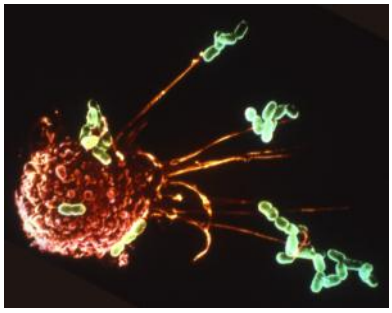
Downscaling analysis: 4th Münster Conference on Single Cell Analysis; November 26-27, 2007

Organizers: Rita Naskar, Ilka Romann, Björn Teichert and Simone König
Interdisciplinary Center for Clinical Research, Core Facility Integrated Functional Genomics, Münster, Germany

Chairs: Stephan Ludwig, Berenike Maier, Dietmar Fischer, Connie Jimenez, Simone König



Introduction

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The Münster Conference Series on Single Cell Analysis brings together scientists of very different fields – physicists, physicians, biologists, chemists – with the common goal of studying events on a cellular, sub-cellular, and molecular level. The impact of mass spectrometry in that area is increasing and, therefore, the conference is monitored in this journal.

The conference started with a keynote by Stephen D. Ginsberg of New York University on single cell gene expression analysis in human post-mortem brain tissues and animal models of neurodegeneration. Modern RNA amplification methods finally allow single cell RNA analysis so that relative gene expression level comparisons across cell types under different experimental conditions and disease states become feasible. In addition to amplification techniques, however, miniaturization is a critical issue in nanoanalytics. Andrea Robitzki (University of Leipzig) talked about novel cell based micro-sensors used as a (i) cardiomyocyte biosensor, (ii) mamma-carcinoma chip, and (iii) an embryonic cell microarray

etc. coupled to a micro-laser manipulation platform and Petra Dittrich (ISAS Dortmund) discussed microfluidic platforms for the analysis of living cells.

Another highlight of the conference was the lecture given by Walter Schubert of Magdeburg University. He recently published the work of his group on the deciphering of the protein network in Nature and talked about the ambitious human TOPONOME project. More interesting tools for the researcher were introduced by Volker Dötsch (University of Frankfurt) discussing conformation and dynamics of proteins inside living cells by in-cell NMR spectroscopy and Jörg Enderlein (University of Tübingen) showing Two-focus Fluorescence Correlation Spectroscopy data on molecular diffusion.

Mass spectrometry is again represented well. Imaging continues to be developed and Connie Jimenez of the University of Amsterdam presented results on peptide profiling of single neurons using proteomic technology. Inductively coupled plasma mass spectrometry, on the other hand, served for the mapping of cancer biomarkers as illustrated by Jaume Seuma (University of Sheffield). A new exciting tool involving ion mobility mass spectrometry was introduced by Keith Compson of Waters Corporation. Ion mobility spectrometry adds another dimension of separation since isobaric ions can now be separated based on their differences in size, charge and shape.

The conference was again accompanied by an industry exhibition featuring the latest technology in sample preparation, microdissection and amplification. Participants came from as far as Africa and the United States which demonstrates the growing impact of the conference.

SPEAKER ABSTRACTS

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Single cell gene expression analysis in human post-mortem brain tissues and animal models of neurodegeneration

Technical and experimental advances in microaspiration techniques, RNA amplification, quantitative real-time PCR (qPCR), and microarray analysis have led to an increase in the number of studies of single cell gene expression. In particular, the central nervous system (CNS) is an ideal structure to apply single cell gene expression paradigms. Unlike an organ that is comprised of one principal cell type, the brain contains a constellation of neuronal and non-neuronal populations of cells. A goal is to sample gene expression from similar cell types within a defined region without potential contamination by expression profiles of adjacent neuronal subpopulations and non-neuronal cells. The unprecedented resolution afforded by single cell RNA analysis in combination with RNA amplification methods and microarrays allows for relative gene expression level comparisons across cell types under different experimental conditions and disease states. The ability to analyze single cells is an important distinction from global and regional assessments of mRNA expression, and can be applied to optimally prepared tissues from animal models as well as post-mortem human brain tissues. This presentation illustrates the potential power of single cell gene expression studies within the CNS in relation to neurodegenerative disorders such as Alzheimer's disease (AD).

Dementia, or progressive memory loss and cognitive decline, describes a syndrome associated with a range of diseases that progressively impair brain functions and rob the afflicted of their ability to learn, reason, make judgments, communicate and carry out daily activities. Dementia knows no social, economic, ethnic or geographical boundaries. The full extent of its impact has only begun to be appreciated. The leading cause of dementia, AD, accounts for approximately 50-60% of all cases. Increasing age is the greatest risk factor for AD. Another clinical pathological entity, termed mild cognitive impairment (MCI,) describes a population whose memory or other aspects of cognition are not normal but who do not meet the clinical diagnosis of dementia. MCI has recently been recognized and may mark the earliest stages where a person shifts from a normal aging course towards dementia and

ultimately AD. The likelihood of developing AD approximately doubles every five years after the age of 65. By age 85, the risk reaches nearly 50 percent. The duration of dementing illness may often vary from 3 to 20 years. It is imperative that we learn more about the causes of neurodegeneration that lead to AD in order to develop rational therapies that delay the onset of MCI, AD, and related dementing illnesses.

Biological substrates or mechanisms underlying the specific loss of certain types of neurons and synapses remain elusive. The overall goal of our research effort is to understand the molecular and cellular events that cause cell-type specific neuronal damage, or selective vulnerability in the brain. One experimental approach that we employ is to study a specific region of the brain called the hippocampus, a structure located in the forebrain that is involved intimately with learning and memory. Cells within the hippocampus are removed (termed microdissected) and cellular and molecular biological studies are performed to assess the cohort of genes within them that may lead to disease pathogenesis. The goal is to identify those messages or transcripts that change over time in relationship to the synaptic and cellular damage and discover a mechanism that explains why specific neurons degenerate, and whether plastic changes occur in the brain to compensate for these losses. By understanding these fundamental processes, we hope to develop a series of therapeutic strategies to prevent neurodegenerative diseases from turning into debilitating and life threatening illnesses.

A multidisciplinary approach of surgical, state-of-the-art molecular biology, immunohistochemical, and imaging techniques are utilized as part of the experimental design. Particular emphasis is placed upon analyzing single neurons *in vivo* as a means of understanding cellular events occurring locally at synaptic and somatodendritic sites. For example, post-mortem normal control, MCI, and AD tissues are combined with regional and single cell mRNA amplification techniques and microarray technology to assess several classes of transcripts simultaneously including glutamate receptors, dopamine receptors, synaptic proteins, cytoskeletal elements, neurotrophins, cell death genes, and transcription factors from individual neurons and their processes. These studies enable a “molecular fingerprint” of the hippocampus as well as specific neurons within the region. Furthermore, these studies are hypothesized to elucidate markers for early cell-specific synaptic and neurodegenerative changes that can be applied to other models of activity dependence and neurodegenerative disorders.

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Frontiers in nano- and microstructures on microarrays for real time and online bio-monitoring of cellular physiology

The demand on innovative cell analysis and/or functional genomics/proteomics is the miniaturisation of devices and the optimisation of real time monitoring for a temporal and spatial high resolution of functional proteome. This report represents an overview and introduction of novel cell based micro-sensors used as a (i) cardiomyocyte biosensor, (ii) mamma-carcinoma chip, and (iii) an embryonic cell microarray etc. coupled to a micro-laser manipulation platform. The goal of the biosensor technology is the development of high-sensitive screening systems that allows the electronic detection of drugs, tissue-secreted factors, effects of proteins on physiology, and receptor-directed cellular alterations by impedance spectroscopy and/or electrophysiological recording. For this purpose, e.g. spontaneously beating neonatal rat cardiomyocytes or human smooth muscle cells were cultured on substrate integrated microelectrodes for measuring activity by extracellular recording of field potential. Each single cell can be addressed and monitored on a single micro-electrode by bioelectronical and/or optical measurements. Therefore, the positive chronotropic effect or activity of angiotensin II and its binding to the AT_1 receptor resulting in an initiation of the signalling pathway can be detected in real time, online and with an extreme high sensitivity (at very low concentrations of 10^{-11}M). Furthermore, at the interface of micro-sensor system technology and cellular bioengineering a multiwell-multi-microelectrode array (MMEA) has been designed and developed for the screening of cellular ischemia or of cytostatica, phorbol-12-myristate-13-acetate (PMA) etc. induced cellular changes in single cells.

The biochip consists of cells as the biological target expressing target molecules for the detection of ligand / receptor interactions via impedance spectroscopy (reflecting the molecular and physiological alterations dependent on the frequency range up to 10^6 Hz by a change of the extracellular resistance). The status of a viable cell (e.g. stem cell, precursor cell etc.) e.g. its proliferation, differentiation, maturation, apoptosis *versus* necrosis or migration can be detected in real time using the described biophysical measurement

systems. For a parallel real time monitoring in viable cells the sensor platform are adapted to a 96 well plate-MMEA for a high content screening and characterisation of viable cells depending on their proteome and *toponome*.

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Microfluidic platforms for analysis of living cells and 3D lipid structures

Living cells stay in permanent contact with other cells, surfaces and solutions. The near environment strongly influences the behaviour of the cell – its viability, differentiation and metabolism. Studying these influences will provide detailed and deep insights into the underlying processes of the cellular life cycle. However, to address cells on an individual level requires tools of the same dimensions, which is perfectly accomplished by microstructured chips. In this presentation, an overview of different approaches and new developments towards cell manipulation and analysis by means of optical and microfluidic methods will be given.

Living cells are complex systems though. A versatile approach for many applications is the replacement of cells by biomimetic objects such as lipid vesicles, which are simple model systems for cells as well as cell organelles due to their similarities in size and membrane composition. The second part of the presentation focuses on methods to form, detect and analyse biomimetic objects in microfluidic chips. In particular, a novel formation pathway for three-dimensional (3D) lipid structures will be presented that combines top-down photolithography techniques and the self-assembling ability of polar lipids. This micro-extrusion process facilitates the formation of spherical, cylindrical, tubular and helical lipid membrane structures. Such 3D lipid structures are useful tools in analytical chemistry, pharmacy, and micro-/nanotechnology since they could serve as sensors, as containers for transport and delivery of sample, and as reaction vessels for conducting biochemical processes. Moreover, they could be employed as building blocks for novel biomimetic soft matter devices.

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The human TOPONOME project: Deciphering the protein network code until 2020

Understanding how proteins are temporally and spatially arranged and how this relates to function is a major post-genomic challenge. We have recently described a multi-dimensional microscopic robot technology which runs cycles of fluorescence protein tagging, imaging, and bleaching *in situ* (Schubert W *et al*. Nature Biotechnology 24, 1270-1278, 2006). It provides a major milestone in proteomics (Proteomics Research highlight "Mapping togetherness", Nature 443, Oct 12, 2006). This technology combines three advances: a technique capable of mapping hundreds of different proteins at light microscopic resolution in one tissue section or cell sample; a method for selecting the most prominent combinatorial molecular patterns by representing the resulting data as binary vectors; and a system for imaging the distribution of these protein groupings in a 'toponome map'. By analysing many cell and tissue types we have shown that this approach reveals new hierarchical properties of protein network organisation, in which the frequency distribution of different protein groupings obeys Zipf's law, and state specific lead proteins control protein network topology and function. The technology offers a rapid route to new diagnostic features and targeted therapies. A toponome initiative has been launched to establish Toponome Centres worldwide, all jointly contributing to deciphering the human toponome (the entirety of protein networks) across human tissues and cell types in health and disease within the next 12 years. The project is open for cooperation and contributions from all sides (www.Toposnomos.com).

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New insights into molecular mechanisms underlying axon regeneration through cell sorting

Following optic nerve injury in mature rats, retinal ganglion cells (RGCs) normally fail to regenerate their axons and soon undergo apoptosis. However, inducing inflammatory reactions in the eye, *e.g*., by injuring the lens, enables many axotomized RGCs to regenerate lengthy axons into the optic nerve. To study the molecular changes that underlie the transformation of mature RGCs from a degenerative state to a regenerative state *in vivo* we pre-labeled RGCs by injecting the fluorescent dye 4Di-10ASP into the superior colliculus, performed optic nerve surgery with or without lens injury, and 4 days later, dissected retinas, dissociated their cells, and isolated RGCs by fluorescence-activated cell sorting. The population of isolated RGCs revealed a purity of 95%. The extracted RNA from these sorted cells was amplified and used for microarray analysis. The mRNA profiling revealed that although axotomy alone significantly alters the expression of a large cohort of genes, switching RGCs to a regenerative state involves the differential expression of a relatively small number of additional genes. A number of the regeneration-specific genes have previously been linked to development or axon regeneration in the PNS, but most were novel. Among these was also an uncharacterized ring finger protein that we named LINA. LINA upregulation in regenerating RGCs was confirmed at the RNA and protein levels. Endogenous LINA expression was also upregulated in differentiating PC12 cells and it's over expression enhanced neurite outgrowth. A point mutation in the ring finger domain decreased neurite outgrowth in a dominant negative fashion. AAV mediated over expression of LINA in RGCs improved survival of RGCs and strongly enhanced axon regeneration into the optic nerve. On the contrary, the dominant negative form of LINA compromised the beneficial effects of intraocular inflammation. This data suggest that LINA plays an important role in axon regeneration of neurons and demonstrates that purification of adult CNS neurons is a powerful tool to achieve new insights into molecular mechanisms underlying diseases or regenerative processes.

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Single cell whole genome amplification: Unleashing a world within a cell

Analyzing genomic DNA at the level of the single cell will provide the ultimate level of variation analysis. Amplifying the picogram quantities of DNA in a single cell has been difficult. The recent advent of commercial kits for whole genome amplification have provided scientists the means to amplify the information from ~3000 cells, affording amplification that is a complete and faithful representation of the original DNA. While advancing the field of DNA variation analysis, these kits still have limitations in fields such as oncology, molecular pathology, and *in vitro* fertilization where analyzing the DNA from a single cell is the optimal choice. The GenomePlex® Single Cell Whole Genome Amplification Kit was created to address these limitations. GenomePlex is a whole genome amplification (WGA) method that allows the researcher to generate a representative amplification of genomic DNA from a single cell.

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Regulation and force generation of the DNA import motor of *Bacillus subtilis*

At high cell concentration *Bacillus subtilis* becomes competent for DNA transformation. Transformation enables the bacterium to acquire novel genetic information including antibiotic resistance traits. Intriguingly, only a fraction of a genetically identical cell population switches into the competent state, where the master regulator for competence is expressed at high level. Thus the population can be considered to divide into a fraction that is "on" (K-state) and into an "off" fraction (B-state). In the K-state cells express the proteins required for DNA import and assemble the DNA import machine.

Using a single cell assay, we have addressed the question how the fraction of competent cells is determined. We used a fluorescence reporter for the expression of the master regulator on competence genes. Our results supported the hypothesis that noise in expression of the master regulator is essential for bi-stability. Furthermore we found that cells can switch into the K-state exclusively during a very well defined "switching-window" and that by tuning the size of this window we were able to control the fraction of competent cells. Basal expression rate of the master regulator was involved in setting the size of the "switching window". Once the cells were in the K-state we characterized the dynamics of

proteins that form the DNA-import machine and characterized DNA import at the single molecule level.

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Two-Focus Fluorescence Correlation Spectroscopy: A versatile tool for precise measurements of molecular diffusion

Thermally induced translational diffusion is one of the fundamental properties exhibited by molecules within a solution. Via the Stokes-Einstein relation it is directly coupled with the hydrodynamic radius of the molecules [1]. Any change in that radius will change the associated diffusion coefficient of the molecules. Such changes occur to most biomolecules – in particular proteins, RNA and DNA – when interacting with their environment (e.g. binding of ions or other biomolecules) or performing biologically important functions (e.g. enzymatic catalysis) or reacting to changes in environmental parameters such as pH, temperature, or chemical composition (e.g. protein unfolding). Therefore, the ability to precisely measure diffusion coefficients has a large range of potential applications, for monitoring e.g. conformational changes in proteins upon ion binding or unfolding. However, many biologically relevant conformational changes are connected with rather small changes in hydrodynamic radius on the order of Angstroms (see e.g. [2]). To monitor these small changes, it is necessary to measure the diffusion coefficient with an accuracy of better than a few percent.

An elegant technique capable of measuring diffusion coefficients of fluorescent molecules at nanomolar concentrations is Fluorescence Correlation Spectroscopy (FCS) which was originally introduced by Elson, Magde and Webb in the early seventies [3]. In its original form it was invented for measuring diffusion, concentration, and chemical/biochemical interactions/reactions of fluorescent or fluorescently labelled molecules at nanomolar concentrations in solution. However, standard FCS is prone to a wide array of optical and photophysical artefacts which make precise quantitative and absolute measurements of e.g. diffusion coefficients rather difficult [4]. The main problem of standard FCS is the absence of

a reliable extrinsic length scale in the measurements, which is, however, necessary for obtaining absolute values of the diffusion coefficient.

Here, we report on our recently developed new technique of 2-focus fluorescence-correlation spectroscopy [5], allowing for measuring the hydrodynamic radius of molecules at pico- and nanomolar concentrations with sub-Angstrom precision. In 2fFCS, the problem of an extrinsic length scale is solved by generating two excitation foci with well defined distance from each other. Several applications of 2fFCS are presented, for example monitoring conformational changes of proteins upon ion binding, or monitoring protein unfolding curves upon chemical and thermal denaturation.

[1] A. Einstein *Investigations on the Theory of the Brownian Movement*, Dover, New York, 1985.
[2]. A. M. Weljie et al., *Protein Science* **12** (2003) 228-236.
[3]. D. Magde et al. *Phys. Rev. Lett.* **29** (1972) 705-8
[4] J. Enderlein et al. *ChemPhysChem.* **6** (2005) 2324-36.
[5] T. Dertinger et al. *ChemPhysChem.* **8** (2007) 433-443.

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Investigation of conformation and dynamics of proteins inside living cells by in-cell NMR spectroscopy

The non-invasive character of NMR spectroscopy allows researchers to investigate the conformation and dynamics of biological macromolecules in their natural environment, for example the cytoplasm of cells. However, many proteins and other macromolecules interact with other cellular components, which increase their rotational correlation time, leading to broad peaks or even complete disappearance of the signals. Several small proteins that in principle should tumble fast are, therefore, invisible in in-cell NMR experiments. Since studying the interaction between the protein of interest and other cellular partners is the key focus of in-cell NMR experiments we have started to develop methods that allow us to observe proteins forming complexes as well. Two different strategies seem very promising. The first one is labeling of methyl groups, in particular of methionines as well as the δ-methyl

groups of isoleucines. Their slow relaxation makes it possible to observe proteins that cannot be studied by amide proton based experiments. For even larger complexes solid state in-cell NMR experiments are the method of choice. Here the main difficulty is the labeling strategy since strong background signals are observed in these experiments.

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From mass spectrometric peptide profiling of single neurons towards imaging mass spectrometry

Neuropeptide profiling is the analysis of the neuropeptides present in a single cell or tissue extract. Mass spectrometry has proven to be a great tool to detect and identify the peptides, which provides information on the processing and post-translational modifications of each peptide. We pioneered the use of MALDI-TOF mass spectrometry for detection and differential display of peptides in single cells (J. Neurochem. 1994), and for semi-quantitative pattern analysis of peptide profiles directly in tissue biopsies (PNAS 1997). In this presentation, I will give a historical overview and present our more recent work on the dissection of PTMs and functions of neuropeptides discovered by our MALDI-TOF-MS-based single cell peptide profiling studies and the use of imaging mass spectrometry at sub-cellular resolution.

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Fine scale measurement and mapping of cancer biomarkers via laser ablation ICP mass spectrometry

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A wide range of analytical technologies have been developed for imaging applications and MS represents a unique sub-set of the 'imaging tool kit' which includes techniques such as SEM, TEM, MRI., μXRF, SR-XRF, X-ray CT, fluorescence, autoradiography etc.. Thus MS in the form of SIMS, MALDI-MS and LA-ICP-MS represents a powerful route for visualization of the spatial (2D/3D) distribution of chemical constituents in diverse sample types. Collectively the laser and ion-beam probing methodologies provide comprehensive measurement and mapping capability across the mass and length scale.

ICP-MS has been used extensively in the life sciences for trace inorganic determinations in diverse sample types. The analytical capabilities of ICP-MS are extended through the use of laser ablation (LA) which permits direct probing of element in solid samples. An opportunity for extending the scope of LA-ICP-MS, particularly in the life sciences, relates to measurement of elemental-tagged antibodies through interrogation of immunohistochemical sections. Such a strategy represents a powerful new approach for quantitative measurement and mapping of peptides and proteins in biological tissue, an activity that traditionally has been the preserve of molecular MS. The presentation will briefly review the status and scope of MS imaging and then give emphasis to bioimaging via measurement of elemental-tagged antibodies. This aspect of research will be illustrated by reference to mapping of β-amyloid peptides (and associated metal ions) in Alzheimer plaques and imaging of cancer biomarkers (HER 2, MUC 1) in breast cancer-derived immunohistochemical sections. Method development studies were designed to benchmark to optical microscopy and selection of fine laser beam (5 μm) and low line raster rate were essential for imaging at the cellular level (MUC 1) whereas wide beam diameter (>100 μm) was suited to scoring of HER 2 status. Most recent work concerning the use of SIMS for interrogation of immunohistochemical sections will also be elaborated.

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MALDI Synapt HDMS System: Advances in high-efficiency IMS/MS

MALDI is a well established ionisation technique for the analysis of large molecules by mass spectrometry. As samples are generally introduced into the mass spectrometer in the solid state, adding additional dimensions of separation, such as liquid chromatography usually requires an intermediate step, *e.g.* fraction collection. Ion mobility separation (IMS), however, can be coupled directly with MALDI MS analysis, as it provides gas phase separation of ions post ionisation. The use of ion mobility spectrometry adds another dimension of separation to the experiment, providing separation of species by their associated drift time, a factor which is dependant on ion size, charge and shape. The Triwave device used for performing the ion mobility separation contains three T-wave regions arranged in series. The Trap T-wave is used for ion storage prior to IMS, the second T-wave, maintained at a higher pressure (typically 0.5 mbar), is used to perform the mobility separation and the Transfer T-wave is used to transmit the separated packets of ions into the TOF mass analyser. Fragmentation can be induced in the Trap T-wave and Transfer T-wave, making it possible to perform a number of novel fragmentation experiments that assist in the characterisation of analytic.

We will give a detailed description of this novel MALDI mass spectrometer and present IMS MS data. For example, using this technique it is possible to isolate low abundance multiply charged peptide ions generated in a MALDI peptide mass fingerprinting experiment and generate fragment ion spectra from co-resident isobaric species.

POSTERS

New biotechnology method for localised analysis of single cells

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Cell polarisation and the localised distribution of specific proteins are hallmarks of cell migration, which drives processes such as organogenesis and cancer metastasis. We have developed a microfluidic-based system for a locally targeted manipulation of the cell using optical tweezers. The microfluidic device includes a chamber for cell growth and channels for the flow of analytes. This system can deliver chemicals to a selected area of the cell surface. Furthermore, the chemicals can include detergents digesting the defined part of the cell membrane, allowing discrimination between the leading and trailing edges of a migrating cell. Such controlled delivery of substances to confined cellular regions has previously not been

possible. A targeted membrane digestion also reduces contamination and enables real-time tracking of membrane changes during migration. In future studies we plan to focus on the analysis of migrational mechanisms during cancer cell metastasis, which may reveal the spatial distribution of proteins, lipids and nucleic acids involved in these processes.

Stem cells for therapy of inherited liver diseases

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Mesenchymal stem cells (MSC) derived from bone marrow possess enormous potential for cell-based therapy of liver disease. *In vitro* and *in vivo* studies have shown that MSC differentiate into hepatocyte-like cells although efficiency of differentiation was generally low. To study the effect of MSC after transplantation into animal models of liver disease, we have isolated, expanded and cryopreserved MSC from human bone marrow. Cells were characterized by flow-cytometry and expressed typical surface markers ($CD45^-$ $CD34^-$, $CD14^-$, $CD29^+$, $CD90^+$, $CD44^+$, $CD166^+$, $CD105^+$). By RT-qPCR anlysis, MSC showed low expression of marker genes associated with pluripotency (e. g. Oct4 and Nanog). However, upon hepatogenic stimulation *in vitro*, MSC differentiated almost completely into hepatocyte-like cells. Cell morphology changed from a spindle-like to a polygonal-shape typically observed in adult hepatocytes. Relative gene expression of early (AFP, CK19, CK7) and late hepatocyte markers (CYP, CX32, CK18, albumin) were significantly up-regulated by 1-5 orders of relative gene expression. Since gene transfer may further enhance the therapeutic effectivity of these cells, e. g. by over-expression of therapeutic transgenes or correction of inherent mutations, we also established retroviral gene transfer protocols. Cells will be transplanted into a rat model of Wilson disease (WD) which leads to copper overload of liver followed by acute and chronic liver disease. Our results suggest that the *in vitro* generated hepatocyte-like cells carry many functions of adult human hepatocytes, which is highly significant for clinical applications.

Are the genomically predicted adipokinetic peptides of *Tribolium castaneum* present in the corpora cardiaca?

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With the ever-expanding range of genomic sequences produced over the past years, several whole insect genomes are known. This genomic information can be “mined” to annotate potentially expressed gene products. We were interested to see how close such predictions come to reality. In our case study, we use the red flour beetle, *Tribolium castaneum*, to analyse the most abundant neuropeptides that are synthesised in the corpora cardiaca, viz. the adipokinetic peptides. Such peptides belong to a large family which is characterised by blocked termini (pGlu and carboxyamide) and the aromatic amino acids Phe and Trp at position 4 and 8. Two octapeptides that fulfil these structural criteria are predicted to be encoded in the genome of *T. castaneum*. Although the adult beetle is minute in size and about 100-fold smaller than an adult yellow mealworm beetle, *Tenebrio molitor*, we were able to dissect the corpora cardiaca. One to three of the glands were put on a MALDI plate, rinsed very briefly with ice-cold distilled water and covered with a droplet of matrix in 60% methanol plus acetonitrile. Four distinct mass peaks at *m/z* 1014.2/1030.2 and 1023.3/1039.3 were observed. This shows the typical AKH mass signature; the mass difference of 16 units represents $[M+Na]^+$ and $[M+K]^+$. In a second set of experiments, a methanolic extract of 30 glands was separated on a C-18 column by liquid chromatography and detected by an LTQXL mass spectrometer (Thermo Fisher). MS/MS sequencing of the peaks of interest revealed two octapeptides: one with $[M+H]^+$ at *m/z* 1001.4 is assigned as pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp amide and the other with $[M+H]^+$ at *m/z* 992.4 is assigned as pGlu-Leu-Asn-Phe-Ser-Thr-Asp-Trp amide. These sequences are consistent with the processed peptides predicted in the genome project.

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SATELLITE EVENTS

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Being fast and efficient – New technologies for reliable sample preparation

Sample preparation is always a crucial step when working with biological samples, especially proteins. It is mandatory to have easy to use and highly reproducible methods in order to achieve scientifically reliable results in down stream applications. In this presentation, we will introduce fast ultra-filtration devices for sample preparation along with passivation methods for increasing recoveries when working with diluted protein samples. For sample fractionation, e.g. prior to 2D-PAGE, an innovative method using ion exchange spin columns based on membrane adsorbers as a matrix will be introduced as a reliable and affordable technology.

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BioMark micro fluidic arrays: A proven method to quantify gene expression within a single cell

Historically, single-cell gene expression experiments have been difficult and expensive to perform. Fluidigm has introduced a new single-cell gene expression technique that, when used with the BioMark System, produces inexpensive, easily reproducible, gene expression results from single-cell samples. The BioMark system which utilizes micro fluidic technology is ideally suited to single cell work by minimizing reaction size and allowing up to 48 individual reactions to be created from as little as 1 ul of sample.

The method is ideally suited for high-throughput cell-line studies to determine individual cell behavior in what has been believed to be homozygous populations. The method is also ideally suited to determine single gene cell expression levels in everything from circulating tumor cells (CTCs) to stem cells. The data we will present includes data from single human cells from 8-cell stage embryos which were collected and analyzed for expression of 46 developmental genes.

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Gene expression profiling of laser capture microdissected samples using SuperAmp™ Amplification method

The analysis of single or a few cells is of increasing importance for the identification of cell-specific marker genes. For example, tumors represent a heterogeneous mixture of neoplastic and non-neoplastic cells. Cells of interest might be represented by a minority of cells and thus, their gene expression profiles might be under the detection limit of conventional methods. The isolation of a certain cell population using laser-assisted microdissection, fluorescent activated cell sorting, or magnetic cell sorting is often a prerequisite to gain insight into cell type-specific gene expression patterns.

The SuperAmp™ Service was developed to enable the microarray-based gene expression analysis of low cell numbers, starting with a minimum amount of one cell. The Laser Microdissection and Pressure Catapulting technology (P.A.L.M. Microlaser Technologies) represents a method for e.g. the isolation of single cells from tissue sections. The aim of the present study was to validate the suitability of the SuperAmp™ Technology for laser-capture microdissected cells derived from cryo-fixed and paraffin-embedded melanoma tissue sections. A total of 36 samples were isolated from sections of cryo-fixed or formalin-fixed paraffin-embedded tissue sections. Sample sizes ranged from 1-1000 cells; the sections were excised from the central regions of the melanoma. Gene expression profiling was performed using two-color PIQOR™ Skin Microarrays.

The SuperAmp™ Technology offers a robust and reliable method for the analysis of gene expression even of small cell numbers such as cells isolated by laser capture

microdissection. The high correlation values support the high reproducibility of the modified global PCR strategy. To our knowledge no comparable method for the microarray analysis of low cell counts is available.

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Laser-microdissection of single living cells opens new fields of applications

Laser Microdissection and Pressure Catapulting (LMPC) is a non-contact method to separate cell areas, single cells or sub cellular compartments out of a fixed tissue section. In recent investigations this technique was modified and optimized for the work with living cells.

The isolation of individual, e.g. fluorescently marked, cells out of a mixed culture and the subsequent cultivation of these captured cells was established on different cell lines in our lab. After capturing selected cells grow out to new cell colonies, not only preserving their viability and proliferation characteristics, but also keeping their genetic information unaltered. This was proved using LMPC on the carcinoma cell line HCT116. After re-cultivation the cells were analysed by CGH (comparative genomic hybridization) and M-FISH (multiplex-fluorescence in-situ hybridization).

Further experiments were done with different stem cell lines to present a solution for the challenge of receiving homogenous cell clones. Embryonic stem cells differentiate into various kinds of cells and it is difficult to obtain a specific differentiated cell type from this assay. To overcome this problem, laser-microdissection techniques are the most convenient way to work only with defined stem cells, especially concerning therapy approaches. LMPC offers a method to receive homogenous cell cultures out of one single isolated cell (clonal expansion). In our experiments it was shown that the cells keep their stem cell character, i.e. by detection of different stem cell markers. For example the expression of CD34 and OCT-4 was unchanged after LMPC and re-cultivation. Additionally these results were proved by FACS (Fluorescent Activated Cell Sorting).

The technology of LMPC represents a progress in the contamination-free isolation and analysis of living cells. It opens a wide field of interesting applications in cell and developmental biology and even drug development research, where homogeneous cell clusters or defined clones are needed.

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Sex hormones and cardiovascular disease; novel ligands, new hopes and clinical implications

Advances in gender based medicine are inherently linked to our improved understanding of sex hormone effects on the cardiovascular system. The talk will update clinicians and basic scientists in the field of cardiology, gynaecology and endocrinology with recent results on the function of newly developed oestrogen- and progestin in heart failure, hypertension and cardiac hypertrophy, which have previously been challenged by the negative outcome of clinical trials on the prevention of cardiovascular disease by conventional hormone replacement therapy. The presentation will assist the audience to make their own judgements whether "New ligands" may translate into "New hopes".