2010 LMU-Harvard Young Scientists' Forum

24 – 27 July 2010

Harvard University

SATURDAY 24 JULY

Northwest Building Auditorium, B103
52 Oxford Street
Cambridge, MA 02138

8:30AM	Depart hotel and dormitory
8:45AM	Continental breakfast at Northwest Building, B1 lobby
9:00AM	Opening Remarks
9:15AM	Faculty Lecture 1: Alan Saghatelian
10:15AM	Break
10:30AM	Student Session 1, Chaired by Stephen Buratowski
	Student 1: Tim Sikorski (Buratowski)
	Student 2: Anna Kochaniak (Walter and van Oijen)
	Student 3: Andreas Schmidt (Endres)
	Student 4: Gaëtan Bellot (Shih)
12:30PM	Lunch
2:00PM	Student Session 2, Chaired by Markus Meister
	Student 5: Anne Kreile (Bonhöffer)
	Student 6: Ryan Draft (Lichtman)
	Student 7: Franz Weber (Borst)
	Student 8: Brendan Lehnert (Wilson)
4:00PM	Break
4:15PM	Faculty Lecture 2: Patrick Cramer
5:30PM	Poster session 1 (all posters present)
7:30PM	Dinner on your own in Cambridge

SUNDAY 25 JULY

Northwest Building Auditorium, B103
52 Oxford Street
Cambridge, MA 02138

9:00AM	Faculty Lecture 3: Dirk Trauner
10:00AM	Break
10:15AM	Student Session 3, Chaired by Daniel Hog
	Student 9: Michael Lazarus (Kahne/Walker)
	Student 10: Anselm Geiger (Griesbeck)
	Student 11: Wenjun Zhang (Walsh)
	Student 12: Philipp Stawski (Trauner)
12:15PM	Lunch
2:00PM	FAS Lab Tours
4:00PM	Faculty Lecture 4: Benedikt Grothe
5:00PM	Poster session 2 (all posters present)
7:00PM	Blue Ribbon BBQ in Tozzer courtyard at Harvard
	(Rain location: Northwest Building)

MONDAY 26 JULY

Cannon Room
Building C
Harvard Medical School
Boston, MA 02115

8:45AM	Board shuttle for Harvard Medical School
9:30AM	Faculty Lecture 5: Stephan Sieber
10:30AM	Break
10:45AM	Student Session 4, Chaired by Thomas Böttcher
	Student 13: Nicholas Kwiatkowski (Gray)
	Student 14: Evelyn Zeiler (Sieber)
	Student 15: Emily Derbyshire (Clardy)
	Student 16: Peer-Hendrik Kuhn (Haass)
12:45PM	Lunch
2:30PM	HMS Lab Tours
4:30PM	Student Session 5, Chaired by Kenneth Blum
	Student 17: Sonia Cohen (Greenberg)
	Student 18: Felipe Ortega de la O (Götz)
5:30PM	Faculty Lecture 6: Alex Schier
6:45PM	Closing remarks
7:00PM	Dinner on your own in Boston

TUESDAY 27 JULY

9:00AM	Depart Northwest Building
10:15AM	Arrive Marblehead
12:45PM	Depart Marblehead
1:00PM	Arrive Gloucester
1:30PM	Whale watch
5:30PM	Depart Gloucester
6:00PM	Dinner at Woodman's
8:30PM	Depart Woodman's
9:30PM	Arrive Northwest Building

SATURDAY 24 JULY TALKS

Investigating peptide processing via peptidomics

Alan Saghatelian
Harvard University, Department of Chemistry and Chemical Biology

Peptides and peptidases play important roles in regulating physiological signaling by shaping the activity of the peptidome. We apply a mass spectrometry (MS)-based peptidomics approach to try to develop a general approach to identify important interactions between peptides and peptidases.

Identification of Promoter Associated Factors with Quantitative Proteomics

Tim Sikorski
Harvard University, Department of Biological Chemistry and Molecular Pharmacology

RNA Polymerase II mediated transcription is known to be a highly dynamic process with respect to the factors required at each stage and the modification state of the proteins involved. The recent wave of genome and proteome scale analyses of active promoters establish that a diverse array of factors occupy the DNA upstream of the transcription start site, and these factors can both positively and negatively regulate initiation. I will present our efforts to isolate promoter associated factors from yeast extracts and to analyze the components using a mass spectrometry based comparative proteomic approach. These catalogs can be used to determine which factors are recruited to various promoters. Using this approach, we have identified novel single stranded DNA (ssDNA) binding proteins that associate with transcription complexes. We have confirmed these interactions occur in vivo, and our findings suggest that ssDNA binding proteins coordinately localize at the transcription bubble throughout the transcription cycle.

Single-molecule imaging of eukaryotic DNA replication

Anna Kochaniak
Harvard University, Department of Biological Chemistry and Molecular Pharmacology

Single-molecule biophysical approaches have been successfully used to study DNA replication in reconstituted prokaryotic systems like *E. coli* involving up to 14 different proteins. However, DNA replication in eukaryotes requires scores of proteins working in concert to replicate megabases of DNA and cannot be reconstituted using current methods. Therefore, to study the activity of eukaryotic replication proteins at the single-molecule level, we have developed a fundamentally new strategy. We have developed the tools to study DNA replication at the single-molecule level in *Xenopus* egg extract, an environment that that closely mimics the cellular context, but is still compatible with our *in vitro* single-molecule fluorescence and manipulation techniques.

Our novel fluorescence-based technique allows us to directly visualize replication initiation and elongation on single DNA molecules in real time. Briefly, a well-stretched lambda phage DNA (48.5kb) is tethered at both ends to a coverslip within a microfluidic flow cell. Addition of *Xenopus* egg extract promotes multiple initiations on each immobilized DNA molecule, leading to complete duplication of the DNA. By supplementing egg extracts with fluorescently-labeled replication factors we are able to mark sites of ongoing DNA replication. Thus, we can follow in real time the firing of individual origins of replication and movement of individual replication machineries. Our dynamic data reveals the location

and timing of origin firing and suggests that more origins fire in this system then previously reported. In the future, we will label various replication proteins to learn about the dynamic composition of the replication fork. This should enable us to measure the stoichiometry and lifetime at the fork of these proteins as well as to track their location over time. All of these parameters can be measured under different conditions, such as replicative stress, in order to probe the dynamics and inner workings of the replisome.

Innate immune recognition of viral RNA by RIG-I-like helicases

Andreas Schmidt
LMU, Division of Clinical Pharmacology

Viral infections are a constant threat to higher organisms. They not only cause severe infectious diseases but can also be a cause for cancer. The cell-autonomous detection of viral but also all other infections is based on the recognition of conserved molecular patterns that are present in the pathogen but not in the host. The innate immune system has evolved so called “pattern recognition receptors” that scan the extracellular and intracellular space for signs of infection. In the case of viral infection some of these receptors recognize structural features of foreign nucleic acids and their aberrant localisation. Recently, a new class of these receptors has been decribed as the RIG-I-like DEXD/H-box helicases (RLHs) RIG-I, MDA-5 and Lgp2. They are expressed in virtually all cells of the body and recognize viral RNA in the cytoplasm of infected cells. Recognition of viral RNA by these helicases leads to the production of type I interferons and inflammatory cytokines and is essential for host defence. We have characterized the ligand-binding properties of RLHs in order to understand how they distinguish self from non-self RNA and how they are activated.

New DNA nanotechnology-based tools for structural biology and single-molecule biophysics

Gaëtan Bellot
Harvard University, Department of Biological Chemistry and Molecular Pharmacology

Molecular self-assembly using DNA as a structural building block has proven to be an efficient route to the construction of nanoscale objects and arrays of increasing complexity. Using the remarkable “scaffolded DNA origami” strategy developed by Paul Rothemund in 2006, we generalized a strategy to build custom-shaped, three-dimensional (3D) objects with precisely controlled dimensions. We are developing the DNA nanotechnology as a tool for structural NMR studies of membrane proteins and single-molecule biophysics. Membrane proteins are encoded by 20–35% of genes but represent <1% of known protein structures to date. Thus, improved methods for membrane-protein structure determination are of critical importance. Residual dipolar couplings (RDCs), commonly measured for biological macromolecules weakly aligned by liquid- crystalline media, provide important global angular restraints for NMR structure determination. For alpha-helical membrane proteins >15 kDa in size, Nuclear-Overhauser effect-derived distance restraints are difficult to obtain, and RDCs could serve as the main reliable source of NMR structural information. In many of these cases, RDCs would enable full structure determination that otherwise would be impossible. However, none of the existing liquid-crystalline media used to align water-soluble proteins are compatible with the detergents required to solubilize membrane proteins. We generated detergent-resistant liquid crystals of 0.8-μm-long DNA nanotubes that enable weak alignment of detergent-reconstituted membrane proteins. This DNA-nanotube liquid crystal will introduce the advantages of weak alignment to NMR structure determination for a number of membrane proteins. To generalize further the method to be compatible with positively-charged protein-micelle complexes and to facilitate measurement of linearly independent restraints to get more structural

information, we are working to generate additional DNA-nanostructure based alignment media. Furthermore, we are applying our DNA-nanotools towards structure determination and mechanistic analysis of mitochondrial membrane proteins and GPCRs.

Plasticity of orientation preference in mouse visual cortex

Anne Kreile
MPI of Neurobiology, Department of Cellular and Systems Neurobiology

Neuronal response properties in the visual cortex are shaped by a combination of intrinsic factors and sensory experience. For example, orientation preference develops independently from visual input, but restricting experience to a single orientation by stripe rearing has been shown to cause an overrepresentation of the experienced orientation and an underrepresentation of the orthogonal orientation. However, due to methodological limitations in earlier studies, the mechanisms underlying the stripe rearing effect could not be completely resolved: According to the permissive hypothesis neurons initially tuned to non-experienced orientations loose responsiveness, while the instructive hypothesis states that single neurons change their tuning towards the experienced orientation. We investigated the plasticity of orientation preference in mouse visual cortex with two-photon calcium imaging, providing single cell resolution and allowing direct testing of these hypotheses. We induced plasticity by stripe rearing visually experienced mice from postnatal day 25 onwards for three weeks with cylindrical lenses (167dpt) of four different orientations fitted to skull mounted goggles.

Following stripe rearing, the goggles were removed, the calcium indicator OGB1-AM was injected into the monocular visual cortex and the responses to drifting gratings were measured with two-photon imaging. In control mice, we found a horizontal bias in the distribution of preferred orientations which decreased with increasing depth in layer 2/3. In stripe reared mice, the distributions of preferred orientations showed a clear shift towards the experienced orientation. Response amplitudes and tuning widths were not affected. The fraction of responsive neurons decreased slightly, indicating that a weak permissive component may be present. The size of the permissive effect, however, was not correlated with the magnitude of the shift in the distribution of preferred orientations in individual animals. Moreover, in lower layer 2/3 there was a clear shift towards the experienced orientation, but no drop in the fraction of responsive neurons. Thus, diverse mechanisms contribute to the changes in preferred orientation following stripe rearing, but the effect is at least partially mediated by an instructive process, causing individual neurons to change their orientation preference.

Synaptic Circuit Refinement in the Developing Neuromuscular System

Ryan Draft
Harvard University, Department of Molecular and Cellular Biology

Neural circuits undergo considerable rearrangement and refinement in early postnatal life. How experience (i.e., neural activity) alters neural circuits in early life is not understood. One approach to this fundamental question would be to ascertain precisely how neural circuits are changed during early postnatal development. To investigate this question we have developed a method to fully reconstruct immature circuits in the mouse neuromuscular system using multi-color ‘Brainbow’ reporter mice and high spectral and spatial resolution confocal microscopy. As a result, we have been able to observe and characterize how an entire set of developing axons interact with each other in several small limb and neck muscles. In contrast to the tortuous branching and absence of any positional topography in the axonal arborizations, there is a striking higher- level organization in the pattern of synaptic connectivity. Namely,

individual motor neurons show a highly significant bias to interact with certain synaptic partners over others during early postnatal life when multiple neurons converge and co-innervate the same neuromuscular junctions. Furthermore, analysis of complete connectomes in neonatal muscles reveals that this bias is part of a systemic single axis ranking of all the motor axons that project to a muscle. That is, the branches of each motoneuron co-innervate neuromuscular junctions most often with neurons that are "adjacent" in this ranking system, and neurons that are far apart in the ranking have fewer co-innervations in proportion to their distance apart. These results may relate to the means by which muscle elaborate tension: arbor size and activity pattern. However, a definitive explanation of what functional properties drive this pattern remains to be resolved.

Spatio-Temporal Response Properties of Optic-Flow Processing Neurons

Franz Weber
MPI of Neurobiology, Department of Systems and Computational Biology

A central goal in sensory neuroscience is the complete description of a neuron's input-output relation. In the classical reverse correlation approach, a neuron is modeled by a linear-nonlinear (LN) model composed of a linear filter (linear receptive field) followed by a static, nonlinear (N) function. These LN models have become a standard for the characterization of sensory neurons. However, they typically cannot capture strong nonlinearities in sensory neurons as changes in the neural gain or selectivity, and, therefore do not generalize to arbitrary stimuli. We studied these issues in large-field, optic-flow processing neurons in the fly. Using novel random motion stimuli with individually moving dots (motion cues), we determined the spatio-temporal receptive field of these neurons. We found that the receptive field can be described by a time-varying vector field that is space-time separable. Increasing the number of presented motion cues leaves the linear receptive field nearly unchanged, however, strongly reduces the neurons' gain and selectivity. To capture these systematic changes in response behavior, we extended the LN-model by a biophysically motivated gain and selectivity mechanism. In this biophysical model, the modulations of gain and selectivity are related to changes in the neuron's membrane conductance and to unbalanced excitatory and inhibitory synaptic driving forces on the dendrite. We estimate all the parameters of this biophysical model directly from the neural responses. The model improves the prediction of responses to varying densities of motion cues and, thus, generalizes over different stimulus ensembles.

A sensitive measure of *Drosophila* hearing

Brendan Lehnert
Harvard University, Department of Neurobiology

Forward genetic screens in *Drosophila* have identified molecules that are essential for proper mechanotransduction, including auditory transduction. However, functional characterization of mutants has been hampered by a lack of high-resolution techniques for assaying primary auditory transduction in this genetic model organism. Some mutants are not deaf, but seem to have poor hearing or altered coupling between the hearing organ and transduction apparatus. This makes it particularly important to accurately measure the threshold and dynamic range of responses to sound in these mutants. To this end, we have developed a novel technique for measuring the activity of *Drosophila* primary auditory neurons. We find that Drosophila have a higher sound threshold and smaller dynamic range than human, though sound transduction occurs with impressive speed. We plan to use this technique, together with sensitive measurements of sound-induced vibrations, to understand the role of particular ion channel proteins in the transduction of sound.

Global mechanisms of gene transcription

Patrick Cramer
LMU, Gene Center

Over the last years, structural biology and functional studies culminated in a movie of RNA polymerase II in the course of mRNA elongation during gene transcription. However, the mechanisms of transcription initiation at promoter DNA and its regulation remain poorly understood. We have recently reported the structure of RNA polymerase II in complex with the central initiation factor TFIIB and models for the closed and open promoter complexes (Nature 19 November 2009). Structure-guided functional studies showed that parts of TFIIB are required for DNA opening and transcription start site selection. In the future, we will combine structural biology with a systemic analysis in vivo, to work towards an understanding of transcriptional gene regulation in eukaryotic cells. We map polymerase over the genome at high resolution, measure mRNA transcription and decay rates on a systemic level, and integrate the data with computational biology tools.

SUNDAY 25 JULY TALKS

Rolf Huisgen and Pericyclic Reactions in Biomimetic Synthesis

Dirk Trauner
LMU, Department of Chemistry and Biochemistry

Rolf Huisgen, whose 90th birthday we celebrated in June, embodies the extraordinary development organic chemistry has undergone since the middle of the last century – and the endurance of this field as a fascinating intellectual playground. His ability to conceptualize mechanisms has led to the development of numerous novel reactions, which have found applications in virtually all fields of chemistry. As such, Rolf Huisgen has done much to endow synthetic chemistry with the power and status it enjoys today.

Although his name is mostly associated with 1,3-dipolar cycloadditions, Rolf Huisgen has made many other contributions to chemical reactivity in the course of his long career. After a brief foray into natural product chemistry during his graduate work, he decided to dedicate himself to topics that seemed more rational and rewarding at the time and began his career with investigations on diazo compounds. This was quickly followed by studies on arynes, medium-sized ring effects, electrophilic azo compounds, azomethine imines and finally, his first papers on 1,3-dipolar cycloadditions as concerted reactions. In his seminal 1963 review of these reactions,[1] Rolf Huisgen achieved what every scientist dreams of: "To see what everybody else has seen but to think what nobody has thought" (Szent-Györgyi). His brilliant conceptualization enabled extensive investigations that have significantly broadened the landscape of this class of reactions now so easily interpreted. In the course of his systematic studies that spanned several decades, more than a dozen new types of 1,3-dipoles were designed and synthesized. These can give access to an almost unlimited variety of five-membered heterocycles, many of which are highly important in biology and medicine.

Rolf Huisgen's work on concerted reactions, however, has gone far beyond 1,3-dipolar cycloadditions. His studies on the mechanism of [2+2] cycloadditions involving ketenes are remarkable for their thoroughness – and his willingness to consider non-concerted pathways. His investigations on the stereochemistry of electrocyclic ring closures demonstrated the predictive power of the Woodward Hoffmann rules and have contributed much to the immediate and enthusiastic recognition of these. I will discuss how Huisgen's discoveries and opinions have influenced our own work on the total synthesis of complex natural products. In particular, I will talk about the occurrence and significance of 1,3-dipolar cycloadditions, electrocyclizations, and Diels-Alder reactions in biosynthesis. Several total syntheses will be presented (e.g. of pycnanthuquinone C, rubioncolin B, variecolortide A, and loline), and the use of 1,3-dipolar cycloadditions (i.e. "click-chemistry") in chemical neurobiology will be discussed briefly.

The Structure of O-GlcNAc Transferase and Its Complex with a Peptide Substrate

Michael Lazarus
Harvard Universty, Department of Chemistry and Chemical Biology

O-GlcNAcylation is a unique mammalian post-translational modification that affects a wide variety of cytoplasmic and nuclear proteins including kinases, tumor suppressors, transcription factors, and histone-modifying proteins.

O-GlcNAcylation is a dynamic modification similar to phosphorylation. Unlike phosphorylation, it is added by a single enzyme, O-GlcNAc Transferase (OGT).

OGT has been implicated in a number of diseases including diabetes, cancer, and neurodegenerative diseases such as Alzheimer's. Despite the importance of OGT, very little is known about the enzyme including how it recognizes substrates.

Here we have determined the crystal structure of human OGT both as a binary complex with UDP and as a ternary complex with UDP and a peptide substrate.

These structures provide insight onto the kinetic and catalytic mechanism of OGT, provide information about how OGT binds peptide targets, and will assist in the development of inhibitors that may have therapeutic value.

Modular design of FRET-based calcium indicators

Anselm Geiger
MPI of Neurobiology, Department of Cellular Dynamics

Genetically encoded calcium indicators (GECIs) are valuable tools for reporting neuronal activity and offer crucial advantages over organic dyes such as targeting specific cell populations, chronic *in vivo* imaging, and the ability to simultaneously monitor large numbers of cells. However, neuronal information coding ranges from sparse spiking, inducing calcium transients with very low concentrations, to fast trains of action potentials resulting in small changes at high calcium levels. Therefore, the demand on the indicator system is very complex and requires both flexible and high-performance indicator properties. Our group developed the FRET-based indicator TN-XXL (Mank *et al.*, Nat. Methods 2008) based on Troponin C as the calcium sensing domain flanked by ECFP as donor and a circular permutated variant of mCitrine as acceptor.

In this talk, I will analyze the modular design of TN-XXL and show how the different components can shape the overall performance of a FRET sensor. The performance of every single component and their interplay can be analyzed by biophysical methods. Having this knowledge at hand, protein engineering by rational design approaches as well as directed evolution can be used to enhance and fine-tune the key properties for the next generation of Troponin C-based calcium indicators.

Identification of the biosynthetic gene cluster for the pacidamycin group of peptidyl nucleoside antibiotics

Wenjun Zhang
Harvard University, Department of Biological Chemistry and Molecular Pharmacology

Pacidamycins are a family of uridyl tetra/pentapeptide antibiotics that act on the translocase MraY to block bacterial cell wall assembly. To elucidate the biosynthetic logic of pacidamcyins a putative gene cluster was identified by 454 shotgun genome sequencing of the producer *Streptomyces coeruleorubidus* NRRL 18370. The 31-kb gene cluster encodes 22 proteins (PacA-V), including highly dissociated nonribosomal peptide synthetase (NRPS) modules and a variety of tailoring enzymes. Gene deletions confirmed that two NRPSs, PacP and PacO, are required for the biosynthesis of pacidamycins. Heterologous expression and in vitro assays of PacL, PacO and PacP established reversible formation of *m*-Tyr-AMP, L-Ala-AMP, and diaminopropionyl-AMP respectively, consistent with the amino acids

found in pacidamycin scaffolds. The unusual Ala4-Phe5 dipeptidyl ureido linkage was formed during *in vitro* assays containing purified PacL, PacJ, PacN and PacO. Both the genetic and enzymatic studies validate identification of the biosynthetic genes for this subclass of uridyl peptide antibiotics and provide basis for future mechanistic study of their biosynthesis.

Photochromic Ligands - New Tools for Neuroscience

Philipp Stawski
LMU, Department of Chemistry and Biochemistry

Optical methods have greatly changed the way electrophysiologist conduct their experiments. By using molecular reporters, which change their fluorescence upon binding of calcium for example, today signals may be read in a lot less invasive manner than puncturing a cell. Moreover, there are more and more tools emerging, that allow to control the fundamental processes involved in neurological activity, i.e. action potentials.

These new neurochemical tools which take advantage of the spacial and temporal resolution of light can mainly be divided into three classes: the light-sensitive proteins like channelrhodopsin and halorhodopsin, caged molecules, and synthetic small photo-switchable molecules which can either act as agonists or blockers. [1]

While the impact of the first two on the neurophysiological community is indisputable, we focus our work on the relatively young class of molecular photoswitches. Although the general ideas were already developed by Erlanger *et al*. in 1972, [2] it took more than 30 years until a small photoswitchable molecule was actually used to control firing of action potentials. [3] Today, two types of these molecules can be identified: photochromic tethered ligands (PTLs, Fig. 1a) and photochromic ligands (PCLs, Fig. 1b). While PTLs require additional genetic engineering for targeting, the latter solemnly rely on substrate-inherent specificity. These approaches have proven especially useful, since they in principle allow to turn any ligand-gated channel into a light-sensitive channel and hence control the electrophysiological properties of a cell up to action potentials.

We here present our past and recent work on PCLs to control ligand gated ion channels such as glutamate receptors.

Figures:

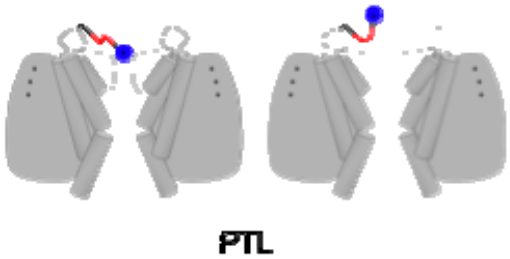

Fig. 1a

Fig. 1b

Literature:

[1] R. H. Kramer, D. L. Fortin, D. Trauner, *Curr. Opin. Neurobiol.* 2009, *19,* 1-9.
[2] E. Bartels, N. H. Wassermann, B. F. Erlanger, *Proc. Natl. Acad. Sci. USA* 1971, *68*, 1820-1823.
[3] M. Banghart, K. Borges, E. Isacoff, D. Trauner, R. H. Kramer, *Nat. Neurosci*. 2004, *7*, 1381-1386.

Sound localization in mammals: new mechanisms - unexpected dynamics

Benedikt Grothe
LMU, Division of Neurobiology

The ability to determine the location of a sound source is fundamental to hearing. However, auditory space is not represented in any systematic manner on the basilar membrane of the cochlea, the sensory surface of the receptor organ for hearing. In order to create a spatial representation of sound, information coming from both ears has to be processed with sub-millisecond precision. Recent studies changed our view of mammalian binaural processing and challenge the "text-book" version of sound localization. Moreover, they revealed unexpected dynamics already at the first stage of binaural processing allowing the system to rapidly adapt to changes in the acoustic environment.

MONDAY 26 JULY TALKS

Natural products and their biological targets

Stephan Sieber, Thomas Böttcher, Isabell Staub
Technical University München/LMU

After decades of successful treatment of bacterial infections with antibiotics, formerly treatable bacteria have developed drug resistance and consequently pose a major threat to public health. Since many antibiotics in clinical development and application still target only a limited set of cellular functions such as cell wall, DNA and protein synthesis, it is a desirable goal to expand the number and breadth of therapeutic targets combined with a deeper knowledge about their mechanism. To address this goal, we applied a chemical proteomic strategy termed activity-based protein profiling (ABPP)[1, 2] that is designed to globally profile enzyme activities in complex proteomes. To identify novel targets for the treatment of multidrug resistant *S. aureus* (MRSA) strains, we utilized small natural product derived biomimetic β-lactone[3, 4], β-lactam[5] and cinnamic aldehyde[6] molecules which were modified with a small tag for the visualization and identification of dedicated targets in complex proteomes by SDS-gel-electrophoresis and mass spectrometry (Figure 1). Structural variations in side chains of selected molecules led to an increased affinity for certain enzymes that played crucial roles in resistance and virulence.[7, 8] The general utility of this approach was demonstrated by the chemical inhibition of a central *S. aureus* virulence regulator that resulted in a drastically decreased expression of major virulence factors which are key players in e.g. sepsis, tissue necrosis, inflammation and toxic shock.[9] Since this virulence regulator is highly conserved in many pathogens, our strategy could represent a global approach for the treatment of infectious diseases by disarming the bacterial virulence repertoire. Disarmed pathogens could then be easily eliminated by the human immune response. A drug based on this concept displays many advantages over conventional antibiotics, such as preserving the useful, cooperating microorganisms e.g. in the digestive tract, and exerting less selective pressure on pathogens, which may result in decreased resistance development.

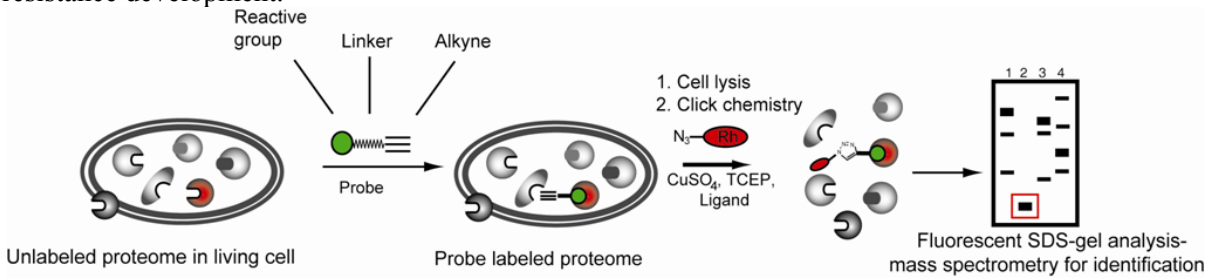

Figure 1: Identification of cellular targets of small molecules.

Literature:

[1] M. J. Evans, B. F. Cravatt, *Chem Rev* 2006, *106*, 3279.
[2] S. A. Sieber, B. F. Cravatt, *Chem Commun (Camb)* 2006, 2311.
[3] T. Böttcher, S. A. Sieber, *Angew Chem Int Ed Engl* 2008, *47*, 4600.
[4] T. Böttcher, S. A. Sieber, *J Am Chem Soc* 2008, *130*, 14400.
[5] I. Staub, S. A. Sieber, *J Am Chem Soc* 2008, *130*, 13400.
[6] M. Pitscheider, S. A. Sieber, *Chem Commun (Camb)* 2009, 3741.
[7] I. Staub, S. A. Sieber, *J Am Chem Soc* 2009, *131*, 6271.
[8] T. Böttcher, S. A. Sieber, *ChemMedChem* 2009, *4*, 1260.
[9] T. Böttcher, S. A. Sieber, *Chembiochem* 2009, *10*, 663.

Small Molecule Kinase Inhibitors Provide Insight into Mps1 Cell Cycle Function

Nicholas Kwiatkowski
Harvard University, Department of Biological Chemistry and Molecular Pharmacology

Mps1, a dual-specificity kinase, is required for the proper functioning of the spindle assembly checkpoint and the maintenance of chromosomal stability. As Mps1 function has been implicated in numerous phases of the cell cycle, it is expected the development of a potent, selective small molecule inhibitor of Mps1 would greatly facilitate dissection of Mps1-related biology. We describe the cellular effects and Mps1 co-crystal structures of novel, selective small molecule inhibitors of Mps1. Consistent with RNAi studies, chemical inhibition of Mps1 leads to defects in Mad1 and Mad2 establishment at unattached kinetochores, decreased Aurora B kinase activity, premature mitotic exit, and gross aneuploidy, without any evidence of centrosome duplication defects. However, in U2OS cells possessing extra centrosomes, an abnormality found in some cancers, Mps1 inhibition increases the frequency of multipolar mitoses. Lastly, Mps1 inhibitor treatment resulted in a decrease in cancer cell viability.

Intracellular virulence of *L. monocytogenes* is abolished by dual inhibition of two copies of ClpP with a vibralactone derived probe

Evelyn Zeiler, Thomas Böttcher, Stephan Sieber
Technical University München/LMU

The bacterial pathogen *Listeria monocytogenes* uses an intracellular mode of infection which makes its treatment with antibiotics particularly challenging. One major obstacle is that the intracellular environment protects these bacteria from the immune response and reduces exposure to antibiotic drugs. In previous experiments we introduced a novel approach to attenuate bacterial virulence by inhibition of the central virulence regulator protease ClpP via monocyclic beta-lactones. The most powerful compounds led to a dramatic decrease in the expression of major virulence factors in *S. aureus* and its multi-resistant strains (MRSA). Although we also observed inhibition of ClpP and a corresponding reduction in LLO and phospholipase C expression in *L. monocytogenes*, its intracellular replication within macrophages was only reduced by 20 % upon lactone treatment even at high concentrations of 1 mM. While our initial β-lactone library was inspired by naturally occurring monocyclic compounds we intended to expand the scope of natural derived lactones to more complex systems which we expected to exhibit altered reactivity profiles, e.g. due to constrained bicyclic ring systems. The natural product vibralactone represents such a desired scaffold and was reported to be a lipase inhibitor. However, a detailed analysis of the dedicated targets and mode of action remained elusive so far. To investigate whether bicyclic vs. monocyclic beta-lactones represent complementary privileged structures for the inhibition of bacterial pathogenesis, we utilized the natural product vibralactone as a bioactive chemical probe to unravel its targets and mechanism of action.

While the monocyclic β-lactones showed only one major labeling event in the whole proteome of *L. monocytogenes* which was identified as ClpP, a molecular probe derived from vibralactone surprisingly labeled a second copy of ClpP. This probe finally led to an almost complete inhibition of virulence in a macrophage infection model. In contrast to *S. aureus*, there are obviously two versions of ClpP involved in the virulence of *L. monocytogenes*, which may have complementary functions or even create mixed complex assemblies as proposed previously.

Targeting liver stage malaria

Emily Derbyshire
Harvard University, Department of Biological Chemistry and Molecular Pharmacology

Parasite resistance has eroded the efficacy of all currently used antimalarial drugs, and the disease's impact – 300-500 million cases per year – continues to grow. All existing drugs target a limited range of processes in the parasite's blood stage and in an effort to develop dramatically different therapies we have developed high-throughput assays for the parasite's liver stage. The talk covers the parasite's life cycle, with special emphasis on the desirability of targeting the liver stage, and the technical issues involved in developing high-throughput screens to quantitatively assess both traversal and infection of human liver cells using a luciferase reporter strain of *Plasmodium berghei*. The traversal screen will discover compounds that inhibit the parasite's migration through liver cells, and the growth assay will discover compounds that block the parasite's growth in its host cell.

Proteolytic processing of the Alzheimer Amyloid Precursor Protein

Peer-Hendrik Kuhn
LMU, Adolf-Butenandt-Institut

Alzheimer's disease is the most frequent neurodegenerative disorder in the world. Patients suffer of a progressive cognitive decline which first of all affects short term memory. Specimens of post mortem brain tissue of these patients show amyloid plaques, extracellular protein aggregates of the hydrophobic and aggregation prone amyloid β peptide (Aβ), as well as intracellular tangles of the tau protein. A sequential processing of the amyloid precursor protein (APP) by the aspartyl protease BACE1 and the gamma-secretase complex liberates Aβ which rapidly aggregates due to its hydrophobic nature. Intermediary forms of these aggregates, like Aβ oligomers are considered to be neurotoxic. On the other hand APP is processed by a metalloprotease activity called α-secretase which cleaves within the amyloid β domain of APP and thereby precludes the generation of Aβ. The identity of α-secretase so far remained elusive. As α-secretase upregulation is considered as a target for AD treatment it is necessary to identify the protease involved in APP α-cleavage and understand its regulation. Therefore we investigated the role of different ADAM proteases in APP processing in primary cortical neurons and cell lines. Finally we could show that only ADAM10 is required for α-cleavage of APP.

Activity-dependent phosphorylation of MeCP2 regulates synapse development and behavior

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Postnatal neurodevelopmental disorders such as autism and Rett syndrome have been suggested to result from disruption of experience-dependent processes including dendritic growth and synaptic maturation. The effects of experience on the developing nervous system are mediated in part by transcriptional programs that regulate the expression of genes involved in these processes of neuronal development. Among the transcriptional regulators that respond to neuronal activity is MeCP2, a chromatin-binding protein that is mutated in Rett syndrome and phosphorylated in response to neuronal activation. To address the hypothesis that the regulation of MeCP2 by neuronal activity is required for proper development of the nervous system and is therefore relevant to the etiology of Rett syndrome, we generated a knockin mouse in which mutation of MeCP2 Serine 421 to alanine prevents activity-dependent phosphorylation at this site without disrupting MeCP2 protein levels in the brain. S421A

knockin mice display defects in dendritic arborization, synaptic development and cognitive function in the absence of other RTT-like phenotypes such as motor dysfunction and early mortality. These findings suggest that postnatal brain development depends on the ability of neuronal chromatin to respond to experience-driven neuronal activity, and that disruption of activity-induced MeCP2 phosphorylation-dependent brain maturation contributes to the pathogenesis of Rett syndrome and related disorders.

Continuous live imaging of adult neural stem cell division and lineage progression in vitro

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Still very little is known about the mode of cell division and lineage progression of individual adult neural stem cells independent of their local niche. Here we describe the first direct observation of stem cell divisions and their subsequent stereotypic lineage trees of cells isolated from the adult subependymal zone in a culture system preserving the neurogenic fate. Stem cells, identified by their astro/radial glial identity and their slow-dividing nature, were observed to generate asymmetrically dividing cells that maintained an astro/radial glia identity. These in turn gave rise to symmetrically fast-dividing cells that lost the glial hallmarks, but had not yet acquired neuronal features. The number of these amplifying divisions was limited to maximal five with each progenitor decreasing its soma size before generating neuroblasts. This highly stereotypic lineage progression was observed in single cells in the absence of mitogenic growth factors indicating that it is to large extent driven by cell intrinsic mechanisms.

Chromatin and sleep: insight from zebrafish

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My lab is interested in the mechanisms that underlie two very disparate subjects: embryogenesis and sleep. We mainly use zebrafish as a model system, because genetic and imaging approaches can be combined to study complex behaviors and developmental processes in a vertebrate. In our embryological studies, we wish to determine how signaling molecules, microRNAs and chromatin regulate the transition from a pluripotent to a specified cell fate. I will present recent studies that suggest a role for chromatin modifications in this process. In our sleep studies, we wish to define molecular and neural pathways that regulate wakefulness and sleep. I will discuss a small molecule screen that defines pathways regulating locomotion and suggests that zebrafish can be used for the behavioral profiling of drugs.

POSTER ABSTRACTS

1. Natural Products and their Biological Targets

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With the emergence of multi-drug resistant bacterial pathogens, infectious diseases pose once again a major threat to human health. We use activity-based protein profiling to identify novel targets in pathogenic bacteria and develop novel lead structures from the repertoire of natural products and derived privileged structures. Thereby we discovered β-lactones as potent inhibitors of the central virulence regulator ClpP, which initiates the expression of virulence factors when the pathogens decide to attack the human host. A chemical knockout of ClpP by the lactones in *Staphylococcus aureus* allowed to abolish the production of important toxins and enzymes which made the bacteria to switch to non-infective in a mouse skin abscess model. As bacteria are disarmed but not killed there is no direct selective pressure that triggers the development of resistances. This successful strategy is now applied to new pathogens, and a novel natural product derived β-lactone also effectively reduces virulence of the intracellular pathogen *Listeria monocytogenes*.

2. The development of a motor program

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We usually don't think much about the complex and exquisitely timed movement patterns necessary to produce even a mundane "Good morning, how are you?" But how do our brains learn the motor sequences that form the very foundation for speech, piano playing or dancing? While elucidating experiments are daunting (if not impossible) in humans, much progress has been made using the songbird as a model system. Both birds and humans acquire their vocalizations, essentially motor sequences, in a similar manner and their respective motor circuits are homologous. In songbirds, the sequence of motor actions resulting in a song is first encoded in a high order premotor area (HVC) and then passed on to a motor cortex equivalent (RA). Current theoretical models posit that the representation in HVC is self-organized early in song development, and is stable during the motor learning phase. In this view the adaptive changes in the motor pathway occur in RA through a process of synapse strengthening and pruning of incoming HVC inputs.

Here I present preliminary results from in-vivo longitudinal HVC recordings in the developing Zebra Finch, a songbird. In both juveniles and adults, the firing patterns of single HVC neurons are temporally aligned to the song, as expected for a premotor area. I analyzed the firing rate corresponding to repeated, well-defined song segments, and aligned and linearly warped them to best match each other. On average, the firing patterns produced by the adult HVC necessitate only half the temporal warping required to align the corresponding patterns in juveniles. Having found the optimal warping, I investigated the finer-scale temporal precision in the premotor output by comparing the pairwise correlation of all firing patterns corresponding to the same song element. Interestingly, there is almost no difference between adults and juveniles, even though there is a significant difference when repeating the same operation on the song itself. In other words, while the song becomes significantly more stereotyped during learning, the fine-scale HVC output seems to stay stereotyped throughout. These findings provide the first experimental

support for the currently accepted motor learning theory, while also pointing out a hitherto unknown, developmentally regulated source of premotor temporal noise.

3. Synaptic location, strength and plasticity in the medial superior olive

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Localization of low-frequency sounds in the horizontal plane in mammals involves a highly precise coincidence detection mechanism recruiting fast excitatory and inhibitory inputs to the medial superior olive (MSO). The sub-cellular localization, strength and short-term dynamics of these inputs to individual MSO neurons are vital to maintaining this precision. Excitation from both cochlear nuclei is thought to be targeted to the dendrites and soma, whereas the inhibition from the medial nucleus of the trapezoid body (MNTB) is apparently to be restricted to the soma. We mapped the receptor distribution, the synaptic input sites and synaptic strength and short-term dynamics to MSO neurons to define the basal synaptic properties that shape this fastest of all coincidence detector circuits.

We used immunohistochemistry, whole-cell voltage-clamp recordings combined with fiber stimulations and/or with UV-uncaging/puffing to determine the map, strength, and dynamics of synaptic inputs and the sub-cellular localization of NMDA, AMPA, GABA-A Gly-receptor (R) All experiments were completed on acute slices from mature Mongolian Gerbils.

We find that GlyRs and NMDARs are biased to the soma. GABA-ARs appear expressed uniformly across the MSO cell membrane, though a lack of synaptic input suggests a possible role for GABA as a volume transmitter. AMPARs are also uniformly expressed along the cell extent. Local synaptic stimulation supports the location of functional GlyR and AMPAR synapses determined by the receptor distributions. The synaptic strength, expressed as charge/fiber is similar for these glycinergic and glutamatergic inputs. During stimulus trains both input types depress equally for frequencies between 0.5 and 300 Hz. The recovery form depression follows also a same time course for glycinergic and glutamatergic inputs. In addition we find that about 2-4 glutamatergic inputs are required to generate an action potential and 2-4 inhibitory fibers provided by the MNTB contact a given MSO neuron. Taken together the basis for the fastest coincidence detection in the mammalian brain appears to be well placed, largely segregated glycinergic and glutamatergic synaptic input sites with balanced strength and short-term dynamics.

4. Slow adaptation currents contribute to spike-response variability in a sensory neuron

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The trial-to-trial variability of neuronal response patterns is a prominent feature of sensory systems and has a profound impact on subsequent sensory signal processing. Fluctuations of the underlying ionic currents represent a major intrinsic noise source that causes neuronal response variability. To characterize these intrinsic stochastic properties of a neuron, direct somatic recordings are well suited. In many systems, however, such recordings are difficult to achieve without severely damaging the sensory transduction machinery. In this study, we therefore introduce an indirect approach to assess the stochastic dynamics of sensory neurons based on interspike interval statistics of the spike train responses.

Spike responses of receptor cells were recorded intracellularly from auditory nerve fibres of Locusta migratoria during simultaneous acoustic stimulation with pure tones of various intensities. The measured interspike intervals (ISIs) show high variability with CVs up to 0.8 depending on sound intensity. With increasing spike frequency the shape of the ISI histograms changes from an inverse Gaussian to a peaked probability density. Additionally, the ISI correlations exhibit a shift from slightly negative values to positive coefficients with increasing spike rate.

By means of simulations of single-compartment conductance-based models we tested different assumptions of possible noise sources which could account for the observed transitions of the ISI histogram shape and correlation.

Simulations of individual channel noise sources revealed inverse Gaussian ISI distributions for fast ion channel fluctuations, like from the mechanosensory receptor channels, while simulations of the slowly changing ion channels mediating spike-frequency adaptation resulted in peaked probability densities. Mixed cases with both fast fluctuations and adaptation channel noise showed a smooth transition between the two limit cases which is in aggreement with our findings from the locust receptor cell responses. This indicates that higher-order statistics can be used to distinguish different kinds of noise sources.

5. Chemical Biology in the Trauner Group

Philipp Stawski, Daniel Hog
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Proteins such as ion channels are sophisticated molecular machines, which are amenable to functional manipulation. Fortunately, structural details of these molecules are available today on atomic resolution and they guide us in our attempt to endow them with new functionalities. This expansion, however, not only requires detailed information on the architecture of these machines, it also demands a sophisticated way of synthesizing the artificial extensions. With powerful chemical methods at our hand, the chemical biology in our group focuses on designing and creating small molecules, which add photosensitivity to endogenous ion channels. Thus, we generate hybrid construct, which can be used to study and dissect neuronal networks. Moreover, we seek to understand the mechanisms by which these molecular photoswitches work by co-crystallizing them with the respective proteins and hence obtain a glimpse of the underlying atomic processes to help us better design future experiments.

6. Activating Toll-like receptors 7 and 9 for the immunotherapy of cancer

Christian Hotz
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Pattern-recognition receptors are constituents of the innate immune system which sense conserved molecular patterns from pathogens and trigger immune responses. Of particular interest in our group are nucleic acid-sensing receptors belonging to the Toll-like receptor (TLR) family of proteins. Using synthetic oligonucleotides designed to activate these receptors, we can initiate a strong immune stimulation that can be targeted for the therapy of cancer. We characterized the in vivo efficacy of TLR7-activating RNA oligonucleotides on two major cell subtypes for antitumor responses, CD8 T cells and NK cells. We also showed that RNA oligonucleotides abolish the suppressive function of regulatory T cells and myeloid-derived suppressor cells, a further mechanism by which these nucleic acids may activate antitumoral immunity. In order to circumvent the induction of tolerance by repeated TLR stimulation, we have developed treatment protocols based on sequential stimulation of different pattern-

recognition receptors or by choosing rational intervals between individual stimulations. In addition, we have recently developed RNA oligonucleotides that lead to regression of established tumors by simultaneously activating three antitumoral mechanisms: activation of innate immunity through both the RIG-I receptor and the Toll-like receptor 7, and intratumoral silencing by RNA interference of an antiapoptotic gene. We thus have a new class of biologicals that may be used to manipulate the immune system for therapeutic benefit.

7. A possible priming mechanism for synaptic structural plasticity involved in Drosophila Long-Term Memory

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Synaptic mRNA localization and protein synthesis are involved in the induction of long-term memory in Drosophila. MicroRNAs (miRNAs) have been suggested to act as local regulators of new protein synthesis involved in synaptic plasticity. We have observed that neural activity controls the biogenesis of miRNAs that have specific targets in neurons, including mRNAs encoding proteins with roles in dendritic structure formation and synaptic function. Our work has focused on let-7-C, a polycistronic miRNA cluster known for its regulatory role during development. These miRNAs are expressed in the Mushroom Body, and are strongly induced by the dopamine signaling pathway that conveys the aversive electric shock stimulus in an odor, electric shock associative conditioning paradigm. We find that the induced miRNAs let-7-C reduce the expression of the protein Abrupt, a developmental regulator of dendrite arbor branching, in a subset of Mushroom Body neurons. Our current effort is focused on determining the role of the regulation of Abrupt by let-7-C in Mushroom Body morphology and in long-term memory formation, maintenance and retrieval. Together, our observations suggest a new pathway by which dopaminergic output modulates miRNA expression in the Mushroom Body of the fly brain to prime synaptic circuitry for change.

8. An exact statistical analysis of visual inference by a neural population amid eye movements

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Sensory information is generally corrupted by neural noise, but also by confounding signals. For example, research on high-acuity visual perception has focused on the limits imposed by variability of retinal spike responses. However, a second limit results from random eye movements during fixation. We study the effects of both sources of variability on Vernier hyperacuity in the estimation of a gap between two parallel bars presented simultaneously in the visual field. Introducing a simple model of neural variability and the statistics of eye movement, we exactly derive the optimal estimator of the gap and its performance and compare it with psychophysical data. A main assumption of our model is that the brain estimates both the eye position and the gap on the exclusive basis of retinal ganglion cell spike trains. We calculate the exact joint probability distribution for the eye position and the gap in our model and derive the optimal Bayesian estimator. The optimal strategy depends on one dimensionless parameter: the root mean squared displacement of the eyes between subsequent spikes in any two ganglion cells, divided by the width of a ganglion cell's receptive field. For slow eye movements, the optimal decoder uses all the spikes to estimate the position of each bar and their separation. For fast eye movements, the decoder uses only near-synchronous spikes arising from each of the bars. Such spikes provide snapshots of the visual stimulus during brief temporal windows, in which blurring due to eye movements is minimal. Nearly synchronous spikes occur naturally in a population of independent neurons described by Poisson

statistics, and they could be enhanced by mechanisms that coordinate spike times among multiple neurons. The optimal estimator bounds the performance of biological neural systems on this task. We also construct simpler estimating schemes that could be implemented by neural circuits of the visual system and analyze their suboptimal performance. By incorporating temporal filtering in the process of spike generation our model explains the psychophysical phenomena of Bloch's law, relating the Vernier threshold to stimulus duration and contrast. Our work provides insight into the fundamental limitation imposed on the visual system by fixational eye movements and suggests how neuronal circuits downstream of the retina may cope with this challenge.

9. Function of dendritic inhibitory synapses in the hippocampal CA1 region

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Inhibition is shaping and controlling neuronal excitation, providing negative feedback and generating some of the brain's rhythmic oscillations. Inhibitory synapses arriving at the soma regulate information transmission by controlling the generation of action potentials while inhibitory synapses onto small dendritic compartments shape dendritic responses to synaptic inputs. Although theoretical predictions exist on the contribution of individual inhibitory synapses to dendritic processing, experimental evidence is still sparse. This study addresses the function of dendritic inhibitory synapses in the CA1 region of the hippocampus. We established an experimental configuration which allows the specific stimulation of individual excitatory and inhibitory synaptic sites on the dendrites of pyramidal cells. Our experimental setup combines three different techniques: 1) two-photon imaging allowing for the identification of putative synaptic connections between inhibitory interneurons and pyramidal cells and for the monitoring of structural changes, 2) two-photon uncaging of glutamate, which is used to evoke excitatory synaptic responses in individual spines of a postsynaptic pyramidal cell, and 3) patch-clamp electrophysiology to stimulate the presynaptic interneuron and monitor functional postsynaptic parameters. We are using organotypic slice cultures of the hippocampus from GAD65-GFP mice, in which a subset of dendrite-targeting interneurons expresses GFP (López-Bendito, Cereb Cortex 2004). By means of the above described configuration, we are starting to explore the local interactions between excitatory and inhibitory synaptic inputs in dendrites of pyramidal cells. We are particularly interested in the question whether (and if so, under which conditions) an inhibitory synapse can veto plasticity of nearby excitatory synapses – a notion with profound implications for our understanding of hippocampal and cortical circuitry.

10. Peptidomics of prolyl endopeptidase in the central nervous system

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Prolyl endopeptidase (Prep) is amember of the prolyl peptidase family and is of interest because of its unique biochemistry and connections to cognitive function. Using an unbiased mass spectrometry (MS)-based peptidomics platform, we identified Prep-regulated peptides in the central nervous system (CNS) of mice by measuring changes in the peptidome as a function of Prep activity. This approach was validated by the identification of known Prep substrates, such as the neuropeptide substance P and thymosin-β4, the precursor to the bioactive peptide Ac-SDKP. In addition to these known substrates, we also discovered that Prep regulates many additional peptides, including additional bioactive peptides and proline rich peptides (PRPs). Biochemical experiments confirmed that some of these Prep-regulated peptides are

indeed substrates of the enzyme. Moreover, these experiments also supported the known preference of Prep for shorter peptides while revealing a previously unknown cleavage site specificity of Prep when processing certain multi-prolinecontaining peptides, including PRPs. The discovery of Prep-regulated peptides implicates Prep in new biological pathways and provides insights into the biochemistry of this enzyme.

11. Cinnamic aldehyde derived probes for the active site labelling of pathogenesis associated enzymes

Max Pitscheider
Technical University München/LMU

Michael acceptor based natural product derived probes are selective and sensitive chemical tools for the identification and characterization of pathologically relevant enzymes in MRSA.

12. The organization and property of sound and wind sensitive neurons in the Johnston's organ of *Drosophila melanogaster*

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The fruitfly *Drosophila melanogaster* responds to sound, wind, and gravity using a pair of sensory organs called Johnston's organ (JO) located in the antennae. There are approximately 480 JO neurons in each JO, and the cell bodies are organized in the ring-like arrays. There are five classes of JO neurons, which project to five distinct regions of the antennal and mechanosensory motor center of the central brain, called zones A, B, C, D and E. Using *in-vivo* calcium response imaging and behavioral analysis, we have shown that zone A and B neurons are sensitive to sound, while zone C and E neurons are sensitive to wind. Importantly, sound and wind sensitive neurons have distinct intrinsic response properties and are activated by different arista movements: The sound sensitive neurons are phasically activated by vibration of aristae, and wind sensitive neurons are tonically activated by unidirectional static deflection of aristae. Two types of wind sensitive neurons, C and E neurons, are sensitive to wind blowing from the front and the rear respectively. Interestingly, when E neurons are activated by wind from the front, C neurons are slightly inhibited. Conversely, when C neurons are activated by wind from the rear, E neurons are slightly inhibited. Our data suggest that this antagonistic activation patterns between C and E neurons are partly due to how cell bodies of these neurons are organized in the JO. Using photo-activatable GFP, we show that the cell bodies of C and E neurons are located at the opposite end of the cell bodies' arrays in the JO. Further analysis of the relative location of cell bodies of sound and wind sensitive neurons suggests that JO neurons are tonotopically organized. This tonotopic organization of JO neurons might also explain why two classes of sound sensitive neurons show different frequency tuning.