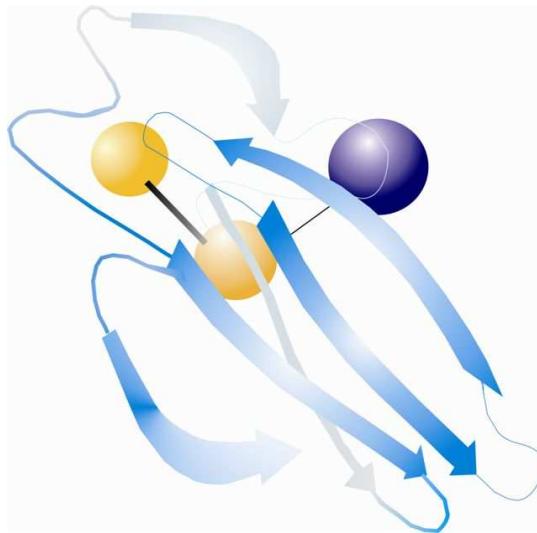


7th Workshop

Molecular Interactions



5th – 7th October, 2011
Berlin

TEAM 2011

Dr. Jens P. Fürste
Free University Berlin



PD Dr. Klaus-Peter Michel
DKFZ Heidelberg

Dr. Susanne Hollmann
University of Potsdam



Dr. Ina Pokorny
University of Potsdam

Dr. Tim Hucho
MPI of Molecular Genetics



Dr. Babette Regierer
MPI of Molecular Genetics

Dr. Stefan Kubick
Fraunhofer Institute for Biomedical Engineering



Heidrun Rudloff
Otto-von-Guericke University Magdeburg

Rougé Lüloff
VWR International GmbH



Dr. Harald Seitz
Fraunhofer Institute for Biomedical Engineering

Local Organising Team

Sarah Schumacher, Matthias Jach, Nadja Bjelopoljak, Jana Ebersbach, Dr. Camilla Völker, Dr. Claudia Flügel

CONTENT

MOTIVATION	3
PROGRAMME	4
ABSTRACTS	7
ORGANISING INSTITUTIONS	29
SUPPORTING INSTITUTIONS	37
POSTER ABSTRACTS	49

**This workshop is being supported by
Deutsche Forschungsgemeinschaft**



**Konrad-Zuse-Zentrum
für Informationstechnik Berlin (ZIB)**
Takustraße 7
D-14195 Berlin-Dahlem
Tel: +49 30 84185-0
E-Mail: board@zib.de
Internet: <http://www.zib.de>

Important Telephone Numbers:
Taxi: 030 26 10 26
Emergency: 112
Police: 110
Organizers: 0175 7147298

MOTIVATION

“Two things are necessary for our work: unrelenting patience and the willingness to abandon something in which a lot of time and effort has been put”.

"Zwei Dinge sind zu unserer Arbeit nötig: Unermüdliche Ausdauer und die Bereitschaft etwas in das man viel Zeit und Arbeit gesteckt hat wieder wegzuwerfen."

(Albert Einstein)

In the ensuing decades of research in molecular genetics, methods have developed rapidly and we collected a lot of information on single molecules and whole genomes. Thanks to the help of modern technologies, it has been possible to decipher whole genomes.

Following the start of the Human Genome Project in 1990, a first draft of human genome sequence was released ten years later and was completed in 2003. Until today, about 4700 genomes have been sequenced: 2685 from viruses and plasmids, 1858 from bacteria, 98 from archaea, and 36 from eukaryotic organisms. Current sequencing projects aim at deciphering the genomes of further organisms from all areas of life. This exponential increase in our knowledge about genetic information raises more far-reaching questions about the function of genes and the interplay of their products: How exactly do the individual molecules of an organism work together to ultimately create a functional living being?

To answer this question, knowledge on regulation of gene expression, on transduction of external and internal signals, and on other determinants such as the concentration and proportion of proteins and messenger substances is required. Functional Genomics combines results from all these research areas. Bioinformatics is increasingly used to combine them to a whole picture. It is therefore certainly justified to state that the research field of systems biology has developed from functional genomic research. The aim of systems biology is to obtain an integrated picture of all processes within a living organism. This includes regulatory processes on all levels; from the genome and the proteome, via the organellar and cellular level right up to the behaviour and biomechanics of the whole organism.

The use and development of sophisticated and complex technologies is characteristic of the so-called "omics" research and its broad sub-fields. The future of functional genomics and systems biology is therefore dependent on the transfer of knowledge within various sub-disciplines of science. This particular concern has been picked up in 2005 with the establishment of our technology-and method-oriented workshop Molecular Interactions.

This year sees the 7th Workshop of Molecular Interactions, under the motto in vivo - in vitro - in silico. The workshop has been growing continuously, hosting an increasingly international audience and internationally renowned speakers. This year's event is organized by scientific co-coordinators and scientists of GoFORSYS, FORSYS, Fraunhofer Institute for Biomedical Engineering, Free University Berlin, University of Potsdam, MaCS, Max Planck Institute of Molecular Genetics and pearls.

We aim to inform young scientists about the latest trends, technological developments and analytical approaches from a practical perspective, and to give them inspiration for their own research. In addition, we would like to provide an opportunity for early career researchers to get into contact with experts from companies and other research institutions.

Finally, a special "Thank You" goes to the DFG and the industrial exhibitors who generously support this event.

Special thanks should also be given to our numerous volunteers, without their help this event would not be possible.

On behalf of the organizing team I welcome you to our 7th Workshop on Molecular Interactions. We wish you a lot of fun, inspiration and many new contacts at the event.



For the organizing team

PROGRAMME

WEDNESDAY, October 5th, 2011

10:30-13:00 PROFESSIONAL INFORMATION EVENT

WELCOME ADDRESS

by the organisers


13:00 Siegfried Neumann *Molecular Diagnostics – Recent Advances and the Practical Needs for Translation from Bench to Bedside*

Session 1 RNA TECHNOLOGIES

Chair: Jens-Peter Fürste

14:00 Jörn Glökler *Life in a Bubble: of Emulsions and Nucleic Acid Libraries*

14:30 Georg Sczakiel *Target RNA influences guide strand loading of biologically active Argonaute-2*

15:00  COFFEE BREAK

Session 2 SINGLE MOLECULE ANALYSIS

Chair: Tim Hucho

15:30 Detlev Schild *Gaining Information From the Time Domain of [Ca²⁺]_i Signals*

16:00 Stefan Prechtel *Is Phenotypic High-Content Screening the Better Pathway Screening Approach to Identify Novel Chemical Entities?*

16:30 Sören Deininger *MALDI Imaging in Clinical Cancer Research*

17:00  COFFEE BREAK

Session 3 BIOTECHNOLOGY

Chair: Babette Regierer

17:30 Peter Guttman *3-D View into Cells with the HZB X-ray Microscope*

18:00 Candan T. Behar *Specific Targeting Molecular Probes: From Materials to Cells*

18:30 Fabio Facchinelli *Establishment of Endosymbiosis and the Making of Synthetic Chloroplasts*

19:00 POSTER SESSION with  and 

THURSDAY, October 6th, 2011

Session 4 SYSTEMS BIOLOGY I

Chair: Ria Baumgrass

09:00 Tobias Scheel *Quantitative Single Cell Analysis of Endogenous Transcription Factor Expression Levels Reveals NFATc2 and c-fos as Limiting Factors for IL-2 Production*

09:30 Mir-Farzin Mashreghi *MicroRNA Expression in Chronically Activated Memory Helper T Lymphocytes*

10:00 Luca Gerosa *Dissecting Transcriptional Circuits from Bacterial Growth*

10:30  COFFEE BREAK


Session 5 TRANSLATIONAL CONTROL

Chair: Stefan Kubick

11:00 Constanze Kaiser *The Interplay between miR2-RISC and the Translation Initiation Machinery*

11:30 Jan Medenbach *Translational Control by Protein Regulated Upstream Open Reading Frames*

12:00 Lisa Roberts *Understanding and Exploiting Novel Mechanism of Viral Translation*

12:30 –13:30  LUNCH BREAK

 Session 6 **DATA MINING AND MATHEMATICAL SYSTEMS** Chair: Peter Deuffhard

- 13:30 Stephan Menz *Direct Hybrid Stochastic-Deterministic Solution of the Chemical Master Equation*
 14:00 Thierry Le Bihan *Quantitative Proteomic Analysis of the Unicellular Alga *Ostreococcus Tauri* under Various Conditions*

14:30 **QUIZ**



15:00 **COFFEE BREAK**

 Session 7 **FUNCTIONAL PROTEOMICS AND GENOMICS I** Chair: Harald Seitz

- 15:30 Robert Wellhausen *Protein Microarrays*
 16:00 Friedrich W. Herberg *Protein Kinase A as a Model for in Vitro, in Vivo and in Silico Studies*
 16:30 Jörg Hoheisel *Proteomics - Antibody Microarrays - Protein Microarrays*



17:00 **COFFEE BREAK**

 Session 8 **FUNCTIONAL PROTEOMICS AND GENOMICS II** Chair: Ina Pokorny

- 17:30 Ingo Dreyer *Long-range Interactions in Transporter Networks in Plants – New Insights from in Silico Approaches*
 18:00 Andreas Vogt *Structural and Functional Protein Network Analysis Predicts Novel Systemic Functions for the G-Protein Coupled Receptor Rhodopsin*
 18:30 Malte Buchholz *Parallelized Functional Characterization of Pancreatic Cancer Candidate Genes on Reverse Transfection Cell Microarrays*



CONFERENCE DINNER
 (registration required for guests)

FRIDAY, October 7th, 2011

 Session 9 **SYNTHETIC BIOSYSTEMS** Chair: Susanne Hollmann

- 09:00 Günter von Kiedrowski *Systems Chemistry: Replicators and Assemblers*
 09:30 Nediljko Budisa *Expanding and Engineering the Genetic Code*
 10:00 Kristian Müller *From Binding Domain to Nano Structure - Selection and Interaction Devices*



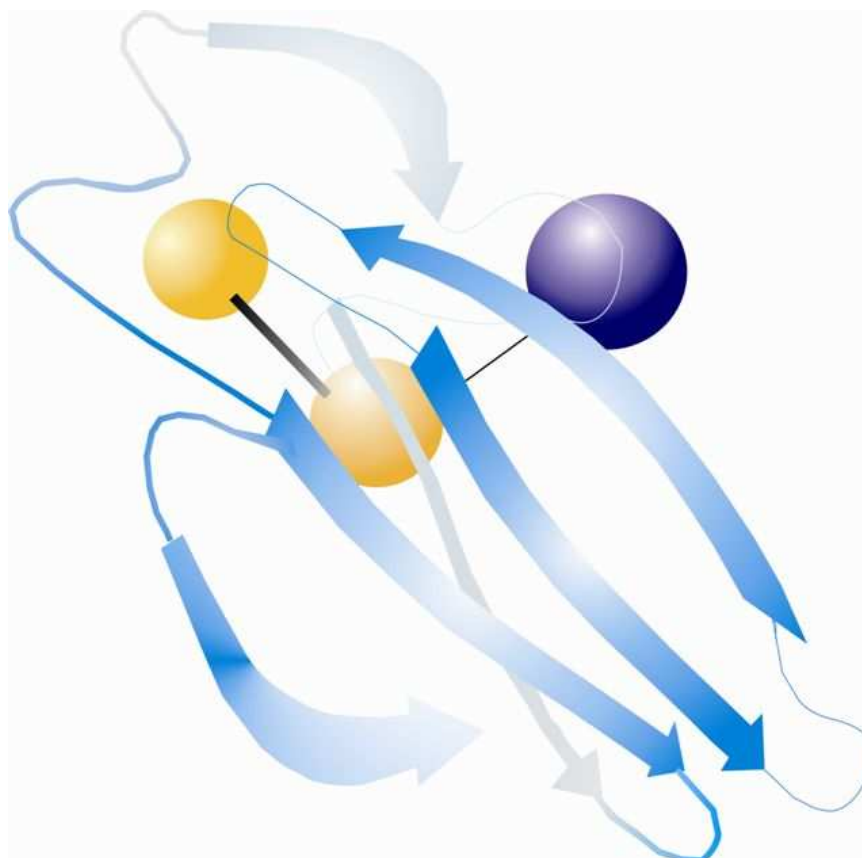
10:30 **COFFEE BREAK**

 Session 10 **SYSTEMS BIOLOGY II** Chair: Klaus-Peter Michel

- 11:00 Hartmut Grammel *Redox Phenomena in Photosynthetic Bacteria – Applications in Systems Biology and Biotechnology*
 11:30 Björn Junker *Towards High-throughput Metabolic Flux Analysis in Seeds of Crop Plants*
 12:00 Timm Schröder *Tracking Stem Cells at The Single Cell Level: New Tools for Old Questions*
 12:30 Melanie Börries *Short and Long-term Correlation Between Transcriptome Dynamics and Protein Synthesis Reveal the Existence of Slaving Principle in Complex Cellular Systems*

13:00 **CLOSING REMARKS**

ABSTRACTS



WELCOME ADDRESS

Molecular Diagnostics – Recent Advances and the Practical Needs for Translation from Bench to Bedside

Siegfried Neumann

Clemens-Schöpf-Institut für Organische Chemie und Biochemie, TU Darmstadt

The recent explosion of biomedical research techniques like gene sequencing by next generation sequencing systems, multiplex analyte quantitation by planar and bead-based microarrays and high-content imaging analyses on cells and tissues with an unprecedented resolution and versatility has lead to a vast accumulation of experimental data on all organizational levels (cells, cellular interactions, physiological processes in health and disease) of living systems. These data sets will ultimately result in identification of molecular causes of diseases. They will change disease definitions and base clinical diagnosis on novel molecular markers in blood, other body fluids and tissue specimen. These molecular markers (biomarkers) as sorted by class are genetic aberrations (aberrant cytogenetics, mutant sequences, copy number variations, haplotypes, SNPs), aberrant protein patterns or changes in metabolites by presence or concentration.

Molecular markers will play an ever increasing role in drug discovery and development, especially in research work on pharmacodynamics. As predictive markers they will be crucial for streamlining clinical trials, by virtue of clear association with response or lack of response to a particular therapy. As an example for this recent and ongoing development this review will present data on the therapeutic significance of the expression of the erb gene family in tumours. Erb-B1 and erb-B2 are special cell surface molecules which are functionally associated with tumour progression. Both of them became the targets for novel drugs and their increased expression is correlated with the potential outcome of the targeted therapy. The overexpression of erb-B1 in combination with K-ras (wildtype gene only) are regularly used as predictive molecular markers for patient stratification of colorectal tumour patients for therapy with therapeutic antibodies to erb-B1.- Thus analyzing molecular markers by specific diagnostic tests for a defined set of selected genes (signatures), by FISH or well defined protein patterns by protein resp. tissue arrays will become good practice in clinical pathology and molecular diagnostics now and in coming years (see for instance 1,2).- More recently and in a more specifically addressed approach progress was achieved by discovery of distinct structural defects in some genes which are involved in driving pathogenesis. Their identification has lead to new therapy approaches which are directly based on the presence of a defined mutation in the diseased cells (for instance a translocation variant of ALK in a subpopulation of patients with non- small cell lung carcinoma disposes to promising treatment results with a novel kinase inhibitor or a mutant version of B-raf the oncogenic effects of which can be antagonized by a specific drug to the mutant protein in advanced melanoma). A targeted therapy in combination with tests for molecular markers (e.g. companion diagnostics) will now become subject to a joint approval by regulatory authorities (3). -This overview will also briefly discuss hurdles for the rapid distribution of freshly discovered candidate molecular markers from basic research groups. One of these challenges to further exploration of basic molecular biology for biomedical practice is a lack of transparency of multiparameter arrays when they try to derive diagnostic conclusions by use of complex proprietary algorithms. The other one is a widely distributed lack of many research groups on a professional strategy for validation of candidate markers or marker sets.

(1) Schweiger, M.-R.: *GenomExpress* 2, 2011, pp. 13. (2) Haney, S.A. :*Europ.Pharm.Rev.* 16, 64 (2011) (3) [http:// www.fda.regulations.gov](http://www.fda.regulations.gov) on „In vitro companion diagnostics devices- draft guidance (July 14,2011)

SESSION 1 – RNA TECHNOLOGIES

Life in a Bubble: of Emulsions and Nucleic Acid Libraries

Jörn Glökler

Max Planck Institute for Molecular Genetics, Berlin, Germany, and Dahlem Centre for Genome Research and Medical Systems Biology gGmbH, Berlin, Germany

Emulsions, best known from everyday products such as milk and cosmetics, have found new applications in biotechnology. By forming distinct compartments, preferably water in oil-emulsions, they can mimic living cells in which genotype and phenotype are kept in tight association. Using this principle, functional enzymes produced from DNA templates by *in vitro* expression, have been successfully selected (1). However, even more daily procedures can profit from reactions in emulsion. The main field of application is amplification of nucleic acid libraries especially for next generation sequencing (2). Some of our recent developments involving emulsion PCR (3) and isothermal amplification of RNA are presented.

(1) Tawfik, D.S. und Griffiths, A.D. (1998) Man-made cell-like compartments for molecular evolution. *Nat. Biotechnol.* 16, 652-656, 10.1038/nbt0798-652. (2) Porreca, G.J., Shendure, J. und Church, G.M. (2006) Polony DNA sequencing. *Curr Protoc Mol Biol*, Chapter 7, Unit 7.8, 10.1002/0471142727.mb0708s76. (3) Schütze, T., Rubelt, F., Repkow, J., Greiner, N., Erdmann, V.A., Lehrach, H., Konthur, Z. und Glökler, J. (2011) A streamlined protocol for emulsion polymerase chain reaction and subsequent purification. *Anal. Biochem.* 410, 155-157, 10.1016/j.ab.2010.11.029.

Target RNA Influences Guide Strand Loading of Biologically Active Argonaute-2

Rosel Kretschmer-Kazemi Far & Georg Sczakiel

Institute of Molecular Medicine, University of Luebeck, Germany, sczakiel@imm.uni-luebeck.de

Active RNA interference (RNAi) in mammalian cells requires loading of the catalytic key component of the RNA-induced silencing complex (RISC), i.e. association of Argonaute-2 (AGO2) with target-specific guide RNA. This short RNA strand may originate from small silencing RNA (siRNA) or microRNA. The process of Ago2-loading, however, is poorly understood and most commonly one assumes that guide strand selection from double-stranded siRNA or microRNA occurs on the basis of thermodynamic rules governing local RNA duplex stability. Here we describe two independent lines of experiments that suggest the involvement of target RNA in Ago2-loading in human cells. (i) Competition of siRNA-induced RNAi by heterologous siRNA requires the competitor-specific target RNA and (ii) Ago2 preferentially associates with the target mRNA-specific single-strand of the same duplex siRNA depending on the presence of the respective matching target. These observations are compatible with the hypothesis of a kind of activation of active RISC by a first round of target suppression/cleavage.

SESSION 2 – SINGLE MOLECULE ANALYSIS

Gaining Information From the Time Domain of $[Ca^{2+}]_i$ Signals

Detlev Schild, Tsai-Wen Chen, Stefan Junek, Mihai Alevra

Dept. Neurophysiology and Cellular Biophysics, Georg-August-Universität Göttingen

In physiological imaging experiments the precise time course of the measured signals and in particular its correlation to other pixels' time courses is largely neglected. Here we show examples how the time structure of imaging experiments can be used to gain so far unobtained information. First, we determined the **background** of a given region of interest (ROI) using the information contained in the temporal dynamics of its individual pixels. Since no information outside the ROI is needed, the method can be used regardless of the staining profile in the surrounding tissue. Moreover, we extend the method to deal with background inhomogeneities within a single ROI, a problem not yet solved by any of the currently available tools. We show that inaccurate background subtraction introduces large errors (easily in the range of 100%) in the assessment of both resting $[Ca^{2+}]_i$ and $[Ca^{2+}]_i$ dynamics. Second, we automatically **determine ROIs** using the correlations of neighboring pixels. This method is however somewhat slow for practical online applications which necessitates more refined algorithms. Third, we introduced **activity correlation imaging** (ACI) which allows the imaging of the activities of neuronal populations while simultaneously visualizing them, each with a different color. To this end we first stain the network's neurons using a membrane permeable $[Ca^{2+}]_i$ indicator (e.g., Fluo-4/AM) and record their activities. We then exploit the recorded temporal activity patterns of all the individual neurons as a means of *intrinsic contrast* using a cross-correlation algorithm. The result is a multi-color visualization of the entire circuitry. We take the *Xenopus* olfactory system (OB) as an example. Odors are detected by olfactory receptor neurons (ORNs) which project to the glomeruli of the olfactory bulb (OB). Using ACI we show that the OB has synchronous modules of M/T cells being connected to the same glomeruli. Furthermore, we investigated the information contained in odor-evoked first-spike latencies. Using simultaneous recordings of dozens of M/T cells with a high temporal resolution and quantitative ensemble correlation techniques we show that latency patterns, and in particular latency rank patterns, are highly odor-specific and reproducible. They reliably predict the odor identity and (with lower accuracy) even the odor concentration on a single trial basis and on short time scales – in fact, more reliably than patterns of firing rates. Furthermore, we show that latency ranks exhibit a better reproducibility at the level of M/T cells than in olfactory receptor neurons. Our results suggest that the latency patterns of M/T cells contain all the information higher brain centers need to identify odors.

Is Phenotypic High-Content Screening The Better Pathway Screening Approach To Identify Novel Chemical Entities?

Stefan Prechtl

Bayer Pharma AG, Lead Discovery Berlin – Screening, High-Content Analysis

Cellular assays are highly recommended during modern pharmaceutical drug research. They are requested for specifying and elucidating discrepancies uncovered during the drug finding process. Cellular assays can be utilized for primary High-Throughput Screening (HTS) campaigns, extensive mechanistic profiling of drug candidates and sophisticated *in vitro* toxicology studies. By deploying a sufficient number of highly sophisticated novel laboratory technologies cellular assay approaches have the potential to provide meaningful decision-relevant data. Disease-focused approaches during early lead discovery phase clearly call increasingly for more complex cellular functional experimental setups which allow for microtiter plate-based screening modalities, multiple wavelength operation and high-resolution imaging. Such High-Content Analysis (HCA) approaches are used to quantify complex drug actions at the level of the individual cell using subcellular resolution and appropriate image analysis algorithms. The utilization of sophisticated HCA approaches during preclinical drug research provides a more specified and detailed insight into complex subcellular processes. As an established scientific tool showing considerably potential to substantially advance pharmaceutical research in the early lead discovery stage HCA is more and more requested for primary screening campaigns analyzing large scale compound libraries of more than 3 million compounds. Such Ultra-High-Content Screening (U-HCS) approaches represent drug screening in a more appropriate physiologically context. Currently, we have performed two U-HCS campaigns in our labs and have demonstrated technical feasibility of these campaigns. Nevertheless, it is important to clarify the outcome of these campaigns when compared to standard cellular HTS approaches. Serious efforts have to be invested in laboratory work, in staff recruitment and material costs during HCA based primary screening. Do these efforts turn to account when novel chemical entities can be identified that are of better drugability? If the biology of the target demands a microscopic approach that can analyze heterogeneous readouts produced by complex intracellular pathways, and if it is clear that this U-HCS approach cannot be replaced in the first round by a simpler classical cell-based assay or even a cheaper biochemical approach, then a U-HCS approach becomes the first choice.

MALDI Imaging in Clinical Cancer Research

Deininger SO, Cornett DS, Paape R, Becker M, Pineau C, Rauser S, Walch A, Wolski E

Bruker Daltonik GmbH, Fahrenheitstr. 4, 28359, Bremen, Germany, Soeren-Oliver.Deininger@bdal.de

MALDI imaging mass spectrometry ("MALDI imaging") is an increasingly recognized technique for biomarker research. After years of method development in the scientific community, the technique is now increasingly applied in clinical research fields. In this workshop the use of MALDI imaging in clinical proteomics will be discussed and put it in context with classical proteomics techniques. The workshop will address specifically the kind of clinical questions that are suitable for MALDI imaging and considerations that are important for successful research. Examples for the importance of careful selection of samples, proper randomization and good study design will be given.

SESSION 3 - BIOTECHNOLOGY

3-D View into Cells with the HZB X-ray Microscope

Peter Guttmann, Stefan Rehbein, Stephan Werner, Katja Henzler, Gerd Schneider

Helmholtz Zentrum Berlin für Materialien und Energie GmbH, Institute for Soft Matter and Functional Materials, Albert-Einstein-Str. 15, 12489 Berlin, Germany

Driven by scientific applications in life science, we developed a new full-field transmission X-ray microscope (TXM) for automated cryo-tomography and spectroscopy. The system operates at the BESSY II undulator U41 at a focusing spherical grating monochromator beamline which provides an energy resolution up to 104. For the first time in soft X-ray microscopy, an elliptically shaped single bounce glass capillary is employed as X-ray condenser which collects the divergent radiation emerging from the exit slit of the monochromator and provides the hollow cone illumination of the object area. This concept has several advantages, it provides higher flux onto the sample compared to diffractive optical devices, its focus position is photon energy independent, and no pinhole is required close to the sample plane. The spatial resolution of the new microscope is 11 nm. For high resolution tomography, we adopted a tilt stage originally developed by FEI for electron tomography which supports automated data collection of cryogenic samples. The stage is able to tilt samples in the X-ray microscope up to $\pm 80^\circ$. Such a large tilt of flat sample holders is impossible with other existing X-ray microscopes. Therefore, the new HZB full-field TXM overcomes two main limitations of previous concepts: It permits spectromicroscopy with high spectral resolution and overcomes the necessity to load the biological specimens into a sample size restricting glass tube for 3-D investigations. In the talk, we present the new TXM and 3-D views into cells.

Specific Targeting Molecular Probes: From Materials to Cells

Candan Tamerler Behar

Genetically Engineered Material Science & Engineering Center & Materials Science & Engineering and University of Washington, Seattle, WA, USA and Adjunct, Molecular Biology & Genetics, Istanbul Technical University, Istanbul, Turkey

Bio-based building blocks are explored for establishing novel routes to develop new performance materials, manufacturing processes and novel biological materials. In our approach, we design, and utilize peptides and multifunctional proteins as molecular tools. Current biotechnological and medical applications such as biosensors, protein arrays, and hybrid nanostructures applicable to imaging and diagnostics require controlled bio-nano interface and oriented assembly of biological molecules. The characteristics of recognition, self assembly and ease of genetic manipulation make inorganic binding peptides an ideal molecular tool for addressable assembly of biomolecules. Building upon the modularity of protein domains, we perform extensive cloning and expression of recombinant proteins fused with inorganic binding peptides as a single multifunctional unit. With an extensive array of multifunctional single molecular units, we tackle different areas of technological interest. Here, we provide examples on the use of peptides as fusion partners to different proteins in creating biocompatible nanomaterials, self immobilization on material surfaces, formation of scaffolds for tissue restoration and cell targeting. The presentation will provide an overview of peptide based functional molecular materials and the approaches carried out in our collaborative groups. Recent developments with examples will be highlighted and prospects will be discussed. *The project supported by GEMSEC-UW, an NSF-MRSEC at the UW, NSF Biomater, TUBITAK-NSF/IRES, TR-SPO.*

E. Yuca, A. Y.Karatas, U.O.S.Seker, M. Gungormus, G. D. Dinler, M. Sarikaya, C. Tamerler, "In vitro labeling of hydroxapatite minerals by engineered proteins", *Biotech& Bioeng*, **108** (5) 1021-1030 (2011)

C. Tamerler, D. Khatyevich, M. Gungormus, T. Kacar, E.E. Oren, M. Hnilova, M. Sarikaya, "Molecular Biomimetics: GEPI Based Biological Routes to Nanotechnologies", *Biopolymer Peptide Science*, **94** 78-94 (2010)

T. Kacar, M. Zin, A. K-Y. Jen, H. Ma, M. Sarikaya, C. Tamerler, *Biotech. & Bioeng*, "Directed self immobilization of alkaline phosphatase on micropatterned substrates via genetically fused metal binding peptide", **103**, 696 (2009)

C. Tamerler, M. Sarikaya, "Genetically Designed Peptide-Based Molecular Materials", *ACS NANO*, **3**(7), 1606-1615, (2009).

C. Tamerler and M. Sarikaya, *MRS Bulletin*, Guest Editors on "Molecular Biomimetics", **33**, 504 (2008)

Establishment of Endosymbiosis and the Making of Synthetic Chloroplasts

Fabio Fachinelli, Andreas P. M. Weber

Institute of Plant Biochemistry, Heinrich-Heine Universität Düsseldorf, Universitätsstrasse 1, D-40225 Düsseldorf, Germany.

In terms of natural selection, the phenomenon of cooperation and altruism may be regarded as paradoxical and risky. However, symbiotic associations drove drastic evolutionary changes, the most crucial among them being the origin of the eukaryotic kingdom. Endosymbiosis is the most intimate form of symbiosis, with one partner (the endosymbiont) living intracellularly within the second symbiotic partner (the host). This interaction is based on a *do ut des* principle where the endosymbiont provides the host cell with nutrients or new metabolic pathways, profiting from the host's nutrient-rich, sheltered environment. Many endosymbiotic relationships exist in nature, predominantly in the aquatic environments involving algae as a source of nutrients derived by the light-driven photosynthetic reactions, turning the heterotroph hosts into a mixotroph or an autotroph (Nowack and Melkonian, 2010). Although all the symbiotic phototrophs benefit from the access to photosynthetic products, the degree of metabolic interdependency and integration varies, ranging from facultative to obligate. The latter is best documented in the acquisition of mitochondria and plastids. The evolution of the eukaryotic cell started with the uptake of an α -proteobacterium by a host cell about 2 billion years ago followed half a billion years later by the engulfment of a photosynthetic cyanobacterium which was ultimately integrated resulting in the modern plastid. While this event, referred to as primary endosymbiosis, is assumed to have occurred only once, sequential endosymbioses involving photosynthetic eukaryotes (secondary endosymbioses) have occurred multiple times. What does it take to establish a permanent and functional plastid in a foreign host? The requirements have been proposed to be the (a) a massive transfer of genes from the cyanobiont to the nucleus of the host cell and (b) the consequent evolution of a protein import apparatus, (c) the coordination of the host and symbiont cell division and (d) the coordination and integration of the metabolic pathways of the two partners (Weber and Osteryoung, 2010). Much effort has been put in order to elucidate the mechanisms at the basis of these processes, and the progress made in fields like molecular and cell biology and also in biotechnology could enable to replicate the process of plastid endosymbiosis in the laboratory by employing a synthetic experimental evolutionary biology approach. Attempts to establish artificial non-photosynthetic and photosynthetic endosymbioses have already been performed several decades ago, although their stability did not exceed few days and no division of chloroplasts or bacteria within their host cells could be achieved. This field of research recently flowered again showing the successful establishment of a synthetic obligate symbiosis by engineering synthetic dual-organism systems of free-living microorganisms (Harcombe, 2010). The next step, the transfer of the organelle of photosynthetic organisms into plastid-free receptor cells, would certainly be a major intellectual and technological challenge. Its success, beyond the potential biotechnological implications, would provide a tool to elucidate the mechanisms at the basis of the early events occurring during the endosymbiotic process and offer insights into the evolution of organelles.

Harcombe, W. (2010). Novel Cooperation Experimentally Evolved between Species. *Evolution* 64, 2166-2172.

Nowack, E.C.M., and Melkonian, M. (2010). Endosymbiotic associations within protists. *Philosophical Transactions of the Royal Society B-Biological Sciences* 365, 699-712.

Weber, A.P.M., and Osteryoung, K.W. (2010). From Endosymbiosis to Synthetic Photosynthetic Life. *Plant Physiology* 154, 593-597.

SESSION 4 – SYSTEMS BIOLOGY I

Quantitative Single Cell Analysis of Endogenous Transcription Factor Expression Levels Reveals NFATc2 and c-fos as Limiting Factors for IL-2 Production

Hanna Bendfeldt¹, Tobias Scheel¹, Manuela Benary², Stefan Frischbutter¹, Gabi Riemekasten³, Hanspeter Herzel², Ria Baumgrass¹

¹German Rheumatism Research Center Berlin (DRFZ), Signal transduction, Berlin, ²HU Berlin, Institute for Theoretical Biology (ITB), Berlin, ³Charité, Medizinische Klinik mit Schwerpunkt Rheumatologie, Berlin

The regulation of Interleukin-2 (IL-2) expression in human Th cells is of great importance for the balance of the immune system: effective immune responses vs. central tolerance. Therefore, precise fine-tuning of IL-2 expression is crucial for adjusting the immune response. So far it is unknown, whether IL-2 producing and nonproducing Th cells are different in the expression levels and activatability of the main TCR-dependent transcription factors. Using transcription factor analysis on single cell level by flow cytometry and mathematical analysis we studied the transcription factor networks regulating IL-2 production in human memory Th cells. We showed that physiological differences in the expression level of NFATc2 and c-fos, but not of c-jun and NF-κBp65, are limiting for the decision whether IL-2 is expressed or not. Particularly, stochastic variation in the expression of c-fos leads to substantial diversity of IL-2 expression in approximately 40% of the memory Th cells. The remaining cells exhibit a high probability of IL-2 expression, thereby ensuring robustness in IL-2 response within the population. Manipulation of c-fos *de novo* synthesis by the small molecular inhibitor U0126 confirms the importance of a certain endogenous c-fos expression level for IL-2 production. A better knowledge of the accurate execution of the IL-2 gene expression program and its perturbation in autoimmune diseases is important for potential therapeutic manipulations such as treatment with exogenous IL-2 protein or anti-IL-2 antibodies.

The work was supported by the German Federal Ministry of Education and Research (BMBF; Forsys-Partner) and by the Deutsche Forschungsgemeinschaft (DFG; SFB-TR52).

MicroRNA Expression in Chronically Activated Memory Helper T Lymphocytes

Anna-Barbara Stittrich*, Claudia Haftmann*, Zhuo Fang, Evridiki Sgouroudis, Anja Kühl, Andreas Bosio, Wei Chen, Hyun-Dong Chang, Nikolaus Rajewsky, Andreas Radbruch* and Mir-Farzin Mashreghi*

German Rheumatism Research Center Berlin DRFZ, AG Prof. Dr. Andreas Radbruch, Charitéplatz 1, 10117 Berlin, Germany

Auto-antigen specific effector memory T helper (Th) lymphocytes are critically involved in the development and maintenance of chronic inflammation in autoimmune diseases. These cells reside at inflamed tissues, function independently of antigenic stimulation and proliferation and thus escape physiological regulation and conventional immunosuppressive therapies. The acquirement of these properties is mediated by a functional imprinting at the transcriptional and epigenetic level. However the exact molecular mechanism, which leads to the transition of a protective into a pathogenic memory effector Th cell, is still elusive. Recent results suggest that microRNA (miRNA) mediated regulation of gene expression may play an important role for the development, function and persistence of chronically activated effector memory Th cells in autoimmune disease. Assuming that Th cells involved in autoimmune inflammation have a history of repeated restimulation by persistent (auto)antigens, we have *in vitro* generated acutely (once) activated and chronically (four times) activated murine memory/effector Th cells. From the miRNA transcriptomes of these *in vitro* generated cells, we have identified miRNAs differentially expressed between once and repeatedly reactivated Th1, Th2 and Th17 cells, by microarrays and high-throughput sequencing of miRNA expression libraries. miR-182 is induced upon activation of naive Th cells. miR-182 expression is regulated by IL-2/Stat5, and T cell receptor signaling. miRNA-182 posttranscriptionally inhibits the transcription factor Foxo1, a

suppressor of Th cell proliferation. The inhibition of miR-182 by antagomirs prevents the downregulation of Foxo1 and results in 70 % reduced expansion of activated Th cells *in vivo* and *in vitro*. In a murine model of arthritis, the inhibition of miR-182 significantly reduces inflammation. Another miRNA, miR-c1, is specifically induced in chronically activated effector/memory Th1 cells. This miRNA targets the pro-apoptotic genes *Bim* and *Pten*. Expression of both genes is reduced in chronically activated memory effector Th1 cells, probably a prerequisite for their survival. Overexpression of miR-c1 in activated Th1 cells results in 50 % down-regulation of endogenous *Bim* and *Pten* expression. Conversely, inhibition of miR-c1 in chronically activated memory Th1 cells by antagomirs results in significantly enhanced cell death. Our results demonstrate a distinct and decisive role of miRNAs in regulating the differentiation, survival and function of memory/effector Th cells in chronic inflammation.

*equal contribution

Dissecting Transcriptional Circuits from Bacterial Growth

L. Gerosa^a(1), K. Kochanowski (1)^a, M. Heinemann (2), U. Sauer (1)

(1) ETH Zurich (Zurich); (2) University of Groningen (Groningen)

Direct and reverse engineering of transcriptional circuits holds promises of understanding and control over cellular processes. Unfortunately, successful engineering is hindered by the complexity of living systems (1). Among the current shortcomings, systems and synthetic biology efforts often assume that transcriptional circuits operate isolated and independently from other actions in cells. However, growing evidences show that gene expression is strongly influenced by orthogonal cellular events as well, primarily by changes in the expression machinery efficiency (2). Therefore, approaches able to decouple expression machinery effects from transcriptional regulation are fundamental towards a more quantitative and dynamic understanding of transcriptional circuits. Here we use the bacterial model organism *E. coli* to investigate the relation between growth-dependent expression machinery and transcriptional circuits. To quantify expression machinery effects, we constructed 12 constitutive (“not regulated by any transcription factor”) GFP-reporter promoters (3) and measured their transcription at 18 different growth rates. Accordingly to theory and previous evidences (4), we found that transcription rate increases linearly with growth-dependent free RNA polymerase abundance (RNAP) and this trend is quantifiable by a promoter-specific kinetic parameter. Surprisingly, we found that this relationship holds in time-course experiments as well, extending its applicability to a range of dynamic processes. We applied such findings to the study of the arginine biosynthesis transcriptional circuit. Given the growth-dependent parameters of involved genes, we were able to infer the gene-specific regulatory parameters and transcription factor activity (5). We found that the response of the arginine repressor ArgR upon arginine depletion is quantitatively very similar under different carbon sources, but transcription is strongly modulated by the different growth rates. When challenged, the model successfully predicted transcription rates of genes simultaneously influenced by arginine depletion and an independently induced growth rate fluctuation. Overall, here we show how expression machinery effects can be decoupled from specific transcriptional regulation by including growth-rate dependent parameters into quantitative models of circuits. We envision that these findings will be applied to the engineering of transcriptional circuits operating in fluctuating environments. More generally, we regard this work as an additional effort to link detailed models of circuits with phenomenological laws of cell physiology.

(1) Kwok R (2010) Five hard truths for synthetic biology. *Nature* 463:288-90. (2) Klumpp S et al. (2009) Growth rate-dependent global effects on gene expression in bacteria. *Cell* 139:1366-75. (3) Zaslaver A et al. (2006) A comprehensive library of fluorescent transcriptional reporters for *Escherichia coli*. *Nature Methods* 3:623-628. (4) Liang S et al. (1999) Activities of constitutive promoters in *Escherichia coli*. *JMB5*. (5) Buchler NE et al. (2003) On schemes of combinatorial transcription logic. *PNAS*. 100:5136-41.

^a contributed equally

SESSION 5 – TRANSLATIONAL CONTROL

The Interplay Between miR2-RISC and the Translation Initiation Machinery

Constanze Kaiser, Francesca Moretti, Agnieszka Zdanowicz and Matthias W. Hentze

European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, D-69117 Heidelberg, Germany

Although major evidence indicates that microRNAs (miRNAs) regulate translation at the initiation step, the exact molecular mechanism(s) of miRNA-mediated translational repression is still incompletely understood. Using a *Drosophila* cell-free system that allows investigation of miR2-mediated translational repression, we sought to elucidate the role of different translation initiation factors in miRNA-mediated regulation. Previously we showed that miR2-mediated repression is stimulated by the presence of a poly(A)-tail on a miR2-target reporter RNA. Moreover, work by others implicated the poly(A)-binding protein (PABP) in miRNA-mediated deadenylation of target mRNAs. By using a combination of PABP depletion and mRNP immunopurification, we demonstrate here for the first time that PABP is required for maximal miR2-mediated repression and that it is displaced from the target mRNA by miR2-RISC. Our data reveal that PABP displacement occurs independently of and prior to deadenylation and translation, suggesting that PABP dissociation is an active process and not the consequence of poly(A)tail removal from a repressed RNA during deadenylation. Comparison of miR2-RISC-mediated and tethered GW182-mediated translational repression suggests that PABP helps to recruit the RISC to the mRNA. Further work on these early events of miRNA-mediated repression to decipher the role of additional translation initiation factors will be presented.

Translational Control by Protein Regulated Upstream Open Reading Frames

Jan Medenbach, Markus Seiler and Matthias W. Hentze

European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, D-69117 Heidelberg, Germany

Analysis of the regulation of *Drosophila msl-2* mRNA by the RNA-binding protein Sex lethal (SXL), which is critical for dosage compensation in *Drosophila*, has uncovered a novel mode of translational control based on common 5' untranslated region elements, upstream open reading frames (uORFs) and interaction sites for RNA-binding proteins. We show that SXL binding downstream of a short uORF imposes a strong negative effect on major reading frame translation. The underlying mechanism involves increasing initiation of scanning ribosomes at the uORF and augmenting its impediment to downstream translation. Our analyses reveal that SXL exerts its effect controlling initiation, not elongation or termination, at the uORF. Probing the generality of the underlying mechanism, we show that the regulatory module that we define experimentally functions in a heterologous context, and we identify natural *Drosophila* mRNAs that are regulated via this module. We propose that protein-regulated uORFs constitute a systematic principle for the regulation of protein synthesis.

Understanding and Exploiting Novel Mechanism of Viral Translation

Lisa Roberts

Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK

The translation of mRNA into protein is the final stage in the process of gene expression. The key regulatory step in this process is the initiation phase which involves binding of the ribosomal subunits to the mRNA and positioning them at the initiation codon. Initiation requires the participation of a number of eukaryotic initiation factors (eIFs) that help to assemble 48S pre-initiation complexes on an mRNA molecule prior to assembly of the 80S initiation complex at the initiation codon. Cytoplasmic mRNAs are capped at their 5' end and the first step in translation is the binding of the initiation factor eIF4F to the 5' cap structure. eIF4F is a complex of three initiation factors - eIF4E is the cap-binding protein, eIF4A functions as an RNA helicase and eIF4G acts as a scaffold to bridge the mRNA to the 40S ribosomal subunit via its interaction with eIF3. As obligate intracellular pathogens, viruses must utilise the host cell's protein synthesis machinery to synthesise new viral proteins and have therefore evolved a number of novel ways to ensure translation of their messenger RNAs (mRNAs) into protein in the presence of high levels of cellular mRNAs. Our work is focused on dissecting some of these novel mechanisms of translation initiation with a view to designing new antivirals or biotechnological exploitation. For example, picornavirus mRNAs have no 5' cap and initiation of translation requires an RNA structure termed an Internal Ribosome Entry Site (IRES) element located in the 5' untranslated region of the viral RNA. In contrast, we have shown that the caliciviruses utilise a novel protein-directed mechanism of initiation mediated by binding of the viral VPg protein covalently attached to the 5' end of the RNA to the eIF4F complex. Our work involves the dissection of the factors involved in IRES and VPg-directed translation, probing of RNA structures important in these processes and understanding how these viruses manipulate the initiation factors to regulate virus protein synthesis. Clearly, a dissection of the distinct novel mechanisms these viruses employ may lead to the identification of targets for the control of these significant human and animal pathogens.

SESSION 6 – DATA MINING/MATHEMATICAL SYSTEMS

Direct Hybrid Stochastic-Deterministic Solution of the Chemical Master Equation

Stephan Menz

Institute of Mathematics & Computer Science, Free University Berlin und Institute of Mathematics, Potsdam University

The chemical master equation (CME) is the fundamental evolution equation of the stochastic description of biochemical reaction kinetics. In contrast to deterministic models it accounts for the discrete and random nature of reaction processes in cells. Due to its high dimensionality, however, in most applications it is impracticable to compute direct solutions of the CME. Instead the CME is indirectly solved by Monte Carlo (MC) simulations of the underlying Markov jump process. Apart from the general problem of estimating the required number of realizations to meet a certain accuracy such indirect approaches become numerically inefficient when the system's dynamics include fast reaction processes or species with high copy numbers. In hybrid approaches, such parts of a reaction network are approximated as continuous processes or replaced by quasi-stationary distributions either in a stochastic or deterministic context. Current hybrid approaches, however, still rely on MC simulations of the stochastic part of the system. This talk first gives a brief recap of the stochastic and deterministic modeling approach of biochemical reaction kinetics. Then, a new direct hybrid approach is presented where a CME for the probability density function of species with low copy numbers is coupled to an evolution equation of deterministically approximated expectations of the remaining species. The performance of the proposed hybrid stochastic-deterministic description is illustrated in application to model systems of biological interest.

This is joint work with J. Latorre and C. Schütte, FU Berlin, and W. Huisinga, University of Potsdam.

Quantitative Proteomic Analysis of the Unicellular Alga *Ostreococcus Tauri* under Various Conditions

Thierry Le Bihan

Centre for System Biology at Edinburgh (CSBE), United Kingdom, thierry.lebihan@ed.ac.uk

Mass Spectrometry (MS) is gaining popularity in various fields of biology for the analysis and the characterisation of complex protein samples. Nevertheless, the generated lists of hundreds to thousands protein names are quite overwhelming and, often meaningless in terms of determining the role or function of a given protein. One possible strategy consists of acquiring a differential protein expression profile resulting from a given perturbation such as chemical treatment, temperature, light stress, pathway inhibition, circadian influences to name a few. This approach provides insight into the function of a subset of proteins whose expression has been altered. Various strategies for quantitative proteomics are available that involve either in vivo or in vitro stable isotope incorporation, or label-free comparisons. In this work, we used *Ostreococcus tauri*, one of the smallest and primitive free-living eukaryotes which have a unique and simple biochemical architecture as a working model. We have characterised the *Ostreococcus tauri* proteome under various conditions using in vivo ¹⁵N incorporation over time in order to evaluate protein turnover. This work involved both the optimisation of the culture media as well as the development of some bioinformatics scripts in order to extract and analyse the level of ¹⁵N under partial incorporation conditions. We also developed a new quantitative strategy, which consists of labelling the N-termini and the lysine ε-amino groups through reductive amination using acetaldehyde (RIDE). Using such a strategy, we evaluated the effect of glufosinate ammonium on the *Ostreococcus tauri* proteome. In this presentation, I will discuss some of the issues encountered with the various quantitative tools used as well as the importance of a robust platform from sample preparation to data analysis.

SESSION 7 – FUNCTIONAL PROTEOMICS/GENOMICS I

Protein Microarrays

Robert Wellhausen

Fraunhofer Institute for Biomolecular Engineering IBMT, Am Mühlenberg 13, 14476 Potsdam-Golm, Phone: 0331 581 875 12, Email: Robert.Wild@ibmt.fraunhofer.de

Post-translational modifications splice variants and polymorphisms are leading to a proteome that is highly variable and differs from cell to cell. Expression analysis of cells and tissues give only an inadequate picture of the protein status of a cell. In contrast to that, protein microarrays are able to track these changes on the level it occurs; the proteomic level. Protein microarrays are a power-full tool not only to unveil e.g. new interaction partners or post-translational modifications but also for relative protein quantification.

Protein Kinase A as a Model for *in Vitro*, *in Vivo* and *in Silico* Studies

Friedrich W. Herberg

Dept. of Biochemistry; Kassel University; 34132 Kassel, Germany, herberg@uni-kassel.de

Protein kinases represent one of the largest classes of proteins in the human genome and play a critical role in intracellular signal transduction. Being involved in many human diseases, protein kinases catalyze the most prominent posttranslational modification (PTM) in a eukaryotic cell, i.e. protein phosphorylation. Numerous protein kinase structures are available in both active and inactive states (1) providing the basis for understanding protein kinase function and regulation in complex cellular signaling pathways. In order to study the molecular basis of fine-tuning of kinase activity, structural information is combined with biochemical, biophysical, cell biological methods. This includes surface plasmon resonance (SPR, Biacore technology), fluorescence polarization (FP) and isothermal titration calorimetry (ITC). These *in vitro* assays are complemented by an *in cell* reporter assay, BRET² (bioluminescence resonance energy transfer) allowing the follow kinase action in living cells also in response to physiological and pharmacological stimuli (2).

cAMP-dependent Protein Kinase (Protein Kinase A, PKA) can serve as a model system for the protein kinase family as well for the development of novel biotechnological and pharmaceutical tools. The modular structure of the enzyme permits in-depth investigation of numerous interactions: Protein-protein interactions are determined between the catalytic and regulatory (R)-subunits and the heat-stable protein kinase inhibitor (PKI). Furthermore, interactions of the (R)-subunits occur via amphipathic helix regions of A-kinase anchoring proteins (AKAPs) thus mediating compartmentalization of PKA. AKAP-PKA interaction surfaces are novel, promising therapeutic targets. Protein-ligand interactions can be quantified very accurately with cAMP, the low molecular weight ligand of PKA, and analogous thereof. High resolution crystal structures of the catalytic and regulatory subunits in complex with natural ligands provide the molecular basis for structure function analysis and for structural based ligand design (1). Since it has been demonstrated that the kinetics of protein interaction as well protein kinase activity are influenced by protein phosphorylation, the dynamics of predicted phosphosites are investigated involving mass spectrometry, SPR and site directed mutagenesis (Hanke in preparation). Furthermore, the role of nucleotides and divalent metal ions on the conformation, activity and interaction patterns of PKA are investigated to provide a detailed picture on mode of action of protein kinases (3).

(1) Kornev, AP, Haste, NM, Taylor, SS, and Ten Eyck, LF Surface comparison of active and inactive protein kinases identifies a conserved activation mechanism. *Proc Natl Acad Sci U S A*. 2006 Nov 21;103(47):17783-8. (2) Diskar M, Zenn HM, Kaupisch A, Kaufholz M, Brockmeyer S, Sohmen D, Berrera M, Zaccolo M, Boshart M, Herberg FW, Prinz A Regulation of cAMP-dependent protein kinases: the human protein kinase X (PrkX) reveals the role of the catalytic subunit {alpha}H-{alpha}I loop. *J Biol Chem*. 2010 Nov 12;285(46):35910-8. (3) Zimmermann, B., Schweinsberg, S., Drewianka, S., Herberg, F. W. (2008). Effect of metal ions on high-affinity binding of pseudosubstrate inhibitors to PKA. *Biochemical Journal* 413(1):93-101.

Financial support: DFG He 1818/6, BMBF Modulation of Pain Switches, EU IP Affinomics and CRAFT Affinity Proteome

Proteomics - Antibody Microarrays - Protein Microarrays

Jörg Hoheisel

Functional Genome Analysis (B070), Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany

As an immediate consequence of the large-scale genomic sequencing efforts, a strong interest has emerged in analysing the function of the DNA-encoded information on a similarly global scale. However, many aspects of modulation and regulation of cellular activity cannot be investigated on the level of nucleic acids but require an analysis of the proteome. Several studies demonstrated a poor correlation of mRNA and protein levels. This is due to post-transcriptional control of protein translation, a number of post-translational modifications of protein as well as protein degradation by proteolysis. Recent estimations suggest that there are more than 200 types of protein modification. The proportion and importance of protein modification is reflected by the fact that 5% to 10% of mammalian genes encode for proteins that modify other proteins. Consequently, the complexity in the human proteome is expected to range from a hundred thousand to several million different protein molecules. With respect to data interpretation, the situation is additionally complicated by the facts that no function is known for very many of the predicted proteins of multicellular organisms and that the dynamic range of protein expression can be very large indeed. With two-dimensional gel-electrophoresis and various processes based on mass spectrometry, powerful techniques exist for the analysis of proteins of an organism or tissue. Yest-two-hybrid analyses also permit global studies for the identification of interaction partners. Nevertheless, many other possibly even more powerful methods are prerequisite to approaching the world of protein analysis in a manner similar to what is already possible for studies at the level of nucleic acids, and beyond. Protein microarrays have an enormous potential of developing into a tool that will allow at the protein level the type of global characterisation of molecule mixtures. Knowledge of genomic sequences and transcriptional profiles do not allow a reliable description of actual protein expression, let alone an analysis of the proteins' structure and biochemical activities, as well as variations thereof, or a quantitative examination of protein-protein interactions. This kind of information, however, is important for a molecular characterisation of physiological or developmental cellular stages and has a broad biotechnical and medical potential. Utilising recently developed processes, we are able to perform such analyses even on a large-scale with nevertheless high reproducibility, a near-single-molecule sensitivity and an accuracy that is as good as ELISA-based assays or DNA-microarray analyses.

SESSION 8 – FUNCTIONAL PROTEOMICS/GENOMICS II

Long-range Interactions in Transporter Networks in Plants – New Insights from *in Silico* Approaches

Ingo Dreyer

Plant Biophysics; Centro de Biotecnología y Genómica de Plantas; Universidad Politécnica de Madrid; Campus de Montegancedo; Carretera M-40, km 37.7; E-28223 Pozuelo de Alarcón (Madrid); ingo.dreyer@upm.es

Molecular interactions are essential processes in cellular life. They do not restrict to short-range biochemical contacts but also involve long-range interactions between proteins that might –at a first glance- not be connected to each other. Illustrative examples for such long-range interactions are transporter networks; especially when the membrane proteins transport charged particles (ions). We analyzed a transporter network of the phloem of plants. By combining diverse experimental approaches with computational cell simulation, we show that K^+ circulating in the phloem serves as a decentralized energy storage that can be used to overcome local energy limitations [1]. Posttranslational modification of a phloem-expressed K^+ channel taps this “potassium battery” [2], which then efficiently assists the plasma membrane H^+ -ATPase in energizing the transmembrane phloem (re)loading processes. The systemic approach involving an iterative interplay between ‘experiment’ and ‘model’ thus allowed uncovering a previously unrecognized role of K^+ in plant growth.

(1) Gajdanowicz et al. (2011) Potassium (K^+) gradients serve as a mobile energy source in plant vascular tissues *Proc. Natl. Acad. Sci. U. S. A* 108, 864-869. (2) Sandmann et al. (2011) The K^+ battery-regulating Arabidopsis K^+ channel AKT2 is under the control of multiple post-translational steps *Plant Signal. Behav.* 6, 558-562.

This study was supported by the German Science Foundation (DFG) by a Heisenberg-Fellowship and the Project DR430/5-2

Structural and Functional Protein Network Analysis Predicts Novel Systemic Functions for the G-Protein Coupled Receptor Rhodopsin

Andreas Vogt, Christina Kiel², Anne Campagna², Andrew Chatr-aryamontri³, Gianni Cesareni³, Luis Serrano², Marius Ueffing¹

¹ University of Tuebingen, Centre for Ophthalmology, Tuebingen, Germany; ² CRG-EMBL System Biology Program, Centre de Regulació Genòmica (CRG) Barcelona, Spain; ³ University of Rome Tor Vergata, Rome, Italy

Understanding complex signal transduction networks is one of the big challenges in modern Biology. Traditionally protein interactions that provide a physical architecture to such networks have been studied by combining biochemical and genetic experiments. Large throughput experiments have added a new level of complexity. However in the majority of these studies network dynamics, the simultaneous regulation of several distinct higher order biological outputs by one network, and the fact that many interactions detected for a particular protein are not compatible simultaneously could not be tackled. In consequence, information on networks remains static, often fails to represent physiology and in many cases lead to wrong interpretations. Combining and integrating several layers of information gathering from biochemistry, in silico network analysis as well as structural biology we have developed an analytical approach to determine the specific quality of a set of interactions. This allows to assess the simultaneous compatibility or competitive nature of protein interactions and to move from analyzing single interactions towards dynamics of protein complexes that can serve as a basis for elucidating physiological principles. The biological system we have chosen for our study is the light receptive GPCR rhodopsin, which is localized in physically highly compartmentalized organellar structures in mammalian photoreceptors called photoreceptor outer segments. By integrating three proteomic datasets, literature mining, computational analysis, and through structural information we have generated a multiscale signal transduction network. Complemented by domain decomposition of protein-protein interactions this network was then qualified for mutually exclusive or mutually compatible interactions and ternary complex formation. The resulting information not only

offers a comprehensive view of signal transduction induced by this GPCR but also suggests novel signaling routes to cytoskeleton dynamics and vesicular trafficking and predicts an unprecedented level of regulation through small GTPases. Further, it demonstrates a specific disease susceptibility of the core visual pathway and a relative robustness of the other functional modules. The approach presents a generic multiscale workflow applicable to the analysis of any cellular pathway. It integrates functional proteomics, structural genomics and in silico biology and opens a new field that we define as structural systems biology.

Funding: BMBF grant QuantPro 0316865A, Dynamo 0315513A

Parallelized Functional Characterization of Pancreatic Cancer Candidate Genes on Reverse Transfection Cell Microarrays

Sandra Melchisedech, Tatjana Honstein, Ramona Kreider, Thomas Gress, **Malte Buchholz**

Clinic for Gastroenterology and Endocrinology, University of Marburg, Germany

In previous high-throughput experiments, we generated expression profiles of ~2000 candidate genes in primary tissues and model systems of pancreatic cancer. 88 high priority candidate genes were selected for experimental functional characterization. The objective of the project discussed here was the analysis of potential roles of the 88 genes in parallelized assays in transformed and non-transformed cell lines, with the aim of selecting highly relevant candidates for further in-depth characterization. The parallelized analyses were performed in the “reverse transfection” format. In this approach, overexpression- and knockdown-constructs are spotted in regular arrays together with transfection reagents on glass slides. Cells are cultivated on the slides, incorporating and expressing the different constructs. The effects are analyzed by fluorescence-based assays. Candidates and controls were downregulated or overexpressed as fusion constructs with fluorescence proteins, respectively, in carcinoma cell lines (PANC-1, SUIT007) as well as non-transformed cells (HEK293) in the presence or absence of serum. Several candidates were identified as target genes for serum stimulation (alteration of subcellular localisation) and/or showed influence on proliferation (Ki67-staining), apoptosis resistance (cleaved caspase 3-assay), cell differentiation (staining for E-Cadherin and Vimentin). A number of these candidates were selected for individual in-depth characterization. Individual assays confirmed the results of the parallelized analyses and revealed additional cellular functions of the selected genes. In conclusion, ‘reverse transfection arrays’ are an efficient technology to identify tumor-relevant genes and characterize important functional roles with high throughput.

SESSION 9 – SYNTHETIC BIOSYSTEMS

Systems Chemistry: Replicators and Assemblers

Günter von Kiedrowski

Organic Chemistry – Bioorganic Chemistry, Ruhr-University, Universitätsstr. 150, 44780 Bochum, Germany, kiedro@rub.de

Self-replication is one of the major principles without life could not exist. The emergence of self-replicating systems on the early earth is generally believed to have taken place before the advent of instructed protein synthesis based on a complex translation machinery. Whether the origin of self-replication is identical to the origin of the hypothetical RNA world or whether it existed at an earlier stage of evolution is an open question that has stimulated chemists to search for chemical systems capable of making copies of themselves via autocatalytic reactions. As self-replication means autocatalysis plus information transfer, the reaction products must necessarily be able to store more structural information than their precursors. Templating as a means to transfer structural information has been exploited since the first successful example of a chemical self-replicating system almost two decades ago¹. Today we have a broad variety of such systems employing oligonucleotides, peptides, and small organic molecules as templates and autocatalytic¹⁻³, cross-catalytic⁴, collectively autocatalytic and non-autonomous (stepwise) schemes of self-replication⁵. A link between the chemical self-replication and nanotechnology was first pointed out by G.M. Whitesides in his debate with Drexler. The ribosome is an example for a nanomachine which may be viewed as a three-dimensionally defined array of 51 modular proteins positioned by the rRNA scaffold. Biomimetic approaches towards the 3D-nanoscaffolding of modular functions may be based on tris oligonucleotides⁶, viz. synthetic 3-arm junctions in which the 3'-ends of three oligonucleotides are connected by a suitable linker. We report on tris oligos as building blocks for the noncovalent construction of nanoobjects. Kinetic control - applied by rapid cooling during self-assembly - was found to favor small and defined nanostructures instead of large polymeric networks.⁶ Maximal instruction was employed as design principles to generate noncovalent 3D-nanoobjects in which both, the topology and the geometry is defined.⁷ Examples include dodecahedral nanoscaffolds composed from 20 tris oligos each bearing three individually defined sequences.⁸ It was demonstrated that the connectivity information in the nanoscaffold junctions can be copied by chemical means.⁹ Chemical copying schemes may be seen in conjunction with "surface-promoted replication and exponential amplification of DNA analogues" (SPREAD)⁵. Applications of such scaffolds include the positioning of modular functions such as multidentate thioether-based gold cluster labels (RUBiGold)¹⁰ which have been tailored for monoconjugability and thermostability. My lecture will introduce chemical self-replication and multicomponent assembly as facets of systems chemistry^{3,11,12} - a nascent field which understands itself as the bottom-up pendant of systems biology towards synthetic biology.¹⁴ The field is clearly inspired by the origin-of-life problem but goes beyond traditional prebiotic chemistry in its mission towards a quantitative dynamic understanding of complex reaction networks with autocatalytic components. Software tools like our SimFit and kinetic NMR titration² may significantly help to decipher signatures of interesting dynamic phenomena such as self-replication, chiral symmetry-breaking, and metabolic autocatalysis found in organic¹³ and biomolecular¹² reaction systems.

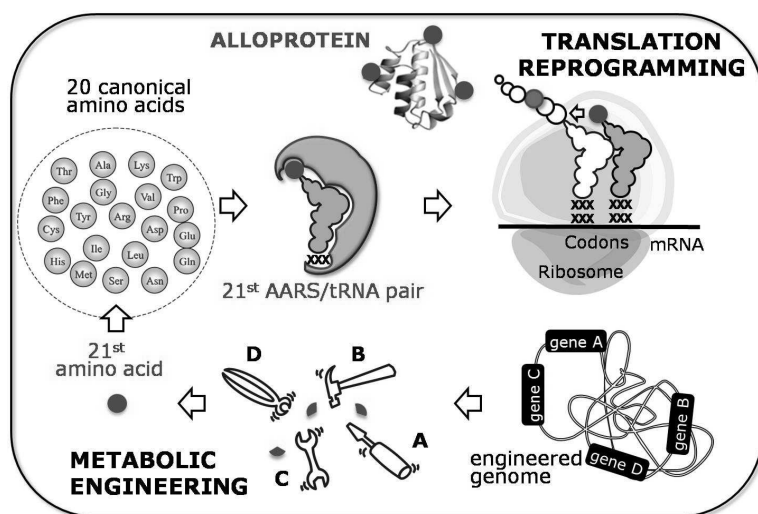
(1) G. von Kiedrowski, *Angew. Chem. Int. Ed. Engl.* 1986, 25, 932. - (2) I. Stahl, G. von Kiedrowski, *J. Am. Chem. Soc.* 2006, 128, 14014 - 14015. - (3) M. Kindermann et al. *Angew. Chem.* 2005, 117, 6908 - 6913. - (4) D. Sievers, G. von Kiedrowski, *Nature* 1994, 369, 221. - (5) A. Luther, R. Brandsch, G. von Kiedrowski, *Nature* 1998, 396, 245. - (6) M. Scheffler et al., *Angew. Chem. Int. Ed. Engl.* 1999, 38, 3312. - (7) G. von Kiedrowski et al., *Pure Appl. Chem.* 2003, 75, 609. - (8) J. Zimmermann et al. *Angew. Chem.* 2008, 120, 3682 - 3686. - (9) L. Eckardt et al. *Nature* 2002, 420, 286. - (10) W. M. Pankau, S. Mönninghoff, G. von Kiedrowski, *Angew. Chem.* 2006, 118, 1923 - 1926. - (11) J. Stankiewicz, L.H. Eckardt, *Angew. Chem.* 2006, 118, 350-352. - (12) E.J. Hayden, G. von Kiedrowski, N. Lehman, *Angew. Chem.* 2008, 120, 8552-8556. - (13) D. Kramer et al., in preparation. - (14) G. v Kiedrowski, S. Otto, P. Herdewijn, *J. Syst. Chem* 2010, 1:1.

Expanding and Engineering the Genetic Code

Michael Hoesl and Nediljko Budisa

Biocatalysis Group, Department of Chemistry, Berlin Institute of Technology/TU Berlin, Franklinstraße 29, D-10587 Berlin, Germany

Genetic code engineering is new research field that intent to reprogram protein synthesis by reassignment of specific codons to non-canonical (mainly synthetic) amino acids. The resulting proteins are congeners with tailor-made properties that are of outstanding interest for both, academia and industrial biotechnology. Our approach successfully combines synthetic chemistry with molecular biology and represents a paradigm for the research areas covered by synthetic biology. We illustrate our holistic approach in this field by giving three examples demonstrating the principles and the applicability of protein engineering with synthetic amino acids. First, in green fluorescent protein, the global replacement of ten proline residues with (4S)-fluoroproline substantially improves the folding and overall stability of the protein. Second, the efficient manipulation of maturation processes is demonstrated by the design of gold fluorescent protein with unique and unprecedented spectral features. The introduction of 4-amino-tryptophan generates an entirely novel chromophore. Third, the incorporation of synthetic protein building blocks with bioorthogonal chemical functionalities, such as terminal azide or alkyne groups, allows the chemical control of post-translational modifications. Using standard methods of organic chemistry (click chemistry), small molecules like sugars can be selectively coupled to the functionalized synthetic proteins. Taken together, the congeneric protein represent possible key players in future pharmaceutical research, materials science and drug design.



The in vivo production of protein-congeners (tailor-made proteins) with synthetic amino acids is possible only with reprogrammed translation. Consequently, vital life processes of intact cells, such as amino acid uptake, activation and tRNA charging, the ribosome cycle and protein folding must be rationally manipulated. In a typical current experiment the synthetic amino acid is added to the growth medium prior to incorporation. It is highly desirable to manipulate the cells by metabolic engineering such that the synthetic amino acid can be biosynthesized intracellularly. This holistic approach is the first step towards the generation of designer cells with a new chemistry of life.

Wiltshi, B., Budisa, N. (2007). Natural history and experimental evolution of the genetic code. *Appl. Microbiol. Biotechnol.* 74:739-753. Hoesl M., G, Budisa, N. (2011) Expanding and engineering the genetic code in a single expression experiment. *ChemBioChem* 12: 552-555. Hoesl, M., Budisa, N. (2011). In Vivo Incorporation of Multiple Noncanonical Amino Acids into Proteins. *Angew. Chem. Int. Ed. Engl.* 50:2896-2902.

From Binding Domain to Nano Structure - Selection and Interaction Devices

Kristian Müller

Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24-25, house 25, 14476 Potsdam, Germany

Analyzing and engineering molecular interaction is a fundamental prerequisite for the understanding and application of biological systems. We integrate research on single binding sites with multivalent display on nano structures using a modular synthetic biology approach from gene to device. For the design and analysis of protein-protein interactions we employ three systems i) TAT-hitchhiker, ii) phage display and iii) protein-fragment complementation. We compared TAT-hitchhiker with phage display and successfully combined it with protein-fragment complementation. For the study and use of interaction principles at a larger scale, we generated two nano-structures, namely DNA origami and virus-like particles. The DNA origami were coupled with ligands and deployed for activation studies of fully modular designer-receptors expressed in eukaryotic cells or modified T-cell receptors. Recombinant Adeno-Associated Virus (rAAV) particles were modified to display binding domains targeting a tumor marker (EGF-R) and to carry genes for prodrug activation. Selective targeting and killing of the tumor cell line A431 was demonstrated.

SESSION 10 – SYSTEMS BIOLOGY II

Redox Phenomena in Photosynthetic Bacteria – Applications in Systems Biology and Biotechnology

Hartmut Grammel^{1,2}, Steffen Klamt^{1,2}, Robin Ghosh³

¹Max Planck Institute for Dynamics of Complex Technical Systems, 39106 Magdeburg, Germany, ²Magdeburg Centre for Systems Biology (MaCS), Magdeburg, Germany, ³University of Stuttgart, Biological Institute, Dept. of Bioenergetics, 70550 Stuttgart, Germany

The formation of intracytoplasmic photosynthetic membranes (PM) by facultative anoxygenic photosynthetic bacteria has become a prime example for exploring redox control of gene expression in response to oxygen and light. The highly pigmented PM can be used as a cellular redox indicator for elucidating how molecular signals affect redox-dependent photosynthetic gene expression. While a number of redox-responsive sensor proteins and transcription factors have been characterized in several species during the last years in some detail, the overall understanding of the metabolic events that determine the cellular redox environment and initiate redox signaling is still poor. The redox state of the ubiquinone (UQ) pool has been suggested to play a key role as a molecular signal. It is particularly interesting that in *Rhodospirillum rubrum* the application of a growth medium containing two substrates, succinate and fructose, was found to dramatically elevate PM under semi-aerobic dark conditions to maximal levels so far attainable only in photosynthetically-grown cells (1). This effect provides an experimental system for elucidating how the growth conditions affect cellular redox signalling. To understand the mechanistic basis for the occurrence of observed phenotypes a mathematical model based on the interactions of the redox sensor proteins was applied. As a valuable complementary tool, a computational model of the electron transport chain (ETC), allows to simulate how environmental factors oxygen and light as well as cytosolic metabolites such as NADH and succinate influence the redox state of UQ and other ETC components and thereby PM expression (2). However, experimental data suggest that PM expression is highly cooperatively regulated by the redox state of the UQ pool (1). These results are probably relevant for redox signalling in bacteria in general. In addition, the high-level expression of PM under semiaerobic dark conditions opens a new perspective for exploitation of *R. rubrum* in bioindustrial applications (3). Potential products derived from PM are photosynthetic pigments (porphyrines, carotenoids), coenzymes (Q₁₀), biohydrogen, biopolymers and others. Central metabolic pathways were analyzed by metabolomics (including 13C-flux analysis) and stoichiometric modelling. Elementary modes analysis and flux balance analysis reveal potential targets for further optimizing the metabolic network.

(1) Grammel, H. and R. Ghosh. 2008. Redox state dynamics of ubiquinone-10 imply cooperative regulation of photosynthetic membrane expression in *Rhodospirillum rubrum*. *J. Bacteriol.* 190 (14), 4912-4921. (2) Klamt, S., H. Grammel, R. Straube, R. Ghosh, and E.D. Gilles. 2008. Modeling the electron transport chain of purple non-sulfur bacteria. *Mol. Syst. Biol.* 4:156. (3) Zeiger, L. and H. Grammel. 2010. Model-based high cell density cultivation of *Rhodospirillum rubrum* under respiratory dark conditions. *Biotechnol. Bioeng.* 105(4):729-739

Funding FORSYS (research units in systems biology) initiative of the German Federal Ministry of Education and Research (grant No. 313922).

Towards High-throughput Metabolic Flux Analysis in Seeds of Crop Plants

Björn Junker

Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben

The field of systems biology has been largely driven by the development of novel techniques that allow for large-scale detection of genes, transcripts, proteins and metabolites. Even though metabolic flux is an important component of systems biology, in plants so far it has been largely neglected, most probably due to the technical and conceptual difficulties that need to be addressed when quantifying fluxes. Steady-State ¹³C Metabolic Flux Analysis (MFA) is a technique that uses the same analytical equipment as metabolomics, but combines this with stable isotope labeling experiments and computer modeling. This methodology allows the establishment of detailed flux maps of central metabolism, including resolution of parallel, cyclic, and reversible metabolic fluxes. We have applied MFA to various plant seeds, which required (a) development of a suitable method for cultivation of seeds or embryos, (b) adaptation of existing protocols for extraction, fractionation, derivatization, and GC-MS analysis to the new material, (c) setup of methods for data extraction and correction, and (d) establishment of a computational model of plant seed central metabolism. While establishing the pipeline, we have especially taken care of increasing the throughput of all steps, so that tens to hundreds of lines can be analyzed in a study, rather than the low number of lines usually analyzed in such studies to date. With this technology at hand, analysis of larger populations of plants comes into reach.

Tracking Stem Cells at the Single Cell Level: New Tools for Old Questions

Timm Schröder

Helmholtz Zentrum München – German Research Center for Environmental Health, Institute of Stem Cell Research, Ingolstaedter Landstraße 1, D-85764 Neuherberg, Germany

Stem cell driven regenerative systems are highly complex and dynamic, consisting of large numbers of different cells expressing many molecules controlling their fates. Therefore, mathematical models are necessary – both to aid the interpretation of experimental data, and to simulate the behavior of stem cell systems based on hypothetical assumptions about their complex cellular or molecular composition. However, the generation of models is hampered by the lack of precise experimental data. Even moderate numbers of unknown variables quickly lead to uncertainties within the models which can render them largely useless for solving biological questions. In particular, it is a major problem that stem cell systems are usually followed by analyzing populations of cells – rather than individual cells – at very few time points of an experiment, and without knowing individual cell identities. Continuous real-time tracking of individual cells would be an important prerequisite to fully understand the developmental complexity of stem cell driven systems. We have therefore developed culture and imaging systems to follow the fate of individual cells over long periods of time. Our approaches also allow the continuous long term quantification of protein expression levels and activity in living stem cells. This novel kind of quantitative data of single cell behavior and molecule expression is used as the basis for the improved generation and falsification of models describing complex stem cell systems.

Short and Long-term Correlation between Transcriptome Dynamics and Protein Synthesis Reveal the Existence of Slaving Principle in Complex Cellular Systems

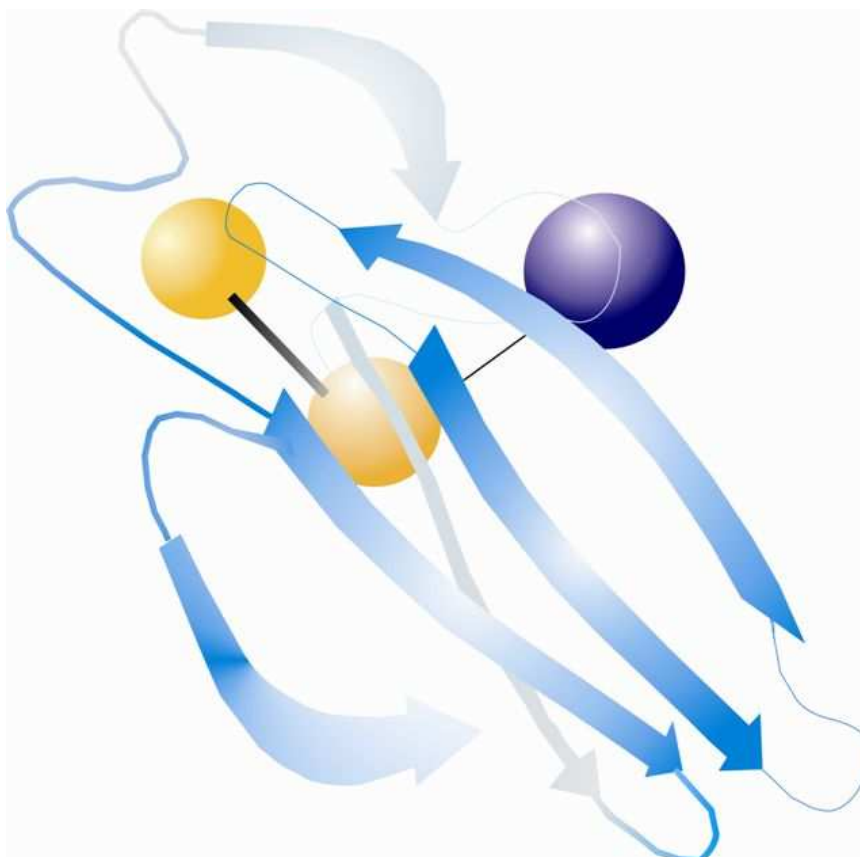
Melanie Boerries and Hauke Busch

Freiburg Institute of Advanced Studies, LifeNet and Center for Biological Systems Analysis, Albert-Ludwigs-University of Freiburg.

The feedback-entangled interaction between gene expression and protein signaling of mammalian cells is a current topic in Systems Biology and the mutual dependence between gene regulation and protein abundance is widely discussed. Here, we suggest that the time scale of observation is a crucial factor in determining the (in-) dependence of the proteome on the transcriptome dynamics. For this, we used PC12 cells as a well-known model for Nerve Growth Factor (NGF) induced differentiation and recorded both the global gene expression kinetics and *de novo* protein synthesis using DNA microarrays and SILAC-based mass spectrometry, respectively. Upon stimulation, PC12 cells differentiate into neuron-like cells within several days. Gene expression kinetics up to 24 hours showed that the decision towards differentiation depends on several events within the first hours after NGF stimulation. Moreover, the transcriptome data predicted several key genes mediating this decision. To gain further knowledge on the mechanistic interaction on the protein level, we study *de novo* protein synthesis during this process to elucidate correlation and mismatches between transcriptome dynamics and time-varying protein synthesis levels. We observed a monotonous increase in the correlation between protein *de novo* synthesis and gene regulation over time. Transcription-independent regulation of protein synthesis was found to be dominant within the first hours after stimulation, hinting at posttranslational effects, for example rate-limiting ribosomal activity. Correlation between the fold change of key genes and protein synthesis increased over time, indicating that on long time scales of several hours the proteome follows the changes in the transcriptome. Assuming protein signaling and gene dynamics to be time-scale separated, these results are reminiscent of the slaving principle known from complex systems theory. It states that slow systems enslave fast system and control their long-term macroscopic behavior. Similarly, in the differentiation process of PC12 cells we find that fast proteome dynamics are independent of the slow changes in gene expression at first, but are controlled by the latter on long time scales. In conclusion we showed for the first time that a dynamic view on both the proteome and transcriptome is necessary in resolving the ongoing debate on cell control through protein signaling as well as gene regulation.

Excellence Initiative of the German Federal and State Governments and BMBF funds this work.

ORGANISING INSTITUTIONS



THE INSTITUTE OF CHEMISTRY AND BIOCHEMISTRY



The three Institutes in the Department of Biology, Chemistry and Pharmacy offer excellent research opportunities, a wide range of study programs, and post-graduate training as well as further education courses. This booklet provides information about research and teaching programs at the Institute of Chemistry and Biochemistry.

The Institute of Biology specializes primarily in molecular botany, microbiology, neurobiology, ecology, biodiversity and plant and animal evolution. In addition to the classical fields of drug development and testing, the Institute of Pharmacy focuses on alternative testing methods and innovative carrier systems.

The Institutes enjoy a high reputation in the scientific world on account of outstanding their excellent, enthusiastic scholars and the wider creative environment of the Berlin research landscape. The Department collaborates in numerous projects not only with the Humboldt-Universität zu Berlin, the Technische Universität Berlin and the Universität Potsdam, but also with leading non-university institutions. The latter network includes the Federal Biological Research Centre for Agriculture and Forestry (BBA), the Federal Institute for Materials Research and Testing (BAM), the Robert Koch Institute, the Leibniz Institute for Molecular Pharmacology (FMP) and the Max Delbrück Centre for Molecular Medicine in Berlin-Buch, the Max Planck Institute of Molecular Genetics, the Fritz Haber Institute, the Max Born Institute, and the Helmholtz Centre Berlin for Materials and Energy. Over and above this, the Institutes cultivate close contact with companies in the private sector, such as Bayer Schering Pharma and Pfizer.

Contact:

Freie Universität Berlin
Institute of Chemistry and Biochemistry
Takustraße 3
14195 Berlin
Tel. +49 30 838-55334

www.bcp.fu-berlin.de
www.chemie.fu-berlin.de

FOCUS AREA OF PLANT GENOMICS & SYSTEMS BIOLOGY

Thanks to substantial financial support by the Federal Ministry of Education and Research (BMBF) the Systems Biology centre GoFORSYS could be established in 2007 in Potsdam-Golm. It develops under the wider umbrella of the „FORSYS – Research Units for Systems Biology“, launched to build interdisciplinary, collaborative and internationally recognized units for Systems Biology at universities in Germany. GoFORSYS is a collaborative initiative of the University of Potsdam’s Institute of Biochemistry and Biology, the Max-Planck Institute of Molecular Plant Physiology and the Max-Planck Institute of Colloids and Interfaces. All located at the Science Park in Potsdam-Golm.

Undoubtedly, GoFORSYS enabled the University of Potsdam and the Max-Planck Institutes to jointly develop a strong focus on Systems Biology, very much to the benefit of our young biology students. In this vein, the University established a new focus area in 2009 - **Plant Genomics & Systems Biology** - and appointed a full professor for Mathematical Modelling and Systems Biology in 2010. In the focus area, different working groups are bundled to create an interface between biology, mathematics, chemistry and physics.

Following a multi-disciplinary approach, the members of the focus area uses experimental and computer-supported approaches to analyse and model biological processes in cells, tissue and organisms. Further appointments in support of Systems Biology are currently ongoing, focusing on metabolism, physiology and investigations in Synthetic Biosystems.

A close-meshed network between university departments as a result of joint research projects and courses is only one attribute of the focus area. It is primarily characterised by established, good relationships to institutions and companies in the region, the nation and other system biology research centres in the world.

As a consequence, Potsdam is extraordinarily attractive for students who are interested in life sciences. The courses of study at Potsdam University profit from being part of this network of high-ranking research institutions in which teams of scientists devote themselves to the burning themes of plant systems biology and molecular life sciences.

The courses of study in this area that existed a few years ago were extremely subject-specific, but today emphasis is placed on an interdisciplinary approach. The barriers between scientific disciplines that were erected because one subject area does not understand how the others think are being overcome. The scientists in the network have the primary aim of teaching young scientists an interdisciplinary approach and creating optimal learning conditions for them. That is why they have set up a Bioinformatics masters programme with Systems Biology as the major area of concentration.

This Masters programme combines life sciences content and computer sciences. Students learn to work in interdisciplinary teams. The course of study is available to people with Bachelor degrees in Computer Sciences as well as students with degrees in Life Sciences.

Contact:

Dr. Susanne Hollmann

Scientific Manager
Focus Area Plant Genomics &
Systems Biology
Co-ordinator GoFORSYS
Susanne.hollmann@uni-potsdam.de

www.uni-potsdam.de/systemsbiology
www.goforsys.de



GoFORSYS
Potsdam-Golm BMBF-FORSYSEinrichtung
zur SYStembio



PEARLS - POTSDAM RESEARCH NETWORK

pearls has been established in January 2009 as a unique network comprising university and non-university research institutions in the region of Potsdam.

Since the establishment of the University of Potsdam in 1991 the capital region of Brandenburg became a renowned research area with the university and more than 20 non-university research institutes representing about 4.000 professors and academic staff. The aim of the new network **pearls** is to support the institutions in the national and international competition for research funds and excellent scientists. **pearls** facilitates multilateral cooperation between institutions and across disciplinary boundaries.

The most important link in **pearls** is an excellent training and support program for young scientists including doctoral students and young researchers. The strategic pillar is the Potsdam Graduate School (PoGS) at the University of Potsdam (www.pogs.uni-potsdam.de). On the basis of international standards Potsdam offers training and attractive research conditions for talented young scientists including postdocs.

pearls promotes the discourse on newly emerging sensitive and innovative research topics and discuss strategic developments. **pearls** is a platform for the acquisition of new research collaborations and the realization of innovative ideas and approaches.

Contact:

Dr. Barbara Gärtner-Rupprecht
pearls – Potsdam Research Network
brupp@pearlsofscience.de
Am Mühlenberg 11
14476 Potsdam
Tel.: +49 331 237 39 44
www.pearlsofscience.org

MAGDEBURG CENTRE FOR SYSTEMS BIOLOGY

The major aim of 'MaCS - Magdeburg Centre for Systems Biology' is the development of novel functional approaches in systems biology and the application of these approaches to the analysis and unravelling of molecular networks, which are involved in signal transduction processes and the overall regulation of selected cellular processes.

MaCS is a project of scientists from the Otto-von-Guericke-University and the Max Planck Institute for Dynamics of Complex Technical Systems in Magdeburg. It is a unit of the 'Dynamic Systems in Biomedicine and Process Technology Research Centre', which is embedded in the Excellence Initiative of Saxony-Anhalt. Currently, MaCS comprises 14 regular academic groups and two junior research groups.

Since MaCS is embedded in the scientific and educational environment provided by the University of Magdeburg and the Max Planck Institute for Dynamics of Complex Dynamical Systems, it serves as a crystallization point for young researchers. This development is fostered by novel PhD programs and novel study programs for systems biology.

Contact:

Head Coordination Office MaCS
Matthias Jach
Institut für Mathematische Optimierung
Universitätsplatz 2
39106 Magdeburg
GERMANY
Phone: +49 (0) 391 67-18555
Fax: +49 (0) 391 67-11171
mjach@mail.math.uni-magdeburg.de
www.mpi-magdeburg.mpg.de/MaCS

FORSYS – RESEARCH UNITS FOR SYSTEMS BIOLOGY



A major objective of the 'FORSYS –Research Units for Systems Biology' initiative, funded by the German Federal Ministry of Education and Science (BMBF) is the development of interdisciplinary and collaborative research units for systems biology at German universities, non-profit research institutions, and industrial companies that will have a high impact on systems biology research in Germany.

To be at the international forefront of systems biology research, the German Federal Ministry of Education and Research initiated an orchestrated program on systems biology in 2001. This program was to fund medium to long-term research initiatives. In 2006/7, the BMBF continued its policy and initiated 'FORSYS – Research Units for Systems Biology', an initiative embedded in the national BMBF support program 'Biotechnology- Taking and Shaping Opportunities'. As a result four FORSYS Centers located in Freiburg, Heidelberg, Magdeburg, and Potsdam were selected (€ 45 million funding from January 1st, 2007 till December 31st, 2011).

FORSYS's major objective is the development of interdisciplinary and work-sharing research units at German universities, non-profit research institutions, and private companies. Starting in 2007, the FORSYS Centers have contributed to a fast growing and increasingly successful systems biology research community in Germany. FORSYS accounts for already existing research activities of the participating institutions on the local, regional, national, and international level. Existing personnel and equipment have been integrated to accomplish maximum synergy. In order to achieve the BMBF's explicit demand for sustainability of systems biology research, FORSYS attains support by contributions of local and state facilities. Once the five year funding period ends the four centers will receive continued funding by the respective state and university. Concomitantly, industrial companies have joined FORSYS and contribute to a sophisticated system biology research in Germany. Besides the development of systems biology research units, FORSYS has boosted teaching activities in the FORSYS Centers. The importance of this goal is reflected by the fact that all FORSYS Centers have set up novel systems biology educational programs for master and bachelor students in the life sciences. Moreover, dedicated PhD programs have been established. These activities strongly account for BMBF's support of selected teaching and student exchange programs.

Since the FORSYS activities are restricted to the four FORSYS centers and their neighboring non-profit research institutions, the BMBF reinforced its support of German systems biology by establishing the FORSYS Partner initiative in 2007/8.

Contact:

Klaus-Peter Michel

FORSYS Managing Office @ DKFZ Heidelberg, INF 580, D-69120 Heidelberg

See www.forsys.net for further information and downloadable materials.

MAX PLANCK INSTITUTE OF MOLECULAR GENETICS

Research at the Max Planck Institute for Molecular Genetics concentrates on genome analysis of man and other organisms to contribute to a global understanding of many of the biological processes in the organism, and to elucidate the mechanism behind many human diseases. It is the overall goal of the combined efforts of all MPIMG's groups to gain new insights into the development of diseases on a molecular level, thus contributing to the development of cause-related new medical treatments.

Genome research, the systematic study of genes and genomes, has changed the way in which research in molecular genetics is pursued. The focus and composition of the MPI for Molecular Genetics reflects this development. The Dept. of Vertebrate Genomics (Lehrach) generates the tools and information to understand the function of most or all genes of man and other organisms. The Human Molecular Genetics Dept. (Ropers) searches for disease genes and their biological function. The Dept. of Computational Molecular Biology (Vingron) exploits the generated data to better understanding of biological and disease processes. The Dept. of Developmental Genetics (Herrmann) uses the systematic functional analysis for understanding developmental mechanisms.

The institute pursues a number of large scale projects. Probably the most prominent national project is the German National Genome Network (NGFN), where all departments of the institute participate and collaborate with each other. Other prominent projects include a number of EU projects, participation in several projects of the German Ministry of Science as well DFG "Sonderforschungsbereiche".

Contact:

Max Planck Institute for Molecular Genetics

Innstraße 63-73 14195 Berlin GERMANY

Phone: (+49 30) 8413 - 0

Fax number: (+49 30) 8413 – 1388

info@molgen.mpg.de

<http://www.molgen.mpg.de/>

The Fraunhofer Institute for Biomedical Engineering (IBMT) with its main building in St. Ingbert (Saarland) covers the multidisciplinary fields of medical technology and biotechnology. The fields of research and development of our branch in Potsdam-Golm are molecular diagnostics, equipment development for lab-on-chip-technology, the development of technologies and logistics for biobanks, but also nanobiotechnology and the preparation of regenerative medicine. The results will help to develop the medical and pharmaceutical industries in the area of molecular diagnostics.

Innovative medical and diagnostic methods, the synthesis of biomolecules or the search for active pharmaceutical compounds cannot do without the competent and reliable processing and characterization of highly complex biological samples. The utility of living single cells, mixtures of cells or cell lysates for the different applications is particularly determined by their vitality and functionality. This requires high standards for the compatibility of technical systems with the demands of the biological samples. In this context and based on concepts of microsystem-, surface- and nanobiotechnology as well as biotechnological and molecular biological processes the department Biotechnology and Biochips pursues different approaches: for the careful handling, the precise control and analysis of living cells lab-on-chip systems are developed. The clever combination of controllable polymer surfaces, dielectrophoretic elements and fluidic microchannels allows for the carrying out of important tasks within the chips: representative examples are the positioning of cells and cell clusters with micrometer accuracy for microscopy, the sorting of heterogeneous cell populations, the activation and differentiation of cells by means of surface-mediated and chemotactic stimuli as well as the gentle and enzyme-free detachment of cells from the cultivation substrate.

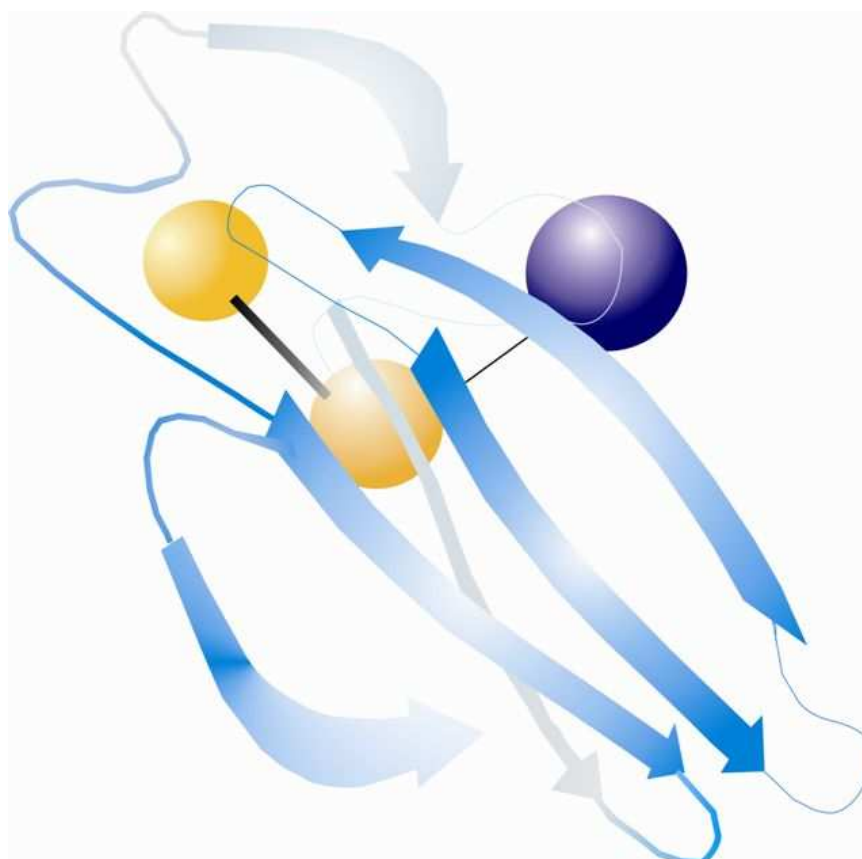
The expertise developed is applied in a second approach to exploit the potential of cell-free protein synthesis for industrial use. This production process, which has recently been established at our location, uses processed cell lysates, thus avoiding many obstacles of the in vivo production of biomolecules within organisms. Through the optimization of the biological processes within defined microfluidic reaction environments we expect substantial improvements in terms of quality and output of this process. At the same time this approach creates the basis for the production of important protein classes, e. g. membrane proteins, such as ion channels or cytotoxic proteins, which so far cannot be produced in sufficient amounts neither using in vivo nor in vitro methods.

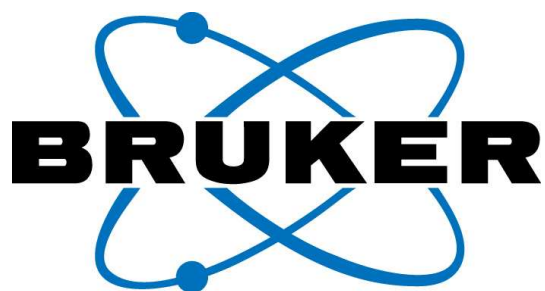
Finally the Extremophile Research group studies cold-adapted snow algae with a focus on their potential use as systems for producing high-quality substances like e. g. antioxidants (carotenoids and vitamins), ice structuring proteins (ISP) or fatty acids. Concomitantly, product-optimized photo-bioreactors are developed, the algal collection CCryo serving as a unique bio-resource used by prospective buyers both from the academic and the private sector.

Contact:

Fraunhofer-Institute for Biomedical Engineering IBMT
Am Mühlenberg 13, 14476 Potsdam-Golm
phone 0331/58187 - 0
fax 0331/58187 – 199

SUPPORTING INSTITUTIONS



BRUKER DALTONIK GMBH

Bruker is a leading provider of high performance analytical systems whose innovative and easy to use product families encompass a variety of Mass Spectrometers and LC systems. Bruker provides qTOFs, Ion Traps, FTMS, and MALDI TOFs as well as nano-LC and unique CaptiveSpray MS Sources to provide outstanding results for Proteomics applications. Delivering premium performance, and backed by decades of Application and Technical Support expertise, Bruker systems lead the industry in protein identification and sequencing, protein quantitation, the identification and analysis of post translational modifications and in MALDI Imaging.

Contact:

Fahrenheitstr. 4
28359 Bremen
Germany
+49 421 2205-433
SALES@BDAL.DE
www.bdal.com

ROBOKLON GMBH

Roboklon GmbH offers products for molecular biological research. Our current product offers include enzymes (such as polymerases, reverse transcriptases and ligases), DNA- and RNA- purification systems as well as selected biochemicals. We pay particular attention towards offering reliable, high quality products at reasonable prices.

As always, we would be happy, if you could consider visiting us at our booth for a cup of espresso. If you want to learn more on our offers, but missed meeting us, our internetshop is open 24/7 at www.roboklon.de.

Contact:

Ingo Fritz
Roboklon
Kantstr. 65
10627 Berlin
Mail@roboklon.de
www.roboklon.de



EPPENDORF

Eppendorf ist ein Unternehmen der Biotechnologie und entwickelt, produziert und vertreibt Systeme für die Life Science-Forschung für den Einsatz in Laboren weltweit.

Das Produktangebot umfasst Pipetten, Dispenser und Zentrifugen sowie Verbrauchsartikel wie Reaktionsgefäße und Pipettenspitzen. Darüber hinaus bietet Eppendorf Instrumente und Systeme zur Zellmanipulation, automatisierte Geräte für Liquid Handling, Komplettausstattungen zur DNA-Vervielfältigung sowie Biochips.

Produktgruppen von Eppendorf:

- Automatisches Pipettieren
- Zellmanipulation
- Zentrifugation
- Photometrie
- PCR
- Pipetten, Dispenser und mehr
- Spitzen, Reaktionsgefäße und Platten
- Temperieren und Mischen
- Vakuumkonzentration
- New Brunswick (Schüttler, CO₂-Inkubatoren, Ultratiefkühlschränke, Bioprozesstechnik)

Contact:

<http://www.eppendorf.de/>

VWR INTERNATIONAL GMBH**Wir sind für Sie da.**

VWR International ist eine weltweit tätige, führende Handelsorganisation, die Produkte und Dienstleistungen für Labor, Reinraum und Produktion anbietet. VWR verfügt über 6.500 Mitarbeiter in 21 Ländern mit direkten Vertretungen auf der ganzen Welt.

In unserem Sortiment finden Sie ausschließlich Produkte höchster Qualität bekannter Hersteller zum einen, als auch unsere Eigenmarke – VWR Collection.

Diese Produkte erfüllen Leistungs- und Produktivitätsanforderungen in den Branchen Pharma, Biotechnologie, Medizintechnik, Halbleiterindustrie und Elektronik, sowie Forschung und Entwicklung.

Die Bedürfnisse unserer Kunden stehen im Zentrum unserer Arbeit. Wir schätzen unsere langfristigen Kundenbeziehungen und die persönlichen Kontakte zu unseren Kunden sind und bleiben uns wichtig.

Unsere Stärken:

- Laborvollversorgung mit ca. 800.000 Produkten von ca. 4700 Hersteller im elektronischen Katalog
- 60.000 Artikel ab Lager verfügbar
- 6 Vertriebszentren in Deutschland mit über 170 qualifizierten Mitarbeiter/innen im Innendienst
- 80 persönliche Ansprechpartner im Außendienst und Telesales
- 51 Produktspezialisten für Life Science, Chromatographie, Geräte, Möbel, Arbeitssicherheit + Reinraum, Chemie, Mikrobiologie, e-Business und Service
- Anwendungstechnische Beratung Laborplanung & Realisation
- Technischer Service inkl. HPLC Geräte-Hotline, Pipettenkalibration und Geräteservice

Contact:

VWR International GmbH
Hilpertstrasse 20a
64295 Darmstadt
Telefon: +49 6151 3972 0
Fax: +49 6151 3972 450
Web: de.vwr.com
info@de.vwr.com

MACHEREY-NAGEL – THE COMPANY



MN history

Since its foundation in 1911, the roots of MACHEREY-NAGEL have been in the field of Filtration (cellulose and glass fiber filters, membranes), Testing, and Chromatography (e.g., chemically bonded silica gels and polymeric phases). This knowledge in analytical separation materials and methods prepared the basis for the Company's involvement in Bioanalysis.

MN worldwide

Operational headquarters, R&D, production, and central marketing are located in Düren, Germany. Subsidiaries focused on local sales and marketing are located in the USA, France, and Switzerland. The worldwide distribution of products is ensured by a net of specialized distributors in more than 150 countries. As a result, our customers can benefit from the advantages of the Company's technologies and products all over the world.

MACHEREY-NAGEL – Bioanalysis

MN Bioanalysis today

Since 1993 the Company has been successfully developing, producing, and world-wide marketing a comprehensive range of ready-to-use kits and consumables for purification of nucleic acids (DNA and RNA) and proteins. MACHEREY-NAGEL has become a worldwide brand of high-value products of sample preparation. Our products cover a broad range of applications, providing solutions for customers with several needs. The Company provides innovative bioseparation technologies and outstanding products for academic, industrial, and clinical research in the area of life science research, genomics, nucleic acid based molecular diagnostics, genetic identity (e.g., with a view to forensics, GMO detection/quantification as well as animal species differentiation), gene expression profiling, gene therapy, and proteomics.

MN Bioanalysis products

These innovative products meet customer needs of time-saving, easy performance, reliable results containing excellent yield and purity, as well as the highest flexibility with regard to sample amount, throughput, and customized applications.

Contact:

MACHEREY-NAGEL GmbH & Co. KG

Neumann-Neander-Str. 6–8 ·

52355 Düren

Germany

Tel.: +49 24 21 969-0

Fax: +49 24 21 969-199

E-mail: info@mn-net.com

THERMO SCIENTIFIC**Thermo**
S C I E N T I F I C**Thermo Scientific laboratory equipment and consumables for research, analysis, discovery and diagnostics**

Thermo Scientific offers the broadest selection of instruments, equipment, consumables and laboratory supplies. Rely on Thermo Scientific products whenever you need to go further, analyze more and move science forward. Discover our broad portfolio of laboratory equipment and consumables, supporting you in every stage of your work.

From routine testing to complex research and discovery, Thermo Scientific Laboratory Equipment and Consumables help to improve productivity and enhance research – even for your toughest application challenges. Your most critical applications require expert solutions. Choose the world leader in serving science.

- Automated Liquid Handling
- Biological Safety Cabinets
- Centrifuges, Rotors and accessories
- Chromatography Columns & Consumables
- CO₂-Incubators
- Cryopreservation Systems
- Hot Plates, Stirrers and Water Baths
- Laboratory Refrigerators and Freezers
- Microbiological Incubators
- Microplate Instruments
- Pipettes
- Plastic Labware for Cell Culture, Bioproduction and Life Science
- Refrigerated/Heated Bath Circulators and Recirculating Chillers
- Shakers
- Ultra-Low Temperature Freezers (-86°C)
- Vacuum Concentrators and Freeze Dryers
- Water Purification Systems

**Contact:**

Robert-Bosch-Straße 1
63505 Langenselbold / Germany
Phone: national toll free 0 800 1 536 376 - international +49 6184 90 6940
E-Mail: info.labequipment.de@thermofisher.com

www.thermoscientific.com

MERCK MILLIPORE

Merck Millipore ist ein führendes Unternehmen in der Life Science Industrie, das fortschrittliche Technologien, Produkte und Dienstleistungen für die biowissenschaftliche Forschung und biopharmazeutische Produktion bereitstellt. Wir arbeiten als strategischer Partner mit unseren Kunden zusammen, um die schwierigen menschlichen Gesundheitsprobleme der Welt zu lösen. Von der Forschung über die Entwicklung bis hin zur Produktion helfen unsere wissenschaftlichen Fachkenntnisse und innovativen Lösungen unseren Kunden, ihre kompliziertesten Probleme zu lösen und ihre Ziele zu erreichen.

Der Geschäftsbereich Lab Solutions von Merck Millipore liefert Produkte für die Forschung sowie analytische und klinische Labors der Industrie, bietet Laborchemikalien, Laborwasser-Produkte und Verbrauchsmaterialien. Für die anorganische Chemie produziert Lab Solutions hochreine Reagenzien, Referenzmaterialien für die instrumentelle Analytik sowie Produkte für die anorganische Spurenanalyse. Der organischen Chemie liefert Merck Millipore die komplette Bandbreite an Basisprodukten für die Synthese, inklusive Bausteinen. Auch die am häufigsten verwendeten Reagenzien und Lösungsmittel gehören zum Portfolio – lieferbar vom Labormaßstab bis zur Massenproduktion. Als Anbieter von Chromatographieprodukten treibt Merck Millipore auch die Entwicklung analytischer Trennverfahren voran. Der Produktschwerpunkt liegt auf der analytischen Hochleistungs-Flüssigkeitschromatographie (HPLC) und auf Innovationen bei den monolithischen HPLC-Säulen der Marke Chromolith® für ultraschnelle Trennungen.

Contact:

Frankfurter Str. 250
64293 Darmstadt
Deutschland
Tel. +49 / (0)6151 / 72-0
Fax +49 / (0)6151 / 72-2000
www.merckmillipore.com/

RiNA GMBH



RiNA GmbH was founded in 1998 as a spin-off from the Institute of Biochemistry, Free University of Berlin and is now privately owned by Prof. Volker A. Erdmann, Leo W. Tristram and RiNA key personnel. RiNA has successfully provided major research & development in the fields of prokaryotic and eukaryotic protein expression systems as well as in Functional RNA molecules such as aptamers and spiegelmers. Currently, RiNA is engaged in several research cooperation projects (e.g. BMBF or EC projects).

Technologies

Cell-free protein biosynthesis

RiNA's protein biosynthesis group has reached an internationally competitive standard and RiNA aims to be one of the world's leading companies in the field of cell-free protein biosynthesis. RiNA's system-platform for cell-free protein biosynthesis has been widely expanded. RiNA specifically provides:

- Highly active cell lysates from bacterial origin suited for the synthesis of proteins up to milligram range.
- A methodology for the improvement of protein solubility and functionality.
- Eukaryotic systems with the ability to synthesize glycosylated and functional membrane proteins.
- Systems for the rapid generation of linear DNA templates with options to introduce e.g. affinity tags, signal peptides.
- A system for the site-specific labeling of proteins, e.g. biotin, fluorescence, PEG, reactive groups.
- A special format for high throughput expression in micro titer plates.
- A System for in vitro transcription

Functional RNA molecules (Aptamers)

In this field of nucleic acids, RiNA develops customized nucleic acids molecules as tools for environmental analysis as well as for diagnostic and therapeutic approaches. The functional RNA group is expanding its expertise in developing aptamers/spiegelmers (RNA/DNA), which have the property to bind target molecules with high affinity and specificity. RiNA's semiautomatic selection procedure will relieve the development of aptamers and new aptamer applications, e.g. aptamer biosensors.

Products & Services

Rapid Translation System

In 2009 Roche Applied Science transferred the complete RTS Protein Expression portfolio to RiNA GmbH, producing according to the same quality Standards originally defined by ROCHE.

Protein Expression Service

A dedicated specific Customer Service for cell-free biosynthesis provides an additional chance for RiNA's customers to receive their desired (highly sophisticated) protein taking advantage of RiNA's total expertise. Based on specialized highly efficient cell-free translation systems RiNA provides a complete solution of synthesizing a broad range of proteins. In order to minimize principal costs and risks, RiNA's Protein Expression Service (PEX) is set up as a Two-Phase-Approach. In Phase I RiNA generates optimal expression templates, evaluates protein yield as well as homogeneity and screens for improved solubility. In Phase II protein synthesis will be upscaled based on the optimized conditions of Phase I. RiNA uses the open nature of the system to adapt reaction conditions to the requirements of a protein allowing for improvement of solubility/functionality and for introduction of modified amino acids (e.g. labels for structural analysis), thus leading to outstanding flexibility. Furthermore, our customers can choose between prokaryotic and eukaryotic translation systems enabling high yield protein synthesis in the milligram range as well as posttranslational modifications.

Aptamer Generation Service

RiNA offers a customized Service for generation of aptamers in Four-Phase-Approach:

- In vitro selection
- Aptamer Cloning & Sequence Analysis
- Characterization of Aptamers
- Production & Delivery

This provides the customer with a maximum scientific and financial control of the project. Additionally, the semiautomatic selection process allows a fast and parallelized aptamer development.

Contact:

www.rina-gmbh.de

GE HEALTHCARE

GE Healthcare provides transformational medical technologies and services that are shaping a new age of patient care. Our broad expertise in medical imaging and information technologies, medical diagnostics, patient monitoring systems, drug discovery, biopharmaceutical manufacturing technologies, performance improvement and performance solutions services help our customers to deliver better care to more people around the world at a lower cost. In addition, we partner with healthcare leaders, striving to leverage the global policy change necessary to implement a successful shift to sustainable healthcare systems.

Our “healthymagination” vision for the future invites the world to join us on our journey as we continuously develop innovations focused on reducing costs, increasing access and improving quality around the world. Headquartered in the United Kingdom, GE Healthcare is a unit of General Electric Company (NYSE: GE). Worldwide, GE Healthcare employees are committed to serving healthcare professionals and their patients in more than 100 countries. For more information about GE Healthcare, visit our website at www.gehealthcare.com.

Contact:

<http://www.gehealthcare.com/dede/>

BRAND



BRAND develops, manufactures and supplies a wide range of measuring and dispensing instruments and Life Science products for use in laboratories in industry, research and education - especially in chemistry, medicine, pharmacy and biotechnology. The product range includes:

Liquid Handling Products

- Microliterpipettes Transferpette[®] electronic, Transferpette[®] Transferpette[®] S
- Repetitive pipettes HandyStep[®] electronic and HandyStep[®] S
- Pipette and filter tips, Dispenser-Tips
- Bottletop dispensers Dispensette[®], seripettor[®] / seripettor[®] pro
- Bottletop burette Titrette[®]
- Direct displacement pipettes: Transferpettor
- Pipette controllers: accu-jet[®] *pro*, macro, micro

Life Science and analysis

- PCR tubes, strips, and plates, also for qPCR
- BRANDplates[®] microplates
- Cuvettes/UV-disposable cuvettes
- Deep-well plates
- BIO-CERT[®] quality level (sterile/free from endotoxins, DNA, RNase and ATP) products
- plastic labware made of PP, PMP, PS and PFA

Services

- OEM plastic injection and blow molding
- Automatic dosing plants/dispensing systems
- Full calibration service
- DKD calibration laboratory for liquid handling and volumetric instruments

Certifications

DIN EN ISO 9001, DIN EN ISO 14001, DIN EN ISO/IEC 17025 (DKD calibration laboratory)

Contact:

BRAND GMBH + CO KG

Otto-Schott-Str. 25
97877 Wertheim/Germany
Tel.: +49-9342-808-0
Fax: +49-9342-808-98000
E-Mail: info@brand.de

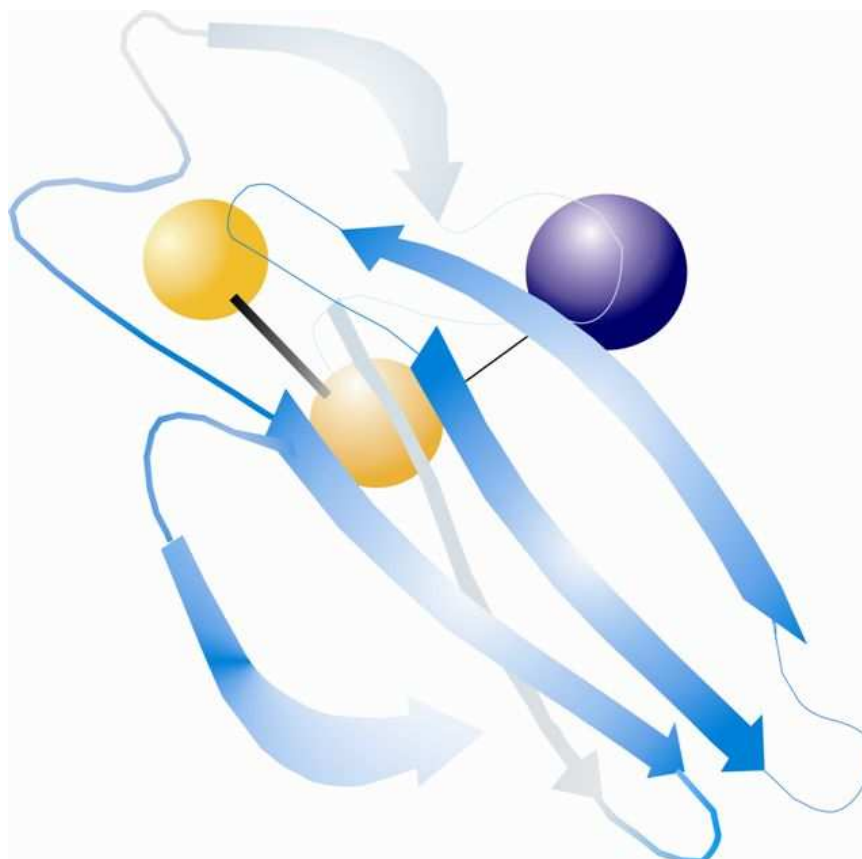
Representative Office Berlin, Brandenburg

Dr. Guido Pahl

Am Gemeindepark 42
12249 Berlin/Germany

Tel.: +49 9342 808 1542
Mobil: +49 175 2779272
Fax: +49 9342 808 91542
E-Mail: guido.pahl@brand.de

POSTER ABSTRACTS



1 A Novel Eukaryotic System for the Production of Functional Antibody Fragments

Marlitt Stech¹, Helmut Merk², Burkhard Micheel³, Jörg Schenk⁴, Walter Stöcklein¹, Doreen Wüstenhagen¹, Claus Duschl¹, Frank Bier¹ and Stefan Kubick¹

¹ Fraunhofer Institute for Biomedical Engineering (IBMT), Branch Potsdam-Golm, Am Mühlenberg 13, 14476 Potsdam, Germany; ² RiNA GmbH, Takustr. 3, 14195 Berlin, Germany, ³ Department of Biotechnology, Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Straße 24-25, Potsdam, Germany; ⁴ UP Transfer GmbH – Hybrotec, clo Fraunhofer Institute for Biomedical Engineering (IBMT), Branch Potsdam-Golm, Am Mühlenberg 13, 14476 Potsdam, Germany

Recombinant antibody fragments are a highly attractive alternative in comparison to full length antibodies. Their small size makes them ideal candidates for therapeutic applications as they are less immunogenic and are able to penetrate tissues. A special subtype of recombinant antibody fragments, called single-chain (sc) antibody fragments, consist of the variable domain of the light antibody chain and the variable domain of the heavy chain linked by a flexible peptide linker. Until now functional antibody fragments are usually produced in a variety of expression systems based on prokaryotic and eukaryotic cells. Here we present a novel strategy for the *in vitro* production of sc antibody fragments in a eukaryotic translation system based on insect cell lysates. These lysates are translationally active and contain intact vesicular lipid structures exhibiting signal peptide cleavage and the glycosylation of cell-free expressed proteins. Here we demonstrate the expression of the recombinant antibody fragment anti-fluorescein (FITC) scFv, which binds specifically to the fluorophor fluorescein isothiocyanate (FITC), in a batch system based on modified insect cell lysates, which are optimized for the expression of proteins with disulfide bridges (RiNA patent application 102011107562.7). Due to the fusion of the sc antibody fragment to a melittin signal sequence, we could show an efficient translocation of the antibody fragments into the vesicles of the insect cell lysate providing an adequate environment for disulfide bonding and therefore functional active antibody fragments. The obtained results demonstrate the production of antibody fragments in cell-free systems in a time-saving and cost-effective manner. Cell-free protein synthesis offers the possibility for co-translational labeling and subsequent selection of binding antibody fragments. In the near future, antibody fragments will be produced in a highly parallel and automated way without the need for animal experiments.

2 Anti-Proliferative Action of *Xylopia aethiopica* Fruit Extract on Human Cervical Cancer Cells

O. Adaramoyea^{*}, J. Sarkara, N. Singha, B. Changkijaa, P. Yadava, S. Kanojiyaa and S. Sinhaa

*a*Drug Target Discovery and Development, Central Drug Research Institute, Lucknow, India and *b*Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria. *aoadaramoye@yahoo.com

The anti-cancer potential of *Xylopia aethiopica* fruit extract (XAFE) and the mechanism of cell death elicited by it, was investigated in various cell lines. XAFE treatment led to a dose-dependent growth inhibition in most cell lines, with selective cytotoxicity towards cancer cells and particularly the human cervical cancer cell line C-33A. In this study, apoptosis was confirmed by nuclear fragmentation and sub-G0/G1 phase accumulation. Cell cycle was arrested at G2/M phase with decreased G0/G1 population. Semi-quantitative gene expression study revealed dose-dependent up-regulation of p53 and p21 genes, and an increase in Bax/Bcl-2 ratio. These results indicate that XAFE could be a potential therapeutic agent against cancer since it inhibits cell proliferation, and induces apoptosis and cell cycle arrest in C-33A cells.

3 Hybrid Systems for Cell-Free Synthesis of Proteins

Andreas Broedel, Birgit Hollmann, Robert Quast, Rita Sachse, Andrei Sonnabend, Marlitt Stech, Doreen Wüstenhagen, Claus Duschl, Frank Bier and Stefan Kubick

Fraunhofer Institute for Biomedical Engineering (IBMT); Branch Potsdam-Golm, Am Mühlenberg 13, 14476 Potsdam, Germany

Recombinant protein production has become an essential tool for providing proteins of interest to either research or therapy. With the advent of the post-genomics era, there is an increasing need for simple and flexible technologies for the efficient synthesis of correctly folded proteins. However, cell-based systems are facing crucial limitations due to time-consuming cloning procedures and the failure to generate functional active proteins, in particular membrane proteins. Cell-free expression systems have recently been developed as promising tools to overcome these limitations. One major benefit is the open design of the system which provides the opportunity to manipulate reaction conditions for the most favorable expression of properly folded proteins. Accordingly, the protein expression process is not only restricted to one specific species but it is possible to combine components of different organisms to achieve desired properties. We have developed a hybrid system for cell-free synthesis of proteins based on translationally active extracts from wheat germ and insect cells. The purpose of the system is to combine the benefits of both systems and neglect their limitations. To date, the eukaryotic systems with highest protein yields are based on wheat germ extracts. However, wheat germ extracts are unable to perform posttranslational modifications, e. g. glycosylation, palmitoylation, myristylation. In contrast, insect based *in vitro* translation systems are able to carry out many types of posttranslational modifications, but yields are far behind the best performing systems. Therefore, hybrid systems are a promising technology to produce high yields of posttranslationally modified proteins.

4 Simulation and Analysis of Electrical/Calcium Signals and their Role in Signal Transduction

Melanie Baumann, Georg Nagel, Dirk Becker*

Molecular Plant Physiology and Biophysics, University of Würzburg, Germany *E-Mail: dbecker@botanik.uni-wuerzburg.de

Various biotic and abiotic stimuli trigger calcium changes and/or membrane potential fluctuations in plant cells. Calcium dependent depolarization of the membrane potential, for example, is observed upon recognition of the *Pseudomonas* flagellin protein by its cognate receptor-like kinase FLAGELLIN SENSITIVE 2 (FLS2). Likewise, calcium and membrane potential transients are observed upon perception of cold and salt stress. Thus, Ca²⁺-based electrical signals represent very early components of signal transduction chains which we suppose to be involved in physiological responses and adaptation towards changing environmental conditions. To investigate the role of calcium and/or electrical signals in these signaling pathways and their impact on e.g. transcriptional re-programming, the current project aims on the generation of "artificial" depolarization/calcium signatures. This is achieved by in planta ectopic expression of the light-controlled cation channel Channelrhodopsin-2 (CHOP2). Upon reconstitution with its photoisomer retinal, blue light dependent activation of Channelrhodopsin-2 (ChR2) allows for specific changes of the membrane potential and/or [Ca²⁺]_{cyt}. The impact of these artificial signatures on adaptation responses will be tested by micro-array/New Sequencing based transcriptional profiling.

5 Exploitation of Antifungal Potential of *Chenopodium Album* for Management of Fusarium Basal Rot of Onion

Saima Rauf, Arshad Javaid

Institute of Agricultural Sciences, University of the Punjab, Lahore. Pakistan

Onion (*Allium cepa* L.), family Alliaceae, is one of the most popular vegetables all over the world. Onion has great economic importance due to its medicinal as well as dietetic values. The average onion consumption is approximately 6.5 kg of onions per person each year across the world. Basal rot disease caused by *Fusarium oxysporum* Schlechtend: Fr. f. sp. *Cepae* (Hans.) Snyder and Hansen is the most destructive soil-borne disease of onion that causes important yield losses in all growing areas of the world. The causal organism infects the basal stem plate of the bulb and eventually kills the entire plant through degradation of the basal plate. The present study was, therefore, designed to investigate the antifungal potential of *Chenopodium album* for the management of *F. oxysporum* f. sp. *cepae*. In laboratory bioassays, methanolic extracts of leaf, stem, inflorescence and root of *C. album* were evaluated for their *in vitro* antifungal activity. Different concentrations of these extracts were tested against *F. oxysporum* f. sp. *cepae*. The maximum antifungal activity was exhibited by leaf and inflorescence extracts resulting in 20–66% and 24–80% reduction in fungal biomass, respectively. The highly effective leaf and inflorescence extracts were further fractionated using different organic solvents viz. n-hexane, chloroform ethyl acetate and n-butanol in order of increasing polarity. Various concentrations of these fractions were evaluated for their antifungal activity against the target pathogen using malt extract broth as growth medium. The chloroform fraction of leaf extract and ethyl acetate fraction of inflorescence extract were found highly antifungal resulting 96–100% and 68–100% reduction in fungal biomass over control. Isolation and purification of antifungal constituents was carried out by thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC). Two compounds viz. LC1 and LC2 were isolated from chloroform fraction of leaf extract while three compounds viz. IE1, IE2 and IE3 from ethyl acetate fraction of inflorescence extract. Antifungal activity of these compounds was evaluated through Minimum Inhibitory Concentration (MIC) technique. Different of the five isolated compounds and a commercial fungicide Mancozeb (80%WP) were used for MIC evaluation. The results of the present study conclude that chloroform fraction of methanolic leaf extract and ethyl acetate fraction of inflorescence fraction contain antifungal compounds that can control the growth of *F. oxysporum* f. sp. *cepae*. The disease can be effectively managed by amending the soil with dry leaf manure of *C. album*.

Bayraktar H, Turkkan M, Dolar FS (2010) Characterization of *Fusarium oxysporum* f.sp. *cepae* from Onion in Turkey Based on Vegetative Compatibility and rDNA RFLP Analysis. *Phytopathology* 10:434-439 **Jabeen K, Javaid A, Ahmad E, Athar M (2010)** Antifungal compounds from *Melia azedarach* leaves for management of *Ascochyta Rabiei*, the cause of Chickpea blight. *Nat Prod Res* 24: in press

6 Modular Virus Construction Kit for Therapy

iGEM Team Freiburg 2010: Hanna Wagner, Volker Morath, Beate Kaufmann, Adrian Fischer, Achim Mall, Max Schelker, Patrick Schindler, Seven Hagen, Tobias Baumann, Katja M. Arndt, Kristian M. Müller

Gene delivery using adeno-associated viruses (AAV) holds great promise for the treatment of acquired and inherited diseases. Taking current knowledge on viral vectors into account, we generated a recombinant, modularized AAV “Virus Construction Kit”. Our system comprises parts for modified capsid proteins, targeting modules, tumor-specific promoters, and prodrug-activating enzymes as well as readily assembled vectors for gene delivery and production of non-replicative virus particles. The viral tropism is altered by N-terminal fusion or by loop replacement of the capsid proteins. Functionality of viruses constructed from our kit was demonstrated by prodrug-induced killing of tumor cells upon viral delivery of a thymidine kinase. Incorporating multiple layers of safety, we provide a general tool to the growing field of personalized medicine and demonstrate its use in tumor therapy.

7 Functionalized Biological Membranes in Eukaryotic Cell-free Systems

Rita Sachse¹, Susanne Fenz², Doreen Wüstenhagen¹, Claus Duschl¹, Frank Bier¹, Thomas Schmidt² and Stefan Kubick¹

¹Fraunhofer Institute for Biomedical Engineering (IBMT), Branch Potsdam-Golm, Am Mühlenberg 13, 14476 Potsdam, Germany, ²Physics of Life Processes, Leiden Institute of Physics, Leiden University, Niels Bohrweg 2, 2333 CA Leiden, The Netherlands

Cell-free expression systems have become a versatile method for the expression of a wide range of structurally and functionally divergent proteins. Various pro- and eukaryotic cell-free systems provide a high number of advantages which can be used for synthesis of difficult-to-express proteins. However, even in cell-free systems integral membrane protein expression can be a challenge due to the hydrophobic nature of their transmembrane domains. Detergent-based methods are used for the improvement of solubility. The correct folding and the functional activity of membrane proteins strongly depend on their integration into a lipid bilayer and their subsequent posttranslational modification. Eukaryotic insect lysates provide endoplasmic reticulum-based vesicles which enable a signal induced translocation and posttranslational modification, e.g. glycosylation and lipid modification. The correct addressing and translocation into microsomal structures has been shown for a broad variety of membrane proteins. Open access to the cytosolic domains of novel synthesized membrane proteins offers an extended number of different analytic approaches. Membrane channel gated ion flux could be supported by vesicles forming separated compartments in cell free systems. In this context biological membrane systems are of major interest for technical applications such as automatization of functional assays. Subsequent membrane modification with synthetic lipids, surface functionalisation and targeted immobilization are favorable techniques to gain technically applicable membrane systems. Electroformation of microsomes is used to gain giant unilamellar vesicles (GUV) from the microsomes harboring membrane proteins of interest. Functionalized lipids allow the vesicles to be immobilized on technical surfaces. Ongoing research is focusing on the activity of proteins incorporated in synthetically modified biological membranes.

8 A Novel Cell-free Eukaryotic System Enabling the Synthesis of Membrane Proteins for Site-directed Chemoselective Modification

Robert Quast¹, Iris Claußnitzer², Michael Gerrits², Doreen Wüstenhagen¹, Claus Duschl¹, Frank Bier¹ and Stefan Kubick¹

¹Fraunhofer Institute for Biomedical Engineering (IBMT); Branch Potsdam-Golm, Am Mühlenberg 13, 14476 Potsdam, Germany, ²RiNA GmbH, Takusstraße 3; 14195 Berlin, Germany

Proteins account for the majority of functional attributes in living systems. The ribosomal translation results in a specific protein structure essential for its function. In theory, the various functions of proteins can be attributed to the properties of the 20 canonical amino acids. In order to expand this natural repertoire numerous non-canonical amino acids (ncAA) have already been successfully introduced into proteins in vivo. Non-canonical amino acids with chemical reactivities differing from those of canonical amino acids enable the posttranslational site-directed conjugation of the synthesized proteins with appropriate functionalized reagents as well as the site-directed crosslinking of proteins to other biomolecules. So called “orthogonal systems” are a prerequisite for the site-directed incorporation of such chemoselective reactive amino acids into proteins. This technique is based on the aminoacylation of suppressor tRNAs with ncAAs by engineered aminoacyl tRNA synthetase-variants. Recently we have developed a cell-free system based on translationally active lysates from insect cells, which contains active vesicular structures thus allowing the synthesis of functional active membrane proteins directly into membrane vesicles using the natural translocation mechanism. Currently we are further developing this system to enable the site-specific incorporation of the chemoselective and bioorthogonal reactive ncAA p-azido-L-phenylalanine into proteins using a combination of the modified system with an orthogonal aminoacyl tRNA synthetase / tRNA pair. This novel cell-free eukaryotic orthogonal system is a powerful tool for the synthesis and site-specific labeling and crosslinking of functional membrane proteins within their natural environment.

9 New Imaging Techniques for Organ Targeted Polyamine Delivery

Olga Lemke

Karlsruhe Institute of Technology (KIT), Institute of Toxicology and Genetics, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany, Phone: +49 721 608 2 2715 or 2148, Email: Olga.Lemke@kit.edu

Positively charged liposomes, nanoparticles, and even smaller structures such as peptides, peptoides and polyamines are today common systems for the systemic application of drugs in living organisms. They mainly serve as packaging devices and support the solubilisation of various drugs. With the combinatorial synthesis of lipophilic polyamines, derivatives were characterized that accumulate in lipid storage depots, so called lipid droplets. Lipid droplets are highly distinct in adipocytes and liver cells and store predominantly triglycerides, cholesterol and fatty acid esters. To respond to biological and medical questions regarding the effects of polyamine accumulation in biological tissues different biological imaging techniques are needed. In vivo bioimaging enables the investigation of compound enrichment in the animal model and especially in distinct organs. Cell imaging can be performed by confocal fluorescence microscopy to even localize the uptake of fluorescent compounds at the cellular level. Today, mainly histochemical staining techniques were used to interpret the tissue distribution of non-labeled compounds or their effect on biological material. However, the staining often cannot prove an influence of the compounds on the metabolism and the immunology of the animal on the molecular level. To overcome this limitation new techniques should be used which allow a simultaneous monitoring of multiple analyses without predefining or labeling selected substances prior to analysis. Fourier transform infrared spectroscopy is an efficient way to locally measure the variation of lipids, proteins, and carbohydrates on tissue sections. Another new method, mass spectrometric imaging, enables the detection of a variety of unlabeled compounds ranging from small molecules to proteins.

10 Mitochondrial-Nuclear Molecular Interactions and Sex Determination in Freshwater Mussels

Don Stewart¹, Sophie Breton², Sally Shepardson³, Richard J. Trdan³, Arthur E. Bogan⁴, Eric G. Chapman⁵, Andrew J. Ruminas², Helen Piontkivska² & Walter R. Hoeh²

¹Department of Biology, Acadia University, Wolfville, NS B4P 2R6 Canada; ²Department of Biological Sciences, Kent State University, Kent, OH 44242 USA; ³Department of Biology, Saginaw Valley State University, University Center, MI 48710 USA; ⁴North Carolina State Museum of Natural Sciences, Raleigh, NC 27699-1626 USA; ⁵Department of Entomology, University of Kentucky, Lexington, KY 40546 USA, don.stewart@acadiau.ca

Many marine and freshwater bivalves have an unusual system of "doubly uniparental inheritance" of mitochondrial DNA. In this system, males are heteroplasmic for an M-type or "male-transmitted" mitochondrial genome, which predominates in gonad tissue, and an F-type or "female-transmitted" mitochondrial genome, which predominates in somatic tissues of males. In contrast, females normally only possess the F-type mt genome. We have shown that in freshwater mussels (Unionoida), (1) dioecious species have retained distinct M and F-type genomes for several hundred million years, (2) there are gender-specific, novel, open reading frames (*orfs*) in the F- and M-type genomes in all dioecious freshwater mussel species examined (i.e., an F-*orf* and an M-*orf*, respectively), (3) that hermaphroditic freshwater mussels lose the M-type genome completely (and concomitantly the M-*orf* is lost as well) and the remaining F-type undergoes macromutations in the F-*orf*. We refer to this highly modified *orf* in hermaphrodites as the "H-*orf*". The precise function of the F-*orf*, M-*orf*, and H-*orf* genes remains unclear, however, using immunoelectron microscopy, we found evidence of the F-ORF protein in mitochondria (as one would expect for a mitochondrially encoded protein) but also on the nuclear membrane and in the nucleoplasm. These extra-mitochondrial localizations represent additional evidence indicating non-oxidative phosphorylation functions for the F-ORF protein and support the hypothesis that this protein is involved in the genetic regulatory network specifying dioecy in freshwater mussels.

11 Modification and Selection of Cyclic Peptides for Therapy

Jessica Eger 1, Katharina Berger 1, Nadine Boehmer 1, Nadja Bjelopoljak 1, Nicole Albrecht 1, Niels Weisbach 1, Niklas Laasch 1, Oliver Zimmer 2, Paul Kaufmann 1, Sabine Meyer 1, Sandrina Heyde 1, Sascha Ramm 1, Sebastian Hanke 1, Stefan Wahlefeld 1, Steffi Sempert 1, Tobias Wenzel 3, Elke Dittmann 1, Katja M. Arndt 1, Kristian M. Müller 1

iGEM Team Potsdam 2011, 1) University of Potsdam, 2) TH Wildau, 3) TU Berlin

One key task of biopharmaceuticals is the binding and blocking of deregulated proteins. Towards this goal, we mutate and select microviridins, which are tricyclic depsipeptides from cyanobacteria. They are small but stable due to their post-translational side-chain crosslinking. Microviridins have a high potential for therapy as they can block disease-relevant proteases. Yet, the possibilities of cyclic peptides are largely untapped since genetic systems for optimization are not well established. Thus, we developed synthetic systems for the mutation, selection and production of such peptides. We use the 6.5 kb microviridin (mdn) gene cluster cloned in *E. coli* plasmids, established random mutagenesis and generated focused libraries of microviridins. For selection against a panel of proteases, we are applying and testing phage display, and we are constructing a novel in-vivo selection device, which links protease blocking to antibiotic resistance.

12 Tannic Acid: A Molecular Longevity-Trigger

Nadine Saul, Kerstin Pietsch, Stephen R. Stürzenbaum, Ralph Menzel, and Christian E. W. Steinberg

1 Laboratory of Freshwater & Stress Ecology, Department of Biology, Humboldt-Universität zu Berlin, Späthstrasse 80/81, 12437 Berlin, Germany, 2 School of Biomedical Sciences, Analytical and Environmental Science Division, King's College London, 150 Stamford Street, London SE19NH, United Kingdom

Low concentrations of the polyphenol tannic acid (TA) prolong the lifespan and enhance the stress resistance of the model organism *Caenorhabditis elegans*. Further biotests with these nematodes revealed that simple antioxidative or antimicrobial actions are not responsible for the beneficial effects. But the observed growth impairment is in line with an energy shift according to the “disposable soma theory” and the dose-response curves speak in favour of a “hormesis effect”, both possible longevity background mechanisms. But what is the molecular basis of the TA mediated effects? Affymetrix DNA microarrays, quantitative RT-PCRs, and studies with *C. elegans* mutant strains were used to shed light on the molecular action of TA. Several interesting genes emerged, such as the cadmium-responsive gene *numr-1*, which expression was highly up-regulated during TA exposure. Since other, usually co-expressed Cd-responsive genes were not affected, *numr-1* seems to have some special, yet unknown, functions. Moreover, *sek-1* is worth mentioning. SEK-1 is a MAPK kinase, which positively regulates pathogen defence and innate immunity. Interestingly, the beneficial impact of TA disappears or even shifts to toxic effects in *sek-1* mutants, even though the expression of *sek-1* in the wild type was found to be slightly down-regulated in response to TA. These and several further differentially expressed genes involved in stress response, growth, development, and reproduction provide a molecular explanation for the observed effects. Moreover, these genes might be key players for the mediation of the “hormesis effect” and the energy shift based on the “disposable soma theory”. At last, this study provides new evidence for the complex mode of action of polyphenols, which turn out, in contrast to former opinions, not to be only simple unspecific antioxidants.

13 Improving *aadA* Gene Expression in *Chlamydomonas* Chloroplasts by Adjustment of Arginine Codons

Caroline Weiß

MLU Halle-Wittenberg

Heterologous protein expression in chloroplasts has become an important field of plant genetic engineering. For obtaining high yields of recombinant protein, codon usage adjustment of genes from various sources is often necessary. It is well known that especially rare Arg codons drastically affect translation. The *aadA* gene, which confers spectinomycin/streptomycin resistance and is frequently used as a selectable marker and reporter gene in chloroplast transformation experiments, contains four rare Arg codons and is usually poorly expressed in chloroplasts. In order to improve *aadA* gene expression, we replaced only these codons by the frequently used Arg codon CGT using site directed mutagenesis. The data indicate that transformants with the codon bias-adjusted *aadA* gene confers significantly higher antibiotic resistance when compared with those carrying the rare codons.

14 RAB Pseudogenes are Novel Regulators of Secretory Membrane Trafficking

Sanchari Roy¹, Vladimir Kurshyev², Reinhard Schneider², Holger Erfle³, Vytaute Starkuviene¹

1 Screening of Cellular Networks, Bioquant, INF 267, University of Heidelberg; 2 Structural and Computational Biology, European Molecular Biology Laboratory, Meyerhofstraße 1, Heidelberg; 3 RNAi Screening Facility, Bioquant, INF 267, University of Heidelberg

Non-coding RNAs (ncRNAs) are characterized as functional transcripts that do not code for proteins (Gorodkin & Hofacker, 2011). Long being considered as 'junk', in recent years they are found to be functional in many processes ranging from transcriptional and post-transcriptional gene silencing to epigenetic control (Wright & Bruford, 2011). They can be functionally categorized into two classes: housekeeping ncRNAs, such as tRNA or rRNA, and regulatory ncRNAs, such as miRNA or siRNA (Costa, 2010). The ncRNAs are generally divided into another two classes 'long' (>200bp) and 'short' (<200bp), based on arbitrary length cut-off. We have focused on one class of ncRNA, namely, pseudogenes to assess their potential function in secretory membrane trafficking. A bioinformatics pipeline has been developed to search for putative transcribed pseudogenes of RAB and ARF GTPases, and 30 of them have been found. 3 pseudogenes were specifically down-regulated and had an influence on secretion of ts-O45-G, transferrin endocytosis and the integrity of the Golgi complex under these conditions. Further work to investigate the co-regulation of parent gene – pseudogene will be necessary, however, our observations strongly suggest that pseudogenes might be novel regulators of secretory membrane trafficking.

15 The Mitochondrial Carnitine/Acylcarnitine Carrier: Identification of Residues that Interact With the Substrates by Site-Directed Mutagenesis and Docking Approach

Lara Console¹, Annamaria Tonazzi^{1,2}, Nicola Giangregorio^{1,2}, Ferdinando Palmieri¹, Cesare Indiveri³.

1 Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari, 70125 Bari, Italy, 2 CNR Institute of Biomembranes and Bioenergetics, via Orabona 4, 70125 Bari, Italy, 3 Department of Cellular Biology, University of Calabria, 87036 Arcavacata di Rende, Italy

The carnitine/acylcarnitine carrier (CAC) catalyses carnitine/acylcarnitines and carnitine/carnitine antiports. It belongs to the mitochondrial carrier protein family [1]. Its 3D structure was constructed by homology modelling on the basis of the ADP/ATP carrier structure, which is the sole resolved by X-ray crystallography. The CAC structural model shows six hydrophobic transmembrane segments (H1–H6) which surround a central cavity closed toward the matrix side by a network of charged residues forming salt bridges [2]. Our studies have shown that two of these amino acids, K35 and E132, play a crucial role in opening the matrix gate and also demonstrated that D179 and R275, that face the cavity, bind carnitine during the transition phase [3]. The binding site for acylcarnitines requires additional hydrophobic amino acid residues interacting with the acyl groups. All the hydrophobic residues facing the water filled cavity have been mutated. The mutants reconstituted in liposomes, exhibited transport activity ranging from 20% to 100% of the WT. Since acylcarnitines are pure competitive inhibitors towards carnitine, their interaction with the transporter has been evaluated using acylcarnitines as inhibitors of ³H-carnitine/carnitine antiport. We have studied the effect of mutations on the inhibition of the carrier by acylcarnitines from 2 to 16 carbon chain length, as percent loss inhibition respect to WT. Mutants V25A, P78A, C89A and A279G highlighted loss of inhibition by all the acylcarnitines tested. Whereas M85A showed reduction of the inhibition by long (C8-C16) acylcarnitines. The experimental data interpreted in the light of the homology model of CAC show that the amino acids involved in the binding of the hydrophobic moiety of acylcarnitines are grouped in a half-shell lying on H1, H2 and H6. The results have been validated from docking analysis using the software ARGUSLAB.

(1) C Indiveri, V Iacobazzi, N Giangregorio, F Palmieri, 1997 *Biochem J* 321, 713-719. (2) E Pebay-Peyroula, C Dahout-Gonzalez, R Kahn, V Trezeguet, GJ Lauquin, G Brandolin, 2003 *Nature* 426, 39-44. (3) N Giangregorio, A Tonazzi, L Console, C Indiveri, F Palmieri, 2010 *Biochim Biophys Acta* 1797, 839-845

16 Engineering Peptides for Targeting the Microphthalmia-Associated Transcription Factor (Mitf)

Tim Kükenshöner, Jean-Christoph Niemöller, Janina Speck, Kristian M. Müller and Katja M. Arndt

Molecular Biotechnology, University of Potsdam, Institute for Biochemistry and Biology, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany

Microphthalmia-associated transcription factor (Mitf) is a basic helix-loop-helix leucine zippers transcription factor involved in key processes of cellular control mechanisms. Mitf forms homodimers binding to E-box and M-box DNA and activate the transcription of various target genes known to be involved in e.g. skin pigmentation. Importantly, Mitf also plays a major role in skin cancer progression of malignant melanoma. In this work, we selected two peptides from rationally designed libraries targeting the coiled coil domains of Mitf and Tfec, respectively, using an *E. coli*-based “hitchhiker” selection system. Both peptides show strong binding to their target sequence as determined by circular dichroism spectroscopy and ELISA. Midpoints of thermal unfolding were shifted by ΔT_m values of 15°C or 25°C, respectively, compared to wild type as elucidated by thermal denaturation measurements. Further characterizations were undertaken to describe the structure and the pattern of the hetero-complexes. With this work, we demonstrated an evolutionary approach to generate peptides with high affinity and specificity to intracellular protein targets.

NOTES

Impressum

Molecular Interactions e.V.
Schloßstr. 25
12163 Berlin
Vereinsregister: VR 28820 B

Layout und Redaktion

Ina Pokorny und Susanne Hollmann
Profilbereich Pflanzengenomforschung und Systembiologie
Universität Potsdam
Karl-Liebknecht-Str. 24-25
14476 Potsdam

Druck

Audiovisuelles Zentrum AVZ
Universität Potsdam
Am Neuen Palais 10
14469 Potsdam

MI 2011 is being supported by

