytes were selected for cation uptake experiments, where a higher Li^+ internalization was obtained compared to negative control (H_2O injected) oocytes, suggesting that the protein fusion is functional in the plasma membrane oocytes. In addition, Two Electrode Voltage Clamp experiments evidenced inward Na^+-dependent currents indicating that an electrogenic process is taking place in NHA2-YFP oocytes.

25 Activation mechanism of the UPR by lipid bilayer stress

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Biological membranes are dynamic assemblies of hundreds of lipids and membrane proteins, which are directly embedded in or peripheral attached to the lipid bilayer. This complex system needs to be tightly regulated as slight aberrancies in the membrane composition can affect cell viability.

Gene deletions in the model organism *S. cerevisiae* which interfere with lipid metabolism results in a highly activated ER stress response, the *unfolded protein response* (UPR), a stress response thought to be only activated by misfolded proteins.[1] The activation mechanism of this

lipid bilayer stress remains to be elucidated, as misfolded proteins and membrane aberrancies activate the UPR in different ways.[2]

We are interested in the mechanism of UPR activation by lipid bilayer stress. To this end, we are using the yeast *S. cerevisae* as a model organism to study the UPR and we are focusing on the juxta-membrane region and transmembrane helix (TMH) of Ire1p as a putative lipid bilayer stress sensor. In order to identify the role of the TMH in lipid sensing, we want to use a Cys-less Ire1p-variant and analyze its potential for dimerization/oligomerization via crosslinking with specific mutated residues in native microsomes derived from cells with a disturbed lipid composition. A further mid-term aim is to purify full-length Ire1p to analyze and characterize the juxta-membrane region and TMH in reconstituted proteoliposomes by site-directed mutagenesis combined with activity assays.

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26 De novo prediction of membrane protein structures using CS-Rosetta with sparse NMR data

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Combining Experiment and Simulation

In silico structure prediction of proteins on the basis of their sequence is a major challenge in computational structural biology, even though the ca. 100.000 entries stored in the Protein Data Bank now give a broad sampling of protein folds. Membrane proteins are particularly challenging cases due to their size and complexity, and high-resolution structural data is often lacking due to inherent experimental difficulties. We have investigated how the recently developed RASREC CS-Rosetta methodology benefits from integrating sparse NMR data for de novo structure prediction of membrane proteins. In particular, we have used the 7-TM protein sensory rhodopsin and the 4-TM disulfide bond formation protein B as test cases for which high-resolution X- ray structures are available and systematically investigated the effect of varying the type of data (chemical shifts, NOE's) and the amount of data (number and type of long-range distance restraints) on the accuracy of the structure prediction. Our work has implications for the prediction of membrane proteins of unknown structure from sparse experimental data.

27 Membrane transport processes analyzed by a highly parallel nanopore chip device

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Membrane proteins are found in all living cells and are of upmost importance in the biochemistry of the cell. Especially passive and active membrane integral transporters are of particular interest, as they control most of the transport of ions and small molecules across the cell membrane. Techniques to elucidate their function are readily available for ion channels, but rarely for membrane proteins that facilitate transport of non-ionic substrates.

We present the development of a new biochip platform to analyze especially those membrane transporters that are inaccessible with other methods. A silicon-based biochip contains 250,000 cylindrical cavities enclosed by a porous SiO_2 top layer with openings in the nanometer range. Large unilamellar vesicles are used to directly applicate reconstituted membrane proteins to the chip surface. Membrane transport can then be followed via fluorescent read-out of each individual chip cavity. The highly parallel time-resolved detection of up to three fluorescent signals enables us to monitor the kinetics of the transport substrate, a control dye and a lipid dye to monitor bilayer integrity.

The presented biochip technique combines highly parallel analysis and small sample consumption with high sensitivity and single protein resolution, creating the first step towards a miniaturized system able to perform high-throughput screenings for pharmaceutical research.

28 Modelling an outward-open state of the Na+/H+ antiporter MjNhaP1 by applying a flexible fitting method and molecular-dynamics simulations

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Sodium proton transport is essential for the viability of the cell by controlling pH and salt concentration, and maintaining volume homeostasis. Transport function of Na^+/H^+ antiporters could be mechanistically explained by the model of alternating access. The bacterial protein structure of the electroneutral NhaP1 of *Methanococcus jannaschii* (MjNhaP1) has been resolved in an inwardopen state, in which the ion binding site is open to the cytoplasm. A detailed understanding of the transport mechanism requires knowledge also of the other relevant conformational states. A cryoelectron microscopy (EM) map of an alternative conformational state of MjNhaP1 was resolved at 8 Å. We used the molecular dynamics flexible fitting (MDFF) method to fit the inward-open crystal structure into the electron density of the cryo-EM map and confirmed that it forms an outward-open conformational state.

29 Leucine zipper system to stabilize low affine dimerization

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Chloroplasts evolved from the engulfment of a free living cyanobacterium by a heterotrophic eukaryotic cell whereby most of the endosymbiotic genome was transferred to the host cell´s nucleus [1]. As a consequence the majority of plastidic proteins is cytosolically translated and has to be post-translationally guided to the chloroplast where it is recognized and transported by two oligomeric complexes spanning the envelope membranes – TOC and TIC (transporter of the outer/inner envelope membrane of chloroplasts, respectively) [2,3,4]. A N-terminal cleavable extension, the so-called transit peptide, being found in a plurality of plastid destined proteins, is needed to guide precursor proteins to their final destination [5]. Despite the unique feature of plants residing two endosymbiotic organelles – chloroplasts and mitochondria – the transit peptide, which is divergent in length and sequence, seems to be sufficient to specifically guide those preproteins to the plastid [6,7]. Since now it is not clear how this specificity is ensured but it is believed that physicochemical properties rather than sequence conservation within the transit peptide is crucial for proper recognition and translocation [5]. Two GTPases of the TOC complex – Toc34 and Toc159 – are believed to be the receptors for precursor proteins [8]. It is

heavily under debate which of the two proteins is the initial receptor and whether GTP hydrolysis is the mode of action regulating the recognition and initial transport mechanism [8,9,10] but it is assumed that both GTPases act in concert by homo- or heterospecific dimerization [11]. Since dimerization of the G-domain of Toc34 is concentration dependent *in vitro*, stabilizing this low affine interaction is of major importance to investigate the role of dimerization and reciprocal activation. To encompass this concentration limiting step, the coiled-coil forming domain of kinesin-1 from *Drosophila melanogaster* is used to increase the possibility that TOC G-domains dimerize due to an increased local protein concentration [12]. Combining this method with the advantage of using different tags, tandem purification protocols allow producing heterodimeric species to investigate their function in regard to e.g. GTP-hydrolysis activation or precursor protein interaction. Furthermore, this method can be used to investigate if dimerization is a trigger for intramolecular conformational changes and therefore a regulatory element for initial precursor protein translocation.

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30 Structure and ion binding of electroneutral Na^+/H^+ antiporters

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Membrane Proteins are essential to transport molecules across biological membranes. This gateway task makes them important drug targets. About 60% of all approved drugs target membrane proteins (1). Transport of ions across membranes is essential for every cell to maintain physiological salt concentrations and to keep pH homeostasis. Na^+/H^+-Exchangers play a key role in this process. Therefore these secondary transporters can be found in all kingdoms of life (2). Na^+/H^+-Exchangers are involved in the development of diseases like epilepsy (3), heart failure (4) and hypertension (5). To understand their way of function, detailed structural and functional information are needed to be able to sketch a mechanism for Na^+/H^+-Exchangers, which could guide drug development. We report crystal structures of two archaeal Na^+/H^+-Antiporters, one of them in a substarate-bound-state, substrate-free-state, at low and high pH (6,7). Further we characterized both antiporters using transport measurements, unexpectedly resulting in substantial differences between both homologues. First they show a completely different pH-dependent activity and second transport of one homologue is allosterically coupled in a pH-dependent manner. Together the data provide a unique view on the substrate-binding site of Na^+/H^+-Antiporters and the CPA1-family of Cation-Proton-Antiporters in general.

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6. D. Wöhlert, O. Yildiz, W. Kuhlbrandt, Structure and substrate ion binding in the sodium/proton antiporter PaNhaP. *eLife* **3**, (2014); published online EpubNov 26 (10.7554/eLife.03579).
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Buidling 42, Room 110 and Foyer
67663 Kaiserslautern

Conference phone
01511 8234828 (Gabi)
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Meeting point in Frankfurt on March 16, 2015 for bus transfer to Kaiserslautern
MPI für Biophysik
Campus Riedberg
Max-von-Laue-Straße 3
60438 Frankfurt
Departure: 10 am

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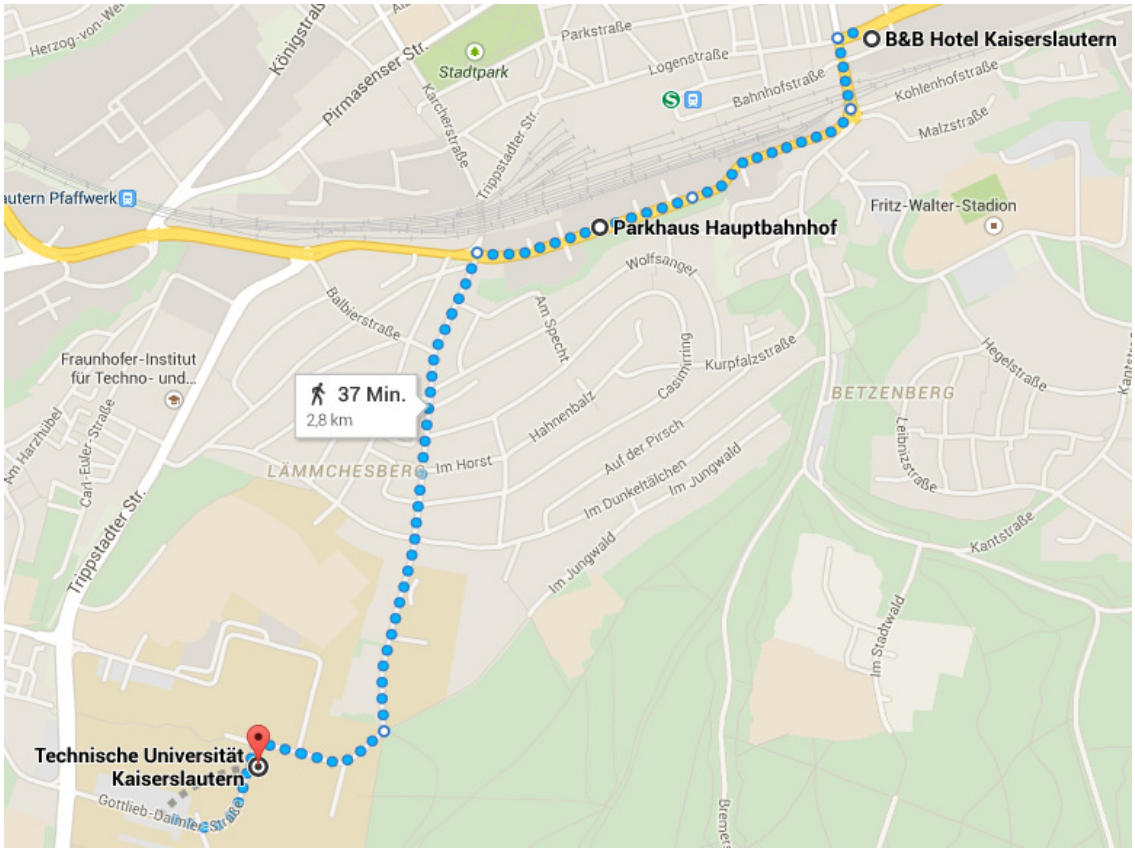
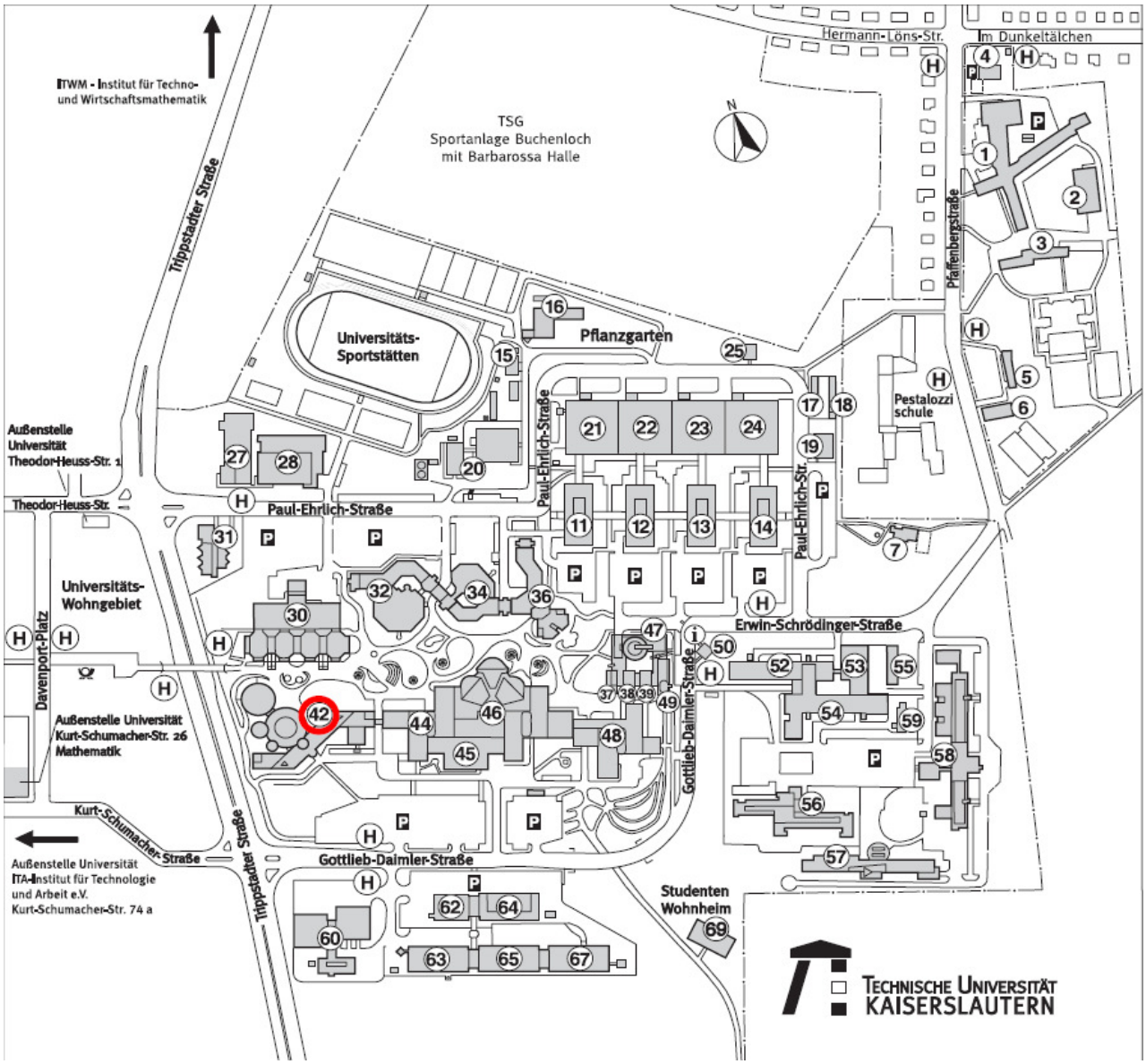
(Chris will welcome trainees from Frankfurt in the hotel at about 12 o´clock and accompany them to the meeting location, either by foot or by bus.)



Site plan University of Kaiserslautern



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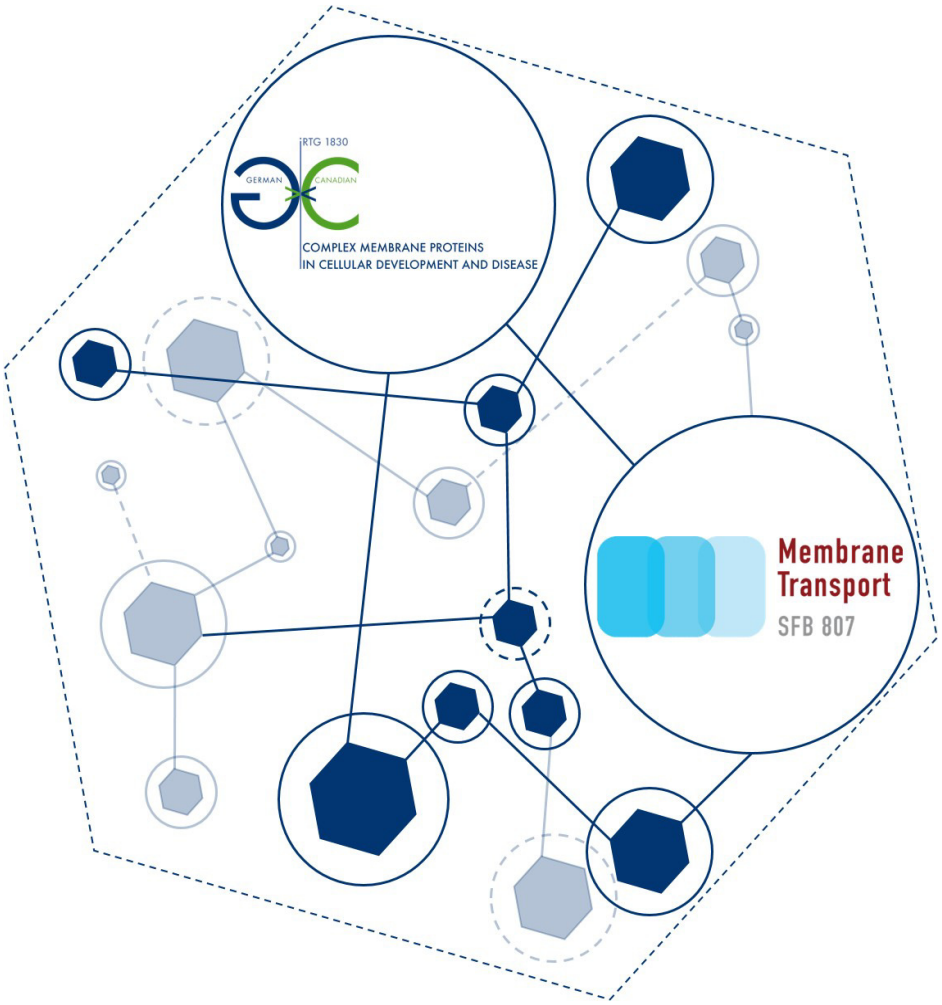
Appendix

2nd Network Meeting
of the Research Training Groups

IRTG 1830
(Complex Membrane Proteins in Cellular Development and Disease)
&
TRAM-MGK
(Transport and Communication across Biological Membranes)



Lab Visits

March 17, 2015

8.30 am – 10 am

Zoology | Membrane Transport

Organisation: Linda Forero Quintero, Zinnia Naoshin

Major Research Interests

Department of General Zoology (Prof. Dr. Joachim W. Deitmer)

Nervous systems are made of two major cell types, neurons and glial cells. In the human brain, glial cells outnumber neurons by a factor of ten, nevertheless, little is still known about glial cells. We are interested in the properties and functions of glial cells and their contribution to the information processing in the brain. In particular, we study glia-neuron communication in rodent cerebellum, in isolated leech ganglia and during metamorphosis of the sphinx moth, using patch-clamp, conventional electrophysiological methods, confocal and two-photon laser scanning microscopy and calcium imaging. In addition, we are interested in glial membrane transport involved in shuttling metabolites between glial cells and neurons, and express glial transport proteins in frog oocytes to study their cooperation.

Department of Membrane Transport (Jr. Prof. Dr. Holger M. Becker)

MCTs are highly expressed in tissues with a large energy consumption like muscle and brain. In these two tissues different MCT isoforms work together to shuttle lactate from glycolytic cell types like astrocytes and white muscle fibers to oxidative cell types like neurons and red muscle fibres, thereby playing a pivotal role in the regulation of energy supply and acid/base homeostasis during changing metabolic conditions. Our research focuses on the interaction between different MCT isoforms and carbonic anhydrase (CA), an ubiquitous enzyme that plays a central role in the regulation of acid/base homeostasis of most cells and tissues. To study the mechanism of the MCT-CAII transport metabolon and its relevance for metabolite and acid/base transport within the living tissue, we apply various molecular biological and electrophysiological techniques including heterologous protein expression, ion-selective microelectrodes and proton-imaging.

Details for visit

A guided tour through the department will be offered. Within the tour, different devices and techniques will be presented.and explained.

Subject	Device | Technique	Description | Keywords
Imaging	Laser scanning microscope Two photon microscope	pH, Na^+, Ca^{2+} and metabolite imaging
Electrophysiological measurements	Patch clamp setups	Study of spontaneous activity in Purkinje neurons
	Oocyte setups	pH, Na^+ and current measurement using single and double barrel electrodes
Protein assays	Mass/IR Spectrometer Multiscan Microplate Spectrophotometer	Western Blot, CA activity measurement, pull down assay

Cell Biology | Cellular Biochemistry

Organisation: Alican Erdogan, Valentina Peleh

Major Research Interests

Department of Cell Biology (Prof. Dr. Johannes M. Herrmann)

One main focus of our group is the biogenesis of proteins of the mitochondrial inner membrane which belongs to the protein-richest membranes of the eukaryotic cell. It accommodates a large number of different integral membrane proteins which, typically assembled in multiprotein complexes, perform a variety of functions like the transport of molecules or generation of ATP. We analyze how these proteins are inserted into the inner membrane, and identify and characterize components that mediate this process.

Department of Cellular Biochemistry (Jr. Prof. Dr. Jan Riemer)

Thiol-Based Redox Processes. Cysteines are rarely encountered amino acid residues in proteins. However, if present they often fulfill important roles such as acting as active centers of enzymes, or forming disulfide bonds with other cysteines to stabilize protein folds or to regulate enzymatic activities. Thus, regulation of the intracellular cysteine redox status is an essential part of cellular homeostasis since oxidative inactivation of active-site cysteines, formation of non-native disulfide bonds, and permanent deregulation of enzymatic activities can have deleterious consequences. We are interested in the reactions of cysteines and the physiological impact of changes in the redox state of cysteines. We aim at understanding these reactions in molecular detail in the model system baker's yeast, in mammalian tissue culture cells and using purified proteins.

Details for visit

A guided tour through the department will be offered. Within the tour, different devices and techniques will be presented.and explained.

Subject	Device | Technique	Description | Keywords
Protein import into isolated mitochondria	Hypoxia chamber, radioactive uptake experiments	Cell culture, Yeast culture, Isolation of Mitochondria/Mammalian Cells

Molecular Biophysics

Organisation: Martin Textor

Major Research Interests

Department of Molecular Biophysics (Jr. Prof. Dr. Sandro Keller)

Membrane-protein folding. We are employing a combination of protein engineering and various biophysical techniques to quantify the thermodynamics and kinetics of membrane-protein folding in various lipid bilayers, detergent micelles, and other membrane-mimetic systems.

Reconstitution of membrane proteins. To put reconstitution on a more rational basis, we are developing novel spectroscopic and calorimetric assays for monitoring the reconstitution of membrane proteins into lipid vesicles. Thus, complex changes in the physical state of a protein/lipid/detergent mixture can be followed in a non-invasive and fully automated manner.

Membrane binding and translocation. A molecule's ability to bind to and translocate across lipid membranes is of uttermost importance for the extracellular application of intracellularly active compounds in cell biology, medicinal chemistry, and drug development. We have developed lipopeptide-based drug-delivery systems and several in vitro approaches to quantify membrane binding and translocation of peptides and proteins as well as a wide range of small molecules.

Details for visit

After the guided tour through the department, different devices and established techniques will be explained. Additionally, an interactive part is planned to get familiar with biophysical methods.

Subject	Device | Technique	Description | Keywords
Biophysical Methods for Membrane Protein Research	Circular Dichroism Spectroscopy, Dynamic Light Scattering, Isothermal Tritration Calorimetry, Membrane Folding and Reconstitution	We will demonstrate how membrane protein reconstitution can be approached more rationally compared with conventional protocols. A novel calorimetric assay involving cyclodextrin-mediated detergent removal for planing and monitoring the reconstitution of membrane proteins into lipid vesicles is explained. Additional biophysical methods are demonstrated interactively with a focus on membrane protein reconstitution. To this end, the membrane protein Mistic is reconstituted into lipid vesicles, the quality of which is checked by dynamic light scattering. Circular Dichroism Spectroscopy is used to verify that the protein retains its native structure within the lipid bilayer, and acrylamide fluorescence quenching is employed to show the reduced accessibility of the reconstituted membrane protein to the aqueous phase.

Plant Physiology

Organisation: Manuel Daumann, Kerstin Duscha

Major Research Interests

Transporters in plant vacuoles (Prof. Dr. Ekkehard Neuhaus)

Vacuoles in higher plants fulfill major functions in storage of assimilates and nutrients, storage of toxic organic and anorganic compounds, and regulation of turgor and stomata functions. For all of these functions an enormous number of solutes have to pass the vacuolar membrane (tonoplast) and transport of these compounds is mediated by channels, primary, and secondary active transporters. Several of these transporters have been identified in the recent past on the molecular level but a large number is still unknown.

Nucleoside transport and metabolism (Dr. Torsten Möhlmann)

Nucleotides are uniquely important since they represent building blocks of genetic information (DNA and RNA), represent major energy carriers and are core elements of cofactors such as NAD, FAD, S-Adenosylmethionine or Coenzyme A, which serve in essential biochemical reactions such as the synthesis of phospholipids and polysaccharides. Additionally, nucleotides are components of secondary metabolites like coffein, cAMP, cGMP and cytokinins. By reviewing the recently published work on nucleotide metabolism, it becomes obvious that many facets of this important biochemical aspect of plant metabolism are still poorly understood. A reason for this is the complexity of a multitude of biochemical reactions, which facilitate de novo synthesis, degradation and interconversion (partial degradation and recycling) of nucleotides, nucleosides and nucleobases. The recycling of nucleosides and nucleobases is known as salvage.

Details for visit

After the guided tour through the department, different devices and established techniques will be explained. Additionally, an interactive part is planned to get familiar with transient protein expression in tobacco.

Subject	Device | Technique	Description | Keywords
Transient protein expression in tobacco; a powerful tool in molecular plant physiology research	transient protein expression, Bimolecular fluorescence complementation (BIFC), confocal laser scanning microscopy	The tobacco protein expression system will be explained and potential subsequent analysis will be shown. Therefore, students get a brief theoretical instruction in transient protein expression in tobacco and will perform plant infiltration on their own. While one part of the group works with these plants, the other part will use preliminary infiltrated plants to analyse the localisation/BIFC using the confocal laser scanning microscopy. Groups will switch afterwards.

Assigned groups

group 1	group 2	group 3	group 4
Miyer Patino-Ruiz	Eva-M. Brouwer	Charlott Stock	David Wöhlert
Michael Urban	Beate Hoffmann	Marina Diskowski	*Peter Eberhardt*
Vedrana Mikusevic	Anne Nöll	Katharina Wiesemann	*Carl Elias Eckert*
Valentina Herbring	Fang Dong	Anna Klinger	*Lucia Groß*
Erik Henrich	Katrin Reichel	Bianca Eisel	*Jean Aymard Mandouckou*
David Urmann	Anastasia Salisowski	Jenifer Cuesta Bernal	*Reinke Müller*
Philipp Graab	Judith Warnau	Tina Zollmann	*Oliver Peetz*
	Ralf-Bernhardt Rues	Christoph Bock	*Kristina Pesek*
			Christoph Ruland
7	**8**	**8**	**9**

time	Zoology | Membrane Transport	Cell Biology | Cellular Biochemistry	Molecular Biophysics	Plant Physiology
8:30-9:00	Group 1	Group 2	Group 3	Group 4
9:00-9:30		Group 3	Group 2	

Contact information

Contact persons within the departments:

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