Student Retreat 2012

- Trier -

Science!

Dear fellow HBIGS students,

Thanks for taking part in the 2012 retreat! We hope you have a great weekend with plenty of fascinating talks on cutting-edge science from Heidelberg, covering a wide range of disciplines. This retreat is not only meant for you to hear about what other PhD students are doing but also to get to know your colleagues personally. Take this unique chance to build up your network, exchange with the other HBIGS students, and establish friendships and collaborations.

Trier was chosen as this year's venue for its historical allure and cultural richness, so you're encouraged fully to take advantage of the opportunities built into the schedule to explore the area and experience some of what the ancient city has to offer.

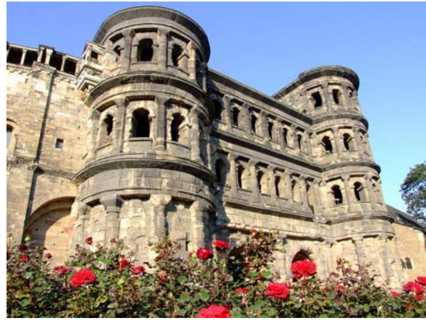
Enjoy your weekend, be inspired, network, and have a great time!

Your Student Speaker team,

Julianne, Patric and Julian

Retreat Venue

Trier is claimed to be Germany's oldest city with more than 2,000 years of history. But Trier is also a city young at heart with a future, a city with a favorable geographical location in the "heart of Europe."



The Porta Nigra is one of the most important monuments, built by the Romans.

Trier, originally "Augusta Treverorum", was founded by the Romans under Emperor Augustus in 17 BC near the tribal sanctuary of the Celtic Treveri.

In AD 293, Emperor Diocletian made Trier, called Treveris at the time, a Roman Imperial residence and capital city of the Western Roman Empire.

Conquered by the Germanic Franks in the 5th century, Trier was allotted to Eastern Francia (eastern German kingdom) at the Carolingian partition in 870.

In the 12th century, the Trier archbishops were simultaneously electors in the Holy Roman Empire. They made Trier the capital of the electoral state, which experienced times of great flowering and profound decline until its dissolution around 1800. After a brief period as a part of France, Trier then was situated in Prussia in 1814 and then in Rhineland-Palatinate after the founding of the Federal Republic of Germany in 1949.

With its 100,000 inhabitants, Trier is today a city independent of the county, a bishop's city and a university city.

Economically, Trier is the center of the wine-growing area on the Moselle, Saar, and Ruwer as well as the site of well-known, even world-renowned, companies in the food and tobacco industry, textile industry, precision engineering, the building industry, and a place of artistic craftsmanship. As

such, it has become a unique vacation and leisure area for people from across Europe.

The channeled Moselle has an industrial and shipment harbor and a freight traffic center. Trier is a shopping center for the Trier area and neighboring countries.

The monuments are stone testimonials to the Roman and electoral eras. The Porta Nigra, the Imperial Baths, the Amphitheater, the Barbara Baths, the recently excavated Forum Baths, and the Roman Bridge over the Moselle make us aware today of the still grand dimensions of a once splendid Roman city. The many guests who visit the city year in, year out are captivated by the unique ambiance where the old and the new coincide.

Hauptmarkt with a 10th century stone cross and a 16th century fountain. lifeslittleadventures.typepad.com

18th Century Saint Paulin Church wikimedia.org

Culturally, Trier is an open-air museum of European architecture with structures from the Roman, Romanesque, and the Gothic eras as well as from the Renaissance, the baroque, and neo-classicism. For centuries, the city has been the center of the Moselle area. Not only home to Trier University and a technical university, the city is also host to the European Academy of the Visual Arts and the Academy of European Law.

(www.trier-info.de/english/trier-in-brief)

Keynote Speaker:

Prof. Dr. Andrea Möller is a full professor of Biology and Science Education at the University of Trier. She holds a Ph.D. in Biology and a “Staatsexamen” (M.A. of Education) in Biology, English, and Educational Sciences from Goethe University Frankfurt. She was a visiting researcher to the Universities of Lund, Sweden and Yale, USA. During her studies she worked part time as a high school teacher in Great Britain, the U.S., and Germany. She has over 8 years experience as a freelance science museum educator and has helped establish several science school labs. Dr. Möller was recently part of a 3-year nationwide project funded by the German Federal Ministry of Education and Research investigating the development of scientific inquiry competence in high school students.

On a more personal note, she has generously donated her time and energy to help develop the HBIGS-sponsored science outreach project, the German National Brain Bee, as one of the neuroscientist judges and an advisor to the organizing committee.

Universität Trier

und ihre Didaktik

uni-trier.de

Schedule

Friday, 18th

Time	*Event*	*Chair*
14:33	Leaving Heidelberg	
19:00	Arrival at the hotel	
19:30	Dinner at the hotel	
20:15-21:00	Keynote Presentation, Dr. Andrea Möller	
21:00-22:00	Talks: ▪ Daria ▪ René ▪ Kristina ▪ Julian	Julianne

Saturday, 19th

Time	*Event*	*Chair*
Until 9:00	Breakfast at the hotel	
09:15-10:30	Talks: ▪ Frauke ▪ Thomas ▪ Petia ▪ Thekla ▪ Julianne	Julian
10:30-10:45	Coffee Break	
10:45-12:00	Talks: ▪ Sumit ▪ Frederik ▪ Martin ▪ Francisco ▪ Patric	Julian
12:00	Lunch at the hotel	

13:15-14:45	Talks: ▪ Julia ▪ Ricardo ▪ Jagadeesh ▪ Wenbo ▪ Stephan ▪ Sahil	Patric
14:45-15:00	Coffee Break	
15:00-16:30	Talks: ▪ Kirti ▪ Nick ▪ Zegeye ▪ Ece ▪ Giuseppe ▪ Christian	Patric
17:00	Wine tasting, followed by dinner at “Das Weinhaus”	

Sunday, 20th

Time	***Event***	
11:00-13:00	Historic city tour meet at “Porta Nigra”	
15:48	Leaving Trier	
19:23	Arriving in Heidelberg	

Abstracts

Name: **Daria Bunina**
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Single cell protein homeostasis: role of competitive transcription on protein abundance

Proteins are the main functional molecules in a cell and changes in their concentrations can have direct functional and phenotypic consequences. Therefore, the concentration of each protein in the cell is tightly controlled. Different mechanisms acting at each level of the cellular machinery, from transcription to translation and proteome turnover, contribute to proteome homeostasis. Recent studies in various organisms have uncovered pervasive transcription of the genome, well beyond the boundaries of known genes, which generates multiple classes of non-coding RNAs. Long non-coding antisense RNAs overlap with many genes. However, the functional impact of such antisense transcription is understood for only a few genes. In this project I will perform a systematic functional analysis of antisense transcription. For this, I will abrogate specifically the antisense transcripts of 170 genes in the yeast S. cerevisiae and investigate the consequences this has for each gene globally on cell fitness and specifically on the regulation of protein abundance of the overlapping gene. This will allow me to gain insights into the function of each antisense transcript and how it impacts gene expression, and to define mechanisms of competitive sense/antisense transcription, which is a key for our understanding of the rational behind the sense/antisense organization of the genome.

Name: **René Schellhaas**
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Production of Glycopeptide Antibiotics – a biomimetic approach

Vancomycin is a glycopeptide consisting of seven amino acids and as one of the so-called last resort antibiotics it is administered against several bacteria that are resistant to common antibiotics. However, even vancomycin-resistant strains are evolving, making it necessary to find new antibiotics.

During the biosynthesis of vancomycin, three P450 mono-oxygenases, OxyA, OxyB and OxyC, are responsible for three oxidative phenol coupling reactions that crosslink aromatic amino acid side chains in the heptapeptide to obtain the functional structure.
This PhD-project aims to develop an in vitro system, able to crosslink aromatic side chains of vancomycin aglycones using synthetic peptides and recombinant P450 enzymes to generate the active conformation of the peptide and thus mimicking vancomycin biosynthesis in vitro. With the help of modified peptides, to investigate effects of structural and chemical characteristics, mechanistic studies concerning the cross linking catalyzed by these P450s will also be carried out. In the longer term, this work will aim to develop new antibiotics based on modified vancomycin aglycones.

Name: **Kristina Haslinger**
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Interactions of Cytochrome P450 enzymes involved in the biosynthesis of Glycopeptide Antibiotics

Glycopeptide antibiotics have been used as last resort drugs against multi resistant Gram-positive bacteria since the 1950's; however the emergence of resistant pathogen strains makes the investigation of their biosynthesis of key relevance. The antibiotic effect of these chemically complex compounds relies on the specific three dimensional shape of the peptide backbone, enforced by cross links between aromatic side chains. These crucial phenolic coupling steps are catalyzed by Cytochrome P450 mono-oxygenases. Since important questions concerning their interplay with other biosynthetic proteins, especially the peptidyl carrier domains of non-ribosomal peptide synthetases, remain unanswered, these P450s are the subject of our current investigations. By applying biochemical and biophysical methods, P450s interactions with their binding partners and substrates are revealed, paving the way to the development of modified antibiotics.

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Structural and Functional Characterization of the RNA helicase DDX1

In eukaryotes and archaea, tRNAs, the essential adapter molecules in translation, can contain intervening sequences such as introns. During their maturation, these tRNAs undergo splicing by an endonuclease complex, which removes the intron from the tRNA-precursor, thereby generating upstream 5' and downstream 3' exon halves (Abelson, Trotta, et al. 1998). Subsequently tRNA exon halves are joined in a ligase reaction (Filipowicz et al. 1983). In mammals and archea, direct ligation of the 2',3'-cyclic phosphate of the upstream exon halve with the 5'-OH of the downstream exon halve is believed to constitute the major pathway (Englert et al. 2011). Recently RtcB in archaea and E. coli (Shuman 2011), the HSPC117 complex in mammals (Popow et al. 2011), respectively, has been identified to be the direct RNA 2',3'-cyclic phosphate ligase.
Among other proteins, the HSPC117 ligase complex contains the DEAD-box helicase DDX1 (Drewett et al. 2001). DDX1 is unique among homologous helicases since it contains a hnRNP U like insertion motif in its RecA like consensus sequence (Godbout et al. 1994).The role of DDX1 during RNA ligation is enigmatic so far and we therefore set out to functionally and structurally characterize the molecular mechanism of DDX1 with a strong focus on its unique hnRNP U insertion motif.

Name: **Frauke Leitner**
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The role of cortical inhibitory neuron types in functional microarchitectural organization

Cellular and network mechanisms for experience-dependent changes in the cellular receptive fields are not well understood. In the whisker-related cortical columns, there are reciprocal interactions between different inhibitory and excitatory neurons that are responsible for organizing cortical activity and controlling the boundaries between barrel fields. Inhibition can be local, lateral, translaminar, within and between cortical columns, and differs in the specific location that inhibitory synapses target on the subsequent dendritic branch. We are chronically recording neuronal activity in anaesthetized mice by in vivo two-photon imaging with a genetically encoded calcium indicator protein (GECI), YC3.60 or GCamP5.

Our goal is to optically record neuronal activity from the same sets of neurons over days and weeks. With a combinatorial approach using viruses and Cre recombinase transgenic mice, we can distinguish between excitatory and inhibitory neurons. With GAD67-Cre, parvalbumin-Cre (PARV-Cre) and somatostatin-Cre (SOM-Cre) mice, we can selectively label different inhibitory neurons types. We have also developed a genetic approach for Inducible Silencing of Synaptic Transmission (INSIST) with tetanus toxin light (TeTxLC). With the INSIST system, we will selectively silence specific inhibitory neuron types to investigate how excitatory and inhibitory neuronal response properties change upon whisker stimulation before and after silencing of synaptic transmission.

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Axonal retrograde transport and its role in establishment of a retinotectal neural circuit

While in the past decades neuroscience research has focused on describing mechanisms and processes in individual cells, in recent years attention was drawn to understanding neural networks and their implication in behavior. Recently, a novel neural circuit was identified in the optic tectum of zebrafish that is responsible for the filtering of visual stimuli (Ref.1). While stimuli by small objects are directed via retinal ganglion cells (RGCs) directly from the retina to the deeper layers of the tectum, for background signals a filtering step involving GABAergic interneurons inhibits activation of deep layer neurons.
We are using this neural circuit to investigate the role of neuronal transport in circuit formation. Microtubule-associated motor proteins are known to play a fundamental role in axonal transport, growth and their loss often leads to neurodegeneration (Ref. 2). Based on a zebrafish mutant line in a dynein heavy chain subunit (dync1h1), that shows a neurodegenerative phenotype, we started to analyze arbor growth, complexity and synaptogenesis in retinal ganglion cells. By employing overexpression of protein subunits that interfere with transport capacities in single cells we are furthermore analyzing these parameters in a cell autonomous way (Ref. 3). Using labeled neurotrophic factors and vesicle markers we started to directly address transport dynamics in wt and mutant cells. Calcium imaging (using optogenetic sensors like Syp-GCaMP5) and the analysis of visual evoked behaviors will allow us to address circuit functionality in vivo. Based on these experiments we try to link loss of transport capacities to the complexity and

functionality of the connections formed between RGCs and their tectal interaction partners.
Ultimately our experiments will deliver fundamental insights into the mechanisms of circuit formation in vivo and help to understand the cause of neurodegenerative disorders in a living and behaving vertebrate model organism.

1. Del Bene, F., Wyart, C., Robles, E., Tran, A., Looger, L., Scott, E. K., Isacoff, E. Y., et al. (2010). Filtering of visual information in the tectum by an identified neural circuit. *Science*, *330*, 669–673. 2. Hirokawa, N., Niwa, S., & Tanaka, Y. (2010). Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. *Neuron*, *68*, 610–638. 3. Tsujikawa, M., Omori, Y., Biyanwila, J., & Malicki, J. (2007). Mechanism of positioning the cell nucleus in vertebrate photoreceptors. *PNAS*, *104*, 14819–14824.

Name: **Petia Djurova**
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New brain-penetrant formulations for drug delivery across the blood-brain-barrier

The central nervous system (CNS) represents a pharmacological and immunological privileged region that results in poor brain-penetration of CNS drug candidates. Here we propose to develop new brain-penetrant formulations for kinase inhibitor leads. CNS targeting liposomal formulations as a drug vehicles support drug accumulation in the targeted tissue and reduce toxic side-effects. Further to that these CNS specific liposomal formulations protect the encapsulated substances from modifications and degradation in the blood stream during their transport to the brain.

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Age-related memory decline is rescued by restoring dnmt3a2 expression

Age-related cognitive deficits and diseases have received much attention in the past decades due to an increase of the average human life span. Studying age-

related memory deficits will help to investigate the basic mechanisms underlying memory function and further promises hope for new therapies. Epigenetic mechanisms are thought to play an important role in regulating gene transcription, which is known to be a crucial process for memory consolidation. A study of Miller and Sweatt (2007) indicates a role of DNA methylation in transcription regulation during memory formation. DNA methylation is catalyzed by DNA methyltransferases (dnmts) and our laboratory has shown that dnmt3a2 is up-regulated by neuronal activity. Further, we have observed that the level of dnmt3a2 is decreased in aged mice compared to young adult mice and that the aged mice have memory deficits. We could show that rescuing the level of dnmt3a2 in aged mice restores their cognitive abilities.

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Transcriptional Regulation of Adult Mammalian Axonal Regeneration

While there are numerous environmental factors that influence the effective or failed regenerative growth in the mammalian peripheral and central nervous systems, respectively, neuron-intrinsic growth programs significantly contribute to the success or failure of the repair. Dorsal root ganglion (DRG) neurons serve as a consistent model for studying the role of cell autonomous mechanisms upon injury. Following a peripheral nerve crush lesion, DRG neurons initiate a genetic process that drives axonal regeneration to reinnervate appropriate targets. Similar damage to the central branch of the same cell, however, fails to trigger any such cellular growth program and repair. However, if a peripheral conditioning lesion (CL) precedes an injury to the central branch, some growth is observed within the inhibitory environment of the CNS. The effect can be demonstrated in vitro, by an increase in neurite growth from dissociated and cultured DRG cells, and in vivo, by an increase in axon sprouting in the lesioned spinal cord. All evidence indicate that the CL effect is dependent upon transcription, and further data show that over 1,000 genes change expression level after a peripheral branch injury, compared to very few after a central branch lesion. Transcription factors likely play a major role in coordinating the abundant genetic changes, as recent microarray analyses suggest. To investigate mechanisms underlying the regenerative program activated by peripheral conditioning lesions, a recently optimized in vitro neurite growth

assay in addition to electroporation transfection of candidate genes into primary neurons will be used as a screening tool.

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Mutational Analysis of Wnt3-Frizzled Recognition

Wnt ligands comprise a large family of secretory cysteine-rich glycoproteins, which act as morphogens during embryogenesis. They play important roles in processes of growth and development of stem cells and in many types of cancer. Although Wnt signaling is increasingly understood at the cellular level, the structural properties of Wnts and their molecular interaction with their cellular receptors remain poorly defined. Activation of the Frizzled receptors by the Wnt–protein regulates expression of key genes, which are responsible for stem cell differentation, cell polarity and growth.

Because of low solubility of Wnt-proteins the Wnt-Fz interaction is poorly studied and there is no X-ray structure of Wnt proteins. Study of the structure of Wnt proteins and Frizzled receptors as well as the structure of the Wnt-Fz complex, is required to understand the specificity of the Wnt-Fz interaction, which is important for designing anticancer effectors and drugs regulating stem cell activity.

Here, we present a mutational mapping of the Wnt3a-Fz8 binding interface. For defining the binding region between Wnt3a and the Fz-CRD domain, we performed a de novo modeling approach. On the basis of this model several sites in Wnt3a relevant for Fz binding were predicted. We have created point mutants in the mouse Wnt3a protein sequence on the basis of our obtained binding model. Each mutant was tested for its signaling activity in a Dual Luciferase TopFlash assay system. By this approach we identified a short region at the C terminus of Wnt3a, which is critical for its activity. Single point mutations in this area dramatically reduced Wnt signaling as compared to wildtype. Combined mutations of several sites completely abolished the signaling activity, but did not reduce the secretion level of the Wnt ligands. These data were confirmed by pull-down experiments using a Fz8-CRD-Fc protein.

Our findings define a region in Wnt3a, which is essential for Fz interaction and signaling activity and form a basis for the design of specific antagonists.

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The role of LAPTM5 in glioblastoma

Glioblastoma is the most frequent primary brain tumor in humans. Despite intense therapy including surgery, radiotherapy and chemotherapy the prognosis remains very poor with a mean progression free survival of 14 months. One of the reasons for this is the ability of the tumor to infiltrate the brain tissue, a characteristic called invasiveness. In order to optimize patient treatment, it is crucial to better understand this mechanism.
In an in-vivo screen aimed to find proteins that mediate glioblastoma invasiveness, we found that the knockdown of a protein called Lysosomal Associated Protein Multispanning Transmembrane 5 (LAPTM5) leads to an invasive phenotype of human glioblastoma cells implanted into mice brain. It has been described that a low expression of LAPTM5 is associated with an increased malignancy in several tumors, such as lung cancer, neuroblastoma and hepatocellular cancer. The expression of LAPTM5 might be regulated by methylation.
The aim of my doctoral thesis is to elucidate the functional role of LAPTM5 in glioblastoma, as well as in therapy resistance and its regulation. In order to do so we will perform in-vitro functional assays with a constitutive knockdown of LAPTM5. This will include experiments evaluating its role in glioblastoma cell migration and invasion, in clongenicity, proliferation, cell cycle and cell death of glioblastoma cells. Additionally we will study the role of LAPTM5 in therapy resistance of glioblastoma cells in-vitro, including radiotherapy and chemotherapy.
In addition to the in-vitro experiments, we will look for the expression of LAPTM5 in human brain tissue and for an association of LAPTM5 expression and patient survival. Finally, we will assess the regulation of LAPTM5. Besides the regulation by methylation, it has been published that LAPTM5 might interfere with the TGFbeta signaling pathway, a well-known pathway in glioblastoma regulation. In the long term the study of LAPTM5 in glioblastoma might lead to a new therapeutic target with a benefit for patient survival.

Name: **Martin Seefeld**
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The mechanisms underlying neuropathic pain (NP) are still inconveniently understood. NP is initiated by dysfunction of the nervous system, causing

functional and structural changes in the central, but also in the peripheral nerve system (PNS).
In order to gain a deeper insight into the contribution of the PNS to the development of NP, we induce NP in different transgenic mice lines by spared nerve injury (SNI) and measure structural changes at distinct time points via two-photon in vivo microscopy. Therefore mice have been generated specifically expressing YFP or GFP in their thick myelinated or nociceptive nerve fibres, respectively. In parallel, behavioural analysis of mechanical sensitivity is conducted at the three territories (saphenous, tibial, sural) of the left hind paw via von Frey monofilaments.
Wallerian degradation occurs in the tibial and saphenous nerve. After 7-8 weeks reinnervation of the tibial and sapheanous digit is measured, and matches the reoccurrence of mechanical sensitivity in the tibial digit. While the sural shows mechanical allodynia shortly after SNI, no significant difference is observed in the saphenous territory.

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Synaptic transmission in thalamic giant synapses

The thalamus relays sensory information from the periphery to the cortex. A trisynaptic pathway connects the whiskers with the somatosensory cortex. The principal nucleus of the trigeminal nerve (Pr5) in the brainstem receives sensory information from the whiskers and sends them to the ventral posteromedial nucleus of the thalamus (VPM), which in turn projects to the somatosensory cortex. Synaptic transmission between Pr5 and relay cells of the VPM is mediated by giant synapses.
In this study we labelled trigeminothalamic (Pr5-VPM) giant terminals by stereotaxic delivery of adeno-associated virus particles (AAV) encoding synaptophysin-EGFP into the Pr5 nucleus of rats (age P12). Pr5-VPM giant terminals were identified in the VPM and directly stimulated after establishing whole-cell patch-clamp recordings from the postsynaptic relay neuron (rats age p24-p30). This allowed us to study for the first time synaptic transmission in identified Pr5-VPM giant synapses.

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Function of Cortico-Thalamo-Cortical Loops

The brain is divided into many functional units. The cortex the highest brain center communicates with the rest of the brain largely through the thalamus, the “distributing” unit of the brain. Albeit a rich reciprocal connectivity between the thalamus und the cortex, research focused mainly on the thalamo-cortical connectivity. However from an anatomical point of view the the cortico-thalamic connections outnumber these. The function of the cortico-thalamic connectivity and the forming loops are largely unknown.
Using channelrhodopsin, a heterologously expressed light-sensitive channel, as a tool to stimulate single synapses, I intend to:

1. investigate the synaptic transmission properties of the cortico-thalamic synapses,
2. characterize changes due to deletion of certain receptors,
3. and continue to describe subsequent implications for the animal in behavioral assays.

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In modern drug discovery, the analysis of the biological activity of candidate drugs is an important task and it has a need for better in-vivo response prediction, which can be achieved by better monitoring drugs effects and by correlating the used in-vitro models as well as possible with the situation in vivo. We focus here on developing a drug testing system with selected improvements to the classical testing methods, in order to then apply this system to candidate drugs.
The first improvement is the use of biosensors that record important metabolic parameters in real-time and directly in the cell culture system, allowing to link changes in biological activity to specific cellular responses and avoiding end-point assays that give information at selected time points. For the moment being, the metabolic parameters monitored are oxygen consumption, pH change and glucose consumption, which could later be complemented with other parameters such as lactate production or glutamine consumption.

Oxygen and pH sensors are available commercially and the development of a functional glucose sensor, based on a modified oxygen sensor that has been coated with the enzyme glucose oxidase, is the first part of this project. It has been possible to develop glucose sensors in different formats (24-well plate, for a cell reactor or a flow-through system) that have a dynamic range of 0,5mM to 30,0mM (depending on the application) and a reaction time around 20min. More efforts have to be made to improve to reproducibility of the sensor and its interference with the measurement.
A computer program allowing the fast and effective analysis of the important amount of data produced is also being developed using the programming language R.
The next planned improvements include the development of a 3D culture technique for the human model cancer or control cell-lines used in this system, to best reflect the diffusion gradients and cell-cell contacts that take place in-vivo. These cells should be supplied by a flow-through fluidics system, mimicking the constant renewal of external conditions achieved in-vivo. This optimised drug testing model can then be used for treatment of cancer and control cell lines with different anticancer drug candidates and potential chemoprotective agents.

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The molecular role of microtubule associated kinases in ciliogenesis

Primary cilia play an essential role in animal development and cellular morphogenesis. Their dysfunction is associated with human diseases and cancer progression. Recently, we identified microtubule-associated kinases as positive regulators of ciliogenesis. We use a combination of molecular/cell biology and protein expression techniques to discover and characterize kinase substrates involved in cilia formation and/or extension. As some of the kinases are mis-regulated in cancer cells, this will contribute to the identification of suitable targets for subsequent small inhibitor compound screenings.

Name: **Jagadeesh Gandla**
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Proteins are considered as working horses of life. The function of proteins is accomplished only when they fold into their native three dimensional structures. Achieving correct protein folding is very crucial for cellular health and viability. Molecular chaperones are a class of proteins that assist in the folding of other proteins. Among these molecular chaperones, group II chaperonins which are found in archaea and eukaryotic cells have a typical ring shaped structure. They are involved in the folding of many different types of proteins. Eukaryotic chaperonin TRiC/CCT is involved in the folding of at least 10% of proteins including cell cycle regulators, tumour suppressors and cytoskeletal proteins like actin. MmCpn60 of Methanococcus mirapaludis have similar structure and allosteric regulation as that of TRiC. My study deals with structural and conformational properties of MmCpn60 by using amide exchange mass spectrometry, to understand the role of MmCpn60 in protein folding.

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Functional study of SSX2IP in ciliogenesis

Primary, non-motile cilium functions as a chemo-sensor or signaling platform thereby contributing to cell and tissue homeostasis. Thus defects in ciliogenesis are likely to underlie a range of cilium-related human diseases. In the previous study, using quantitative differential proteomics, we identified SSX2IP as a novel microtubule associated protein and a component of centriolar satellites that co-localized and worked together with PCM1 to govern centrosome integrity during cell division and to maintain genome stability. To study the function of SSX2IP in ciliogenesis, we have performed RNA interfere assay to knockdown the expression level of SSX2IP and found that the primary cilia is shorter than the control cells. In this project, we will systematically characterize interaction partners of SSX2IP at basal bodies and investigate the function of SSX2IP in the process of cilia formation. To know the function of SSX2IP in ciliogeneis, we will perform partial rescue assay in the cells after interference by transfection and expression of truncated human SSX2IP variants and test the recovery of cilium formation after rescue to see the recovery effect on ciliogenesis. We would also like to see the changes of the SSX2IP complex components in different truncated SSX2IP transfected cells and observe

the localization of the truncated forms of SSX2IP so that we can understand the binding pattern and the relationship between the complex formation and ciliogenesis. Through all the above studies, we will obtain more information of the function of SSX2IP in the process of ciliogenesis.

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Golden GATEway – A combinatorial cloning approach to generate fusion and recombination constructs

The design and generation of DNA constructs is still one of the main tasks for most biological researchers. Understandably, the design is often based on available cloning strategies. However, DNA constructs are getting more and more complex which limits standard cloning procedures. Many transgenes include in-frame fusion proteins like FRET sensors or recombination elements (for example loxP sites flanking an open reading frame). Here, we present an efficient method that greatly facilitates the generation of these complex constructs.

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CAF1/Pacman under scanner - removal of the polyA-tail under stress conditions

Gene expression is regulated at multiple levels, wherein cells integrate extra- and intracellular signals in order to properly decode the information in the genome. The control of mRNA degradation is a major mechanism by which gene expression can be rapidly turned on and off. The removal of poly-A tails is a key event that initiates degradation of most mRNAs. There is substantial evidence that under stress conditions, many mRNAs are stabilised, yet the mechanism remains elusive. In this study, we investigate the regulation of the major cytoplasmic deadenylase in metazoans, CAF1. Using two different assays, 1) a tethering approach whereby human (h)CAF1a is forced to bind a reporter mRNA within cells, and 2) an in-vitro deadenylation assay, for which hCAF1a is purified from cells that were subjected to

stress, we observed that hCAF1a is regulated. Surprisingly, different stress treatments cause hCAF1a to be regulated differently. Mass spectrometry followed by mutation analysis confirmed the role of post-translational modifications on hCAF1a. Co-immunoprecipitation experiments showed that a Serine residue on hCAF1 was important for interactions with BTG2, an activator of hCAF1a. Phosphorylation on this residue under anisomycin-induced stress abolished interactions with BTG2. Summarizing, hCAF1a with its activator BTG2 play an important role in removal of the polyA-tail, and these interactions are controlled via post-translational modifications to prevent abnormal mRNA decay under stress.

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Screening for transcription factors influencing EGF-induced migration

Migration and invasion belong to the hallmarks of cancer leading to metastasis and dissemination of cancer. MCF-10A cells are non-tumorigenic breast epithelial cells. They are known to undergo a change from resting to migratory phenotype in presence of Epidermal Growth Factor (EGF). In our current study, we aim to identify transcription factors responsible for this complex phenotypic switch. To address this question, we performed a focused RNAi screen. Effects of candidate transcription factor knockdown on EGF-induced migration of MCF10A cells were analyzed applying an automated wound healing assay. The screen has identified 3 accelerators and 3 inhibitors of migration which are currently in the process of validation.

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Transcriptional targets of Dpp signaling mediating growth

A fundamental but unresolved problem in developmental biology is the question of how developing growing tissues know when they have reached their correct final size and therefore should stop growing. The developing wing of Drosophila melanogaster is one model system that has been extensively used to address this question. The tissue forming the wing is initially specified as a group of 30-50 cells,

called the wing disc, which proliferates during larval and early pupal development, until it reaches the final size of 50,000 cells and stops growing. All the information required for a wing disc to know when it has achieved the correct final size is autonomously contained within the disc itself.
One signaling pathway that strongly influences wing tissue size is Dpp. Dpp is a gradient morphogen member of the TGFβ family growth factors. Flies lacking Dpp in the wing have extremely small, vestigial wings, whereas overactivation of the Dpp pathway leads to excessive tissue overgrowth, clearly indicating that Dpp signaling is required to support tissue growth, also indicating that the size of the wing correlates with the activity of the Dpp pathway.
The goal of my project is to find out, how Dpp signaling promotes tissue growth. Moreover, identifing, which the Dpp target genes that regulate growth are, since none has been identified yet.

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Reversal of Cardiac Hypertrophy by an N-Terminal HDAC4 Cleavage Product

The heart responds to stress signals by hypertrophic growth, which is accompanied by an increase in the size of cardiac myocytes, sarcomeres assembly and activation of a fetal cardiac gene programme. Nucleo-cytoplasmic shuttling of class IIa HDACs has been implicated as an underlying key mechanism for cardiac remodeling in response to cardiac stress signaling. β-adrenergic signaling plays a critical role in pathological cardiac remodeling, characterized by activation of fetal genes. Transcription factors like MEF2 are required for this process and can be repressed by HDAC4. The non-phosphorylated form of HDAC4 localizes to the nucleus where it binds MEF2 and represses MEF2 target genes. β-adrenergic receptors activate CaM Kinase II (CaMKII), which phosphorylate HDAC4, triggering its nuclear export, relieving MEF2 repression, and promoting pathological cardiac remodeling. Recently we have shown that protein kinase A (PKA) also targets HDAC4 and induces limited proteolysis of HDAC4 resulting in an N-terminal cleavage product that acts as a transcriptional repressor of MEF2. This signaling pathway overcomes CaMKII-induced and HDAC4-dependent induction of the fetal cardiac gene program. Based on these findings we now expressed the N-terminal HDAC4 cleavage product in cultured cardiomyocytes and in mice to test its therapeutic ability to reverse cardiac hypertrophy.

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A "Turn on" Fluorescence Probe Library for Unraveling the Stereoselectivity of a Bio-catalytic Diels-Alder Reaction

The Diels-Alder reaction is one of the most important C-C bond forming reaction in organic chemistry. Many efforts had been given to control the enantio- and diestereoselectivity of the reaction. Diels-Alderase (DAase) ribozyme catalyses the Diels-Alder reaction between anthracene and N-pentylmaleimide (NPM) with multiple turnover and 1.100 fold rate acceleration. A new generation of Antracene-BODIPY based dye library was developed to follow the catalysis of DAase. These probes' fluorescence emission increases up to 90-fold upon reaction with the NPM. With the help of these probes, we found that DAase catalyses the Diels-Alder reaction with >90% de and >99% ee. The stereochemistry of the products was determined unambiguously by the ROESY (Rotating frame Overhauser Effect NMR SpectroscopY). Preliminary results showed that the diastereoselectivity can be explained by the dimer formation of the DAase.

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Developing Anti-Angiogenic Drugs Based on the FGF2/Tec Complex

FGF2 (18 kDa) is a potent mitogen involved in the regulation of cell proliferation and differentiation. It is a strong pro-angiogenic molecule acting during development and supporting tumor growth and metastasis. Following secretion, it exerts these functions by activating high-affinity FGF-receptors on cell surfaces of target cells. FGF2 does not contain a signal peptide but rather has been shown to make use of an unconventional, ER-Golgi independent secretory mechanism. Unconventional secretion of FGF2 occurs by direct translocation across the plasma membrane. This process involves sequential interactions with the phosphoinositide PI(4,5)P2 at the inner leaflet, and heparan sulphate proteoglycans at the outer leaflet of the plasma membrane. FGF2 membrane translocation requires the protein to be folded, and depends on tyrosine phosphorylation of FGF2 mediated by Tec kinase. To develop inhibitors of FGF2 secretion that may exert anti-angiogenic activity, we analyzed a combinatorial library of 80,000 small molecules

for compounds that block binding of FGF2 to Tec kinase. Following multiple rounds of various validation assays, 15 compounds were found to impair this interaction. Most of these compounds also blocked Tec kinase-mediated phosphorylation of FGF2 and about half of these compounds significantly inhibited FGF2 secretion. Future efforts will focus on the synthesis of chemical derivatives of the active lead compounds and the analysis of their anti-angiogenic potencies in animal models.

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Role of the lipid kinase phosphatidylinositol 4-kinase IIIα in Hepatitis C virus replication

Hepatitis C virus (HCV) belongs to the family Flaviviridae and affects 170 million people worldwide. As a positive-strand RNA-virus, it causes rearrangements of internal cellular membranes to form vesicles accommodating the replication complexes. In several studies, the lipid kinase phosphatidylinositol 4-kinase IIIα (PI4KIIIα) was identified as an essential host factor for HCV replication. PI4KIIIα is localized at the endoplasmic reticulum and synthesizes phosphatidylinositol 4-phosphate (PI4P), which is recruited to the sites of viral RNA replication as well as the kinase itself. Enzymatic activity of PI4KIIIα is stimulated by interaction with the viral non-structural protein 5A (NS5A), thereby causing induced intracellular levels of PI4P in HCV positive cells. NS5A is a phosphoprotein existing both in a basal (p56) and a hyperphosphorylated (p58) form favoring RNA replication or viral particle assembly, respectively. Remarkably, the loss of RNA replication caused by knockdown or inhibition of PI4KIIIα correlated with elevated levels of NS5A p58 suggesting that PI4KIIIα might be involved in regulation of NS5A phosphorylation. These findings were supported by overexpression experiments of PI4KIIIα resulting in decreased levels of NS5A p58. However, it is still unclear, whether PI4KIIIα directly or indirectly influences NS5A phosphorylation and which domains of the kinase in general are important for HCV RNA replication. Additionally, the viral RNA-dependent RNA polymerase NS5B also seems to be required for PI4P induction, but not for modulation of NS5A phosphorylation by PI4KIIIα.
Future work in this direction will address functional mapping of PI4KIIIα to identify regions essential for HCV RNA replication, induction of PI4P and modulation of NS5A phosphorylation. Furthermore, the sites of NS5B which contribute to PI4P induction will be elucidated by mutagenesis experiments. Eventually, the mechanism of the NS5A-PI4KIIIα interaction shall be identified and might give an insight into the strong dependency of HCV on this host factor.

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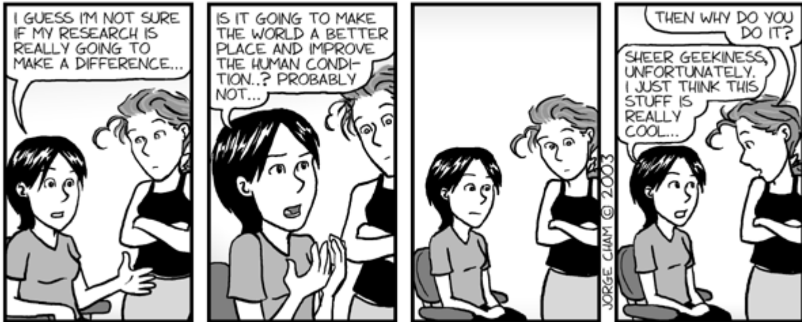
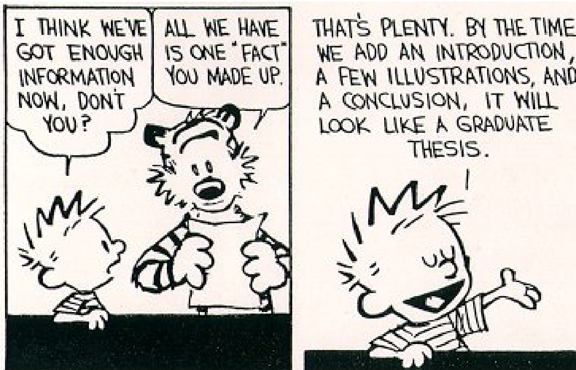
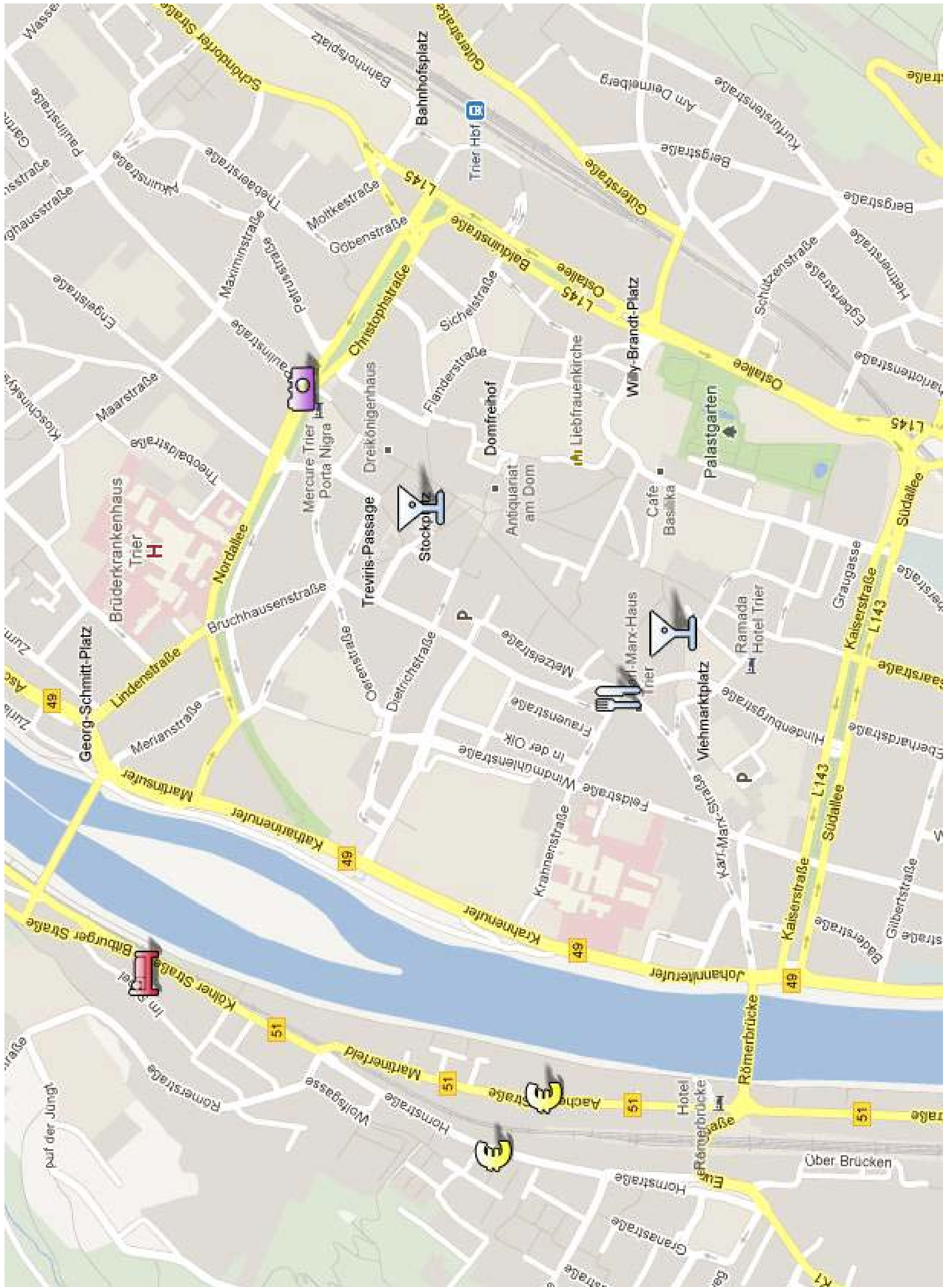




Calvin & Hobbes



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Trier Hbf
Schöndorfer Straße
Güterstraße
Am Deimelberg
Bergstraße
Kurfürstenstraße
L145
Moltkestraße
Gobenstraße
Theobaldstraße
Thebäerstraße
Paulinstraße
Maximinstraße
Petrusstraße
Christophstraße
Balduinstraße
Ostallee
Willy-Brandt-Platz
Schützenstraße
Egbertstraße
Hermesstraße
Sichelstraße
Flanderstraße
Dreikönigenhaus
Domfreihof
Liebfrauenkirche
Palastgarten
Mercure Trier Porta Nigra
Maarstraße
Engelstraße
Brüderkrankenhaus Trier
Nordallee
Treviris-Passage
Stockplatz
Antiquariat am Dom
Cafe Basilika
Südallee
Graugasse
Kaiserstraße
L143
Ramada Hotel Trier
Karl-Marx-Haus
Viehmarktplatz
Hindenburgstraße
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Bruchhausenstraße
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Feldstraße
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Krahnenstraße
Meriansstraße
Martinsufer
Katharinenufer
Krahnenufer
Johanniterufer
49
51
Bitburger Straße
Kölner Straße
Martinerfeld
Aachener Straße
Römerstraße
Wolfsgasse
Hornstraße
Auf der Jüngt
Hotel Römerbrücke
Römerbrücke
Eurener Straße
Über Brücken
Granastraße
Gilbertstraße
Bäderstraße

Hotel: Feilen-Wolff
Kölner Straße 22, 54294 Trier

Grocery stores
Lidl and Kaufland

Restaurant: Das Weinhaus
Brückenstraße 7
54290 Trier

Partytime… Excellent!!!
these two areas seem to be the places to go for a fun night out

Porta Nigra
City tour: Sunday 11am-1pm

Train connection

Friday	Time	Platform	Train
Heidelberg Hbf	14:33	3	S2
Kaiserslautern Hbf	15:59	3	
Kaiserslautern Hbf	16:03	8	RE 12156
Saarbrücken Hbf	16:56	5	
Saarbrücken Hbf	17:04	12	RE 12017
Trier Hbf	18:11	12 Nord	

Sunday			
Trier Hbf	15:48	11 Süd	RE 12014
Saarbrücken Hbf	16:54	12	
Saarbrücken Hbf	17:02	5	RE 12161
Kaiserslautern Hbf	17:54	8	
Kaiserskautern Hbf	17:58	10	S2
Heidelberg Hbf	19:23	7	

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Molecular & Cellular Biology
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Retreat 2012

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