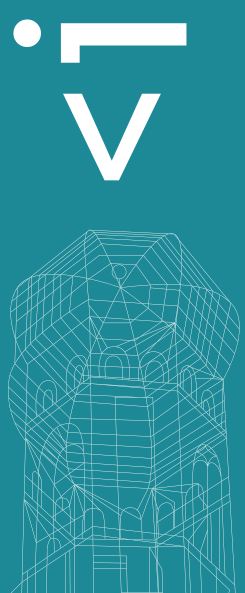
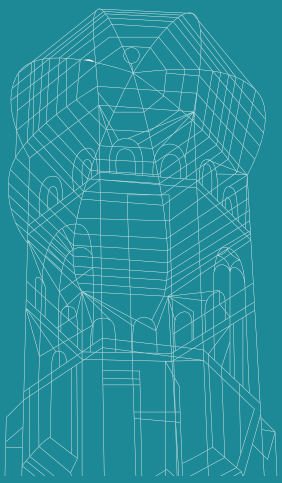
The Munich Life Science Symposium for Young Scientists

<interact>

2012

Life.
Science.
Community.





March 29th | Pre-event | Old City Hall
March 30th | Symposium | LMU Main Building

IMPRESSUM

Published by <interact> 2012

c/o Michael Breckwoldt
Institute of Neuroscience, TUM
Biedersteiner Str. 29
80802 Munich, Germany

Phone: +49-89-4140-3330
Email: Michael.breckwoldt@lrz.tum.de

@ info@interact-munich.org
www.munich-interact.org

Editors
Irina Ionescu, Katrin Schneider, Bianca-Sabrina Targosz, Katharina Thanisch, Christina Wolf

Design
Katharina Thanisch, Christina Wolf

Welcome to <interact> 2012!

Dear Young Scientist,

we are happy to welcome you to the <interact> symposium 2012 at Ludwig-Maximilians Universität München! This year the symposium hosts 400 registered participants and over 100 scientific contributions.

Over the last five years the <interact> symposium has established a platform for young scientists to present their scientific work and to extend their network. This year the talks will be subdivided in parallel sessions framed by short methodological tutorials and poster presentations. Highlights of the symposium will be the keynote lectures by Ada Yonath, Nobel Prize in Chemistry 2009 for solving the ribosome structure, and by Gero Miesenböck who did pioneering work in the new field of optogenetics.

To celebrate our 5th anniversary, this year's <interact> includes for the first time a special pre-event, held in the relaxing atmosphere of the Old City Hall. Our pre-event speaker Herbert Jäckle, Vice President of the Max-Planck Society, will give an inspiring outlook towards "Science in the year 2050" which is followed by a reception. The key goal of <interact> is to foster communication and discussion. Take the opportunity to talk about your work, exchange your ideas and get valuable input. In order to connect and to get to know each other also join our traditional symposium party which will complete the day. We hope you will enjoy the event. We wish you two inspiring and exciting days!

Your organizing team

Little guide of the day

Location

- You will find a map of the location on page 23 and public transport information from page 159 to 165.
- On the ground floor, an unsupervised cloakroom is available (A002).
- Keynote lectures will be held in the Große Aula.
- Parallel sessions (PS) will take place in four rooms designated PS I to IV.
- WLAN access will be provided in the rooms listed. For connection details refer to page 156.
  - Große Aula
  - Lichthof
  - Dekanatsgang
  - Speerträger
  - Senatssaal
- Lunch and dinner will be served at the Lichthof. For menu details refer to page 173.

Talks

- Talks are subdivided into seven categories:
  - New Methods
  - Neurobiology
  - Microbiology
  - Regulation of Gene Expression
  - Translational Research
  - Systems Biology
  - Cell Biology

- We have one morning and one afternoon session with parallel talks in four categories, each (overview on pages 18 to 21).

- Pick one category for the morning session and one for the afternoon session. Please, do not disturb the speakers and the audience by changing rooms during the session.

Poster Sessions

- Posters will be presented in the Senatssaal, the Thomas-Mann-Halle and in the Dekanatsgang.

- Posters are subdivided into four categories:
  - New Methods
  - Neurobiology
  - Molecular & Cell Biology
  - Bioinformatics & Translationa Research

- Poster abstracts are sorted in each category by first author name in alphabetical order (index, pages 66 to 72).

- Poster presentations are devided into two poster sessions. Odd numbers will be presented in the morning, even numbers in the afternoon.

Methods Seminar

- After the talk session in the afternoon, we proudly present four parallel method seminars in the PS rooms.

- If you want to change the room before the seminar, you can do this within the 10 min break.

Industry & Institution Stalls

- Inform yourself at the stalls of companies and institutions in the Senatssaal and at the Speerträger.

- We will have two 20 min workshops by Molecular Devices. They will present the SpectraMax®Paradigm®Microplate Reader during the poster sessions at the Senatssaal (page 150).

Awards

- You can vote for the best poster and for the best talk. We need your voice even if you haven't seen all posters or heard all the talks (page 157).

- You'll find the voting sheets in your booklet.

- Please drop them off before 5.00 pm at the registration desk.

- Every voter will recieve our <interact> 2012 cup.

- Winners will receive great prizes and are announced after dinner.

Party

- The party will take place after the announcement of the award winners at the CADU (Cafe an der Uni) (page172).

- Everyone is invited. You will find a coin for one free drink in your goodie bag.

Content

Introductory Note by the President of the LMU, Prof. Dr. Bernd Huber

Ladies and Gentlemen,

It is a great pleasure for me to welcome you to Ludwig-Maximilians-Universität München for the <interact> Symposium 2012. LMU is proud to host the fifth of these interdisciplinary life science conferences, which were initiated and are organized entirely by Munich-based doctoral students.

Among the scientific disciplines represented in the Munich area, the Life Sciences are a particularly thriving and successful field.
The <interact> Symposium not only benefits from this highly dynamic community, but makes a very valuable contribution to it by bringing together young Life Scientists to present their research, develop and share new ideas, and converse with internationally renowned experts.

The impressive list of distinguished keynote speakers reflects the high regard this conference enjoys within the scientific community.
The fact that it is now open to postdocs and Master's students, as well as members of International Max-Planck Research Schools from all over Germany, further increases its significance as a forum for wide-ranging scientific discussions and exchange.

I wish you all a very successful conference in these inspiring surroundings. I hope your discussions will be fruitful and that you will find many occasions for networking with your colleagues.

— Prof. Dr. Bernd Huber

Introductory Note by the Mayor of Munich, Christian Ude

The European metropolitan area Munich is, with its total of 13 universities, academies and colleges, the second-largest academic center in Germany. The excellent rank and reputation enjoyed by this area across Germany as well as internationally is supported by the numerous academic and non-academic research facilities in and around Munich. I would like to mention among others the two Munich elite universities, the Ludwig-Maximilians-University and the Technical University of Munich, the institutes of the Fraunhofer Society and of the Max Planck Society as well as the numerous biotech and pharmaceutical enterprises based in Munich. The close network between academic and non-academic research has led to the selection of the Munich Biotechnology Cluster as a "top cluster" by the Federal Ministry of Research and Education in 2010. At present, access to science and the exchange between scientists continue to be actively promoted in Munich.

The Munich <interact> Symposium, an event organized by Munich PhD students for young scientists from all over Germany, takes place this year at Ludwig-Maximilians-University and is celebrating its fifth anniversary. As in past years, <interact> offers PhD students, young scientists and students of the life sciences a platform for promoting communication and networking among the various research areas. It is therefore with great pleasure that I act this year again as patron for this event. I wish the symposium a successful 5th meeting.

— Christian Ude

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Pre-event

For the first time, there will be a pre-event with a keynote lecture by Herbert Jäckle, Vice President of the Max-Planck Society. The event takes place on March 29th at the Old City Hall at Marienplatz. After the talk we will have time for discussion and the opportunity to socialize with the other young scientists during a wine and cheese reception. For the lovers of Bavarian culture there will also be "Bier und Brezn".

Schedule

06.15 - 07.15 pm	*Check-in*
07.15 - 07.30 pm	*Welcome Words*
07.30 - 08.30 pm	*Keynote Talk: Herbert Jäckle*
	Science 2050: A glance back into the future
08.30 - 09.00 pm	*Discussion*
09.00 - 11.00 pm	*Cheese & Wine, "Bier und Brezn"*

Wikipedia - Richard Huber

Presse- und Informationsamt München

Herbert Jäckle

A short biographical sketch

Dr. Herbert Jäckle is Director at the Max-Planck-Institut für biophysikalische Chemie (Göttingen, Germany) and Vice-President of the Max Planck Society. He studied Chemistry and Biology (Universität Freiburg) and spent his postdoc at the University of Texas at Austin (USA). He held positions as staff scientist at the EMBL (Heidelberg), as research group leader (Max-Planck-Institut für Entwicklungsbiologie, Tübingen) and as professor for genetics (Ludwig Maximilian Universität, München).

Dr. Jäckle is a member of EMBO, the Academia Europaea, and German Academies of Sciences (Leopoldina and Göttingen). He obtained several scientific awards (including the Gottfried Wilhelm Leibniz Prize, the Otto Bayer Prize and the Louis Jeantet Prize for Medicine), and serves on Advisory Boards both in academia and industry (e.g. EMBL, Biocenter of the University Basle, Boehringer Ingelheim Foundation, DeveloGen AG).

Using the fruitfly Drosophila melanogaster as a model organism, Dr. Jäckle's research is focussed on molecular mechanisms (biochemical pathways and regulatory networks) involved in embryonic pattern formation (segmental body organization, formation of organs). More recent work ("molecular physiology") aims to understanding the genetic and molecular basis of cellular and organismal energy homeostasis. Herbert Jäckle is author of more than 200 scientific articles.

Keynote Talk

Science 2050: A glance back into the future.

Vice-President of the Max Planck Society and Director at the Max Planck Institute for Biophysical Chemistry, Göttingen

Schedule Symposium

08.00 – 08.45 am	*Check-in*
09.00 – 09:15 am	*Welcome Words*
09.15 – 10.20 am	*Keynote Talk I : Gero Miesenböck*
10.20 – 10.40 am	*Refreshments*
10.40 – 11.45 am	*Morning Parallel Sessions*
11.45– 01.00 am	*Coffee Break and Poster Session I*
01.00 – 02.00 pm	*Lunch*
02.00 – 03.20 pm	*Afternoon Parallel Sessions*
03.30 – 04.00 pm	*Methods Seminar*
04.00 – 05.15 pm	*Coffee Break and Poster Session II*
05.15 – 06.15 pm	*Keynote Talk II: Ada Yonath*
06.15 – 06.30 pm	*Final Words*
06.30 – 07.30 pm	*Dinner*
07.30 - 07.45 pm	*Poster & Talk Awards*
07.45 - open end	*Party*

Gero Miesenböck

A short biographical sketch

Gero Miesenböck is Waynflete Professor of Physiology and Director of the Centre for Neural Circuits and Behaviour at the University of Oxford. A native of Austria, he received a medical degree from the University of Innsbruck in 1993 and then moved to the United States as a postdoctoral fellow with James Rothman. Before coming to Oxford in 2007, he held faculty appointments at Memorial Sloan-Kettering Cancer Center in New York and at Yale University.

Abstract

Lighting Up the Brain

Department of Physiology, Anatomy and Genetics, University of Oxford, gero.miesenboeck@dpag.ox.ac.uk

An emerging set of methods enables an experimental dialogue with biological systems composed of many interacting cell types - in particular, with neural circuits in the brain. These methods are sometimes called "optogenetic" because they employ light-responsive proteins ("opto-") encoded in DNA ("-genetic"). Optogenetic devices can be introduced into tissues or whole organisms by genetic manipulation and be expressed in anatomically or functionally defined groups of cells. Two kinds of devices perform complementary functions: light-driven actuators control electrochemical signals; light-emitting sensors report them. Actuators pose questions by delivering targeted perturbations; sensors (and other measurements) signal answers. Optogenetic approaches are beginning to yield previously unattainable insight into the organization of neural circuits, the regulation of their collective dynamics, and the causal relationships between cellular activity patterns and behavior.

Ada Yonath

A SHORT BIOGRAPHICAL SKETCH

Prof. Ada Yonath studied at the Hebrew University, earned a Ph.D. degree from Weizmann Institute of Science (WIS) and completed her postdoctoral studies at Carnegie Mellon and MIT, USA. In the seventies she established the first laboratory for protein crystallography in Israel, which was the only laboratory of this kind in the country for almost a decade. Currently she is the Kimmel Professor of structural biology at WIS, and the Director of the Kimmelman Center for Biomolecular Structure and Assembly. In 1986-2004 she also headed the Max-Planck Research Unit for Ribosome Structure in Hamburg, Germany.

She is a member of the US National Academy of Sciences (NAS); the American Academy of Arts and Sciences; the Israel Academy of Sciences and Humanities; the European Academy of Sciences and Art; the European Molecular Biology Organization; the Korean Academy for Science and Technology, and the International Academy of Astronautics. She holds honorary doctorates from Oxford University in UK, New York University in USA, Oslo University in Norway, Fujou University in China and the Hebrew, Open, Tel Aviv, Ben Gurion and Bar Ilan Universities in Israel, and is an honorary supreme Prof. of KEK, Japan.

Her awards include the 1st European Crystallography Prize; the Israel Prize; the Paul Karrer Gold Medal; the Israel Prime Minister EMET award; the Rothschild Prize; the Louisa Gross Horwitz Prize; the Paul Ehrlich-Ludwig Medal; the Linus Pauling Gold Medal; the Anfinsen Prize; the Wolf Prize; the Massry Award and Medal; the UNESCO Award for Women in Science; the Albert Einstein World Award for Excellence; the Erice Prize for Peace; the DESY pin; the Eminent Scientists Award of the Japan Society for Promotion of Science; Honorary Supreme Prof of KEK, Photon Factory, Tsukuba, Japan; the Exner Medal, Austria; the Indian Prime Minister Gold Medal; the President of Panama medal; the WISH Award, Lausanne; the Maria Sklodowska-Curie Medal, Poland; the Nobel Prize for Chemistry.

Yonath is using X-ray crystallography supported by molecular biology, mutagenesis and biophysical methods to investigate protein biosynthesis. She is focusing on the ribosome, the cellular particle translating the genetic code into proteins, on its origin and on its inhibition by antibiotics.

Ribosomes are the universal cellular universal machines with stunning intricate architecture accompanied by inherent mobility, which facilitate their smooth performance as polymerases that translate the genetic code into proteins. The site for peptide bond formation, which is composed solely of RNA moieties, is located within a universal internal semi-symmetrical region connecting all of the remote ribosomal features involved in it functions. The elaborate architecture of this region positions ribosomal substrates in appropriates stereochemistry for peptide bond formation, for substrate-mediated catalysis, and for substrate translocation and for nascent chain elongation. The high conservation of the symmetrical region implies its existence irrespective of environmental conditions and indicates that it may represent a prebiotic RNA bonding machine, which is still functioning in the contemporary ribosome.

ABSTRACT

The spectacular ribosome architecture clues about its origin

Department of Structural Biology, Weizmann Institute, Rehovot, Israel, ada.yonath@weizmann.ac.il

Morning Parallel Sessions

PS 1 — New Methods I

10:40 -11:00 — David Brucker
Neurotax-Optical needle endoscopy for fluorescence guide biopsy sampling in brain tumor diagnostics

11:00 -11:20 — Claudia Wolff
Molecular profiling of protein networks in cancer tissues using reverse phase protein arrays

11:20 -11:40 — Juan Antonio Aguilar-Pimentel
New high-throughput strategies for detection of immunodeficient mouse models using a systemic screening approach

PS 2 — Neurobiology

10:40 -11:00 — Shahaf Peleg
A little tweaking of histone acetylation for treating memory loss in aging

11:00 -11:20 — Catherine Sorbara
In vivo imaging of axonal transport reveals early transport-block in animal model of multiple sclerosis

11:20 -11:40 — Jenia Jitsev
Learning from positive and negative rewards in a spiking neural network model of the basal ganglia

Microbiology — PS 3

Malte Gersch — 10:40 -11:00
Structure and inhibition of the bacterial virulence regulator ClpP

Daniel Sauter — 11:00 -11:20
Disarming the cell - how pandemic HIV conquered the world

Madlen Pogoda — 11:20 -11:40
Characterization of conserved region two (CR2) deficient mutants of the cytomegalovirus egress protein pM53

Regulation of Gene Expression — PS 4

Lisa Marcinowski — 10:40 -11:00
Real-time transcriptional profiling of cellular and viral gene expression during lytic cytomegalovirus infection

Jörg Renkawitz — 11:00 -11:20
Monitoring Homology Search during DNA Double-Strand Break Repair

Anne Krämer — 11:20 -11:40
Identification and functional analysis of radiation-induced microRNA changes in endothelial cells

Afternoon Parallel Sessions

PS 1 — New Methods II

14:00 - 14:20 — Christoph Lippert
FaST linear mixed models for genome-wide association studies

14:20 - 14:40 — Tamas Schauer
Dissecting gene activity within the Drosophila head

14:40 - 15:00 — Anselm Geiger
Biophysical characterization and modular design of FRET based biosensors

15:00 - 15:20 — Minh Schumacher
Imaging calcium levels in immune-mediated axon degeneration

PS 2 — Systems Biology

14:00 - 14:20 — Daniel Schulz
Comparative Dynamic Transcriptome Analysis (cDTA) reveals mutual feedback between RNA synthesis and degradation

14:20 - 14:40 — Desislava Boyanova
Systems biological analysis of the human platelet proteome network

14:40 - 15:00 — Marco Y. Hein
A systems biology perspective of protein complexes by quantitative interactomics and deep proteome sequencing

15:00 - 15:20 — Stefan Brandmaier
Performance, Reliability and Robustness - A comparison of several experimental design strategies

Translational Research

PS 3

Nicole Teichmann — 14:00 - 14:20
The novel allosteric MEK1/2 inhibitor BAY86-9766, a promising therapeutic agent for the treatment of pancreatic cancer

Marc Taenzer — 14:20 - 14:40
A novel epigenetic biomarker for predicting 5-FU response in colorectal cancer

Mihaela Culmes — 14:40 - 15:00
Analysis of DNA Methylation, DNA Methyltransferases, and Histone Modifications in Human Atherosclerotic Lesions

Adanela Musaraj — 15:00 - 15:20
Metabolic and Respirators Acidosis in Cerebrospinal Fluid, studied in COPD Albanian patients

Cell Biology

PS 4

Shun-ichi Yamashita — 14:00 - 14:20
Induction of peroxisome degradation by overloading of peroxisome membrane protein in mammalian cells

Cornelia Schönbauer — 14:20 - 14:40
Spalt mediates an evolutionarily conserved switch to fibrillar muscle fate in insects

Eva Rath — 14:40 - 15:00
Mitochondrial unfolded protein response protects mitochondria under inflammatory conditions

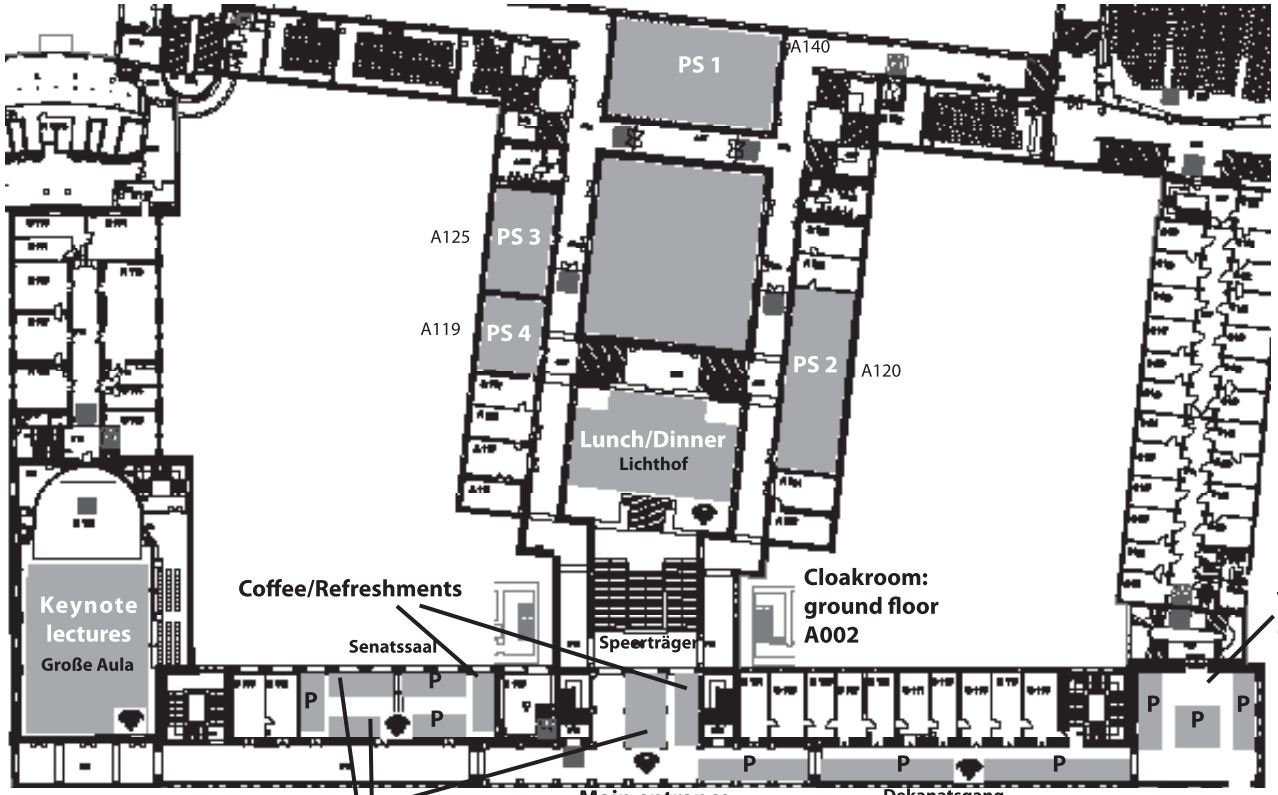
Paulina J. Paszkiewicz — 15:00 - 15:20
Selection and depletion of T cells – engineered with a truncated EGFR marker

Afternoon Methods Seminars

15:30 -16:00	Methods Seminars

PS 1	**Andreas Binder** *Introduction to Adobe Illustrator for Scientific Illustrations*
PS 2	**Marco Düring** *Statistics in biology: Why the t-test is usually the wrong choice!*
PS 3	**Philipp Baaske** *Biomolecular <interact>ions: some like it hot*
PS 4	**Günter Jäger** *Mayday - integrative analytics for expression data*

Map


First Floor LMU Main Building
PS 1
A140
A125
PS 3
A119
PS 4
PS 2
A120
Lunch/Dinner
Lichthof
Keynote lectures
Große Aula
Coffee/Refreshments
Senatssaal
Speerträger
Cloakroom: ground floor A002
Thomas-Mann-Halle
P Poster
WLAN (SSID: con, unprotected)
PS: parallel session
Companies
Main entrance
Dekanatsgang

Speakers

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LMU

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Claudia Wolff

TUM

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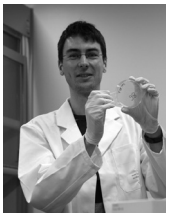

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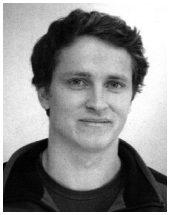

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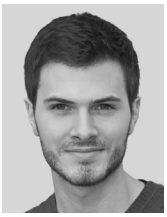

A systems biology perspective of protein complexes by quantitative interactomics and deep proteome sequencing

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Helmholtz Center Munich

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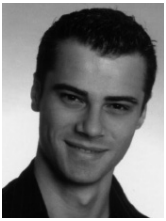
Mayday - integrative analytics for expression data



Talks & Methods Seminars

Neurotax-Optical needle endoscopy for fluorescence guided biopsy sampling in brain tumor diagnostics

David Brucker[1], Werner Goebel, Yvonne Kienast, Ann Johannson, Gesa Palte, Adrian Rühm, Sabina Eigenbrot, Markus Goetz, Friedrich-Wilhelm Kreth, Andre Ehrhardt, Herbert Stepp, Klaus-Martin Irion, Jochen Herms
1 davidbrucker@med.uni.muenchen.de
LMU Munich

Proper treatment of brain tumors requires histological sampling through stereotactic biopsy . MRI-guided tissue sampling in the brain is commonly used, but bears the risk of incorrect sampling location or fatal damage to cerebral blood vessels.
Here, we present a novel contact endoscopic probe that allows selective fluorescence detection of tumors and cerebral blood vessels. With an outer diameter of only 1,5mm, the probe is suited for minimal invasive biopsy procedures. We used 5-ALA to selectively label tumor cells and ICG to detect blood vessels. The capability of the system was evaluated in vitro, followed by in vivo studies in a mouse model of glioma. Eventually, a successful application in clinical pilot case was carried out.
We expect sterotactical intervention to become safer and more precise through fluorescence based approaches.

Molecular profiling of protein networks in cancer tissues using reverse phase protein arrays

Claudia Wolff, Katharina Malinowsky, Karl-Friedrich Becker
Institute of Pathology, Technische Universität München, Munich, Germany

Mapping tumour cell protein networks in routinely processed clinical samples, such as formalin-fixed and paraffin-embedded (FFPE) cancer tissues, will be critical for realizing the promise of personalized molecular therapy as only a subset of patients will respond. The quantification and characterisation of deregulated signalling molecules (e.g. human epidermal growth factor 2) are very promising candidates for the identification of new suitable therapy targets and for the selection of those patients who will receive the greatest benefit from individualised treatments. In this regard the reverse phase protein array (RPPA) is a very promising new technology for quick and simultaneous analysis of many patient samples allowing relative and absolute protein quantifications. Importantly, it requires only very small amounts of routine clinical material (e.g. biopsies). Although very challenging, we have recently established a method for efficient protein extraction from routine FFPE tissues. In combination with the RPPA technology new avenues for extract-based precise quantification of biomarkers for therapy decisions in routinely used cancer tissues are now open. We have successfully applied our approach to identify deregulated signalling networks and suggest new biomarkers in cancer tissues of the breast, endometrium, ovary, pancreas, and oesophagus.

Wolff C, Schott C, Porschewski P, Reischauer B, Becker KF. Successful protein extraction from over-fixed and long-term stored formalin-fixed tissues. PLoS One. 2011 Jan; 6(1):e16353.
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New high-throughput strategies for detection of immunodeficient mouse models using a systemic screening approach

Dr. rer. nat. Juan Antonio Aguilar-Pimentel[1], Dr. Thure Adler[2], Dr. Valerie Gailus-Durner[3], Dr. Helmut Fuchs[4]

1 Technische Universität München, Department of Dermatology and Allergy Biederstein, Munich, Germany
2 Technische Universität München, Institute for Medical Microbiology, Immunology, and Hygiene, Munich, Germany
3 Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Experimental Genetics, Munich, Germany
4 Center of Allergy and Environment (ZAUM), Technische Universität München and Helmholtz Zentrum München, German Research Center for Environmental Health, Munich, Germany

The large-scale generation of mouse mutant lines has been well established by now, thus raising the potential of mouse models for immunologic and allergic diseases as another primary source of information. To identify phenotypic alterations in mice, a systemic screening platform able to detect new phenotypes under baseline and/or challenge conditions with a large number of animals in a short time frame together with a limited amount of biologic sample is required. The aim of this study was to establish such high-throughput technologies for detecting Immunodeficient phenotypes in the German Mouse Clinic. Immunoglobulin levels in murine plasma are used as first-line screening parameter. Mutant lines showing an interesting phenotype are subjected to a more in-depth assessment using challenge platform. This includes a model of sensitization and aerosol challenge with an antigen. Alterations of all immunoglobulin isotypes can be rapidly monitored from a single plasma sample by using a Luminex bead-array technology. Cell fractions (eosinophils, neutrophils, MΦ, T and B lymphocytes, NK cells) from bronchoalveolar lavage (BAL) are analyzed by one-step, single staining multi-color flow cytometry. A second one-step staining is used for the immune phenotyping of the lymphocyte compartment. Additionally, a single-step quantification of multiple cytokines from BAL fluid is performed. All methods proofed to be highly useful for the high-throughput phenotypic Immune response analysis in large cohorts of animals. We have successfully established a systemic phenotypic screening platform to identify distinct gene functions in mutant mouse lines that enhance Immune/allergic disease in the murine model. These high-throughput technologies are likely to provide important advances with regard to pathophysiology, diagnosis, and therapy of human diseases.

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A little tweaking of histone acetylation for treating memory loss in aging

Dr. Shahaf Peleg
LMU Munich

Thanks to incredible advances in technology and medicine, human life expectancy has dramatically increased. Compared to our ancestors, who lived for an average of 30 years during Roman times, we can now look forward to an average life nearly three times as long. As the fraction of elderly in the population increases, new health challenges have surfaced, which demand our attention as scientists. One of them is age-associated memory impairment (AAMI), which manifests itself to various degrees of severity among the vast majority of the older population and dramatically decreases our quality of life. Advanced forms of AAMI can result in dementia, including Alzheimer's disease. Extremely little is known about the molecular mechanisms that contribute to AAMI, so – hardly surprisingly – there is no effective therapy for it. Here I will discuss recent results suggesting that deregulated hippocampal histone acetylation might be one of the causes for AAMI and therefore may serve as a novel target for treating it.

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In vivo imaging of axonal transport reveals early transport block in animal model of multiple sclerosis

Catherine Sorbara[1], Ivana Nikic[1], Doron Merkler[2], Martin Kerschensteiner[1], Thomas Misgeld[3]
1 Research Unit Therapy Development, Institute of Clinical Neuroimmunology, Ludwig-Maximilians University, Munich, Germany
2 Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland
3 Chair of Biomolecular Sensors, Center for Integrated Protein Sciences (Munich) at the Institute of Neuroscience, Technische Universität München, Munich, Germany

Multiple Sclerosis (MS) is an inflammatory disease of the human central nervous systen and a major cause of disability in young adults due to the progressive loss of axons. Recently, our lab describes a process entitled Focal Axonal Degeneration (FAD) that underlies axon loss in a mouse model of MS. In the current study we wanted to understand when and how the function of axons is affected during the damage process. The function we chose to analyse is axonal transport. This plays a key role in maintaining neuronal viability and tranport disturbances have been proposed to contribute to neurodegeneration. When and how axonal transport deficits arise in neuroinflammatory conditions is incompletely understood.

To address this question we first inverstigated the accumulation of multiple transport cargos in an animal model of MS. Our data show that while accumulations of synaptic vesicles and SPP are only detected in the advanced stages of axon damage, mitochondrial accumulations can already be detected prior to changes in axon morphology. This suggests that disturbances in axonal transport of mitochondria are an early step in the pathogenesis of axon damage. To directly investigate this hypothesis, we developed a method to image axonal transport on mitochondria in the mouse spinal cord in vivo using multiphoton imaging and transgenic mice with fluorescently labelled mitochondria. Our data show that axonal transport rates are dramatically reduced in acute neuroinflammatory lesions but recover early in disease remission. Axonal trasport is nearly abolished in intermediate and advanced stages of axon damage and is even substantially reduced in normal-appearing axons in or close to neuroinflammatory lesions at the peak of disease. Interestingly, tranport deficits primarily affect the anterograde transport while the retrograde transport in normal-appearing axons remains largely unchanged.

Learning from positive and negative rewards in a spiking neural network model of the basal ganglia

Jenia Jitsev[1], Nobi Abraham[1], Abigail Morrison[2], Marc Tittgemeyer[1]
1 Department of Cortical Networks and Cognitive Functions, Max-Planck-Institute for Neurological Research, Gleueler Str. 50, 50931 Cologne, Germany
2 Functional Neural Circuits Group, the Faculty of Biology and Bernstein Center Freiburg, Albert-Ludwig University of Freiburg, Hansastr. 9a, 79104 Freiburg, Germany

A fundamental property of the nervous system is its ability to learn from consequences of self-generated behavior by adapting itself to both maximize reward and avoid punishment. The basal ganglia is a network of subcortical nuclei that is very likely involved in reinforcement learning. Although vast experimental evidence has been gathered regarding the role of the basal ganglia in reward-based learning, it remains unclear how various local mechanisms of synaptic plasticity implement reinforcement learning in the basal ganglia network and lead to behavioral changes at the system level. Aiming towards a minimal realistic model of learning from both positive and negative rewards, we implemented a spiking actor-critic network model of the basal ganglia that incorporates the segregation of both the dorsal and ventral striatum into populations of D1 and D2 medium spiny neurons (MSNs). This segregation allows explicit, separate representation of both positive and negative expected outcomes within the respective population. Based on recent experiments [1], D1 and D2 MSN populations were assumed to have distinct, opposing dopamine-modulated bidirectional synaptic plasticity. We implemented the network in the NEST simulator [2] and performed experiments involving the application of delayed rewards in a grid world setting, in which a moving agent must reach a goal state while maximizing the total reward obtained [3]. We demonstrate that the network can learn both to approach the delayed positive reward and to consequently avoid punishment. Thus, the spiking neural network model highlights the functional role of D1/D2 MSN segregation within the striatum and explains the necessity for the reversed direction of dopamine-dependent plasticity that occurs at synapses converging on distinct types of striatal MSNs.

This work was supported by the German Research Foundation (KFO 219), by the Helmholtz Alliance on Systems Biology, Neurex and the Junior Professor Program of Baden-Württemberg.

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Structure and inhibition of the bacterial virulence regulator ClpP

Malte Gersch[1], Anja List[1], Felix Gut[2], Vadim Korotkov[1], Gerhard Klebe[2], Michael Groll[1], Stephan A. Sieber[1]

1 Center for Integrated Protein Science Munich (CIPSM), Technische Universität München, Department of Chemistry, Lichtenbergstr. 4, 85747 Garching, Germany

2 Department of Pharmaceutical Chemistry, Philipps University Marburg, Marbacher Weg 6, 35032 Marburg, Germany

IIn 2011, multiresistant Staphylococcus aureus (MRSA) was already found in one of four clinical isolates in Germany. The rising threat through these bacterial pathogens establishes an urgent need for novel ways of treatment for infectious diseases. A new concept focuses on the reduction of bacterial virulence rather than viability.(1)

The barrel-shaped ClpP protease is a highly conserved virulence regulator in bacterial pathogens. Its inhibition by β-lactones was shown to lead to a drastic decrease in the expression of virulence factors in S. aureus.(2) We will present work that focuses on two aspects: The structural characterization of the tetradecameric ClpP forms the basis for an improved understanding of protein function and inhibition. It was recently shown that SaClpP is able to adopt a compressed, inactive conformation.(3) We have solved the 2.3 Å resolution structure of SaClpP in its closed, active conformation as well as the structure of the S98A mutant. Comprehensive mutational analysis was able to pinpoint key residues involved in this catalytic switch and in intersubunit interaction. By probing the active site serine with a β-lacton probe, we could show that the tetradecameric organization is essential for a proper formation of the active site. Our data suggest that a highly conserved hydrogen-bonding network links oligomerization to activity. A comparison of ClpP structures from different organisms provides suggestive evidence for the presence of a universal mechanism regulating ClpP protease activity.

We then used the active conformation for docking studies to rationalize inhibitor binding. A hydrophobic pocket next to the active site accommodates aliphatic β-lacton substituents and leads to an ideal positioning of the warhead for reaction with the active site residue. A screen of enantiopure bisubstituted β-lactones provided insights into the stereo preference of the protease which is in accordance with molecular modelling. These results have provided guidance to the design and synthesis of improved β-lactone-based inhibitors.

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Disarming the cell - how pandemic HIV conquered the world

Daniel Sauter, Anke Specht, Daniel Unterweger, Michael Vogl, Susanne Engelhart, Ravindra K. Gupta, Michael Schindler, Jörg Votteler, Frederic Bibollet-Ruche, Martine Peeters, Beatrice H. Hahn, Theodora Hatziioannou, Frank Kirchhoff
Institute of Molecular Virology, Ulm University Medical Center

With more than 60 million infected people world-wide, HIV-1 is the major causative agent of AIDS. Until today, four different groups of HIV-1 (M, N, O and P) have been described. Each of them represents an independent cross-species transmission of simian immunodeficiency viruses from chimpanzees (SIVcpz) or gorillas (SIVgor) to humans [1]. Interestingly, only HIV-1 group M is responsible for the global AIDS pandemic, whereas the other groups are rare and remained largely restricted to Western Africa. The reasons for these differences in spread are unknown.

We hypothesized that efficient counteraction of the human antiviral factor tetherin, which inhibits the release of budding virions [2,3], might explain the particularly effective spread of HIV 1 group M in the human population.

Our results demonstrate that many SIVs including SIVcpz and SIVgor, the direct precursors of HIV-1, employ their Nef proteins to antagonize the tetherin orthologues of their cognate host species [4]. Human tetherin, however, contains a deletion in its cytoplasmic tail that renders it resistant to Nef and therefore poses a significant barrier to zoonotic transmissions of SIVs to humans [4-6]. This finding challenges the dogma that SIVcpz did not have to overcome major barriers to efficiently infect humans because humans and chimpanzees are genetically closely related. Furthermore, we found that only pandemic HIV-1 group M mastered the tetherin hurdle perfectly by switching from Nef to Vpu-mediated anti-tetherin activity. In contrast, Vpu proteins of non-pandemic HIV-1 group O and P strains do not antagonize tetherin and those of rare group N gained moderate anti-tetherin activity but lost their second function: the degradation of the viral receptor CD4 [4,5,7].

This fitness advantage of HIV-1 group M may explain why only one out of four independent cross-species transmissions of SIVs from apes to humans is responsible for the global AIDS pandemic.

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Characterization of conserved region two (CR2) deficient mutants of the cytomegalovirus egress protein pM53

Madlen Pogoda
Max von Pettenkofer-Institute, LMU

Dominant negative (DN) mutants are powerful tools to study protein-protein interactions of essential proteins. A systematic genetic screen of the essential murine cytomegalovirus (MCMV) protein pM53 revealed the accumulation of inhibitory mutants within conserved regions (CR) 2 and 4. The strong inhibitory potential of the CR4 mutants allowed description of a phenotype. The DN effect of the subtle mutations in CR2 was too weak for analysis. Here we constructed M53 alleles lacking CR2 in total or partially and analyzed the DN effect on MCMV replication upon conditional expression. Overexpression of CR2 deficient pM53 inhibited virus production about 10,000-fold. This was due to interference with capsid export from the nucleus and with viral genome cleavage/packaging. In addition, the fate of the nuclear envelopment complex (NEC) in the presence of DN pM53 overexpression was analyzed. The CR2 mutants were able to bind to pM50 albeit to a lesser extent than the wild type protein and re-localized the wild type NEC in infected cells. Unlike the CR4 DN, the CR2 DN mutants did not affect the stability of pM50.

Real-time transcriptional profiling of cellular and viral gene expression during lytic cytomegalovirus infection

Lisa Marcinowski[1], Michael Lidschreiber[2], Lukas Windhager[3], Martina Rieder[1], Jens Bosse[4], Bernd Rädle[1], Thomas Bonfert[3], Ildiko György[5], Olivia Prazeres da Costa[6], Philip Rosenstiel[7], Caroline Friedel[3], Ralf Zimmer[3], Zsolt Ruzsics[1], Lars Dölken[8]

1 Max von Pettenkofer-Institute, Ludwig-Maximilian-University, Munich, Germany
2 Gene Center and Department of Biochemistry, Ludwig-Maximilians-University, Munich, Germany
3 Institute for Informatics, Ludwig Maximilians-University, Munich, Germany
4 Department of Molecular Biology, Princeton University, Princeton, USA
5 School of Biomedical and Biological Sciences, Centre for Research in Translational Biomedicine, Plymouth University, Plymouth, UK
6 Institute for Microbiology, Immunology and Hygiene, Technical University, Munich, Germany
7 Institute of Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany
8 Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK

During lytic viral infection cellular gene expression is subjected to alterations induced by both viral and antiviral mechanisms. In this study, we employed metabolic labelling of newly transcribed RNA using 4-thiouridine (4sU-tagging) coupled to qRT-PCR, microarray analysis and RNA-Seq to study the real-time kinetics of transcriptional activities during lytic murine cytomegalovirus (MCMV) infection.

Microarray profiling on newly transcribed RNA during the first six hours of murine cytomegalovirus infection revealed discrete functional clusters of cellular genes regulated with distinct kinetics at surprising temporal resolution. Among others, this included a rapid inflammatory/interferon-response, a transient DNA-damage-response and a delayed ER-stress-response which were all rapidly counter-regulated by the virus implying the involvement of novel viral regulators. It is important to note that most of these changes (the rapid counter-regulations in particular) were inherently undetectable by conventional total RNA purifcation. Promoter analysis revealed strong associations of distinct transcription factors with each functional cluster.

Furthermore, metabolic labeling and purification of newly transcribed RNA provides access to the real-time kinetics of viral gene expression in the absence of any interfering virion-associated RNA. Both, qRT-PCR and RNA-seq analysis on newly transcribed RNA derived from various time frames of whole lytic virus life cycle revealed a peak of viral transcriptional activity at 1-2 hpi, including transcription of immediate-early, early and even well characterized late genes. This peak of transcription was subject to rapid gene silencing until the onset of DNA replication which indicates the involvement of so far undisclosed molecular mechanism in the regulation of viral gene expression. In summary, this study pioneers real-time transcriptional analysis during lytic herpesvirus infection and highlights numerous novel regulatory aspects of virus-host-cell interaction.

Monitoring Homology Search during DNA Double-Strand Break Repair

Jörg Renkawitz, Marian Kalocsay, Stefan Jentsch
MPI Biochemistry

DNA double-strand breaks (DSBs) are the most toxic lesions in the genome as they can cause genomic rearrangements, chromosome loss, cell death and cancer. Cells respond by initiating either error-prone non-homologous-endjoining or error-free homologous recombination (HR). DSB repair by HR is intriguingly elegant, as it uses undamaged homologous DNA sequences as templates for repair. Most of the past research focused on the components of HR, the basic mechanism, and its regulation. However, despite this detailed knowledge, the fundamental question how the search for the undamaged homology within the genome is brought about remained enigmatic. In fact, whether such a homology search can occur genome-wide, and even whether it exists at all, is intensely debated. Here, we present time-resolved chromatin immunoprecipitations of repair proteins combined with genome-wide high-resolution tilling arrays to detect homology search in vivo. We found that homology search indeed exists, and that it proceeds primarily via a scanning mechanism, which starts at the break and continues along the broken chromosome. However, elements thought to instruct chromosome architecture can guide the search to distant chromosomal locations. Moreover, the search can be even steered to other chromosomes via centromeres. We thus propose that homology search-relevant parameters like linear distance, chromosome architecture and proximity determine recombination efficiency. It seems likely that these principles are also critical for undesired translocations and gene fusions that can cause abnormalities and cancer.

Identification and functional analysis of radiation-induced microRNA changes in endothelial cells

Anne Kraemer, Dr. Zarko Barjaktarovic, Dr. Soile Tapio, Dr. Michael Atkinson, Dr. Simone Moertl
Helmholtz Center Munich

Due to normal tissue damage, especially of endothelial cells, only limited doses of radiation can be applied in tumor therapy. Therefore, a better understanding of the processes governing radiosensitivity of endothelial cells would be desirable for improved radiation therapy. We have demonstrated that the sensitivity of endothelial cells to ionizing radiation is dependent upon the processing of microRNAs (miRNAs) (Kraemer et al.). We identified a phenotypic effect from global inhibition of the miRNA response to radiation by knockdown of two miRNA-processing enzymes (Ago2 and Dicer). Subsequently, we conducted a screening to identify specific miRNAs responding to ionizing radiation. The effects of 7 of these upregulated miRNAs on survival and cell cycle regulation after radiation were individually measured by transfection with specific anti-miRs. Out of 7 analyzed miRNAs, 3 (miR-216a, miR-518d-5p and miR-525-3p) had an impact on the cellular response to irradiation, enhancing apoptosis rate and reducing survival. Furthermore, we were able to identify proteins targeted by these miRNAs through proteome analysis of anti-miR transfected cells using 2D-DIGE. GO term analysis assigned the majority of changed proteins to cell cycle regulation, apoptosis and DNA repair. Ingenuity pathway analysis categorizes the deregulated proteins to biological networks of cell death, lipid metabolism and biochemistry of small molecules. Currently, work is in progress to validate the specific miRNA-3'UTR interactions by cotransfection of 3'UTR luciferase reporter constructs along with a specific miRNA mimic. Our data suggest that miRNA-mediated gene silencing has an essential function in the radiation response. In the future, targeting miRNAs could be an important tool to sensitize tumors during radiation therapy by protecting normal tissue.The research leading to these results is supported by the BMBF Grant NUK007C (KVSF).

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FaST linear mixed models for genome-wide association studies

Christoph Lippert[1], **Jennifer Listgarten**[2], **Ying Liu**[2], **Carl M. Kadie**[2], **Robert I. Davidson**[2]
1 MPI Tübingen
2 Microsoft Research

IMotivation: With the advent of cheap high-throughput profiling of large numbers of genomes, the statistical mapping of genetically regulated phenotypes has gained considerable importance. Results from such genome-wide association studies (GWAS) can be highly confounded, if genetic relatedness among samples from natural populations is not accounted for. Linear mixed models have been shown capable of correcting for several forms of genetic relatedness, such as population structure, family structure, and cryptic relatedness [1].

Methods: We describe factored spectrally transformed linear mixed models (FaST-LMM) [2], an algorithm for GWAS that scales linearly with cohort size in both run time and memory use.

Results: On Wellcome Trust data for 15,000 individuals, FaST-LMM ran an order of magnitude faster than current efficient algorithms [3,4]. Our algorithm can analyze data for 120,000 individuals in just a few hours, whereas current algorithms fail on data for even 20,000 individuals.

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Dissecting gene activity within the Drosophila head

Tamás Schauer[1], Carla E. Margulies[1,2], Petra Schwalie[3], Paul Flicek[3] and Andreas G. Ladurner[1,2*]
1 Genome Biology Unit, EMBL Heidelberg, Meyerhofstrasse 1, 69117 Heidelberg, Germany
2 Department of Physiological Chemistry, Adolf Butenandt Institute -- Faculty of Medicine, Ludwig--Maximilians--Universität (LMU), Butenandtstr. 5, 81377 Munich, Germany
3 EMBL--EBI, Hinxton, Cambridge, CB10 1SD, UK
*To whom correspondence should be addressed.
E--mail: andreas.ladurner@med.uni--muenchen.de

Metazoan cell--types show differential gene activity patterns. This pattern changes upon environmental perturbation depending on the function of the tissue. I have developed methods that allow us to investigate chromatin structure and transcription activity at genome--wide resolution within various cell--types of the fly head.

I used genetically encoded, cell--type specific, GFP--tagged reporters to map
1) RNA polymerase II activity (Pol II) and
2) H2Av incorporation (Carla Margulies)
in cephalic neurons, glia, adipose tissue of adult and embryonic Drosophila.

We obtained a few thousand differences of Pol II peaks between neurons, glia cells and adipose tissue. I validated neuronal or glial Pol II peaks by expressing GFP under the control of juxta--posed enhancer trap insertions. I co--stained neuronal (ELAV) and glial (REPO) marker proteins and found that the cell--type specific Pol II peaks reflect the correct spatial expression pattern.

Interestingly, I find a novel function of the histone variant H2Av marking cell--type invariant genes. This is a class of genes that cluster together and overlap with house--keeping genes. H2Av bound genes are associated with the constitutively active chromatin domain. Furthermore, H2Av incorporates into the chromatin of these genes already in the embryonic stages. This suggests an epigenetic marking of house--keeping genes throughout development.

The new tools will allow us to systematically map changes in gene activity within the complex anatomy of the brain.

Biophysical characterization and modular design of FRET-based biosensors

Anselm Geiger
MPI Neurobiology

Genetically encoded calcium indicators (GECIs) have become instrumental in imaging signaling in complex tissues and neuronal circuits in vivo. During the last decade several sets of FRET-based GECIs have been developed and continuously improved offering access to sensors with high affinity and strong signal output. The common design principle of this indicator class is the fusion of a calcium binding domain between a pair of fluorescent proteins evoking a FRET signal upon conformational change. Despite their importance, structure-function relationships of these sensors often remain largely uncharacterized due to their artificial and multi-modular composition. In this talk, I will first characterize in detail the biophysics of the most recent FRET-based GECI developed in our group - TN-XXL - and give an outlook on further engineering potential. Subsequently, I want to provide a general primer to designing FRET-based biosensors. The emerging field of GECIs soon became a role model for various other sensor classes such as sensors for phosphorylation, ATP/ADP ratio and mechanical tension or even specific protein complex assembly. Researchers developing new biosensors based on a known conformational change can now profit not only from a toolbox of fluorescent proteins but also from tried and tested routes of sensor optimization such as rational design, directed evolution and high-throughput screening.

Imaging calcium levels in immune-mediated axon degeneration

Minh Schumacher, Christoph Mahler
Kerschensteiner lab, Institute of Clinical Neuroimmunology Klinikum der Universität München & Misgeld lab, Institute of Neuroscience TUM

Multiple sclerosis, is an inflammatory disease of the central nervous system where immune-mediated axon damage leads to progressive neurological deficits in young adults[1,2]. Neuroinflammatory axon damage is mediated by a process called 'focal axon degeneration' (FAD), where axons show focal swellings followed by axon fragmentation. Notably focal axonal swellings can still be reversible[3]. In this project we investigate the role of calcium during FAD. Intra-axonal calcium levels are assessed with 2-photon in-vivo imaging using thy1-CerTNL15 mice, which express a genetically encoded calcium sensor in a subset of neurons[4.] We use in-vivo imaging to show that axonal calcium levels predict the subsequent fate of an axon in acute experimental autoimmune encephalitis (EAE) lesions. Furthermore, we address the mechanisms that lead to the elevation of intra-axonal calcium levels. We show that reactive nitrogen species (RNS) are able to induce high calcium levels and subseqeunt axon degeneration in healthy axons which can be effectively inhibited by pharmacological blockage of the Na+/Ca2+ exchanger (NCX). Currently we are testing whether this selective block of the NCX can prevent FAD in EAE.

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The novel allosteric MEK1/2 inhibitor BAY 6-9766 (RDEA119), a promising therapeutic agent for the treatment of pancreatic cancer

Nicole Teichmann, Marija Trajkovic-Arsic, Arne Scholz, Roland M. Schmid, Rickmer Braren, Jens T. Siveke
TUM Medicine

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the western world. Due to difficult early diagnosis and resistance to known chemotherapies and ionizing radiation, PDAC patients have a poor prognosis with a median survival <6 months. Therefore, new chemotherapeutic agents and improved mouse models for better prediction of clinical efficacy of new therapies are necessary. In this study we used a genetically engineered mouse model of PDAC for preclinical evaluation of a novel highly selective MEK1/2 inhibitor BAY 86-9766.

Experimental design: To mimic molecular and morphological characteristics of human PDAC, we generated mice with pancreas specific activation of oncogenic Kras and concomitant deletion of p53 (Ptf1a+/Cre, Kras+/LSL-G12D, p53loxP/loxP; CKP) using a Cre/loxP approach. Those mice develop lethal tumors by 8 weeks of age. Primary mouse pancreatic tumor cell lines derived from PDACs isolated from CKP mice were used to investigate effects of BAY 86-9766 in vitro. To assess the in vivo efficacy of BAY 86-9766, CKP mice with a defined tumor burden were treated daily with 25 mg/kg of BAY 86-9766 from 40 days of age till death. Tumor progression was monitored by measurements of tumor volume via non-invasive T2-weighted magnetic resonance imaging on a clinical 1,5T MRI device.

Results: Treatment of different human and mouse pancreatic cell lines with BAY 86-9766 resulted in a dose-dependent inhibition of MEK1/2 kinase activity as measured by phosphorylation of the target protein ERK1/2 by Western blot, with concentrations between 10-300 nM required for complete inhibition. Additionally, a dose of 30 nM BAY 86-9766 was already sufficient to induce apoptosis within 4 h to 12 h in primary CKP cell lines. Cells also manifested an impaired proliferation after 48 and 96 h of treatment as determined by BrdU-Assay. In vivo, BAY 86-9766 prolonged the survival of CKP mice significantly with a median survival advantage of 20 days. Moreover, tumors regressed dramatically already after 1 week of therapy. However, in most animals, tumors relapsed albeit with different morphological features.

Conclusions: These preclinical data provide compelling evidence that the novel MEK1/2 inhibitor BAY 86-9766 is a promising future therapeutic agent for the treatment of pancreatic cancer in clinical practice. Nevertheless the escape mechanism of the relapsing tumor will be further investigated for an improved therapy strategy of this aggressive cancer type.

A novel epigenetic biomarker for predicting 5-FU response in colorectal cancer

Marc Taenzer
Klinikum rechts der Isar, TUM Medicine

We examined checked hypermethylation in an initial cohort of 74 patients (tumor and non-tumor samples each) and in a panel of six colon cancer cell lines. Although more than two thirds (around 70%) of the tumor samples had a high degree (over 30%) of methylation compared to healthy tissue, a substantial number of patients showed this hypermethylation also in normal tissue (probably due to age dependent epigenetic defects). Thus, the overall tumorspecific hypermethylation was only around 50%, and did not correlate with clinical factors such as TNM status. However, in vitro experiments with colorectal cancer cell lines showed a higher resistance towards Fluorouracil (5-FU), which is a key component of standard chemotherapy (typically administered with leucovorin and oxaliplatin/irinotecan as FOLFOX or FOLFIRI regimen). These results could be reproduced in 4 additional patient cohorts with a total of 220 patients for the human situation. We concluded therefore that tumors with hypermethylation in this region mark a patient subgroup which is at a much higher risk for nonresponse towards 5FU (around 6fold). These patients (45%) represent the large part of Non-Responders to conventional chemotherapy (70%); this is independent of the mode of assessment (e.g. histological or radiological) for response.

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Analysis of DNA Methylation, DNA Methyltranferases, and Histone Modifications in Human Atherosclerotic Lesions

Mihaela Culmes[1], Verena Grabher-Meier[1], Renate Hegenloh[1], Hans-Henning Eckstein[1], Ernst Wagner[1], Rudolf Napieralski[1,2], Manfred Schmitt[2], Jaroslav Pelisek[1]

1 Clinic of Vascular Surgery, Technical University Munich, Klinikum rechts der Isar

2 Clinic of Obstetrics and Gynecology,Technical University Munich, Klinikum rechts der Isar

Background: DNA and histone methylation is a major epigenetic modification regulating gene expression and maintaining chromosomal structure. Experiments in animal models demonstrated already that DNA hypomethylation might be a pre-step in the onset of atherosclerosis. It is however unknown, whether are epigenetic changes related to the development of atheroslerotic vulnerable lesions, or whether they just represent a consequence of the ongoing pathological processes. For this reason, the aim of the present study is to investigate the state of global methylation and the expression of DNA and histone methyltransferases in early and advanced human carotid atherosclerotic lesions. Furthermore, possible changes in DNA methylation were also compared in serum between healthy individuals and patient with carotid atherosclerotic plaques.

Material&Method: 26 carotid plaques from patients with high graded carotid atherosclerosis were included in our pilot study. Within each carotid plaque the early (I-III according AHA classification, n=10) and advanced (V-VI, n=16) stage of atherosclerosis were compared. All carotid plaques were characterized histologically to assess the plaque morphology and were immunostained for DNA and histone methylation markers. DNA was isolated from formalin fixed tissue embedded in paraffin and also from fresh harvested serum; DNA extraction was followed by bisulfite conversion. Global genomic methylation was determined by sybr green based real time PCR of LINE1 and Sat alpha sequence and normalized to Alu1 expression.

Results: Carotid plaques in the advanced atherosclerotic plaque (V-VI) reveals significant decrease of the global DNA methylation status compared with the carotid artery in the early stage of atherosclerosis (I-III) for up to 40% ($p<0.05$).Changes in the global DNA methylation were observed also in serum of patients with advanced carotid lesions with a significant decrease for up to 60% ($p<0.05$) compared to values in serum of healthy patients. Moreover, different expression pattern was observed also for various histone methylation markers in the immunostaining samples in different stages of atherosclerosis.

Conclusion: Epigenetic changes analyzed in our study were significantly associated with the stage and progression of atherosclerosis both in serum and carotid tissue. Hence, epigenetics seems to play an important role during atherosclerotic plaque progression, which corresponds with epigenetic changes in serum. Further investigations are needed for a better understanding of the epigenetic mechanisms during the progression of atherosclerosis.

Metabolic and Respiratory Acidosis in Cerebrospinal Fluid, studied in COPD albanian patients

Dr. Adanela Musaraj
Medicine Department, Aleksander Moisiu Durres University

Severe acidemia due to metabolic or respiratory acidosis, or both, can contribute to the high mortality of intensive care unit (ICU) subjects. Comparing the experimental values from arterial blood and cerebrospinal fluid for acid-base balance parameters and also for electrolytes can simplify the correcting diagnoses and treatment for an intensive care unit subject. Blood samples and CSF samples were taken from 35 patients of ICU to determine the acid-base balance and electrolyte levels. The samples did differ significantly regarding pH, pCO2, TCO2, Na+ and Ca+ ($p<0.05$). 23 of the patients form the study group, has metabolic and respiratory acidosis in arterial blood and also in Cerebrospinal Fluid. Regarding the results of the acid-base balance values, for the other 12 patients of this group, they showed metabolic acidosis combined with respiratory alkalosis in Cerebrospinal Fluid. On the basis of the data gathered from this study, we think the physician may not predict correctly the acid- base balance in Cerebrospinal Fluid, thus in the brain milieu. These differences may be relevant for the patient strategy treatments, for the delicate nature of the brain tissue and the role it plays.

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Comparative Dynamic Transcriptome Analysis (cDTA) reveals mutual feedback between mRNA synthesis and degradation

Daniel Schulz, Mai Sun, Björn Schwalb, Nicole Pirkl, Stefanie Etzold, Laurent Larivière, Kerstin Maier, Martin Seizl, Achim Tresch, Patrick Cramer
LMU Gene Center

To monitor eukaryotic mRNA metabolism, we developed comparative Dynamic Transcriptome Analysis (cDTA). cDTA provides absolute rates of mRNA synthesis and decay in Saccharomyces cerevisiae (Sc) cells with the use of Schizosaccharomyces pombe (Sp) as internal standard. cDTA uses non-perturbing metabolic labeling that supersedes conventional methods for mRNA turnover analysis. cDTA reveals that Sc and Sp transcripts that encode orthologous proteins have similar synthesis rates, whereas decay rates are five-fold lower in Sp, resulting in similar mRNA concentrations despite the larger Sp cell volume. cDTA of Sc mutants reveals that a eukaryote can buffer mRNA levels. Impairing transcription with a point mutation in RNA polymerase (Pol) II causes decreased mRNA synthesis rates as expected, but also decreased decay rates. Impairing mRNA degradation by deleting deadenylase subunits of the Ccr4-Not complex causes decreased decay rates as expected, but also decreased synthesis rates. Extended kinetic modeling reveals mutual feedback between mRNA synthesis and degradation that may be achieved by a factor that inhibits synthesis and enhances degradation.

M. Sun*, B. Schwalb*, D. Schulz*, N. Pirkl, S. Etzold, L. Larivière, K. Maier, M. Seizl, A. Tresch, P. Cramer. (* joint first authorship) Comparative Dynamic Transcriptome Analysis (cDTA) reveals mutual feedback between mRNA synthesis and degradation. (submitted)
B. Schwalb, D. Schulz, M. Sun, B. Zacher, S. Dümcke, D. Martin, P. Cramer, A. Tresch. Measurement of genome-wide RNA synthesis and decay rates with Dynamic Transcriptome Analysis (DTA). Bioinformatics.

Systems biological analysis of the human platelet proteome network

Desislava Boyanova, Santosh Nilla, Ingvild Birschmann, Thomas Dandekar, Marcus Dittrich
University of Würzburg

The increasing amount of available proteomics data in recent years has led to the development of new methods for analysis of large-scale proteomic datasets. Nonetheless, it is still a challenge to interpret data in a biologically meaningful way. Therefore, bioinformatical approaches such as network analysis may prove useful for investigating protein signaling.

Our work aims at elucidating signaling transduction of human platelets by integrating multiple sources of data. We introduce an online platform (PlateletWeb) for systems biological analysis of human platelet proteins in the context of integrated networks. Assembly of platelet proteins from over 20 proteomic studies and databases resulted in around 5000 platelet proteins. Addition of drug data allowed the analysis of drug targets in relation to the number of their interaction partners, which indicated highly connected platelet proteins are more often affected by drugs. We also supplied kinase-substrate information identifying tyrosine kinase substrates as the most highly enriched in platelets. Kinases acting on experimentally validated phosphorylation sites with unknown kinase association were predicted thereby increasing the amount of sites with known regulation. By extraction and visualization of interaction and phosphorylation events from a list of proteins PlateletWeb provides a systematic analysis of signaling regulation in platelets. We investigated a new phosphorylation site in the docking protein DOK1 involved in integrin regulation and proposed a hypothesis for its functional relevance during integrin activation.

In summary, network analysis approaches are a valuable tool for the systems biological understanding of human platelets. Hypothesis-generating research of large datasets is only possible by investigating protein signaling in a network context. This analysis can be particularly useful in cases where experimental testing options are sparse.

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A systems biology perspective of protein complexes by quantitative interactomics and deep proteome sequencing

Marco Y. Hein, Nina C. Hubner, Jürgen Cox, Ina Poser, Anthony A. Hyman, Matthias Mann
MPI Biochemistry

Protein-protein interactions are involved in all aspects of life at the molecular level. Large scale protein-protein interaction data are rich resources for both systems biologists trying to model cellular networks and for biologists looking for new binding partners of their proteins of interest. However, these datasets have often been of limited utility due to high false positive rates and missing additional information.

We detect protein-protein interactions by quantitative GFP-BAC interactomics (QUBIC), a technology which is both robust and sensitive (Hubner et al., 2010). QUBIC makes use of libraries of cell lines which express GFP-tagged proteins from bacterial artificial chromosome (BAC) transgenes under endogenous control, thus avoiding artifacts of ectopic expression (Poser et al,. 2008). Protein complexes are being immunoprecipitated under mild conditions, thereby preserving weak interactors, and then subjected to streamlined liquid chromatography-mass spectrometry analysis. Specific interactors are determined by a sophisticated label-free quantification algorithm and robust statistical testing.

We recorded interaction patterns for a large number of bait proteins and then combined the interactome data with deep proteome data. Our fully quantitative approach allows the estimation of both the stoichiometries of interaction partners as well as their relative expression levels in the proteome. This helps to classify interactors as either core complex components or accessory binders and provides hints to whether a protein resides exclusively in one specific complex or fulfils additional functions. This work uniquely adds a missing dimension to the cellular context of protein interactions and should provide a useful basis for a systems biology view of the cell.

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Performance, Reliability and Robustness - A comparison of several experimental design strategies

Stefan Brandmaier, Igor Tetko
Helmholtz Center Munich

The goal of the design of experiments is to reduce a large collection of relevant instances to a smaller set of representative and informative instances. Thereby experimental design is an adequate way to efficiently manage resources and to save costs in experiments, aiming to systematically explore the space of interest. The selected instances are usually experimentally measured and afterwards used, to build a statistical model on them. Apart from a good performance, reliability and robustness, both against small variations in the dataset and structural outliers are required from that model. A variety of approaches aiming to realize a meaningful selection is available, but for chemical applications, they all work in the descriptor space.

We developed three adaptive approaches for a representative subset selection and compared it to several commonly used experimental design strategies. Like Bayesian approaches, adaptive approaches take information about the target property into consideration, but instead of using preliminary available information, they make use of the accumulating information, that is gathered in a stepwise procedure. The adaptive approaches we implemented combine an iterative refinement of the descriptor space with known sampling concepts, like similarity and dissimilarity selections. We evaluated the approaches on a variety of chemical datasets and our results show, that stepwise approaches can significantly improve the performance of a selection and the resulting models. Furthermore, they provide a higher adaptiveness to small variations in the dataset, which makes them more capable of structural outliers, than the classic approaches. Additionally, we found indications, for a correlation between the flexibility of a selection approach and the balance between the performance and the reliability of the resulting model.

Induction of peroxisome degradation by overloading of peroxisome membrane protein in mammalian cells

Shun-ichi Yamashita, Yukio Fujiki
Kyushu University

Autophagy is one of intracellular degradation systems in eukaryotic cells and responsible for regulation of cellular activities. Several groups of organelles such as mitochondria are indeed degraded by organelle-specific autophagy. Peroxisome is an intracellular organelle and functions in various metabolic pathways including biosynthesis of ether-phospholipids and beta-oxidation of very long chain fatty acids. In mammalian cells, peroxisomes are induced by the treatment with peroxisome proliferators (PPs) such as Wy-14,643 and 4-phenylbutyric acid. The induced peroxisomes, not all of peroxisomes, are mainly degraded by peroxisome-specific autophagy. As a step to getting mechanistic insight into peroxisome degradation, we attempted to set up a monitoring system for peroxisome degradation in mouse embryonic fibroblasts using the PP-induction and its withdrawal system. However, only a small range of changes in peroxisome number was detectable in such system. In this study, we recently developed a novel system to monitor peroxisomal degradation. Upon overloading Pex3 onto peroxisome membrane, peroxisomes were drastically degraded. Furthermore, in the course of this degradation, LC3 and p62 were colocalized with peroxisomes, where peroxisomal matrix proteins were recruited into the lysosomes, indicative of autophagic degradation pathway. Together, our new monitoring system is apparently very useful for tackling molecular mechanisms of peroxisome degradation in mammalian cells.

Spalt mediates an evolutionarily conserved switch to fibrillar muscle fate in insects

Cornelia Schönbauer, Jutta Distler, Nina Jährling, Martin Radolf , Hans-Ulrich Dodt, Manfred Frasch, Frank Schnorrer
MPI Biochemistry

Human body muscles are composed of distinct muscle types that differ in their contractile properties according to their functions. These include fast and slow muscle fibres as well as a rhythmically beating muscle, the heart. Such functional differences correlate with a characteristic composition of muscle contractile filaments. How these different muscle types are specified and molecularly constructed remains elusive.

Many insect species possess indirect flight muscles that enable fast and powerful wing oscillations at frequencies up to 1000 Hz, together with power outputs as high as 100 W per kg. To achieve these specific parameters, indirect flight muscles contain stretch-activated myofibrils that display a unique fibrillar morphology and contain only sparse sarcoplasmic reticulum. This is in stark contrast to all other, more slowly contracting insect body muscles that display a tubular morphology and, similar to vertebrate skeletal muscles, contain large amounts of sarcoplasmic reticulum. In a genome-wide muscle-specific RNAi screen we have identified the conserved transcription factor spalt-major (salm) as a master regulator of fibrillar flight muscle development in Drosophila. salm expression is induced specifically in the myoblasts that will form the indirect flight muscles by the transcription factor vestigial (vg). salm is required and sufficient to induce fibrillar muscle fate and switches the entire transcriptional program from tubular to fibrillar fate by regulating expression and splicing of key sarcomeric components specific to each muscle type. Interestingly, we find that spalt function is conserved in insects separated by 280 million years of evolution leading us to speculate that Spalt might also be important for the function of the vertebrate heart, a stretch-activated muscle sharing some features with insect flight muscle.

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Mitochondrial unfolded protein response protects mitochondria under inflammatory conditions

Eva Rath, Emanuel Berger, Nadine Waldschmitt, Elena Lobner, Dirk Haller
Chair for Biofunctionality, ZIEL - Research Center for Nutrition and Food Science, Technische Universität München, Gregor-Mendel-Str. 2, 85350 Freising-Weihenstephan, Germany

We have recently shown that mitochondrial unfolded protein response (mtUPR) is activated in intestinal epithelial cells (IEC) under intestinal inflammation. UPRs aim to restore protein homeostasis and represent autoregulatory pathways adjusting organelle capacity to cellular demand. Since mitochondrial alterations and dysfunction are implicated in the pathogenesis of inflammatory bowel disease (IBD), we characterized the role of mtUPR under stress conditions. Using a selective trigger for mtUPR, we identified dsRNA-activated protein kinase (PKR) as cytoplasmic mediator of mtUPR in IEC. Downstream, PKR was able to directly phosphorylate eukaryotic translation initiation factor (eIF2) α and triggered the activation of the transcription factors (TF) cJun/AP1 and CHOP via MEK and JNK. Increased expression of mitochondrial chaperonin (CPN) 60, a surrogate marker of mtUPR, was also associated with induction of PKR in primary IEC from IBD patients and murine models of colitis. Thus, we employed Pkr-/- mice to further investigate the role of mtUPR in IEC. These mice appear to be phenotypically normal however, colonic IEC displayed an increased ratio of mt/nuclear DNA, indicating elevated mitochondrial abundance. Interestingly, acute stress evoked by administration of 1% DSS for 3 days led to a decrease in mt/nuclear DNA ratio and decreased mRNA levels of nuclear encoded CoxIV as well as mtDNA-encoded CoxI in Pkr-/-, but not Wild type mice. Yet, only Wild type mice showed induced Cpn60 and PKR levels. Prolonged treatment with 1% DSS revealed Pkr-/- mice to be largely protected from DSS-induced colitis. These contradictory findings might indicate a more effective clearance of dysfunctional mitochondria in Pkr-/- mice, leading to reduced oxidative stress and tissue protection. Our data suggest mtUPR to be important for mitochondrial homeostasis in IEC and underline the critical role of mitochondrial stress signaling during the onset and perpetuation of intestinal inflammation.

Selection and depletion of T cells – engineered with a truncated EGFR marker

Paulina J. Paszkiewicz[1], **Prof. Dr. Stanley R. Riddell**[2], **Prof. Dr. Dirk H. Busch**[1]
1 Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Germany
2 Fred Hutchinson Cancer Research Center, Seattle, USA

Harnessing components of the immune system to combat cancer is an appealing approach. T cells of the adaptive immune system have the unique features of specific recognition of the target, multiple mechanisms of eliminating a foreign organism and the potential to provide long-lasting immunity. These properties render the T cell a promising candidate for immunotherapy. After purification of a well-defined T cell subset from a patient's blood, cells are genetically engineered to express a receptor specific for an antigen that is exclusively expressed on the surface of tumor cells. Using gene-modified T cells in therapies bears some potential risks due to impure cell products and adverse effects if transferred cells recognize healthy tissue by mistake. We want to address these safety issues by equipping the engineered cells with an additional marker – a truncated epidermal growth factor receptor (tEGFR) - that facilitates both cell selection and depletion.

For obtaining highly pure cell products for transfer into the patient, we utilize the Streptamer technology that allows for positive selection of cells based on reversible labeling with anti-EGFR Fab multimers followed by complete removal of the multimers. For in vivo depletion of engineered cells, the tEGFR marker could be targeted by the clinically approved anti-EGFR antibody (Erbitux), which mediates antibody-dependent cell cytotoxicity. First studies of this safety mechanism in different animal models have confirmed the feasibility of rapid and efficient elimination of EGFR-modified cells after a single Erbitux injection.

There is a huge interest in developing a universal safety switch, as gene-modified cells have been successfully applied for tumor therapy in recent clinical studies. Our strategy could be easily transferred to any T cell-based therapy and would therefore represent a promising concept to improve the safety of T cells as therapeutic regimens.

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Introduction to Adobe Illustrator for Scientific Illustrations

Andreas Binder
LMU Munich

Unlike pixel-based (bitmap) images such as photographs, scans or microscopic images, vector-based graphics can be fully edited and freely scaled. They are therefore the preferred option to use in technical or scientific drawings. One of the most powerful and widely utilized vector-based graphic editors is Adobe Illustrator. Over the years Illustrator has gained a very large set of tools and options, making it very flexible and versatile, but also a bit more difficult to get into than other programs.

For scientists Illustrator can be very helpful in the creation of presentations, figures and drawings as well as posters. Several features such as layers, art boards and the coordinate system make complex and precise illustrations much easier to handle than other drawing programs.

This presentation will introduce some basic features of the Illustrator interface as well as tools for handling standard tasks such as arranging microscopic images. In a short tutorial I will cover some more advanced functions and tips useful for the easy generation of professional-looking drawings.

Statistics in biology: Why the t-test is usually the wrong choice!

Marco Düring
Institute for Stroke and Dementia, Klinikum der Universität München

Statistics play a vital part in a scientists everyday work. "Are my observations just random noise or am I looking at a real effect?" "Does my treatment group behave differently than my control group?" Among many others, these are the questions that biologists hand over to statistical testing. However, there are many pitfalls and caveats when applying statistical methods. This is especially true in the field of biology, where the number of observations is typically small.

This seminar aims to provide an intuitive approach to statistical methods. We will focus on suitable methods for biological experiments. And participants will learn why the t-test is usually the wrong choice.

Biomolecular <interact>ions: some like it hot

Philipp Baaske
NanoTemper Technologies GmbH

The analysis of bio-molecular interactions, such as protein-protein, protein-nucleic acids or small molecule binding, not only helps to develop therapeutics or serves as diagnostic technique, but also provides novel insights into basic cellular processes. Here I show a new label-free and preparation-free technique to analyze the affinity of bio-molecular interactions based on the recently invented method of microscale thermophoresis (MST).

Label-free MST uses the directed movement of molecules in optically generated microscopic temperature gradients. This thermophoretic movement is determined by the entropy of the hydration shell around the molecules. Almost all interactions between molecules and also virtually any biochemical process relating to a change in size, stability and conformation of molecules alter this hydration shell and can thus be determined and quantified by MST. In this talk I exemplify MST by investigating the binding of ligands to membrane receptors (GPCRs), quantifying protein-DNA interactions and screen for small molecule binders to the kinase p38. All groups of interactions were readily accessible by label-free MST and the measured affinities confirmed values reported in the literature.

NanoTemper's Microscale Thermophoresis is a game changing technology, its speed, affinity resolution and minute sample consumption are unmatched. MST enables to measure interactions considered as immeasurable before.

Mayday - integrative analytics for expression data

Günter Jäger
University of Tübingen

Since their inception in the 1990s, microarrays have revolutionized biological research. Today they are a standard method for large scale analyses of gene expression and epigenomics. During a normal microarray experiment, several methods have to be used in combination to deal with the increasing complexity and inherent noisiness of the data. Which methods are used and in which order depends on the nature of the data, the experimental conditions and on observations made during the analysis itself. Thus, there is a need for an integrative framework combining many of these methods to efficiently analyze the data.

Mayday is a comprehensive platform for the analysis and the visual exploration of microarray data. Many built-in methods for clustering, machine learning and classification are provided for dissecting complex datasets. Plugins can easily be written to extend Mayday's functionality in a large number of ways. Mayday can import data from several file formats and database connectivity is included for efficient data organization. An important focus of exploration of high-dimensional data, such as microarray data, lies on visualization. Numerous interactive visualization tools, including box plots, profile plots, principal component plots and a heatmap are available, can be enhanced with metadata and exported as publication quality vector files. The advantage of Mayday is the tight integration of both, analysis and visualization as well as the various visualization techniques themselves. This combination of automatic and visual analysis leads to a visual analytics approach that provides more insights in the structure of the data.

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Disarming the cell - how pandemic HIV conquered the world

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Design and evaluation of a multifunctional nano-carrier system for targeted drug delivery in gastro-intestinal cancer

Isabella Almstätter[1], Olga Mykhaylyk[2], Marcus Settles[1], Christian Plank[2], Rickmer Braren[1]
1 Institute of Radiology, Molecular Imaging, Klinikum rechts der Isar, Technische Universität München, Munich, Germany
2 Institute of Experimental Oncology and Therapy Research, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

In this project a nano-carrier formulation for targeted drug delivery after systemic application in gastro-intestinal cancer will be developed, characterized and validated. Genetically engineered mice bearing pancreatic ductal adenocarcinoma (PDAC) will be used as a model system. The nano-carrier targeting will be bi-functional, mechanical and biological. By encapsulation of magnetic nano particles (MNPs) into liposomes, the nano-carrier system of choice, accumulation at a specific target region could be achieved mechanically by application of an external magnetic field. Surface functionalization of the liposome with a ligand molecule specifically binding a pancreatic tumor cell surface structure (Sarantopoulos et al.) should enable biological targeting, followed by internalization of bound liposomes via endocytosis. After accumulation, the release of the chemotherapeutic agent from the liposomes will be induced by focused hyperthermia application (deSmet et al.). Thereby higher doses of the therapeutic drug at the target site could be achieved. Liposome delivery visualization and follow-up of tumor growth kinetics will be performed by magnet resonance imaging (MRI) and fluorescence molecular tomography (FMT) and the results will be correlated with histology. Results of the in vitro characterization of specific MNPs and MNP-loaded liposomes will be presented. Determined parameters are saturation curves and cell viability assays in primary murine PDAC cell lines and several physical parameters of liposomes including size, zeta potential and magnetic responsiveness. In addition first results of tissue-mimicking MRI phantoms will be shown (Mykhaylyk et al.).

Molecular imaging of integrin aVb3 for in vivo detection of precursor lesions and pancreatic cancer. Sarantopoulos A, Trajkovic-Arsic M, Themelis G, Kalideris E, Beer A, Pohle K, Braren R, Schmid RM, Siveke JT. submitted
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Imaging of Dose and Bioactivity of Drugs in the Lungs of Mice

Nirav Barapatre, Friedrich Prade, Otmar Schmid
Helmholtz Center Munich

One of the key therapeutic approaches in the lung diseases is the delivery of drugs directly to lungs through its airways. The efficacy of drugs will depend on the amount of the drug and the location of its deposition. Hence, the quantification of drug amount and its spatial distribution in lungs are indispensable factors in the validation of any drug in preclinical research.
Optical microscopy is, in general, the method of choice for determining drug dose and distribution in biological experiments, because in vivo analysis can be performed with it. It can broadly be classified into fluorescence and bioluminescence imaging. Both the processes involve visible light. However, the biological tissue interacts strongly with the visible light. Due to absorption and scattering of the light, the quantification and mapping of the drug dose becomes increasingly difficult.
We discuss the challenges in doing in vivo optical imaging of the drug dose in the lungs of mice and present innovative solutions.

Nanobodies: A Versatile Tool to Learn More about Your Protein

A. Buchfellner[1,2], L. Yurlova[2], K. Zolghadr[2], T. Romer[2], U. Rothbauer[3], H. Leonhardt[1]
1 Dept. Epigenetic, Biocenter Martinsried, LMU Munich
2 ChromoTek GmbH, IZB, Munich
3 NMI, Tübingen

Conventional antibodies are invaluable research tools, but their application is restricted mainly to fixed-sample and in vitro investigations due to their complexity and size (app. 150 kDa for the IgG molecules). A promising alternative to conventional antibodies are the naturally occurring heavy-chain antibodies of the Camelids. The antigen-binding fragments of these antibodies (VHH) are as small as 12-15 kDa, extremely stable and can be expressed at high levels in heterologous systems. In this work we introduce three different applications of VHHs.

Firstly, when fused to fluorescent proteins (FP) and expressed in living cells, VHHs enable real time analyses and visualization of endogenous cellular structures and processes. These VHH-FP binding molecules are called "Chromobodies". This antigen detection technique is superior to the traditional system of target-protein-FP fusions, as it allows labeling of endogenous antigens and does not alter the target protein concentration in the cell.

Secondly, VHHs can be applied in immunofluorescence in a similar manner as conventional antibodies. Here we generated VHHs recognizing GFP and RFP and labeled them with the corresponding chemical dyes. Employing these so-called "Boosters" helps to overcome the often occurring insufficient photostability and low quantum efficiency of FPs, as well as their sensitivity towards chemical treatments, making the samples suitable for Super Resolution Microscopy.

Finally, VHHs can facilitate biochemical analyses of your proteins of interest. For this, a VHH binding domain, raised against a target protein can be coupled to agarose or magnetic beads. As demonstrated here with the "GFP-Trap", this method ensures a quick and quantitative immunoprecipitation of the protein of interest and their interacting partners from cell extracts without contaminating the samples with heavy and light chains of conventional antibodies.

Development of a Physiologically-based Model of the Circulation and respective Regulatory Systems

Karina Claassen[1], Dr Michael Block[2], Prof. Dr Tobias Preusser[1,3]
1 Jacobs University Bremen, School of Engineering and Science, Campus Ring 1, 28759 Bremen, Germany
2 Bayer Technology Services GmbH, BTS-TD-ET Systems Biology, Building 9115, 51368 Leverkusen, Germany
3 Fraunhofer MEVIS, Universitätsallee 29, 28359 Bremen, Germany

Aims: This work is aimed to develop a regulatory whole body (wb) physiology-based pharmacokinetic (PBPK) model of the Circulation and respective regulatory systems, even as for a cardiovascular drug and its effects.

Objectives: The physiological unit of CVS is responsible for supply of all organs with blood and plays a key role in the absorption, distribution, metabolism and excretion (ADME) processes of drugs. These processes are highly dependent on blood flows. Therefore modeling can aid the drug development pipeline [1] and give new insights into pathological and physiological conditions [2].

Methods: Physiologically-based pharmacokinetic (PBPK) models integrating the knowledge about the physical and physiological processes affecting the pharmacokinetics of a drug were used to develop a circulation model, a model for the RAAS and a model of an RAAS effecting drug. Specific information about physiological parameters of the RAAS and PK parameters are included to the model developed with the generic modeling and simulation software platform consisting of PK-Sim® and MoBi® (Bayer Technology Services GmbH).

Results: The results of the analysis of pharmacokinetic and pharmacodynamic relationships under drug administration of a RAAS inhibitor are discussed in detail.

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Probing the conformation of the ISWI ATPase domain with genetically encoded photoreactive crosslinkers and mass spectrometry

Ignasi Forné, Johanna Ludwigsen, Axel Imhof, Peter Becker, Felix Mueller-Planitz
Protein Analysis Unit and Molecular Biology Unit, Adolf-Butenandt Institute and Center for Integrated Protein Science, Ludwig Maximilian University, Munich, Germany

We present a strategy for rapidly gaining structural information about a protein from crosslinks formed by genetically encoded unnatural amino acids. We applied it to ISWI, a chromatin remodeling enzyme involved in chromatin assembly, DNA replication and transcription. ISWI is part of the vast Snf2 family of helicase-related proteins, many of which constitute the catalytic cores of chromatin remodeling complexes. Structural information about this family is scarce, hampering our mechanistic understanding of chromatin remodeling. Making use of cells that harbour a special tRNA/aminoacyl-tRNA synthetase pair, several residues within the ATPase domain of ISWI were individually substituted with the UV-reactive unnatural amino acid p-benzoyl-p-phenylalanine. Intramolecular crosslinks could be mapped with amino acid precision by high resolution tandem mass spectrometry and the novel bioinformatic tool "Crossfinder". Most crosslinks were fully consistent with published crystal structures of ISWI-related ATPases. A subset of crosslinks, however, disagreed with the conformations previously captured in crystal structures. We built a structural model using the distance information obtained from the crosslinks and the structure of the closest crystallized relative, Chd1. The model shows the ATPase lobes strongly rotated against each other, a movement postulated earlier to be necessary to achieve a catalytically competent state. The minimal requirements for solubility and protein amounts make the described approach ideal for studying structures and conformations of proteins that are not amenable to conventional structural techniques.

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Studying Neuronal Network Plasticity in the Lateral Amygdala using Voltage-Sensitive Dye Imaging

Florian Hladky, Charilaos Avrabos, Matthias Eder
MPI Psychiatry

The amygdala as part of the limbic system is a brain structure which lies in the anterior medial portion of each temporal lobe. Through a large variety of connections with brain regions involved in the processing and expression of emotions, the amygdala is thought to be one of the brain's key structures in coordinating these processes. In the context of psychiatric research, altered signal propagation through the amygdala could be causally involved in the manifestation of anxiety disorders.

The amygdaloid complex is composed of several subnuclei, such as the central nucleus or the lateral nucleus (LA), which are interconnected for computations of sensory information. The LA represents one of the major input regions of the amygdaloid complex, in which afferents carrying signals from the neocortex and the thalamus converge to be further processed. To investigate dynamics in signal propagation through the LA in response to specific stimuli, we developed a voltage- sensitive dye imaging (VSDI) assay in horizontal brain slices obtained from adult mice. In combination with advanced data processing, this assay allows us to detect changes in neuronal excitability with a micrometer spatial resolution and a scope spanning the entire brain circuit (LA) under study.

Our experiments revealed that high-frequency stimulation (100 Hz for 1 s) of cortical projections to the LA induces both long-term potentiation and long-term depression of neuronal excitability at numerous sites of the LA network. The present work exemplifies the high plastic nature of the LA and, most importantly, illustrates the capability of its network to respond spatially segregated to the same stimulus in opposite ways.

A novel, highly efficient method for the quantification of plastic particles in sediments of aquatic environments

Hannes K. Imhof[1], Johannes Schmid[2], Reinhard Niessner[2], Natalia P. Ivleva[2], Christian Laforsch[1]
1 Department of Biology II, Ludwig-Maximilians-University Munich (LMU), Grosshaderner Str. 2, 82152 Planegg-Martinsried, Germany
2 Institute of Hydrochemistry (IWC), Chair for Analytical Chemistry, Technische Universität München (TUM), Marchioninistr. 17, 81377 Munich, Germany

Although plastic debris is constantly accumulating in aquatic environments, the impact on aquatic ecosystems is not yet fully understood. Once plastic litter enters natural environments, UV radiation, mechanical abrasion, biological degradation and disintegration cause the formation of tiny fragments. Those persist and are not completely degraded in any human scale of time. Harmful consequences of plastic debris for aquatic organisms have been already shown. Next to mechanical impairments of swallowed plastics mistaken as food, many plastic associated chemicals are proven to be carcinogenic, endocrine-disrupting or acutely toxic. Additionally, plastic particles can adsorb and therefore accumulate persistent organic "pollutants" such as polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene. This is even more likely for microscopic plastic fragments. Hence, it is indispensable to identify and quantify the types of plastic debris of different size classes in aquatic habitats for future risk assessment studies.

A first important step in order to assess the consequences of plastic debris in aquatic ecosystems is a reliable, verified and standardized method to quantify the amount of plastic particles. Hence we developed an accurate method based on density separation in a ZnCl2 solution (at density 1.6 - 1.7kg/l), which allows for an extraction and quantification of plastic particles from sediments of aquatic environments. Furthermore, we constructed a Plastic Sediment Separator (PSS) which enables a reliable separation of different types and size classes (including S-MOPP) of plastic particles from sediment samples. Our study is the first providing validated recovery rates of 100% for large micro-plastic particles (L-MPP, 1-5 mm) and 95.5% for small micro-plastic particles (S-MPP, <1 mm). Subsequent identification of the particles with spatial resolution down to 1 µm can be performed using Raman microspectroscopy.

Hence, our new method can be used for a reliable separation, identification and quantification of plastic fragments ranging from mesoplastic particles (20-5 mm) to S-MPP (<1 mm) from samples of aquatic sediments and even plankton samples. This will help to foster studies quantifying the increasing contamination of aquatic environments with plastic particles, which is a crucial prerequisite for future risk assessment and management strategies.

Quantitative interaction proteomics to determine protein-protein interactions in S. cerevisiae.

Eva Keilhauer, Marco Hein, Nils Kulak, Matthias Mann
MPI Biochemistry

Proteomics is a broad field of science that by various methods seeks to analyze the proteome, which is the entire set of proteins of a cell, tissue or organism in a specific state and at a specific time point including all post-translational modifications. In the last decade, particularly mass spectrometry-based proteomics has evolved as a very powerful proteomics method, and is more and more becoming the method of choice for many scientists.

Mass spectrometry-based proteomics can be applied for various questions; in this work we focused on studying protein-protein interactions. Almost all cellular processes are executed by proteins and based on their interactions with each other, therefore understanding these interactions is essential to gain insights into cellular processes, functions and physiology.

The budding yeast S. cerevisiae is an important eukaryotic model organism for biological research, and discoveries made in this organism can give interesting insights into the biological mechanisms of human cells. It is also a powerful tool for investigating protein-protein interactions as it can very easily be genetically modified.

With this work, we provide a new mass spectrometry-based method for studying protein-protein interactions in yeast. The method relies on automated anti-GFP immunoprecipitation of GFP-tagged bait proteins and their interaction partners followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) in a label-free quantitative format. The method development involved amongst other finding an efficient lysis method for yeast that preserves protein complexes, the optimization of the whole method for studying yeast protein complexes and finding appropriate tools for statistical analysis. Our new method offers various possibilities for investigating protein-protein interactions and can therefore contribute to better understand the complex biological mechanisms that rely on these interactions.

Development of immunospot assay for Dengue fever detection based on fluorescent nanoparticles

Elisangela Linares[1], Prof. Dr. Claudio Pannuti[2], Prof. Dr. Lauro Kubota[3], PD. Dr. Stefan Thalhammer[1]
1 Helmholtz Zentrum München
2 Laboratory of Virology, São Paulo Institute of Tropical Medicine, Brazil
3 Institute of Chemistry, University of Campinas, Brazil

Dengue fever is a mosquito-borne infection that in recent decades has become a major international public health concern with 50 million cases worldwide every year. An early detection of the infection can lower the mortality rate from more than 20% to less than 1%. The viral non-structural glycoprotein (NS1)is used as a high potential analyte in early diagnostics. (1) Up to now, enzyme linked immunosorbent assay (ELISA) is the gold standard method to detect Dengue NS1 protein, however it is time consuming, and takes up to 5 hours. For routine of clinical analysis laboratory in epidemic periods, the test duration is relatively long when hundreds of samples should be tested in the same day. In our work we describe a fast and simple method based on a fluorescent immunospot assay to detect Dengue NS1 protein in 45 to 60 minutes. The test uses mouse anti-NS1 IgG labeled with fluorescent particles (λmax: 567nm) and requires only 4 µL of patient serum. The fluorescent signal can be measured with a fluorometer or alternatively with an UV-lamp. The detection limit (5.2 ng/mL) is comparable to ELISA tests, which is also in the range of nanograms per milliliter. A comparison of 83 samples with a commercial ELISA revealed a sensitivity of 81% and specificity of 88% for our method. In addition, the use of fluorescent particles provides a higher sensitivity (0.758 a.u. mL/ng) than an assay using only a single fluoresceinisothiocyanat (FITC) dye molecule per IgG (0.539 a.u. mL/ng), besides avoiding bleaching effects. Based on the results, the presented method provides fast, specific and sensitive results and has the potential to substitute available methods for Dengue NS1 detection.

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Cytogenetic Submicroliter Lab-on-a-Chip System for Personalized Medicine

Agata Michna, Heike Schittl, Daria Grönlinger
Helmholtz Zentrum München

Advances in micro- and nanotechniques enabled a new generation of laboratory instruments. These instruments are dedicated for personalized medicine as they provide a faster and more sensitive biochemical analysis than conventional laboratory techniques. Here we present a modular system for analysis of smallest amounts of biological material up to the single cell level, based on fundamental principles of micro- and nanotechnology. The system comprises an isolation tool, a single particle transfer system [1] and a processing component. Samples are isolated by laser microdissection and precisely released at any desired position in a volume as little as 50 nl. Further processing of the sample is performed with the lab-on-a-chip system. The heart of this device is a piezoelectric chip with a lithographic structured surface. Actuation of liquids on the planar chip is achieved by surface acoustic waves generated by interdigital transducers on the chips' surface. The device was proven to perform reliable sample isolation and sensitive DNA analysis of forensic material [2] and medical samples at the single cell level [3]. Due to its modular character the system can be easily adapted to other scientific questions like the identification of new targets for personalized therapy. Furthermore the system offers the possibility to overcome current detection limits in the field of -omics.

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QSPR approach to predict non-additive properties of multicomponents mixtures. Application to the azeotropic behavior of binary liquid mixtures

Ioana Oprisiu[1], Dr. Gilles Marcou[2], Dr. Dragos Horvath[2], Dr. Alexandre Varnek[2]
1 Helmholtz Zentrum Muenchen Institute for Structural Biology, Ingolstaedter Landstrasse 1, Neuherberg
2 Laboratoire d'Infochimie UMR 7177 CNRS, UdS, 4, rue B. Pascal, Strasbourg, France

Quantitative structure-property relationships (QSPR) is an approach linking chemical structure with some biological activities, chemical reactivity or physicochemical properties. Usually, in QSPR studies each compound is represented by an ensemble of parameters (molecular descriptors) calculated directly from its chemical structure which are used in mathematical equation (model) Property = f(descriptors).

Generally, QSPR approach is limited to individual compounds. Here, we present a new technique allowing one to extend conventional QSPR modeling to 2-component non-additive mixtures, and its application to the azeotropic behavior of binary liquid mixtures. An azeotrope is a homogeneous liquid mixture of two or more components that behave as a pure substance. Consequently the temperature of boiling azeotrope is a constant at a given pressure. This mixture, unlike zeotrope is not separable by fractional distillation. Prediction of azeotrope formation is required in many academic and industrial applications

Classification models were developed on the data set of 400 binary liquid systems forming both azeotropes and zeotropes measured at atmospheric pressure. Special "mixture descriptors" were suggested to build predicting models using Support Vector Machine (SVM) and Voting Perceptron approaches performing a 5-fold external cross-validation. Thus, all mixtures from the parent set were predicted with reasonable accuracy (BA>0.82). Additional validation has been performed on a set of 94 new mixtures, containing no new pure compounds, with BA>0.8. The validation shows that obtained models are well suitable to fill the "missed data" in the mixture matrix formed by 66 individual liquids and containing 2145 their possible combinations. Thus, azeotropic behavior for 2145 – 400 = 1745 new mixtures have been predicted.

Neuroimaging of enteric neurons in human intestinal biopsies

Daniela Panzitta, Dr. rer. nat. Sabine Buhner, Prof. Dr. rer. nat. Michael Schemann
TUM

The enteric nervous system (ENS) consists of ganglionated networks of more than 100 Mio. nerve cells and is located in the wall of the gastrointestinal (GI) tract. The ENS is able to autonomously regulate complex gastrointestinal processes including motility, secretion, and immune functions (Schemann & Neunlist, 2004). Malfunctions of ENS control are increasingly recognized as underlying factors in GI disorders particularly in the irritable bowel syndrome (IBS) (Gershon, 2005).

Previous studies could show altered nerve activation by supernatants from biopsies taken from IBS patients in comparison to healthy controls. The application of supernatants from biopsies of IBS patients significantly increased the activity of nerve cells in the submucous plexus of human colon. It could be shown that the mast cell products histamine and proteases as well as serotonin were the main components mediating these effects. The concentration of these components was increased in comparison to healthy controls. (Buhner et al., 2009) So far there is no evidence if also the enteric nerves of IBS patients are affected by the disease. As the nerve cells are exposed to an increased amount of excitatory mediators over a certain period, nerve adaption could lead to an altered behavior of the enteric nervous system.

With this project we will for the first time directly measure nerve activity in routine biopsies from IBS patients using multisite optical recording methods (Neunlist et al., 1999; Michel et al., 2011). The response pattern to standardized stimuli, such as synaptic stimulation and postsynaptic activation by serotonin, histamine and proteases, enable us to identify altered nerve activity in IBS. In order to identify nerve sensitizing components we will antagonize the actions of immune mediators. This study aims to reveal the biomarker potential of mucosal biopsy supernatants in order to improve diagnosis and treatment of diseases associated with altered mucosa-nerve signaling.

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Binding Constants in Complex Liquids and Living Cells

Maren Reichl, Susanne Seidel, Dieter Braun
LMU Munich

Thermophoresis, the motion of molecules in temperature fields, is very sensitive to changes in size, charge and solvation shell of the molecules. Binding events alter these molecular properties. We are capable of creating localized temperature fields via infrared laser sources under a fluorescence microscope. This has opened new possibilities for basic research and drug discovery. In a recently developed technology, Microscale Thermophoresis (MST), these temperature fields are used to perform biomolecular interaction studies.In contrast to conventional methods, MST is performed in free solution and almost does not have limitations on molecular size or weight. It requires lower sample volumes and allows faster measurements in almost any buffer or even in complex biological fluids like cell extracts or blood serum.

Binding constants are expected to be very different in vivo than in vitro. Molecular crowding and interactions with competitive binding partners in the complex fluid of the cytoplasm are encountered everywhere in biological systems and only partially reproducible in vitro. Thus we are developing a new technique to measure binding affinities directly inside living cells.

Vertical thermophoresis in combination with total internal reflection fluorescence (TIRF) microscopy has the potential to measure binding affinities in the binding equilibrium. The chamber bottom is heated optically and the coverslip with cells growing on it acts as a heat sink. We achieve temperature gradients of 0.1 to 1 K/µm while only heating 2 to 20 K in total. In this temperature gradient the intracellular molecules experience vertical thermophoresis, move into the evanescent field and there they are detected with TIRF. The advantage compared to MST is the spatially pixel-wise resolution within the cell.

Online database of toxicological structure alerts

Elena Salmina, Iurii Sushko, Vladimir V. Potemkin, Igor V. Tetko
Helmholtz Center Munich

Identification of chemicals that are able to exert adverse effects on human, other living organism or environment is an important feature of the modern toxicology. This problem is especially important considering the new legislations such as REACH in European Union. Assessment of toxicity with direct in-vivo animal tests is very costly and time consuming. An alternative, which could reduce costs and avoid unnecessary animal tests, is using predictive computational models. One of the simplest yet powerful techniques for detecting potentially toxic chemicals is using substructure patterns also known as structure alerts or toxicophores. We have developed a platform for collecting and storing toxicological alerts from literature and for screening chemical datasets against these alerts. In our system, an alert is uniquely identified by a SMARTS pattern, a toxicological endpoint and a publication where the alert was described. Additionally, the system allows storing complementary information such as name, comments, mechanism of action, etc. Most importantly, the platform can be easily employed for fast screening of large chemical datasets against the toxicological alerts, providing a detailed profile of the chemicals grouped by alerts and endpoints. Such a facility can be used for decision making regarding whether a compound should be tested experimentally, validated with available QSAR models or eliminated from consideration altogether. The system is open and tightly integrated with the Online Chemical Modeling Environment (OCHEM) [1]. Any user on the Web can introduce new alerts, browse and edit alerts introduced by other users or screen his/her datasets against all or some alerts. The datasets filtered by alerts can be used at OCHEM for other typical tasks: export in a wide variety of formats, creation of QSAR models, additional filtering by other criteria, etc. The platform can be accessed on the Web at http://ochem.eu/alerts and it is open for any user following a simple registration procedure.

AlGaN/GaN semiconductor biosensors for radiation dosimetry and imaging applications in biophysics

Martin Schmid[1], Dipl.-Phys. Markus Hofstetter[1], M.Sc. John Howgate[2], PD Dr. Ian D. Sharp[3], Prof. Dr. Martin Stutzmann[2], PD Dr. Stefan Thalhammer[1]
1 Helmholtz Zentrum München, Institute for Radiation Protection, Ingolstädter Landstraße 1, Neuherberg, D-85764, Germany
2 Walter Schottky Institut, Technische Universität München, Am Coulombwall 3, Garching, D-85748, Germany
3 Joint Center for Artificial Photosynthesis, Lawrence Berkeley National Laboratory, 1 Cyclotron Rd, Berkeley, CA 94720, USA

X-ray as part of ionizing radiation plays an important role in our daily life. More than 50% of our mean annual exposure is due to medical diagnostics and therapy. Unwanted radiation for instance, Gamma-rays is part of the natural radiation background. There is a massive need for detector systems in these fields of application. Besides their stability to harsh environmental conditions the sensors have to show their reliability against radiation hardness, high quantum efficiency and good energy resolution. The "gold standard" tool for dosimetry applications is the ionization chamber, but also semiconductor materials are widely used and the list of applications is growing rapidly. In our research group, we are interested in GaN thin film structures with high electron mobility channels (HEMT) to be used as x-ray detectors.

The devices are operated with 40-300 kV Bremsstrahlung x-ray sources and within an air kerma dose rate range of 1uGy/s up to 10mGy/s. Our experiments did not show a radiation-induced damage of the sensor material even for high-accumulated air kerma doses and furthermore, the photoconductive device provides a large gain. For the previously mentioned conditions this gain is in the order of 105 [1].

To demonstrate the feasibility of our devices, medical imaging was performed using conventional finger phantom models and for resolution tests lead-slit phantoms were used. This was done with a computer controlled two-dimensional translation stage and a sensor fixed onto it. A maximum spatial resolution far below 1 mm can be achieved.

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Statistical Issues in Dynamic transcriptome analysis (DTA/cDTA): Bias Correction, Parameter Estimation, Validation

Björn Schwalb, Mai Sun, Daniel Schulz, Nicole Pirkl, Stefanie Etzold, Laurent Larivière, Martin Seizl, Kerstin Maier, Dietmar Martin, Patrick Cramer, Achim Tresch
LMU Gene Center

Standard transcriptomics measures only total cellular RNA levels. These levels result from a dynamic equilibrium of mRNA synthesis and decay processes. Our understanding of gene regulation would be greatly improved if we could measure RNA synthesis and decay rates on a genome-wide level. To that end, the Dynamic Transcriptome Analysis (DTA) method has been developed. DTA uses non-perturbing metabolic labeling that supersedes conventional methods to measure these rates in a precise and non-perturbing manner. We have recently extended DTA to comparative Dynamic Transcriptome Analysis (cDTA). cDTA provides absolute rates of mRNA synthesis and decay in Saccharomyces cerevisiae (Sc) cells with the use of Schizosaccharomyces pombe (Sp) as internal standard. cDTA is provided with a statistical methodology and all required bioinformatics steps that allow the accurate absolute quantification and comparison of RNA turnover. DTA/cDTA can be applied to reveal rate changes for all kinds of perturbations, e.g. in knock-out or point mutation strains, as responses to stress stimuli or in small molecule interfering assays like treatments through miRNA or siRNA inhibitors. The DTA/cDTA approach is in principle applicable to virtually every organism.

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QSPR prediction of melting point using Online Chemical Modeling Environment

Iurii Sushko, Sergii Novotarskyi, Robert Koerner, Igor Tetko
eADMET GmbH

Melting point is an important physico-chemical property and it can be used as an additional model parameter to improve predictions for other properties , e.g. solubility of chemical compounds. It is recognized that melting point is particularly difficult to predict using QSPR models in comparison to other physico-chemical properties, such as boiling point.

This study aims to perform a comprehensive QSPR modelling based the biggest up to our knowledge publicly available dataset with more than 35,000 experimental measurements. The generated models are based on 6 machine learning methods and 9 types of molecular descriptors, both 2D and 3D descriptors. Each machine learning method was invoked with each descriptor type to identify the approaches having the highest prediction accuracy.

A particular focus has been made on the applicability domain of the created models. How to estimate whether a model is applicable to a particular compound? What is the expected prediction accuracy for this compound? These questions are thoroughly investigated in the study based on specific metrics of the prediction accuracy referred to as "distances to models".

All the models were developed using a publicly available platform, Online Chemical Modeling Environment (OCHEM, www.ochem.eu) [1]. OCHEM was used to perform all the steps of the modelling: preprocessing of the structures, optimisation of 3D conformations, calculation of molecular descriptors, running machine learning methods, validation the models and assessment of the applicability domain. The models as well as the data used train them are available online under http://ochem.eu/models/Q9271. These models can be easily employed for prediction of melting points for the new chemicals.

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Evolutionary Engineering of Minimal Domain FRET-Based Calcium Indicators

Thomas Thestrup[1], Julia Litzlbauer[1], Tsai-Wen Chen[2], Douglas Kim[2], Oliver Griesbeck[1]
1 Max Planck Institute of Neurobiology
2 Janelia Farm Research Campus

The successful application of FRET-based indicators has extended to many fields in biology. In neurobiology, genetically encoded calcium indicators (GECIs) are valuable tools for reporting neuronal activity and offer crucial advantages over organic dyes such as targeting specific cell populations and chronic in vivo imaging of large populations of cells (1, 2).
Our approach, the FRET-based GECIs, utilize a Calcium-binding moiety Troponin C (TnC) fused between a pair of fluorescent proteins (FPs). The FRET signal output is achieved by the rearrangement of the overall conformation of the indicator upon Ca2+-binding. Modulation of the FPs and Ca2+-binding moiety allows for flexibility to optimize the indicators for diverse in vivo purposes.
Here we demonstrate the directed evolution of minimal domain FRET indicators based on the novel Troponin C (tsTnC) from the fast twitching Toadfish swimbladder muscle (3).

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How Subvisible Particles Get Invisible – Relevance of Refractive Index for Protein Particle Analysis

Sarah Zölls[1,2], Manuel Gregoritza[1], Ruedeeporn Tantipolphan[1], Michael Wiggenhorn[1], Gerhard Winter[2], Wolfgang Friess[2], Andrea Hawe[1]
1 Coriolis Pharma, Am Klopferspitz 19, 82152 Martinsried, Germany;
2 Ludwig Maximilian University, Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Butenandtstr. 5-13, 81377 Munich, Germany

Purpose: The aims were to develop a method for the refractive index (RI) determination of protein particles and to investigate the influence of the RI on the outcome of subvisible particle analysis of therapeutic protein formulations.

Methods: Protein particles were generated by stirring an IgG formulation (1 mg/mL) or by heating an HSA formulation (1 mg/mL). These particles and standard particles (polystyrene and silica) were analyzed by light obscuration (LO) and Micro-Flow Imaging (MFI) in formulations of increasing sucrose (0-50%) and/or protein concentrations (up to 200 mg/mL). The RI of protein particles was determined by mixing the particles with sucrose solutions of different concentrations providing an RI gradient and light scattering was quantified using UV absorption/emission.

Results: The RI of protein particles was determined as 1.41. The diminished RI difference between particles and surrounding liquid caused by high sucrose and/or protein concentrations, resulted in an apparent reduction of particle size and number determined by LO and MFI. A significant decrease of the apparently measured concentration of protein particles was observed already at pharmaceutically relevant sucrose concentrations (5%) and protein concentrations (100 mg/mL) in LO whereas concentrations in MFI were influenced to a lower extent. Silica particles (RI 1.42) showed an effect only at higher sucrose concentrations, but became completely invisible (= not detectable anymore by LO and MFI) at the RI match of particles and liquid.

Conclusions: The RI difference between particles and surrounding liquid had a strong influence on the performance of LO and MFI. Already at pharmaceutically relevant sucrose and protein concentrations significantly lower particle concentrations were measured than actually present in the formulation. Therefore, increased attention is required in the data evaluation of particle concentrations in formulations containing high sugar or protein concentrations.

Genome-wide approach to find genes involved in pain and relief learning of Drosophila

Mirjam Appel, Dr. Claus-Jürgen Scholz, Samet Kocabey, Sami Gödekdağ, Sinead Savage, Dr. Hiromu Tanimoto, Dr. Ayse Yarali
MPI for Neurobiology

Animals and man form two opposing kinds of memory from a traumatic experience. For example, fruit flies learn to avoid an odour as a signal for 'pain' if it preceded an electric shock during training; whereas they approach an odour as a signal for 'relief' if it followed the shock during training (Tanimoto et al. 2004).40 inbred strains have been generated from a natural population and transcriptomically analyzed (Ayroles et al. 2009). We first characterize these 40 strains for pain and relief learning and then we look for associations between transcript abundance and either kind of learning. Candidate genes will be varified using reverse genetic methods. Later on varified genes can then be used for analyzing the neural circuits underlying either kind of learning Their human homologues may make good therapeutic targets for psychiatric conditions.

eQTL analysis of glucocorticoid regulated gene expression: new insight in the genetics of mood and anxiety disorders

Janine Arloth, Andreas Menke, Torsten Klengel, Bertram Müller-Myhsok, Elisabeth B Binder
MPI Psychiatry

Abnormal regulation of the hypothalamus pituitary adrenal axis, the main stress-response systems, is a key neurobiological characteristic of major depression. Especially glucocorticoid receptor (GR) function has been shown to be disturbed in depression. Genetic variants that alter the transcriptional effects of GR activation might thus be interesting candidate polymorphisms for this disorder.

The aim of this study was to identify SNPs associated with glucocorticoid (GC)-induced gene expression changes of nearby genes (cis-eQTL analysis).

In a first step, we identified 2,364 response eQTLs, namely loci that were only associated with variation in GC-stimulated gene expression, but not with baseline gene expression changes. Fifty-six % of these response eQTLs were located within 200 kb from the probe ends- as compared to baseline eQTLs that showed 81% cis-association within 200 kb, indicating a more long range regulation of gene expression by GCs. In a second step, we tested for an enrichment of transcription factor (TF) binding sites nearby eQTL SNPs. We identified the GR response element (GRE) as one of the most enriched TF binding site and observed differences in the affinity of GREs between the opposite SNP alleles, with the minor alleles being on average associated with a reduced affinity to the predicted GRE. In a last step we integrated our data with results from GWAS for mood and anxiety disorders from publically available data sets (from http://www.genome.gov/gwastudies/ and http://geneticassociationdb.nih.gov/). We found an overlap of SNPs in significant response eQTLs and SNPs associated with traits related to mood and anxiety disorders. Interestingly, the majority of these overlapping SNPs does not alter the gene expression of the closest gene but of more distant genes. For example, SNPs within the CLOCK gene moderate the gene expression of PAICS, which is 947 kb upstream of the CLOCK gene. Finally, we combined depression susceptibility loci from a recent meta-analysis of GWAS for unipolar depression and that GC response eQTLs were significantly more likely to be associated with the disease as baseline eQTLs.

In summary, our data suggest that GC-stimulated eQTLs could expand our understanding of the genetic basis of stress-related disorders, including major depression, in which GR-function plays an important pathophysiologic role.

Establishing the hippocampal cytokine profile in a mouse model for posttraumatic stress disorder

Joanna Gutmann[1], Leonie Herrmann[1], Christine Huber[1], Kathrin Henes[1], Prof. Florian Holsboer[1], Dr. Carsten T. Wotjak[1], Dr. Ulrike Schmidt[1]
1 Max Planck Institute of Psychiatry, Munich, Germany

Posttraumatic stress disorder (PTSD) is a severe and debilitating psychiatric disease.
Alterations in the immune system like elevated serum levels of the proinflammatory cytokines TNFα, IFNγ, IL-1β, and IL-6 have already been associated with stress-associated psychopathologies in humans. Employing the PTSD mouse model established by Siegmund and Wotjak (2007), we detected increased hippocampal levels of immunoglobulin G (IgG) in a pilot experiment. In order to screen for additional alterations in the immune system in PTSD, we now aim at establishing the hippocampal cytokine expression profile of the footshocked and control mice by using a cytokine antibody array which simultaneously detects expression levels of 97 cytokines. For further validation by enzyme-linked immunosorbent assay (ELISA), we selected promising candidates, such as leptin which has already found by others to be increased in serum of PTSD patients. In order to identify mechanisms leading to altered cytokine expression, we analyzed the nuclear factor kappa B (NFκB) pathway since cytokines are the main target genes of this pathway which plays a key role in regulating the immune response. Here, we present preliminary data of the expression levels of IKKα, IKKβ, IKBα, and p65 assessed by Western blotting. The results demonstrated here contribute to elucidating the role of the immune system in PTSD pathogenesis.
Furthermore, identifying mechanisms regulating immune system proteins like cytokines might open up novel treatment options.

Promoter characterization of TMEM132D - a susceptibility gene for anxiety-related phenotypes

Leonie Herrmann, Bozidar Novak, Elisabeth Binder, Rainer Landgraf, Jan Deussing, Dietrich Trümbach, Florian Holsboer, Theo Rein, Ulrike Schmidt
MPI Psychiatry

Transmembrane protein 132D (TMEM132D) was identified as a susceptibility gene in a genome-wide association study in panic disorder patients. This result was corroborated by the finding that its expression is correlated positively with anxiety-related behavior in HAB/LAB mice which in addition indicates that TMEM132D might also be a candidate molecule for other anxiety disorders, e.g. for posttraumatic stress disorder (PTSD).

To explore the regulation of TMEM 132D gene expression, we performed a promoter characterization: Analyzing progressive 5'deletion mutants in a reportergene assay, we identified the first 600 bp upstream of the start codon as the most potent promoter activating region. Site-directed mutagenesis of selected transcription factor binding sites and overexpression of the corresponding transcription factors in a cell culture model revealed that most of the transcription factors analyzed exhibit inhibitory effects on TMEM132D expression.

To elucidate the functional significance of these transcription factors, we determined their mRNA expression levels in a model of oligodendrocyte maturation, as TMEM132D has been found to be upregulated in mature oligodendrocytes. Here, we show that upregulation of TMEM132D is accompanied by a downregulation of a variety of inhibitory transcription factors during oligodendrocytic maturation.

In summary, the results presented here elucidate the regulatory mechanisms of TMEM132D gene expression in the context of oligodendrocyte maturation which might contribute to the enlightenment of the pathomechanisms of anxiety disorders.

Safety first: different helmets for different predators

Quirin Herzog, Christian Laforsch
LMU Munich

Predation is a strong force of selection driving evolution. Due to it's rarely stable nature it is known to cause adaptations in the form of plastic responses in phenotypes. These changes are known as inducible defenses and widespread in many different taxa, including bacteria, plants, vertebrates and invertebrates. They are especially well studied in the genus Daphnia (Crustacea), a group of planktonic freshwater organisms that has been established as model organisms. However so far, with few exceptions, research on inducible defenses focused on European and American species. In this study, we tested the response of the endemic African species Daphnia barbata Weltner, to infochemicals (kairomones) of two different predators, the backswimmer Notonecta glauca and the tadpole shrimp Triops cancriformis. We show that D. barbata reacts to both predators with an array of inducible morphological defenses. Apart from a longer tail spine and a dorsal ridge armed with longer thorns, their unique subtriangular helmet was significantly longer and differed in shape depending on the predator used to induce. Furthermore, we demonstrate their specific adaptive value through direct predation experiments. In this study we not only provide first evidence for inducible defenses in the formerly disregarded species D. barbata, but also show for the first time that different predators can induce different helmet shapes in the same species.

Experimental tools to study the role of glia in CNS homeostasis

Malte Kremer[1], Dr. Sara Batelli[2], Dr. Christophe Jung[2], Dr. Gerald M. Rubin[3], Prof. Ulrike Gaul[1,2]
1 GeneCenter Munich, LMU, IMPRS, Janelia Farm Visitors Program
2 GeneCenter Munich, LMU
3 Janelia Farm Research Center, HHMI, Ashbrun, VA

The nervous system consists of two main cell types: neurons and glia. In vertebrates, glia constitute the majority of all cells in the brain but, to date, their functions remain less characterized compared to those of neurons. Studies in different animal models are now revealing a deep interconnection between these two cell populations at every step of nervous system development and in every aspect of nervous system function. Preliminary analysis provides evidence for a high degree of morphological, functional and molecular homology between fly and vertebrate glia, making Drosophila a powerful model for studying the role of this cell type in the nervous system homeostasis.

A detailed investigation of glial functions in the (adult) Drosophila brain in detail requires subtype specific manipulation at defined developmental stages. Therefore, we identifoed glia subtype specific drivers and comprehensively characterize them with respect to their morphology, localization in the CNS, cell number and region-specific specialization. Here, we first want to present the results of this anatomical study. Second, we want to introduce our strategy for adult specific gene manipulation. We will finish with an outlook how these new genetic tools help to investigate adult glial subtype specific function and why we believe these tools will ultimately pave way to investigate the role of glia in nervous system homeostasis and the possible contribution in neurodegenerative disease.

Mechanosensitivity as a property of isolated enteric neuronal networks

Eva Kugler, Florian Zeller, Michael Schemann, Gemma Mazzuoli
TUM Humanbiologie

The enteric nervous system located within the gut wall controls all vital gut functions autonomous fully independent of the central nervous system. One of its network - the myenteric plexus - controls motility and thereby intestinal transit. In the gut mechanical activity is permanently present and causes distortion of the myenteric plexus by the movement of the muscle layers. Enteric neurons react to these mechanical stimuli, but their mechanotransduction modality has not been identified. We therefore investigated the mechanosensitive properties of primary cultured enteric neurons, a model that allows one to study neuronal mechanosensitivity directly, without interference from other cells. We performed studies using cultures from 26 guinea pigs and 14 human intestine samples. The neurons were mechanically probed using a micrometric carbon fiber and neuronal activity was detected with ultra-fast recordings of voltage sensitive dye signals.

We found that cultured myenteric neurons of the guinea pig and human reproducibly fire action potentials in response to mechanical stimulation with a micrometric carbon fiber, in guinea pig 72 (50/92) % responded with an action potential frequency of 2.8 (1.1/6.1) Hz and in human culture 75 (50/100) % responded with an action potential frequency of 1.6 (0.6/4.2) Hz. Excitatory responses were elicited while probing the soma and neuronal processes. Loading and unloading stimuli evoked a similar response, showing that these neurons respond to dynamic changes. The response patterns clearly indicate that the neurons rapidly adapt to the stimulus.

We describe for the first time mechanosensitive properties of isolated enteric neuronal networks. It is striking that the majority of enteric neurons seem to function as rapidly adapting mechanosensors. Both the soma and the neurites encode the mechanical stimulus. Our findings set the basis to understand how the enteric neurons regulate reflex pathways.

Role and Regulation of Guanosine Exchange Factors on Neuronal Polarity

Alejandro Lopez-Tobon, Kristina Mahnken, Andreas W. Püschel
University of Münster

The establishment of a polarized axo-dendritic axis is essential for the organization and function of the nervous system. The process of neuronal polarization requires the regulation of cytoskeleton dynamics, which is in part mediated by small GTPases. The activation of small GTPases relies upon the binding to Guanosine Exchange Factors (GEFs). One of the first intracellular processes that determines which neurite will become the axon is the recruitment of the small GTPases Rap1B and Cdc42 to a single neurite, which subsequently leads to the downstream cytoskeletal effects that stimulate the accelerated axonal outgrowth. However, the GEF involved in linking Rap1B and Cdc42 remains to be determined.

The structural and functional homology to a conserved polarity signaling pathway on budding yeast, suggests that the PIX family might act as the missing link between Rap1B and Cdc42 in neuronal polarity. Our preliminary results show that bPix localizes to the tip of the axon in stage 3 neurons. Moreover bPix is able to interact with Rap1B and Cdc42 in vitro. In hippocampal primary cultures the knockdown of bPix leads to the loss of axons, while its overexpression increases axon length. Further studies are required to establish whether bPix is functionally regulating the activity of Cdc42 and Rap1B as a GEF during the establishment of neuronal polarity.

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Comprehensive analysis of gene expression regulated by fosB gene in neuron and glia

Hiroko Nomaru, Noriko Yutsudo, Yusaku Nakabeppu
Kyushu University

fosB gene produces two mature transcripts, fosB and ΔfosB mRNAs by alternative splicing, thus encoding multiple subunits of AP-1 (activator protein-1) transcription factors. ΔFosB and Δ2ΔFosB proteins, lack the C-terminal 101-amino-acid region of FosB protein encoded by the fosB mRNA. Because the region contains the motifs responsible for the interaction with TATA-box binding protein (TBP) and TFIID complex, it is likely that the fosB gene products, FosB, ΔFosB and Δ2ΔFosB may differentially modulate the expression of various AP-1 targets.

The basal expression of fosB is detected in throughout brain, especially cerebral cortex and hippocampus, and is highly inducible upon various brain stimuli. We recently found that fosB-knockout (KO) mice exhibit depressive behavior and adult onset epilepsy, while fosBd/d mutant mice expressing only the DfosB mRNA exhibit increased locomotor activity.

In the present study, we isolated neurons, astrocytes and microglia from cortex and hippocampus and compared the expression levels of fosB and DfosB mRNAs. We found that expression levels of fosB and DfosB mRNAs vary according to types of cells or brain regions, and especially those in cortical neurons are twenty times higher than those in hippocampal neurons. In addition, the ratio of fosB and ΔfosB mRNAs also varies according to types of cells.

We then compared gene expression profiles in wild-type, fosB-KO and fosBd/d mutant microglia and astrocyte by microarray analysis. In the microglia, 8 genes exhibit significantly different expression levels more than 1.5 fold among the three samples, and in the astrocyte, 20 genes also exhibit different expression levels. Among the genes changed expression levels in microglia and astrocyte, only ERCC2 genes expression is decreased both microglia and astrocyte from fosB-KO and fosBd/d mutant mice. This result is also consistent with microarray data of mouse hippocampus.

Phenotypic plasticity in Daphnia: A proteomic approach to ecology

Kathrin Otte, Thomas Fröhlich, Georg Arnold, Christian Laforsch
LMU Biocenter

Predation is a key factor in the evolution of prey species and the dynamics of prey communities. In both animals and plants, different defensive mechanisms have evolved in response to this selection pressure. Phenotypic plasticity in defensive traits appears to be an appropriate mechanism to cope with the variable hazard of a frequently changing predator system. A textbook example for these so called inducible defences is the waterflea Daphnia.

However, our understanding of the mechanisms underlying plastic responses in Daphnia is still in its infancy. Given that Daphnia is emerging as the key model invertebrate system in ecological genomics, our study aims to discover the molecular basis of predator induced defences. Therefore, we use proteomic complementary approaches like gel-based 2D fluorescence difference analysis (DIGE) and mass spectrometry-based iTRAQ technology. Currently, we investigate candidate proteins responsible for the formation of inducible morphological defences in Daphnia.

As proteomic and transcriptomic approaches are complementary to relate gene expression data to the organism's phenotype, our overall aim in this collaborative project is to combine both methodologies in the long run to gain insight into the evolution of genes involved in the formation of predator induced defences.

Polar magneto-aerotaxis in *Magnetospirillum gryphiswaldense*

Felix Popp, Julia Hofmann, Denis Bartolo, Dirk Schüler
LMU Biology

The paradigmatic concept of random walk motion observed in most prokaryotes is greatly simplified in freely swimming magnetotactic bacteria (MTB) which contain a chain of nano-sized magnetic particles. Passive alignment with the Earth's magnetic field forces the bacteria onto a nearly linear track. In addition, most MTB possess the selectable trait to follow the magnetic field lines in a preferred swimming direction (either N- or S-seeking) depending on the prevailing habitat conditions. To date, the underlying molecular mechanism of how magnetic polarity is integrated with other taxis mechanisms is not understood.

M. gryphiswaldense is a bipolarly flagellated gradient organism which is capable of polar swimming behaviour if grown under selective conditions. Automated video tracking of wild type cells revealed swimming episodes in alternating directions which are interrupted by short reversals. The reversal frequency did not change significantly in polarised cultures.

We identified four chemotaxis gene clusters containing conserved genes cheAWYBR in the genome of *M. gryphiswaldense*. Whereas deletion of operons 2-4 did not impact on chemotaxis, only loss of CheOp1 had a clear effect on aerotaxis. This indicated a possible link between polarity and chemotaxis at the genetic level. In magnetospirillum cells having an apparently symmetrical morphology, polarity might be established by asymmetric localization of constituents of the chemotaxis machinery. Therefore, we studied the intracellular localization of fluorescent protein fusions to chemotaxis proteins CheA and CheW. Since these proteins localised to variable positions in the cell they are unlikely to determine polarity.

Swimming polarity is currently being studied quantitatively by a microfluidic assay using fluorescence-labelled cells and in competition assays. This will also reveal the putative selective advantage of magnetotaxis.

In vivo pathogenesis of demyelination in an animal model of multiple sclerosis

Elisa Romanelli, Stephanie Potz, Ivana Nikic, Doron Merkler, Martin Weber, Derron Bishop, Thomas Misgeld, Martin Kerschensteiner,
LMU Munich

Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the human central nervous system (CNS). Although demyelination represents the major histopathological hallmark of the disease, currently we know only little about mechanisms that mediate it. We have now used an in vivo imaging approach, previously developed in our lab (Kerschensteiner et al., 2005 Nature Medicine; Misgeld and Kerschensteiner, 2006 Nature Reviews Neuroscience; Nikic et al., 2011 Nature Medicine) to study how myelin is removed in Experimental Autoimmune Encephalomyelitis (EAE), the most commonly used animal model of MS. Here we can show that during demyelination in vivo single myelin sheets are pulled from the myelin and form bulb-like structures that we called "myelinosomes". We confirmed the presence of myelinsomes in experimental and human neuroinflammatory lesions by confocal microscopy and serial-sectioning electron microscopy. We are currently investigating the molecular mechanisms that underlie the formation of myelinosomes and in particular the contribution of anti-myelin antibodies. We hope that in the long run a better understanding of the mechanisms that mediate inflammatory demyelination will help us design better therapeutic strategies for MS.

Dissecting the regulation of Rap1 GTPases during neuronal polarisation

Bhavin Shah, Daniela Lutter, Prof. Andreas Püschel
Institute for Molecular and Cell biologie, Münster

Polarization is an essential process during neuronal differentiation whereby a single axon and multiple dendrites are generated. In cultured hippocampal neurons from rat embryos, polarization can be divided into 5 stages. Initially a neuron forms several neurites, each having the potential to form an axon. During the transition from stage 2 to stage 3, one of the neurites is selected to become the axon and grows rapidly to form an axon while the other neurites mature to form dendrites. We have identified a signalling pathway that involves the sequential activation of PI3K, Rap1, Cdc42 and the Par polarity complex during the polarization process. We showed that Rap1 GTPases are essential for axon formation and act upstream of Cdc42 to initiate axonogenesis in cultured neurons. However, the upstream factors that regulate Rap1 activity still remain unknown.

In vivo, neurons originate from the ventricular zone (VZ) and subsequently migrate towards the cortical plate (CP). They transiently show a multipolar morphology in the intermediate zone and become bipolar when they resume their migration towards the cortical plate. It remains to be shown that the signalling pathways identified in cultured neurons also regulate this process in vivo. Here, we study the role of Rap1 in vivo using conditional knockout mouse. Our results show that Rap1 is essential for axon formation in hippocampus. We also identified a GEF that acts upstream of Rap1 and is essential for axon formation in cultured hippocampal neurons.

Jens C Schwamborn & Andreas W Püschel, Nature Neuroscience 7, 923 - 929 (2004)

A Large-Scale Behavioural Screen For Neurons Responsible For Electric Shock And Sugar Response In Drosophila

Vladimiros Thoma, Christine Damrau, Stephan Knapek, Thomas Templier, Hiromu Tanimoto
MPI Neurobiology

The ability to sense stimuli and elicit proper responses is crucial for the survival of all animals. Up to date, the neuronal networks responsible for driving such responses remain poorly characterized. To comprehensively characterize such networks, we investigated behavioural responses of transgenic Drosophila lines to broadly used appetitive (sugar) and aversive (shock) stimuli. In these flies, subsets of neurons can be conditionally blocked. Therefore, the importance of these neurons in driving responses to the stimuli utilized can be investigated.

Dissecting The Neural Circuit Of Aversive Visual Learning In Drosophila Melanogaster

Katrin Vogt[1], Christopher Schnaitmann[1], Simon Triphan[2], Hiromu Tanimoto[1]
1 Max-Planck-Institute of Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany
2 Department of Neurobiology and Genetics, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

To understand neurobiological mechanisms for visual processing and memory, we developed a new conditioning assay for aversive visual associative learning in adult flies. Flies are trained en masse to associate one of two different visual stimuli (e.g. blue and green light) with electric shock punishment. In the test phase, both punished and control visual stimuli were presented, and the difference in flies' visual preference was measured as associative memory. The trained flies showed significant conditioned avoidance of a punished cue. Since the setup is based on a similar existing appetitive conditioning assay we can directly compare appetitive and aversive visual memories. Furthermore we want to map the neuronal circuit for color perception in the fly brain by using this classical conditioning setup. Thereby we started investigating the role of the mushroom body, a prominent structure in the central brain of the fly, in visual learning.

Psychological and biological mechanisms of stress reactivity and stress-coping in post-traumatic stress disorder (PTSD)

Zaba M[1,2], Kirmeier T[1], Ionescu IA[1], Gall-Kleebach D[1], Schubert C[1], Uhr M[1], Wollweber B[1], Höhne N[1], Ising M[1], Schönfeld S[2], Schmidt U[1]
1 MPI Psychiatry
2 Dresden University of Technology

Post-traumatic stress disorder (PTSD) develops as a consequence of maladjustment to a traumatic event. This disorder is accompanied by re-experiencing, avoidance, and hyperarousal symptoms. PTSD treatment encompasses both medication and psychotherapy. However, there is still a need to develop drugs being effective specifically against PTSD symptoms, and psychotherapy methods enabling therapeutical success to occur more rapidly.

To better understand PTSD and to improve its treatment, in this study stress reactivity and stress-coping are examined. For the stress response the hypothalamic-pituitary-adrenal axis depicts a major biological system. There is evidence that the mechanism is sensitized in PTSD patients in terms of decreased basal cortisol levels and increased negative feedback regulation. Gene expression patterns associated with PTSD have also been found. However, the research in this field is sparse and characterized by contrary results. Additionally, there is evidence that PTSD patients use maladaptive coping strategies, such as suppression or avoidance. Changes in cognitive processing, such as dissociation or attention bias, were also found to be related with PTSD.

In this study we compare PTSD patients with healthy controls with respect to stress reaction and stress-coping differences between them. We conduct Trier Social Stress Test (TSST), which is a well-established method to induce stress reaction in humans, and analyze stress response both from the psychological and biological perspective. From the psychological perspective, we assess changes in feeling, stress perception, and dissociation symptoms. From the biological perspective, changes in stress hormones levels (adrenocorticotropin, cortisol), in the autonomous nervous system (heart rate, blood pressure), in the immunology parameters, as well as differences in gene expression patterns, are examined.

Gene expression analysis in neoadjuvant treated gastric cancer: a role for Notch and Wnt signaling

Lukas Bauer[1], Rupert Langer[1], Karen Becker[1], Katja Ott[2], Alexander Novotny[3], Alexander Hapfelmeier[4], Heinz Höfler[1], Gisela Keller[1]

1 Department of Pathology, Technische Universität München
2 Department of Surgery, Universität Heidelberg
3 Department of Surgery, Technische Universität München
4 Department of Medical Statistics and Epidemiology, Technische Universität München

Neoadjuvant treatment of gastric cancer offers the opportunity to investigate residual tumor cells after chemotherapy (CTX). We analyzed the expression of cancer stem cell (CSC) related genes in residual gastric tumors for an association with overall survival and compared corresponding pretherapeutic biopsies and resected tumors for CTX associated expression changes.

An initial mRNA expression screening of 44 genes selected due to their relevance for differentiation and development or as putative CSC markers was performed. Resected specimens from 63 gastric cancer patients treated with neoadjuvant platinum/5-FU based CTX that demonstrated a partial tumor regression (i.e. 10-50% residual tumor cells) were included. Selected genes were additionally compared between corresponding biopsies and resected specimens from patients with partial and minimal/no tumor regression (i.e. >50% residual tumor cells).

Genes involved in Wnt and Notch signaling pathways, such as GSK3B, CTNNB1 and NOTCH2 demonstrated a prominent association with overall survival (p=.006, p=.043 and p=.072, respectively) in the initial screening. A combined expression pattern of these three genes identified a group of patients that hat a significantly increased overall survival (p<.001). Comparison between biopsies and resected specimens revealed an increase of NOTCH2 and LGR5 expression in tumors with partial response (p=.002 and .017) and of POU5F1 in both partial and minimal/non responding tumors (p=.028 and .002).

In tumors with partial regression after neoadjuvant CTX, the expression of genes involved in CSC associated signaling pathways, such as the Wnt and Notch pathway, showed a prognostic significance, which may be used for risk stratification in this patient group. The comparison of expression levels between corresponding biopsies and resected specimens revealed gene expression alterations that might be related to chemotherapy resistance of residual tumor cells.

Analysis of morphological changes in serially observed bovine embryos

Andrea Beck[1], Myriam Reichenbach[2], Horst Dieter Reichenbach[3], Felix Habermann[4], Georg Arnold[5], Eckhard Wolf[1,2]
1 Lehrstuhl für Molekulare Tierzucht und Biotechnologie, LMU München
2 Bayrisches Forschungszentrum für Fortpflanzungsbiologie (BFZF) e.V.
3 Institut für Tierzucht, Bayrische Landesanstalt für Landwirtschaft, Grub
4 Lehrstuhl für Anatomie, Histologie und Embryologie, LMU München
5 LAFUGA, Genzentrum, LMU München
6 Lehrstuhl für Molekulare Tierzucht und Biotechnologie, LMU München; Bayrisches Forschungszentrum für Fortpflanzungsbiologie (BFZF) e.V.

Time-lapse monitoring has recently become an important method for determining the developmental potential of oocytes and early embryos. In this study, a monitoring system for cattle embryos was established in order to analyze the most critical stages in early embryo development for proteome and 3D multichannel CLSM analysis. Oocytes were recovered from abattoir ovaries. After 23 h maturation, the oocytes were in vitro fertilized and subsequently cultured for 7 d. To guarantee an identification of the individual embryo, a special Well-of-Well culture dish was used. While being cultured, the embryos were photographed every 5 min using the Primo Vision system, with regard to time of the first and second cleavage as well as normal and abnormal cleavages. This way, it is possible to obtain developmental time profiles for individual embryos that can be correlated with different endpoint analyses. In order to collect embryos with a high developmental capacity, it is necessary to determine parameters for early embryonic development. In the present study the relationship between the duration of the first cell cycle and the embryo developmental rate was high. In addition a wide time window for the first two cell cycles and an early cleavage in embryos that reached later embryonic stages was observed.

Ku-dependent DNA double-strand break repair analyzed by transmission electron microscopy

Christina Brunner, Yvonne Lorat, Claudia E. Rübe
Department of Radiation Oncology, Saarland University, Germany

DNA double-strand breaks (DSBs) generated by ionizing radiation (IR) pose a serious threat to the preservation of genetic and epigenetic information. Severe consequences can arise if DSBs are not efficiently recognized and repaired. An essential step of the repair pathway non-homologous end-joining (NHEJ) is the high-affinity binding of DNA-PK (consisting of two Ku70-Ku80 heterodimers and DNA-PKcs) to DSBs. Thereby, the Ku dimer holds the broken ends in physical proximity in preparation for religation. Because Ku itself is a substrate of DNA-PKcs and becomes phosphorylated upon binding, we apply an antibody which detects the phosphorylated and thus activated form of the protein.

Repair factors like gH2AX or 53BP1 that accumulate at damaged sites in focal structures termed IR-induced foci (IRIF) can be studied by immunofluorescence microscopy. In contrast, Ku proteins cannot be detected by IF since they do not cluster around the DNA break. We therefore established a transmission electron microscopy (TEM) approach to study the affinity and kinetics of Ku proteins at IR-induced DNA lesions at the single molecule level.

To consolidate that the phosphorylated form of Ku70 (pKu70, Ser 5) analyzed in our approach binds to DSBs, we show that it is not found within chemically induced single-stranded DNA lesions.

Corresponding to the literature, we noticed that pKu70 is recognized in clusters of 2 or a multiple of 2 molecules in the nucleus of irradiated cells. To determine if this reflects the binding of two Ku-dimers to double-stranded DNA ends, we develop an in vitro DNA-protein binding assay. Therein, DSBs are induced in vector DNA by restriction enzyme digestion. They are then bound by a recombinant Ku complex, which is finally highlighted by immunogold-labeling using our TEM approach.

The Role of the NF-kappaB Pathway in LMP1/CD40 expressing B cells in vivo

Kristina Djermanovic[1], Sabine Schmidl[1], Cornelia Hömig-Hölzel[1], Caroline Hojer[1], Falk Weih[2], Marc Schmidt-Supprian[3], Ursula Zimber-Strobl[1]
1 Department of Gene Vectors, Helmholtz Center Munich
2 Fritz-Lipmann-Institute
3 Molecular Immunology and Signal Transduction, Max Planck Institute of Biochemistry

CD40 is a member of the tumor necrosis factor (TNF) family and is expressed on almost all antigen presenting cells, including B cells. In B cell lymphomas, such as the Hodgkin lympoma, CD40 is highly upregulated indicating that aberrant CD40 signalling plays a critical role in oncogenic processes that contributes to lymphomagenesis. Therefore, we established a transgenic mouse strain that expresses B cell specific the fusion protein LMP1/CD40, which is composed of the intracellular domain of CD40 and the transmembrane domain of the Epstein-Barr-viral protein LMP1, allowing to investigate constitutively active CD40 signal in B cells. The constitutive CD40 signal leads to a splenomegaly and enlarged lymph nodes due to enhanced proliferation and prolonged survival of these B cells. At the age of 80 weeks more than 50% of these mice develop tumors. LMP1/CD40 expressing B cells show constitutive Erk and Jnk phosphorylation. They also display a selective activation of the non-canonical NF-kappaB pathway, which is known to be highly active in various carcinomas as well as in Hodgkin lymphomas, whereas canonical NF-kappaB pathway was comparable to wild type level. Therefore, we want to further investigate the role of the NF-kappaB pathway in lymphomagenesis of LMP1/CD40 mice. By crossing the LMP1/CD40 mouse to conditional RelBfl/fl mouse and to a IKK1fl/fl mouse we inactivated the non-canonical NF-kappaB pathway. The inhibition of the canonical NF-kappaB pathway was achieved by inactivation of Nemo and IKK2 in LMP1/CD40 expressing B cells. The inactivation of Nemo in LMP1/CD40 expressing B cells affected the expansion of LMP1/CD40 expressing B cells severley and diminished the splenic weight to wild type level, whereas the depletion of RelB had no effects on the phenotype of the LMP1/CD40 mice. We have also evidence that Nemo plays a crucial role in the activation of the MAPK (Mitogen-activated-protein-kinase) Erk in LMP1/CD40 expressing B cells.

A long way to chaperone a kinase: The binding of Hsp90 to its kinase specific co-chaperone Cdc37

Julia M. Eckl, Daniel Rutz, Klaus Richter
TUM

The molecular chaperone Hsp90 is a highly abundant protein inside the cell and is involved in protein folding, cell signaling and tumor suppression. To facilitate these functions Hsp90 cooperates with a defined set of co-chaperones, which help to regulate the ATP turn-over rate at the N-terminal domain. One prominent group of clients are the protein kinases, which interact with Hsp90 in presence of the phosphorylated form of the Cdc37 co-chaperone. Cdc37 itself binds with high affinity to Hsp90 and inhibits its ATPase activity. Until now it remains still unclear how the Hsp90-Cdc37-kinase complex formation occurs. To investigate this issue we first started to analyze Hsp90 and Cdc37 interaction sites by generating different deletion and point mutations. Using biophysical methods like analytical ultracentrifugation, SPR and ATPase assays we could delimit the conformational state of Hsp90 and the binding site of Cdc37. Further on we are going to examine the dependency of Caenorhabditis elegans kinases that have a high homology to human kinases, to clarify the complex formation with Hsp90-Cdc37 and also their maturation with other co-chaperones.

Transposon silencing by endo-siRNAs – a hint to precursor production

Katharina Elmer[1], Prof Dr Klaus Foerstemann[1]
1 Gene Center Munich LMU

In flies deleterious invasion of transposons in somatic cells is repressed by endo-siRNAs. Endo-siRNAs are 21nt long double stranded RNAs which show perfect complementarity to their target sequence. Their precursors derive from endogenous transcripts and are processed in the cytosol by a distinct pair of RNaseII/RNA binding proteins. Introduction of artificial transposons into the fly genome by stable transfection of high copy reporter genes, can also lead to an endo-siRNA response, showing that there is no need for a pre-existing pool of small RNAs or pseudogenes as a template for their production. I could show in cell culture experiments that the number of endo-siRNAs produced against artificial transposons is dependent on their copy number, that there might be a threshold level for efficient silencing of repetitive elements and that silencing is dependent on a 3' poly A signal. Genes that are deliberately encoded in high copy numbers, as for example histone genes, might therefore be protected from silencing by their unusual 3'UTR.

Endothelial barrier protection by hawthorn extract WS® 1442: Identification of the bioactive phytochemical compounds

Simone Fuchs, Elisabeth A. Willer, Evelyn Hartung, Angelika M. Vollmar, Robert Fürst
Department of Pharmacy, Center for Drug Research, Pharmaceutical Biology, University of Munich, Germany

We have recently discovered that the hawthorn (Crataegus spp.) extract WS® 1442, widely used for the treatment of mild forms of heart failure, efficiently prevents inflammation-induced endothelial hyperpermeability in vitro and in vivo. WS® 1442 characteristically affects key mechanisms of the endothelial barrier function, such as F-actin cytoskeleton, the adherens junctions (VE-cadherin), the contractile machinery (myosin light chain), and the intracellular calcium concentration. Mechanistically, the extract blocks the barrier destabilizing Ca2+/PKC/RhoA pathway and activates the barrier protective cAMP/Rap1/Rac1 signaling. Acquiring first insights into the bioactive principles of the extract, we previously examined four different phytochemical fractions (A-D). It turned out that only fraction B (small phenolic compounds and flavonoids) and C (oligomeric proanthocyanidins) affect Ca2+ and cAMP pathways, respectively.

Intention of the present study was to further identify the bioactive compounds responsible for these effects. We tested various sub-fractions (B1a-g, B4a-f, C3a-f, C5a-d) of fraction B and C (preparative reversed-phase HPLC, kindly provided by Dr. Willmar Schwabe GmbH & Co.KG, Karlsruhe, Germany) in cultured human endothelial cells (HUVECs). Concerning fraction B, we identified four sub-fractions (B1e-f, B4d-e) out of thirteen that revealed a clear reduction in the thrombin-induced rise of cytosolic calcium (ratiometric imaging). Regarding fraction C, a clear augmentation of cortactin phosphorylation (confocal microscopy) can be observed by the sub-fractions C3b-f, C5a and C5c-d. Moreover, these mechanisms can be assigned to distinguished chemical structures, namely procyanidin B2 (dimer) and procyanidin C1 (trimer).

In summary, we found that the isolated representative procyanidins indeed beneficially affect the cAMP signaling. Presently, fraction B undergoes further investigations in order to identify the precise components responsible for the strong impact on the barrier disrupting calcium pathway. Most importantly, we could for the first time identify distinct bioactive compounds of the multi-component drug WS® 1442 and allocated them to different signaling pathways.

Classification of permanent and transient protein-protein interactions

Florian Goebels[1], Prof. Dmitrij Frishman[1,2]
1 Department of Genome Oriented Bioinformatics, Technische Universitat Munchen, Wissenschaftszentrum Weihenstephan
2 Institute for Bioinformatics and Systems Biology/MIPS, HMGU German Research Center for Environmental Health

There is a variety of methods for distinguishing between permanent and transient protein-protein interactions (PPI). However they all require a known three-dimensional structure of the protein complex and are therefore not applicable to millions of interactions determined by high-throughput techniques such as yeast two-hybrid or affinity purification combined with mass-spectrometry. We have developed a method for classifying PPIs into permanent or transient based on protein sequence features and the topological properties of interaction networks . Our predictor achieves 87.5% precision on a test set of 77 manually classified PPIs. Efforts are currently underway to provide an exhaustive classification of all experimentally determined protein interactions for model organisms.

Relationships between structural proteins and biomechanical AAA wall properties

Verena Grabher-Meier[1], Jaroslav Pelisek[1], Mihaela Culmes[1], Renate Hegenloh[1], Andreas Maier[2], Michael Gee[2], Wolfgang A. Wall[2], Hans-Henning Eckstein[1], Christian Reeps[1]
1 Clinic of Vascular Surgery, Klinikum rechts der Isar der Technischen Universität München, Germany
2 Institute for Computational Mechanics, Technische Universität München, Germany

Introduction: The association between structural proteins and mechanical properties of vessel wall during formation and progression of abdominal aortic aneurysm (AAA) has not yet been reliably defined. Extracellular matrix (ECM) components contribute essentially to the stability of aorta and pathophysiological changes in ECM are the leading cause of aneurismal wall expansion. The aim of the study is therefore to investigate the relationships between structural proteins of ECM and mechanical properties of AAA wall.

Methods: Selected patients assessed for open repair of AAA underwent computed tomography (CT) and these data were used for 3-dimensional (3D) reconstruction of AAA. Wall specimens from different locations were harvested during surgery and 3D image was used to determine the exact sample excision site. Small longitudinal segments were removed, fixed with formalin, embedded in paraffin, and examined for the content of elastin, collagen, and proteoglycans. The remained tissue, corresponding to the histopathological part, was analysed biomechanically by uniaxial tensile tests. Elastic properties were assessed by cyclic sinusoidal loading followed by destructive testing in order to determine the failure load.

Results: Preliminary results confirm that elastin content is significantly diminished in AAA tissue specimens compared to healthy aorta (2.4±2.9% vs. 54.8±7.6%, p=0.021). Furthermore, the total amount of proteoglycans in AAA samples is markedly decreased as well, however, without the postulated level of significance (34.2±17.2% vs. 56.7±1.8%, p=0.090). In contrast, the amount of collagen was similar in both study groups (44.9±10.1% vs. 37.2±1.3%, p=0.153). Mechanical properties of AAA tissue specimens correlated well with the structural composition of the vessel wall, especially with the content of collagen.

Conclusion: Significant differences were observed in the structural composition of vessel wall between AAA specimens and control aorta. Furthermore, the composition of ECM correlated with the mechanical properties of our tissue samples. Thus, determination of the content of structural proteins within AAA wall might contribute to predict its progression and also the risk of rupture.

The role of alveolar macrophage cell death in sterile, persistent pulmonary inflammation

Nunja C. Habel[1], Stephanie Hirn[1], Furong Tian[1], Anke-Gabriele Lenz[1], Oliver Eickelberg[1,2], Tobias Stoeger[1]
1 Comprehensive Pneumology Center, Institute of Lung Biology and Disease, Helmholtz Zentrum München, Germany,
2 Institute of Experimental Pneumology

Alveolar macrophages (AM), due to their close contact to inhaled environmental stress factors such as nanoparticles, and their phagocytic activity, play a key role for lung clearance and innate immunity. AM display ambivalent properties. According to their polarization state, they can trigger inflammation and also participate in its resolution. Defects in this tightly balanced response can lead to persistent lung injury, whereby inflammasome activation as well as AM cell death play a central role.

Here, we study AM dynamics during the inflammatory process caused by carbon nanomaterial exposure as an environmental model. We further compare nanoparticle response to the well-established model of bleomycin-induced lung injury, to elucidate common mechanism of persistent lung inflammation.

Investigation of the environmental model revealed sustained inflammation in murine lungs after a single dose of carbon nanotube (CNT) instillation. The response induced by the same dose of spherical carbon nanoparticles (CNP) was completely resolved after three months. At this point of time, free particle agglomerates are still apparent in the alveolar lumen upon CNT, but not CNP instillation. Additionally, we found a concurrent increase of macrophage-recruiting CCL2 and AM numbers. Cell death of nanotube-laden AM might explain this observation. In vitro we excluded necrosis as cause of particle release. Interestingly, analysis of apoptosis mediators (caspase3, caspase9, caspase8 and Fas-receptor) in lung tissue and bronchoalveolar lavage revealed significant expression mainly in AM of CNT exposed mice. Moreover the expression seems to be restricted to particle-laden cells (AM colocalizing with CNT agglomerates) and caspase-9 and -3 show highest signal intensity after 90 days.

We thus hypothesize that CNT induce a vicious cycle of AM recruitment, repeated CNT-phagocytosis, and subsequent apoptotic AM cell death. Furthermore, a single dose of bleomycin, which caused sustained lung inflammation, also induced apoptosis like cell death in AM at the in vitro level.

Because apoptosis is actually regarded as a cell death pathway preventing inflammation, further investigations of inflammasome activation and pyroptosis should elucidate the cause of persistent lung inflammation in either model.

Small RNAs and the AU-rich binding protein Tis-11 in Drosophila melanogaster

Stephanie Helfer, Klaus Förstemann
Gene Center Munich

Small RNAs like microRNAs (miRNAs) and short interfering RNAs (siRNAs) are 21 to 23 nucleotide long single-stranded molecules that are assembled together with proteins of the Argonaute family (Ago) into an effector complex called RISC (RNA induced silencing complex). Thus they are able to regulate cognate mRNAs through reduction of their stability or inhibition of their translation.

AU-rich elements (AREs), on the other hand, are a class of mainly destabilizing motifs in the 3'UTR of some mRNAs. They also target the RNA for degradation by binding different cellular proteins (AU-rich binding proteins, AU-BPs). This mechanism is described as AU-rich mediated decay. The AU-rich binding protein Tis-11 is the homologue of mammalian Tristetraprolin (TTP) in Drosophila. Tis-11 binds AREs of RNAs via its zinc-finger domain and accelerates deadenylation and the decay of the mRNAs. A genomewide screen in Drosophila cell culture showed that tis-11 is also involved in the RNAi pathway.

In Drosophila cell culture we could demonstrate that the correct functionality of Ago2-loaded RNA species depends on Tis-11, while it has no influence on Ago1-loaded RNAs. The effects of Tis-11 and Ago2 are not additive, thus the two factors act in the same pathway. Co-immunoprecipitation experiments displayed both isoforms of Tis-11 as associated with a small fraction of Ago2 and also with the RNaseIII enzyme Dcr-2. Furthermore neither miR-277 nor the hairpin-derived endo-siRNA (CG4068B) are stably bound to Tis-11. Generally miR-277 is predominantly loaded in RISC with Ago2 and only a minor fraction in RISC with Ago1. Our data showed that the loading into the effector complexes and also the distribution of the microRNA isn't altered when tis-11 is inhibited. But after depletion of tis-11 we observed that the processing of mature miR-277 is diminished compared to the control.

Synthesis and Mass Spectrometric Quantification of tRNA Modifications

Antje Hienzsch, David Pearson, Thomas Carell
LMU Chemistry & Pharmacy

Accurate transcription of DNA into mRNA and its precise translation by tRNA and rRNA into proteins are key factors for the fidelity of protein synthesis. Nature has developed different mechanisms to increase the efficiency and fidelity of these processes. One such mechanism involves the introduction of modified nucleosides into RNA species. Currently more than 100 RNA modifications are known, including simple methyl derivatives of the four canonical RNA bases as well as heavily modified RNA nucleotides. Most of them are found in tRNAs. The role of individual modifications in the translation process has been, in some cases, well investigated. The tRNA modifications have different functions in individual tRNAs, for example by contributing to tRNAs stabilization and folding, promoting decoding and enhancing efficient translation, or by establishing the identity of a certain tRNA. All these factors result in an increase of the accuracy and fidelity of the translation process. While individual studies about tRNA modifications provide useful information, we were interested in investigating tRNA modifications on a more global level, such as in complete tRNA extracts from tissues or cells. The aim was to isolate whole tRNA extracts and quantitatively analyze their modification levels. To this end, we established an HPLC-MS based quantification method that allows in principle all tRNA modifications in one tRNA sample to be measured.

Therefore, important ribonucleoside modifications as well as their isotope labelled analogues were synthesized. These natural and isotope labelled RNA modifications were then used in quantification experiments for tRNA from various organisms and mammalian tissues. In this process we isolate and purify whole tRNA extracts from cells or tissues, digest defined amounts of tRNAs to single nucleosides, then add labelled nucleosides as internal standards. The final samples are measured by LC-MS and analysed.

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Green Tea Extract significantly improves cell viability, functionality and reduces oxidative stress in human osteoblasts

Nina Holzer[1], Dr. med. Helen Vester[1], Dr. rer. nat. Claudine Seeliger[1], Dr. Steffen Benzing[2], Dr. med. Markus Neumaier

1 Klinikum rechts der Isar, Department of Experimental Traumatology, Technical University Munich, Germany

2 Kabi Innovation Centre - Clinical Affairs EN, Fresenius Kabi Deutschland GmbH

Background:

Bone metabolic disorder is closely related to a surplus production of free radicals (ROS) and chronic systemic inflammation. The resulting poor bone quality leads to an increased susceptibility to fractures and higher rates of postoperative implant failure. For improvement of bone quality, new therapeutic approaches for reduction of oxidative stress and inflammation are strongly demanded. Aim of this study was to analyze the impact of Green Tea Extract (GTE) on the viability of osteoblasts after ROS formation and the effect of GTE on the formation of extracellular matrix (ECM).

M&M:

Human osteoblasts were isolated, cultured and treated with 1mM H2O2 for induction of oxidative stress. Incubation with GTE was performed before, after and simultaneous to the incubation of H2O2. The amount of ROS was measured by flow cytometry. Cell damage was analyzed by LDH leakage and cell viability by MTT assay. The formation of ECM after was visualized with von Kossa and Alizarin Red staining. Moreover, Alizarin Red was quantified.

Results:

H2O2 treatment of human osteoblasts (N=3, n=2) resulted in immediate and dose-depending ROS production. Viability of the osteoblasts was significantly reduced and LDH leakage was significantly increased after treatment with H2O2. All three set ups, pre-, co- and post-incubation with GTE significantly reduced ROS formation and improved the viability of H2O2 treated osteoblasts. The stimulation with low doses of GTE (1µg and 10ng per ml) over 21 days improved the production of ECM in the osteoblasts.

Conclusion:

We can show that incubation of osteoblasts with GTE can significantly reduce oxidative stress and improve the cell viability. GTE has also a beneficial effect on osteoblasts ECM what implies the increase of bone mineral density. Therefore, GTE supplementation could be a prophylactic possibility to recover bone quality in osteopenic patients and to support fracture healing especially in elderly patients.

A Conserved GA Element in TATA-Less RNA Polymerase II Promoters

Friederike Hoeg, Martin Seizl, Holger Hartmann, Fabian Kurth, Dietmar E. Martin, Johannes Söding, Patrick Cramer
LMU Gene Center

In eukaryotes, transcription of protein-coding genes relies on RNA polymerase (Pol) II, the general transcription factors (GTFs) TFIIB, -D, -E, -F, and –H, and coactivators such as Mediator and the Spt-Ada-Gcn5 acetyltransferase complex (SAGA).

During activation, gene-specific transcription factors recruit coactivator complexes and TFIID, thereby facilitating pre-initiation complex (PIC) formation at the promoter. A subunit of TFIID, the TATA box-binding protein (TBP), binds the TATA box, which is located upstream of the transcription start site (TSS), and nucleates PIC assembly. However, TATA boxes with the consensus sequence TATAWAWR occur in only 13% of yeast promoters, and 10% of human promoters.

Since TATA-less promoters require TBP for function, and bind TFIID, alternative pathways for PIC assembly were proposed. In metazoans, many TATA-less promoters contain a downstream promoter element (DPE) and an initiator (INR), which interact with components of TFIID to facilitate PIC assembly. Although the GTFs are highly conserved throughout eukaryotes, alternative core promoter elements could not be identified in yeast. A TATA box is not strictly required for TFIID-dependent activity of a yeast model promoter. Indeed, recent studies of the TATA-less yeast RPS5 promoter showed that functionally redundant AT-rich stretches within the core promoter region promote TFIID-dependent transcription.

Based on these observations, we hypothesized that TATA-less core promoters contain DNA elements that are functionally similar to the TATA box in promoting PIC assembly and Pol II transcription. Here we used a combination of bioinformatics, in vivo reporter gene assays, and in vitro biochemistry to identify and functionally characterize a region in TATA-less yeast core promoters that is bound by TBP, required for Pol II transcription and contains a novel conserved promoter element, the GA element, or GAE.

Amyotrophic Lateral Sclerosis: Engaging neurodegeneration on the proteome level

Daniel Hornburg
MPI Biochemistry

Besides DNA methylation, epigenetic information is mainly encoded by histone post translational modifications (PTMs) which contribute to the establishment of cell type specific gene expression, inheritance of chromatin states, genome stability and differentiation. Histone PTMs are often associated with certain chromatin states: one of the best-known modifications, trimethylated lysine 9 on histone H3 (H3K9me3), marks dense chromatin regions, whereas the acetylated counterpart is largely underrepresented in silent heterochromatin. Recently, a member of the UHRF family, UHRF1 (also known as Np95 or ICBP90), was found to interact with all three DNA methyltransferases and to bind hemimethylated DNA via its SRA domain. Genetic ablation of UHRF1 leads to remarkable genomic hypomethylation, a phenotype similar to dnmt1-/- embryonic stem cells. Furthermore, UHRF1 specifically recognizes H3K9me3 histone peptides via an aromatic cage embedded in the tandem Tudor domain. The second member of the UHRF family, UHRF2, shows high primary sequence similarities to UHRF1. However, the function of UHRF2 is still unknown. Using in vitro and in vivo techniques, we address the question whether the sequence similarities between the two UHRF family members correspond to functional similarities.

More than just cell death regulators: inhibitor of apoptosis proteins (IAPs) as modulators of endothelial permeability

Michael C. Hornburger, Bettina A. Mayer, Elisabeth A. Willer, Stefanie Leonhardt, Angelika M. Vollmar, Robert Fürst

Department of Pharmacy – Center for Drug Research, Pharmaceutical Biology, University of Munich, Butenandtstr. 5-13, 81377 Munich, Germany

A broad variety of diseases associated with an inflammatory component, such as sepsis, asthma or artheriosclerosis, shows an increased endothelial permeability as common pathological pattern. Drugs interfering with the pathologically impaired endothelial systems that govern vascular barrier function are widely lacking. Inhibitor of apoptosis proteins (IAPs) were originally found as regulators of programmed cell death in tumor cells. We recently discovered a crucial role for IAPs in inflammatory signaling processes in endothelial cells. Here, we hypothesized that IAPs might play a role in the regulation of endothelial permeability.

We revealed that a small molecule IAP antagonist (ABT) prevented thrombin receptor-activating peptide-induced endothelial hyperpermeability, as assessed by measuring macromolecular permeability (Transwell®assay) and transendothelial electrical resistance. ABT diminished thrombin-evoked stress fiber formation and activation of the myosin light chain, suggesting an inhibition of cell contraction. Moreover, ABT prevented the thrombin-induced disruption of endothelial adhesion junctions (VE-cadherin phosphorylation). Checking different signaling pathways upstream to these events, we could neither detect any influence of ABT on Ca^{2+}-levels (Fura-2 measurements) nor activation status of protein kinase C (PKC), mitogen-activated protein kinases (p38, ERK) and the small GTPases Rac1 (pulldown assay). However, we could show that ABT inhibits the activation of RhoA, an important mediator of endothelial barrier disruption.

In summary, we for the first time provide evidence that IAPs participate in the regulation of endothelial barrier integrity, probably by interfering with the activation of RhoA. Thus, IAP inhibitors could represent a novel pharmacological approach for the prevention of hyperpermeability-associated disorders.

*ABT (full name A-410099.1) was kindly provided by Abbott Bioresearch Corp., Worchester, MA, USA.

Nuclear architecture in human hematopoetic cell types – an analysis using high-resolution microscopy

Barbara Hübner, Mariana Lomiento, Yolanda Markaki, Sergio Ferrari, Thomas Cremer
LMU Biology

Current evidence suggests that the regulation of cell cycle, cell type and differentiation specific gene expression patterns may not only depend on local epigenetic modifications of chromatin but also on dynamic global changes of higher order chromatin organization. The current gap of knowledge how nuclear architecture may be causally correlated with differences in gene expression patterns is emphasized by the lack of generally agreed models of the functional nuclear organization.

Using high resolution microscopy (3D-SIM and TEM) we compared the nuclear architecture of human hematopoetic cells during differentiation. Normal hematopoesis provides an ideal system as it includes well defined differentiation stages ranging from multipotent stem cells and progenitor cells (CD34+) to precursor cells (monoblast, myeloblast) to functionally differentiated cells (monocytes, granulocytes).

Between nuclei of these five cell types we found major differences, e.g. regarding size and shape as well as patterns of clustered chromatin and the interchromatin compartment (IC). However, all studied cell types show the same principle of nuclear organization as predicted by the chromosome territory - interchromatin compartment (CT-IC) model: active chromatin characterized by H3K4me3-staining is preferentially located at the surface of chromatin domains, termed the perichromatin region (PR), and possible sites of transcription indicated by polymerase II are found almost exclusively in this region.

Mechanism of the interaction between Pex14p and LC3-II

Li Jiang[1], Sayuri Kuge[2], and Yukio Fujiki[23]
([1]Grad. Sch. of Life Sci., Kyushu Univ., [2]Dep. of Bio. Fac. of Sci. Kyushu Univ. Grad. Sch., [3]JST, CREST) Fukuoka, 812-8581, Japan

Pexophagy, the selective degradation of peroxisomes by autophagic machinery, has been well studied in yeast. In mammalian culture cells, it can be also induced experimentally by switching the culture medium to the starvation condition. Pex14p, which is identified as an essential peroxisomal membrane protein for matrix protein import in binding to soluble receptor Pex5p-cargo complex, is involved in the mammalian autophagic degradation of peroxisomes and interacts with the lipidated form of LC3, termed LC3-II, an essential autophagy marker, under the starvation condition in CHO-K1 cells, whereas the binding of Pex5p to Pex14p observed under the normal condition is greatly reduced. However, the molecular mechanism underlying the interaction between Pex14p and LC3-II is largely unknown.

To verify whether Pex14p directly binds to LC3-II, a binding assay system using purified endogenous LC3-II and recombinant His-Pex14p was established. As a result, His-Pex14p preferentially bound to the purified LC3-II under the starvation condition. Moreover, Pex5p was identified to be an inhibitory factor for this interaction. Next, to determine LC3-II binding domain in Pex14p, we also reconstituted a conjugation system for synthesis of LC3-II in vitro. Synthesized LC3-II was then pulled down by GST-fusion proteins each with full-length and the region encompassing amino-acid residues at 106-140 of Pex14p. We found that the transmembrane domain (110-138) was important for the LC3-II binding. Notably, this domain is not the binding region consisting of amino-acid residues at 31-70 for Pex5p. Accordingly, our data suggest that LC3-II and Pex5p do not directly compete in binding to Pex14p. We are now analyzing how Pex14p preferentially binds to Pex5p under the nutrient-rich condition, whilst Pex14p interacts with LC3-II under the starvation condition in vivo.

DDX27 as a novel interaction partner for the PeBoW-complex

Markus Kellner, Michaela Rohrmoser, Lars Israel, Kaspar Burger, Bastian Mühl, Thomas Harasim, Anita Gruber-Eber, Axel Imhof, Dirk Eick
Helmholtz Center Munich

Hundreds of factors participate in the process of ribosome biogenesis in mammalian cells. Three of them, Pes1, Bop1 and WDR12 form the trimeric PeBoW-complex. The complex is essential for the processing of the pre-rRNA and maturation of 28S and 5.8S rRNA. We searched for proteins that interact with PeBoW. The complex was immunoprecipitated with antibodies directed against each of the three components which all enriched the complex quantitatively. The co-precipitated proteins were determined by mass spectrometry. The DEAD box RNA helicase DDX27 could be confirmed as true interaction partner. Antibodies specific for DDX27 co-precipitated the PeBoW-complex, and vice versa, PeBoW-specific antibodies precipitated DDX27. The PeBoW-complex migrates in native gel electrophoresis in two distinct, DDX27-positive and DDX27-negative, forms. Together this leads to the assumption that DDX27 is not an integral part of the complex, but forms with it a more transient secondary complex. We demonstrated that the helicase takes part in rRNA maturation, as knock down of the protein diminishes this process. Further immunoprecipitation experiments with DDX27 deletion mutants revealed its N-terminus as site needed for interaction with PeBoW. Besides, the C-terminus appears to be responsible for nucleolar localisaion.

XPA as damage sensor in Nucleotide Excision Repair

M.Sc Sandra Koch, Dr. Ralf Strasser, Dr. Stefanie Schorr, Dr. Sabine Schneider, Prof. Dr. Thomas Carell
LMU Munich

Nucleotide excision repair (NER) is the principal genome maintenance system in mammals. The repair system presents a promiscuous lesion recognition mechanism that enables the removal of DNA lesions caused by UV-radiation or other environmental carcinogens. By incision of the damaged strand on both sides of the lesion the damaged oligonucleotide can be removed. A defective NER response in mammals leads to a high predisposition to skin cancer (*Xeroderma Pigmentosum*). XPC/Rad4 is known to detect disrupted duplex structures[1] whereas XPA/Rad14 is thought to be required for the binding of bulky adducts generated by reaction of metabolically activated aromatic compounds. Since the exact mechanism of the DNA damage recognition is not fully understood, we first investigated the binding of the human protein XPA and its yeast homolog Rad14 to DNA lesions with EMSA (Electrophoretic Mobility Shift Assay) studies and then co-crystallized the enzyme with synthetic oligonucleotides containing bulky adducts (AAF-dG and dU-Fl).

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Reduction of 5-Hydroxymethylcytosine in human astrocytomas and glioblastomas

Dr. Theo F. J. Kraus, Daniel Globisch, Gesa Kolck, Dr. Sabina Eigenbrod, Mirko Wagner, David Widmann, Prof. Dr. Thomas Carell, Prof. Dr. Dr. h.c. Hans A. Kretzschmar
LMU Medicine

Epigenetics, the study of mechanisms that influence gene expression and silencing but are not directly based on the coding sequence, exert essential influence on regulation of the embryonic stem cell differentiation and cell cycle control. Important fields of epigenetics are histone modifications, nucleosomal repositioning and DNA-methylation. One of the most commonly studied epigenetic modifications is the 5-methylation of cytosine (5mC) by the DNA-methyltransferases. Recently, new epigenetic modifications have been found: 5-hydroxymethylcytosine (5hmC), 5-formylecytosine and 5-carboxymcytosine. The biological relevance of these modifications are still unknown, but it has been suggested that they play essential roles in cellular reprogramming and tumor formation. The world health organization (WHO) classifies brain tumors according to their degree of anaplasia and malignancy as grades I-IV, indicating that grade IV tumors are highly malignant with a poor prognosis. Glioblastomas, WHO-grade IV tumors, are brain tumors with a very poor outcome. The mean survival of patients is less than 12 months. Etiologically some tumors develop from lower grade tumors and some develop de novo. To obtain a first insight into the relevance of 5hmC in the development of glioblastomas, we investigated low grade gliomas as well as high grade gliomas by means of immunohistochemistry using antibodies against 5hmC and mass spectrometry and compared the amounts of 5hmC with normal brain tissue. We found significantly lower amounts of 5hmC in tumors compared to brain. Interestingly we did not see any significant differences in the amount of 5mC. Furthermore we performed immunofluorescence stainings with 5hmC and the proliferation marker Ki67. We saw that Ki67 positive cells stain negative for 5hmC. Summarizing, we show that 5hmC is expressed in normal brain as well as in brain tumors but the number of 5hmC positive cells is significantly lower in tumors than in normal brain tissue.

The tubulin antagonist pretubulysin shows strong vascular-disrupting properties in vitro

Verena Kretzschmann[1], Angelika Ullrich[2], Stefan Zahler[1], Angelika Vollmar[1], Uli Kazmaier[2], Robert Fürst[1]
1 Department of Pharmacy, Pharmaceutical Biology, University of Munich, Butenandtstr. 5-13, 81377 Munich, Germany
2 Institute of Organic Chemistry, Saarland University, Post Box 151150, 66041 Saarbrücken, Germany

Vascular-disrupting agents (VDAs) have emerged as a novel promising class of anticancer therapeutics. We aimed to elucidate the vascular-disrupting potential of the new tubulin-depolymerizing agent pretubulysin (PT). PT is a synthetically accessible precursor of tubulysin, a myxobacterial compound that has recently been found to exert potent tumor cell death-inducing properties. In this study, we focused on the action of PT on endothelial cells in vitro in comparison to the lead VDA combretastatin A-4 phosphate (CA4P). We investigated the effects of PT on key features of vascular disruption using human dermal microvascular (HMEC-1) and human umbilical vein endothelial cells (HUVECs). PT induced a concentration-dependent disassembly of established endothelial tubes on Matrigel in vitro as well as in an ex vivo aortic ring model. Furthermore it rapidly increased endothelial permeability within 1 h, as measured by impedance sensing and Transwell® assays. Moreover, using immunocytochemistry and confocal microscopy, we found that PT leads to a disruption of microtubules and cell junctions (VE-cadherin, claudin-5), and to a strong induction of F-actin stress fibers. Interestingly, PT showed in all assays a potency comparable to CA4P. Regarding cytotoxicity, PT-treated cells did neither undergo apoptosis or necrosis within 24 h, nor reduce their metabolic activity after 1 or 24 h. HUVECs were even able to reassume their normal morphology after washing out of PT/CA4P. In summary, PT exhibits the typical features of a microtubule-targeting VDA: it disrupts endothelial tubes, causes barrier breakdown and induces cytoskeletal rearrangements without inducing cell death. Since PT shows similar effects than the lead VDA CA4P, this natural compound might represent an interesting novel VDA. These findings warrant investigations into the in vivo potential of PT.

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The TGFß co-factor Smif is regulated by phosphorylation

Sebastian Kuhn, Justus Duyster
TUM Medicine

The TGFβ pathway plays a critical role in regulating many biological processes including cell growth, differentiation, embryonic development and immune function.

After phosphorylation of receptor-associated Smads (R-Smad) by the ligand activated TGFβ-receptor complex, R-Smads bind the common Smad4 and translocate into the nucleus, where they regulate the expression of TGFβ-target genes. Smif binds to Smad4 in a TGFβ dependent manner and is an important transcriptional cofactor, which further regulates the activity of the R-Smad-Smad4 complex.

Proteomic large scale screenings by several research groups demonstrated that Smif is a multiphosphorylated protein. Here we show that the Smif phosphorylation status varies depending on extracellular stimuli. EGF and other promitogenic stimuli such as FGF2 or IL3 lead to the phosphorylation of Smif. In addition, also UV irradiation or other cellular stress can lead to Smif phosphorylation. By the use of siRNA mediated knockdown we identified different MAP kinases responsible for Smif phosphorylation after promitogenic or stress stimulation. Most importantly, we can demonstrate that phosphorylation of Smif modulates its transcriptional activity using several TGFß dependent reporter constructs. Mutation of the Smif phosphorylation sites led to the abrogation of this effect demonstrating that stress or mitogen induced phosphorylation of Smif is capable to modulate the TGFß response.

It is known that binding of additional cofactors to the R-Smad/Smad4 complex leads to cell specific transcription regulation. To fine-tune the response on the transcriptional level, the Smad complex can assemble with other transcription factors and additionally with cofactors to further define the amplitude and specificity of transcriptional activity. Here we describe an additional layer of modulation of the TGFß response involving the MAP kinase mediated phosphorylation of the cofactor Smif.

Replication of Stabilized Bioisosteric Formamidopyrimidine Analogues inside Various DNA Polymerases

Ulrike Lischke, Tim H. Gehrke, Sabine Schneider, Prof. Dr. Thomas Carell
LMU Munich

During aerobic respiration in the cell or exposure to chemical agents reactive oxygen species (ROS) are formed. ROS can constantly damage the DNA within the cell resulting in base lesions. Those oxidatively generated lesions are one of the main reasons for mutagenesis. Next to the oxidized forms of purines the formamidopyrimidines are one of the most common DNA lesions formed after the attack of DNA by ROS.1 Oxidative lesions are responsible for a variety of genetic diseases, cell apoptosis, progression of cancer and aging.2 In order to study the miscoding potential of these lesions we synthesized configurationally stable analogues of the naturally occurring formamidopyrimidine lesions 3, 4 and incorporated the carbocyclic β-configurated bioisosteric nucleosides into oligonucleotides at defined sites using automated solid phase synthesis. The properties of the carbocyclic analogues during replication were determined via primer extension studies in vitro with different high and low fidelity polymerases. Additionally co-crystallization studies with these lesion analogues and various DNA polymerases are in progress to gain snapshots of different stages during the DNA replication process.

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A Thermal Trap for DNA Replication and Polymerization

Christof Mast, Dieter Braun
LMU Munich / Systems Biophysics

Normally, genetic information is protected from free diffusion into the environment by cell walls. The cells provide all conditions for the replication and mutation of genetic material - the basic prerequisite for Darwinian evolution. We modeled a fundamental principle in the laboratory, which allows for continuous evolution of genetic material without cell membranes. We filled a thin capillary with DNA and nucleotides, solved in buffer as an aqueous solution. A moving infrared laser spot then generated a thermal gradient realizing thermophoresis as well as a cyclic convection of the solution. The superposition of both effects caused the double-stranded DNA molecules to migrate to the cold area, while simultaneously cycling the DNA between the cold and warm section of the capillary. In the hot area, the DNA separates into single strands. These are then elongated by a polymerase enzyme in the cold region to two double-stranded copies of the original template DNA. Therefore, a simple temperature gradient drives both, an exponential replication as well as the selective accumulation of information. This is relevant as presumably similar thermal conditions prevailed in rock pores near hot undersea springs of prehistoric oceans. Our experiment shows how a simple disequilibrium setting may allow life to evolve. Furthermore, we are interested in the combination of polymerization/degradation reactions and thermal trapping. Results show a massive boost of accumulation yielding towards a hyper-exponential selection and creation of longer polymers compared to shorter ones.

A Thermal Trap for DNA Replication
Christof B. Mast and Dieter Braun Physical Review Letters 104, 188102 (2010)

Crystal structure of the C-terminal globular domain of oligosaccharyltransferase from Archaeoglobus fulgidus at 1.75 Å resolution

Shunsuke Matsumoto
Division of Structural Biology, Medical Institute of Bioregulation, Kyushu University

Protein N-glycosylation occurs in the three domains of life. Oligosaccharyltransferase (OST) catalyzes the transfer of glycan to asparagine in the N-glycosylation sequon. The catalytic subunit of OST is called STT3 in eukaryotes, AglB in archaea, and PglB in eubacteria. The genome of a hyperthermophilic archaeon, Archaeoglobus fulgidus, encodes three AglB paralogs. Two of them are the shortest AglBs across all domains of life. We expressed the C-terminal globular domain of the smallest AglB to identify minimal structural unit. We found that the Archaeoglobus AglB lacked a ß-barrel-like structure, which had been found in Pyrococcus AglB and Campylobacter PglB. In agreement, the deletion of the barrel-like structure in the Pyrococcus AglB did not change the OST activity. The Archaeoglobus AglB contains a kinked helix that was found in the other two structures, but the kinked helix is interrupted by an inserted sequence. This finding revised the sequence alignment, and revealed that the Archaeoglobus AglB possesses the DK motif that was previously identified in the Pyrococcus AglB, but a variant containing the insertion. The mutagenesis study confirmed the contribution of the DK motif to the Archaeoglobus AglB activity. By contrast, the Campylobacter PglB possesses the MI motif at the spatially equivalent position. Thus, OST is classified into two groups: the DK-type including eukaryotes and major archaea, and the MI-type including eubacteria and the remaining archaea. Because the DK/MI motif participates in the recognition of +2 Ser/Thr residue in the sequon, this classification provides a useful framework to the OST studies.

Substrate specificity in small RNA silencing pathways

Milijana Mirkovic-Hoesle, Klaus Foerstemann
Genzentrum München

RNA silencing (RNAi) is a conserved mechanism for posttranscriptional gene regulation executed by double-stranded RNAs (dsRNAs) suppressing specific transcripts in a sequence-dependent manner. Two classes of these guide molecules are known: microRNAs (miRNAs) and small interfering RNAs (siRNAs). The latter include exogenous RNAs (exo-siRNAs) produced during viral infection or transgenic RNAi and endogenous small RNAs (endo-siRNAs) derived from transposable elements. Exo-siRNA biogenesis depends on Dicer 2 (Dcr 2) and a double-stranded RNA binding protein (dsRBP) called R2D2 while endo-siRNAs are produced by Dcr2 and a specific splice variant of Loquacious, Loqs-PD. The puzzling conclusion is that the cells must be able to distinguish the precursor despite the fact that their structure is identical. How is this possible?
I will use Drosophila melanogaster as a model organism to combine genetic studies with next-generation sequencing approaches to respond to this question.

Identification of the earliest branch point for myelo-erythroid development in adult hematopoiesis

Kohta Miyawaki
Department of Medicine and Biosystemic Science, Faculty of Medicine, Kyushu University

In murine hematopoiesis, hematopoietic stem cells (HSCs) have been identified within the LSK fraction of bone marrow cells, which is defined by the absence of lineage-associated surface markers (Lin) and high expression of Sca-1 and c-Kit. The first commitment step at the myeloid versus lymphoid bifurcation has been proposed outside the LSK fraction, where myeloid or lymphoid lineage-committed progenitors such as common myeloid progenitors (CMP) and common lymphoid progenitors (CLP) are prospectively isolated. However, recently, by utilizing mice harboring a fluorescent reporter for GATA-1 transcription factor, we found that the upregulation of GATA-1 occurs within the LSK population; The GATA-1+ LSK population was capable of generating only myelo-erythroid cells but lacks lymphoid potential, suggesting strongly that the earliest myeloid development occurs at the LSK stage.

To isolate the earliest myelo-erythroid LSK progenitors in normal mice without utilizing the GATA-1 reporter system, we conducted expression profiling of GATA-1+ LSK cells by cDNA microarray analyses, and identified a cell-surface antigen (X) specifically expressed in GATA-1+ LSK cells. By using this new surface marker X, we could successfully purify a cell population from normal C57B6 mice which gives rise exclusively to granulocyte-macrophage (GM) and megakaryocyte-erythroid (MegE) colonies, but lacks lymphoid potential. Furthermore, X+LSK cells display very potent GM and erythroid expansion as compared to the original CMPs, suggesting that X+LSK might reside upstream of CMPs. The gene expression analysis of the X+ LSK population at single-cell level revealed that this population expresses GM and MegE-associated genes at a high level, but not lymphoid genes, suggesting that the gene expression pattern clearly reflects its lineage differentiation potential. Thus, the X+ LSK population might represent the earliest stage for myelo-erythroid development, upstream of conventional CMP.

The functional analysis of multinucleated giant cells

Ronny Müller, Julia Ritter, Fernando O. Martinez, Laura Layland, Clarissa Prazeres da Costa, Katja Specht, Siamon Gordon, Laura Helming
TUM

Multinucleated giant cells (MGCs), first described in tuberculosis, can be detected in many other types of diseases. While it is known that MGCs originate from fusion of macrophages, the current knowledge about the function of these remarkable cells is limited and it is unclear whether they are beneficial or detrimental for disease outcome. The aim of our study is to shed light on the presumptive function of MGCs and therefore the functional consequence of macrophage fusion. Our analysis is based on the direct comparison of MGCs with non-fused macrophages isolated under the same experimental conditions, e.g. the same tissue or cell culture dish. We established a cell culture system for the formation of MGCs from murine bone marrow derived macrophages as well as a novel method to separate these in vitro generated MGCs from non-fused macrophages. In addition, we are using the Schistosoma mansoni mouse infection model as well as tissue samples from tuberculosis patients to study MGCs under physiological conditions. Using these models, we found that MGCs show increased expression of degradative metalloproteinases when compared to non-fused macrophages. Furthermore, we could detect increased mRNA expression of several genes including carbonic anhydrase II, the dendritic cell marker CD11c and the fusion regulator DC-STAMP. On the other hand, when stimulated with LPS, MGCs showed an attenuated cytokine response. Overall, our data show that when compared to non-fused macrophages, MGCs exhibit a distinct phenotype in terms of their capacity for degradation, cytokine secretion and adhesion.

The *mamXY* operon is involved in controlling magnetite formation and magnetosome chain positioning in *Magnetospirillum gryphiswaldense*

Oliver Raschdorf[1], Dr. Frank Müller, Emanuel Katzmann, Prof. Dr. Mihály Pósfai[2], Prof. Dr. Dirk Schüler
1 Ludwig-Maximillians-Universität München, Department Biologie I, Bereich Mikrobiologie, Biozentrum der LMU, D-82152 Martinsried, Germany
2 Department of Earth and Environmental Sciences, University of Pannonia, POB 158, 8200 Veszprém, Hungary

Magnetotactic bacteria (MTB) use intracellular chains of membrane-enveloped magnetite crystals, called magnetosomes, to orientate along magnetic fields. The sequential steps of magnetosome synthesis involve intracellular differentiation and include vesicle formation, magnetite nucleation and mineralization as well as magnetosome chain alignment and are subject to tight genetic regulation. Most of the genes implicated in magnetosome formation are organized in four operons that are clustered within a genomic magnetosome island. Despite of recent progress in characterization of these genes, the function of the *mamXY* operon has not been well investigated so far. To close this gap, we created unmarked deletions of all four individual genes within this operon and analyzed the phenotype of the mutants mainly by transmission electron microscopy. The mamH-like gene encodes for a unique membrane-spanning protein affiliated to the group of MFS transporters but fused to a putative ferric reductase-like domain. The *ΔmamH-like* mutant forms magnetite crystals with heterogenic size, structure and cellular distribution. The mutant also displays a delay in production of ferrimagnetic magnetosomes. A similar phenotype was observed upon deletion of *mamX*, indicating a function in the same cellular biomineralization process. Deletion of the MTB-specific *mamY* gene however, did not influence mineralization but led to mislocalization of magnetosome chains. Fluorescence microscopy revealed that MamY localizes as a filamentous structure coinciding with the expected position of the magnetosome chain. The protein may therefore directly participate in targeting magnetosomes to their assigned position by an as yet unknown mechanism. Unexpectedly, deletion of *ftsZm*, coding for a truncated homolog of the major bacterial cell division protein FtsZ, did not show any obvious cell division phenotype, and in contrast to previous reports also no biomineralization defects. In conclusion, our data suggests that the proteins encoded within the *mamXY* operon play a major role in magnetosome biomineralization and chain positioning.

Identification of posttranslational modifications of the RNA Polymerase II CTD via Mass Spec analysis

Roland Schüller, Dr. Ignasi Forne, Prof.Dr. Axel Imhof, Prof.Dr. Dirk Eick
Helmholtz Zentrum München

Mammalian RNA polymerase II largest subunit Rpb1 contains a unique and highly repetitive domain at the carboxy-terminus (CTD) with the consensus heptad Tyr(1)-Ser(2)-Pro(3)-Thr(4)-Ser(5)-Pro(6)-Ser(7). Each single residue of this sequence could potentially be modified, and the phosphorylation of serine-2, serine-5 and serine-7 are already well characterised. To get more detailed informations in both, quantity and quality of the modification pattern within the CTD, CTD mutants were established to make the whole sequence accessible to Mass Spectrometry.

Mass Spec analysis of four mutants showed that it is difficult to catch the longer peptides with more than 30 amino acids in length and therefore the sequence coverage of the proximal part of the CTD is incomplete. Some peptides of the distal part of the CTD however have a very high output in the Mass Spec analysis. These frequent peptides were analysed in more detail in order to obtain dominant modification patterns based on which a protein fishing assay can be established, finally. So far, the overall modification frequency is rather low limited to three phosphosites per CTD-peptide and there are some dominant phosphorylation site patterns in double- and triple combinations of high frequent CTD-peptides.

Superagonism at the muscarinic acetylcholine receptors

Ramona Schrage, Prof. Dr. med. Klaus Mohr
University of Bonn

Endogenous messenger compounds and synthetic ligands bind to specific target molecules in the organism. G protein-coupled receptors are embedded into the outer cell membrane and translate external messenger molecule binding into intracellular activation of adaptor molecules and subsequent downstream signalling.

Superagonism means that a synthetic ligand activates the receptor with higher maximum effect than the endogenous activator (1). For the superfamily G protein-coupled receptors superagonism has rarely been described in literature although it might be beneficial for both research and clinical applications (2, 3). We suggest that superagonism may have been overlooked for methodical reasons, because the cellular response to receptor activation is often limited. We applied analytical procedures that allow to conclude from the cellular response on the signalling competence of an activator bound receptor protein. This approach identifies superagonism at the muscarinic acetylcholine receptor. A prototypal activator, iperoxo, requires 100-1000 fold lower concentrations for receptor activation than the physiological activator acetylcholine in a variety of assays.

More importantly the power of this ligand to induce a signalling competent receptor conformation clearly exceeds acetylcholine.

Taken together we present a novel approach to identify superagonism of artificial receptor activators at G protein-coupled receptors.

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The Spt5 C-terminal region recruits yeast 3′-RNA cleavage factor I

Amelie Schreieck, Andreas Mayer, Michael Lidschreiber, Kristin Leike, Dietmar Martin, Patrick Cramer
LMU Gene Center

During transcription of protein-coding genes in yeast, RNA polymerase (Pol) II associates with many factors, including elongation factors, RNA-processing factors, and chromatin-modifying enzymes. Many of these factors are recruited by binding to the C-terminal repeat domain (CTD), a tail-like extension of the largest Pol II subunit that is highly phosphorylated during elongation. Some elongation factors also bind the body of Pol II, including TFIIS and Spt5.

Spt5 is the only Pol II-associated factor that is conserved in all three kingdoms of life and has recently emerged as a platform that recruits factors to elongating Pol II. Spt5 co-purifies with over 90 yeast proteins that are involved in transcription elongation, RNA processing, transcription termination, and mRNA export (1). Recruitment of factors can be mediated by the repetitive C-terminal region (CTR) of Spt5. The CTR forms a repeat structure similar to the Pol II CTD, in S. cerevisiae it consists of 15 hexapeptide repeats of the consensus sequence S[T/A]WGG[A/Q]. Similar to the Pol II CTD, the CTR of Spt5 can be phosphorylated by the kinase Bur1 in yeast (2). CTR phosphorylation promotes transcription elongation, and is important for the co-transcriptional recruitment of the Paf1 complex (2).

Here we report a new role of the Spt5 CTR in the recruitment of 3′-RNA processing factors. Chromatin immunoprecipitation (ChIP) reveals that the Spt5 CTR is required for normal recruitment of the pre-mRNA cleavage factor (CF) I to the 3′-end of yeast genes. RNA contributes to CFI recruitment, as RNase treatment prior to ChIP further decreases CFI ChIP signals. Genome-wide ChIP profiling detects occupancy peaks of CFI subunits around 100 nucleotides downstream of the poly-adenylation (pA) site of genes. CFI recruitment to this defined region may result from simultaneous binding to the Spt5 CTR, to nascent RNA containing the pA sequence, and to the elongating Pol II isoform that is phosphorylated at serine 2 (S2) residues in its CTD. Consistent with this model, the CTR interacts with CFI in vitro, but is not required for pA site recognition and transcription termination in vivo.

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Nuclear MV C protein interferes with interferon β transcription

Konstantin Sparrer, Dr. Christian Pfaller, Prof. Karl-Klaus Conzelmann
LMU München

Transcriptional induction of interferon beta (IFN-β) through pattern recognition receptors is a key event in the host defense against invading viruses. Infection of cells by paramyxoviruses like measles virus (MV, Genus Morbillivirus) is sensed predominantly by the ubiquitous cytoplasmic helicase RIG-I recognizing viral 5'-triphosphate RNAs, and to some degree by MDA5. While MDA5 activation is effectively prevented by the MV V protein, the viral mechanisms for inhibition of MDA5-independent induction of IFN-β remained obscure. We here identify the 186 amino acid MV C protein, which shuttles between the nucleus and cytoplasm, as a major viral inhibitor of IFN-β transcription in human cells. Activation of the transcription factor IRF3 by upstream kinases and nuclear import of activated IRF3 was not affected in the presence of C protein, suggesting a nuclear target. Notably, C proteins of wildtype MV isolates, which are poor IFN-β inducers, were found to comprise a canonical nuclear localization signal (NLS), whereas the respective sequence of all vaccine strains, irrespective of their origin, was mutated. Site-directed mutagenesis of the C proteins from a MV wildtype isolate and from the vaccine virus Schwarz confirmed a correlation of nuclear localization and inhibition of IFN-β transcription. A functional NLS and efficient nuclear accumulation is therefore critical for MV C to retain its potential to downregulate IFN-ß induction. We suggest that a defect in efficient nuclear import of C protein contributes to attenuation of MV vaccine strains.

Identification of proNGF - NGF and their receptors in the ovary

Sabine Ströbl, Dieter Berg, Ulrike Berg, Gregory A. Dissen, Sergio Ojeda, Artur Mayerhofer
LMU Medicine

Neurotrophins play important roles in survival, differentiation and death of neuronal but also non-neuronal cells, including ovarian cells. At least in rat, the prototype neurotrophin nerve growth factor (NGF) promoted the development of preantral follicles and appears to be involved in the regulation of ovulation and ovarian angiogenesis mainly via activation of its TrkA receptor. Mature NGF readily binds to this tyrosine kinase receptor and to the p75 neurotrophin receptor (p75NTR). The NGF precursor (proNGF), however, differs from NGF by its binding affinities and prefers the p75NTR and a co-receptor, sortilin. Secreted proNGF is cleaved by a matrix metalloproteinase (MMP7) into its mature form. While NGF was reported in human ovary and in follicular fluid (FF), the presence of proNGF is not examined. Hence we studied proNGF and NGF, MMP7 - p75NTR/TrkA/sortilin expression in human and mxxxxx ovary and in IVF-derived human FF and cultured human granulosa cells (hGCs).

Immunohistochemistry (IHC) identified proNGF in granulosa cells and in FF in mxxxxx and human antral follicles. ProNGF was also detected in FF derived from IVF-patients (Western Blot), indicating that proNGF is released from hGCs in vivo. MMP7, able to cleave proNGF and thus to generate NGF, was detected in mxxxxx and human ovarian sections by IHC. MMP7 was also present in human FF and in cultured hGCs (Western Blot, RT-PCR). Furthermore, the proNGF/NGF receptors p75NTR and TrkA, as well as sortilin, the recently described proNGF receptor connected to cell death, were found (Western Blot, RT-PCR).
Taken together the results raise the possibility that changing concentrations of the ligands (proNGF/NGF) and/or changes of receptors (p75NTR, TrkA, sortilin) may influence the fate of ovarian follicles. Studies to explore the impact of proNGF and NGF on survival and death of hGCs are under way.

Circulating MicroRNAs as Biomarkers in Radiation Therapy of Head and Neck Cancer Patients

Isolde Summerer, Anne Kraemer, Dr. Horst Zitzelsberger, Dr. Maximilian Niyazi, Dr. Michael Atkinson, Dr. Simone Moertl
Helmholtz-Zentrum München

MicroRNAs (miRNAs) are present in stable extracellular forms in body fluids such as liquor or blood plasma. Changes in the levels of circulating miRNAs of cancer patients are associated with malignant progression, prognosis and have proven useful for the prediction of therapy responses. This study is intended to investigate if therapeutic irradiation induces changes in the plasma miRNA profiles of head and neck cancer patients that give information on the clinical outcome. We aim to correlate any changes with clinical radiation response parameters such as tumor regression or normal tissue reactions in order to establish plasma miRNAs as non-invasive biomarkers in radiation therapy. MiRNA expression profiles of blood plasma samples from 7 head and neck cancer patients (squamous cell carcinoma) were analyzed using TaqManTM array microRNA cards. Out of 378 investigated miRNAs approximately 100 were detected in human plasma. After exposure to 2 x 2Gy, on average 15 miRNAs showed increased expression, while 24 miRNAs showed lower expression levels compared to the plasma sample of the same individual collected before radiation therapy. Despite the varying miRNA profiles of the patients 20 miRNAs were identified as responding to irradiation in at least 4 out of 7 patients. Out of these miRNAs miR-29c and miR-425 were also found to be deregulated after 2Gy in-vitro irradiation in the human cell line CAL-33. As this cell line is derived from a tongue squamous cell carcinoma a role for these miRNAs in the radiation response of the tumor is indicated. The identification of radiation-responsive miRNAs in human blood plasma may provide a minimally invasive way to predict the radiation therapy response of tumor patients, thus potentially leading to a more individualized treatment in the future.

QSAR modeling for the evaluation of Aryl Hydrocarbon receptor activators

Ahmed Abdelaziz, Aleksandr Safanyaev, Igor V. Tetko
Chemoinformatics group, Institute of Structural Biology, 85764 Neuherberg, Germany
Helmholtz Zentrum München

The Aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor that responds to exogenous and endogenous chemicals with the induction/repression of expression of large battery of genes1 and production of a diverse spectrum of biological and toxic effects2 in a wide range of species and tissues.

The physicochemical characteristics necessary for binding of a chemical to the AHR have been examined by many laboratories for more than 30 years and have led to limited modeling of AHR ligand binding characteristics and to the identification of numerous AHR ligands. However, a greater diversity of novel AHR ligands and classes of ligands has been identified through the application of AHR-based bioassay approaches for chemical characterization and screening.

Data were collected from Pubchem BioAssay [AID: 2796]3. In which, 324858 chemical substances were evaluated to identify compounds that act as agonists to the AHR. This cell-based assay measures the ability of compounds to activate AHR signaling.

To evaluate the structural characteristics of small molecules responsible for AHR binding and toxicity, quantitative structure–activity relationships (QSARs) were developed using the free tool (OCHEM.eu) by ASNN, SVM and MLR analyses. In this study, a cross-validated qualitative model was developed and Applicability domain of the models was estimated. For the subset of most confident predictions the achieved accuracy can reach as high as 95%.

Fragmental analysis of the chemical structures was applied to better understand the origin of AHR binding affinity. Analysis confirms several chemical groups of halogenated aromatic hydrocarbons (HAHs) and polycyclic aromatic hydrocarbons (PAHs) as activators of the AHR.

The high accuracy of in silico techniques suggests a potential application for QSAR in predictive toxicology.

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Acoustic inversion in optoacoustic tomography

Dr. Xosé Luís Deán Ben, Dr. Daniel Razansky, Prof. Vasilis Ntziachristos
Helmholtz Zentrum München

Optoacoustic tomography has recently demonstrated powerful performance in small animal imaging and initial clinical trials in terms of the high spatial resolution, versatile contrast and dynamic imaging capabilities it can provide. The quality of the images rendered in optoacoustics depends on the accuracy of the reconstruction algorithm employed, for which usually an ideal acoustic media is assumed. The propagation of acoustic waves is however affected by several acoustic phenomena so that distorted images are retrieved with standard algorithms. In this poster, we showcase the effects of three different acoustic phenomena, namely acoustic scattering, small speed of sound variations and acoustic attenuation, in the optoacoustic signals. Subsequently, we modify the reconstruction algorithms accordingly in order to avoid the associated artefacts and distortion in the images.

Modeling toxicity of nanoparticles using Online Chemical Modeling Environment

Natalia Golovina
Helmholtz Zentrum München

In the recent decades, nanomaterials have deeply integrated into our everyday's life. There are numerous examples of already established and possible applications of using nanoparticles such as textile, cosmetics, optical, pharmacy, electronics, etc. Although the nanotechnology field is growing rapidly, the potential harmful effects of nanomaterials on human's health or the environment have not yet been identified. Thus, there is a clear need for assessment of such potentially dangerous toxic effects of nanomaterials.

The classical way of assessing toxicity, e.g. by performing in-vivo experiments of hydrobionts, is very expensive and time consuming. Performing such tests for all possible nanoparticle types, sizes and concentrations is practically infeasible. A cheap and efficient alternative to such tests is using predictive computational models, for example Quantitative Structure-Activity Relationship (QSAR) models.

Using QSARs for nanoparticles is a new and still developing area of research. Within our study, we have collected toxicity data for a number of nanoparticles (currently, metals and metal oxides) for different species: daphnids, planaria worms, mussels. Additionally, we have collected the information for different nanoparticles sizes, under different concentrations and exposure intervals. The data has been uploaded to the Online Chemical Modeling Environment (www.ochem.eu) and is publicly accessible by everyone on the Web. In our studies, we plan to use this data to develop predictive QSAR models for nanoparticles toxicity. Several models calculated using measured properties of nanoparticles are presented. The further work will include development of new descriptors to characterize nano-particles according to their chemical composition and size.

Predictive in-silico modeling of human CYP450 inhibition

Sergii Novotarskyi, Igor Tetko
Helmholtz Zentrum München

Cytochromes P450 (CYP450) are a superfamily of enzymes, involved in metabolism of a large number of xenobiotic compounds. CYP450 are involved in metabolism of a large amount of drugs, currently present on the market. It is estimated that over 75% of currently marketed drugs are metabolized by CYP450. Of these reactions over 90% are facilitated by CYP1A2, CYP2C9, CYP2D6 and CYP3A4. This makes these enzymes particularly interesting targets, e.g. in early stage drug discovery.

We take an in-silico data-driven approach to CYP450 inhibition prediction. The study was performed on datasets of high chemical diversity obtained from PubChem BioAssay database, which include measurements of inhibition activity against five major CYP isoforms. The combination of novel docking-derived descriptors allowed to build QSAR models with predictive abilities of over 90% of correctly predicted inhibitors. Applicability domain methods allowed to determine subsets of most confident and least confident predictions. For the subset of most confidently predicted compounds the prediction accuracy is sufficient for early stage drug discovery screenings. The subset of least confidently predicted compounds can be used in experimental design, since it represents the understudied areas of chemical space.

The fragment-based dataset and model interpretation approach allowed to identify molecular fragments (indole, benzodioxole, quinazoline and pyrimidine fragments) strongly associated with CYP inhibition activity. This finding provides a testable hypothesis on the importance of these fragments for CYP inhibition that can be verified experimentally.

Prevalence and antibiotic resistance of Staphylococcus aureus among Long-term Care Facilities residents in Krakow, Poland

Monika Pobiega[1], Dorota Romaniszyn[1], Jadwiga Wójkowska-Mach[1], Agnieszka Chmielarczyk[1], Piotr Heczko[1]

1Chair of Microbiology, Jagiellonian University Medical College, 18 Czysta Street, 31-121 Krakow, Poland

Long-term Care Facilities (LTCF) residents represents a population at risk for carrying meticillin-resistant Staphylococcus aureus (MRSA) because of greater age, age-associated morbidity and high rate of hospital contacts. Understanding the epidemiology of community and healthcare associated MRSA is essential for guiding new control programs. The presented results are from the first Polish study about infections and colonization among LTCF residents. The aim of this study was to investigate the prevalence and antibiotic resistance of MRSA isolates from LTCF-residents. Anterior nares and (if present) wound swabs from every enrolled resident were taken and cultured. Bacterial DNA was extracted with Genomic Mini (A&A Biotechnology). The presence of mecA gene was detected by multiplex-PCR. Isolates were tested using disk diffusion antimicrobial susceptibility methods. Staphylococcus spp. growth was identified among 70 of 193 cultures. Of the isolates, 57 came from nasal swabs and 13 from wounds. The general prevalence of MRSA was 15.1% corresponding to 29 MRSA carriers. Among isolates from nasal colonization, 12 exhibited resistance to erythromycin (21.0%), 30 to norfloxacin and ciprofloxacin (52.6%). Five isolates (4 MRSA, 1 SA) were resistant to 7 antimicrobials tested. The prevalence of MRSA reported in our study is higher than rates recorded in other European surveys (7.6% in Germany[1], 0% in Sweden[2]). Higher rates have been reported in England (20%)[3]. The majority (87.5%) of isolates in Sweden [2] were resistant to fluoroquinolones compared to 58.1% of isolates in this study. In Californian nursing homes[4] about 95% of MRSA isolates are resistant to erythromycin and 74% to clindamycin. Here the proportions were lower, 42.4% and 27.3% respectively. These data raises serious concern about the therapeutic options available for physicians treating residents of LTCFs. Standard infection control policies regarding isolation of high-risk patients are needed to minimize the risks of transmission.

1. Pfingsten-Wurzburg S, Pieper DH, Bautsch W, Probst-Kepper M. Prevalence and molecular epidemiology of metillin-resistant Staphylococcus aureus in nursing home residents in northern Germany. J Hosp Inf 2011,78:108-112.
2. Olofsson M, Lindgren PE, Ostgren CJ, Midlöv P, Mölstad S. Colonization with Staphylococcus aureus in Swedish nursing homes: A cross-sectional study. Scand J Infect Dis 2012;44(1):3-8.
3. Baldwin NS, Gilpin DF, Tunney MM, Kearney MP, Crymble L, Cardwell C et al. Cluster randomised controlled trial of an infection control education and training intervention programme focusing on meticillin-resistant Staphylococcus aureus in nursing homes for older people. J Hosp Infect. 2010;76(1):36-41.
4. Reynolds C, Quan V, Kim D, Peterson E, Dunn J, Whealon M et al. Methicillin-resistant Staphylococcus aureus (MRSA) carriage in 10 Nursing Homes in Orange County, California. Infect Control Hosp Epidemiol. 2011;32(1):91-3.

Modeling higher-order nucleotide dependencies within DNA binding sites

Matthias Siebert, Holger Hartmann, Johannes Söding
LMU Gene Center

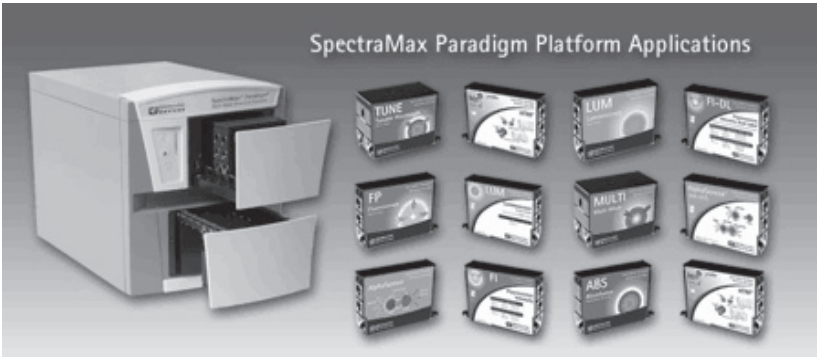
DNA-binding proteins combine multiple readout mechanisms to achieve DNA-binding specificity. In particular, DNA structure (e.g. bend, kink, narrow minor groove) can extensively contribute to protein-DNA recognition (shape readout). Since DNA structural features are based on nucleotide dependencies in the sequence (e.g. the di-/tri-/tetra-nucleotide composition), they can be suitably described by sequence-based models. The most popular model for representing and predicting protein binding sites is the Position-specific Weight Matrix (PWM). Although working well in many cases, the PWM assumes that the nucleotides of the binding site contribute independently to the binding energy, and thus cannot describe nucleotide dependencies. To overcome this limitation we make use of Interpolated Markov Models (IMMs) that are able to model higher-order dependencies while minimizing the susceptibility to over-training the more complex models. This is achieved by combining probabilities from sequence contexts of varying lengths using only those contexts for which sufficient data are available to produce good estimates. Importantly, no prior knowledge about the dependencies is necessary. In ligned sequences we adapt the EM learning scheme explored by MEME. We model core promoter elements like the TATA box, and the MTE/DPE motif in Drosophila melanogaster and discover significant contributions from higher-order nucleotide dependencies to the binding energy of the binding sites. When comparing IMMs with diverse model complexity to the PWM model we show that incorporating higher-order dependencies improves the prediction and interpretation of binding sites.

Molecular Devices
Workshop Senatssaal (12:15 and 4:15pm)

Workshop

SpectraMax® Paradigm® Microplate Reader

The SpectraMax® Paradigm® Multi-Mode Microplate Detection Platform is the only user upgradeable microplate reader on the market that allows for real-time system configuration by the user in less than two minutes. Unlike standard readers its modularity allows you to modify the instrument configuration to your needs. If your detection requirements change, you can simply add or replace a detection cartridge. The detection cartridges are offered for a wide range of applications: Absorbance, Fluorescence Intensity, FRET, Fluorescence Polarization, Luminescence, Alpha Screen, HTRF as well as custom-made cartridges. Molecular Devices is constantly working on new cartridges to meet future application needs of our Paradigm® customer base. With the current setting of the system, users can analyse up to 1536-well microplates under controlled temperature conditions. Additional, an inlet for CO2 gassing allows environmental control for cell culture experiments.



Workshop program (20 minutes)

The workshop will cover a short introduction into the system's working principle and a live SpectraMax® Paradigm® demonstration. We will show the dynamic concentration range of the system using a common fluorescent dye. This will include reader optimization steps to achieve the best possible signal.

Presentation of Industry & Academia

At **Active Motif**, *we develop innovative cell biology-based research tools and biocomputing resources that help researchers elucidate the function, regulation and interactions of the genes and their encoded proteins. Our goal is to provide our customers with high-quality products complemented by superior service & support. Active Motif is especially committed to helping researchers study important events in regulation of the genome through the development of high-quality products centered on chromatin biology and epigenetics.*

BASF - The Chemical Company

The Bavarian Ministry for Sciences, Research and the Arts established three different research networks, with the main focuses on **molecular biosystems (BioSysNet)**, **functional genome research (BayGene)** *and* **green gene technology (FORPLANTA)**. *The common goal of these government funded measures is, to concentrate different disciplines within Bavaria. By this the Bavarian state can attract new, young researcher and keep well-respected scientists at its site.*

Biomol *is a key player in the research reagent market. Based in Hamburg, Biomol supplies biochemicals, assay kits, proteins and hundred thousand antibodies, pinpointing research areas like gene regulation, signal transduction, inflammation and apoptosis. Biomol presents with pleasure its product highlights at the Interact.*

eBioscience *is a lead supplier in flow cytometry and offers besides a large selections of antibodies, also immunoassays and proteins for Life Science research and diagnostic. Frequently first to market eBioscience develops more than 800 innovative products every year including antibodies, fluorochromes and further reagents for immunology, oncology, cell biology, stem cell biology and diagnostics. In addition a broad spectrum of reagents for the analy*

sis of cytokines, growth factors and other soluble proteins as well as bead-based multiplex immunoassays for the flow cytometer, ready-to-use ELISA kits, matched antibody pairs, proteins and standards enables eBioscience to provide complete solutions for biological systems analysis.

Eurofins MWG Operon is a Member of the Eurofins Group and an international provider of genomic services established around the following core business lines:

DNA & RNA Synthesis
Next Generation Sequencing
Custom DNA Sequencing
Gene Synthesis
siRNA Synthesis

Our main mission is focussed on customer convenience and high quality services in industrial scale for the life science industries and academic research institutions around the world.
www.eurofinsdna.com

Eppendorf *is a biotech company which develops, produces and distributes systems for use in all spheres of life science. At the interact 2012 Eppendorf presents a part of the company's broad range of high quality products including pipettes, dispensers and centrifuges as well as consumables such as micro test tubes and pipette tips. In addition to this, Eppendorf displays instruments for the fields of cell growth, detection and storage plus the new instrument for PCR- the Eppendorf mastercycler ® nexus.*

The **Graduate Center LMU** *is the central unit for all matters related to doctoral studies at LMU Munich. It aims to optimize the conditions for all doctoral students at the university; both those participating in doctoral programs and individual doctoral students. The GraduateCenterLMU offers an expanding range of training courses for doctoral students, monitors the development of supervision conditions and supports the faculties in the conception, implementation and quality assurance of structured doctoral programs.*

It was founded in 2008 as part of the "LMUexcellent" institutional strategy, which is funded by the Excellence Initiative of the German federal and state governments. Meanwhile the center has established a comprehensive range of services and advice for doctoral students, professors and coordinators of doctoral programs.

metaBIOn *is a "one-stop-shop" in the field of molecular biology, with products ranging from custom synthesis to biochemicals. Founded in September 1997 as a company for applied biotechnology in Martinsried near Munich, metaBIOn has managed to become one of the leading biotechnology custom synthesis services, offering a wide range of different biomolecules (DNA oligonucleotides, PTO oligonucleotides, ZNATM oligonucleotides, RNA oligonucleotides, peptides, polyclonal and monoclonal antibodies etc.) designed for the scientist's specific application. For a consistently high quality standard, metaBIOn's oligo production is certified according to DIN EN ISO 9001 and EN ISO 13485 regulations, allowing a strict quality control at every synthesis and purification step, assuring permanent and constant high quality products and ultimately benefiting the customer.*

Molecular Devices, *is one of the world's leading providers of high-performance bioanalytical measurement systems, software and consumables for life science research, pharmaceutical and biotherapeutic development. Included within a broad product portfolio are platforms for high-throughput screening, genomic and cellular analysis, colony selection and microplate detection. These leading-edge products enable scientists to improve productivity and effectiveness, ultimately accelerating research and the discovery of new therapeutics.*

Molecular Devices will host a 20 minutes workshop at 12:15 and 4:15 pm in the Senatssaal.

The workshop will cover a short introduction into the SpectraMax® Paradigm® system's working principle and a live demonstration. We will show the dynamic concentration range of the system using a common fluorescent dye. This will include reader optimization steps to achieve the best possible signal.

NewEnglandBiolabs–*enablingtechnologiesinthelifesciences*
New England Biolabs is a world leader in the production and supply of reagents for the life science industry. NEB offers the largest selection of recombinant and native enzymes for genomic research and continues to expand its product offerings into areas including Next-Generation-Sequencing, proteomics, cellular analysis and drug discovery. NEB can be considered one of the companies who helped to shape today's biotechnology industry. The company was amongst the first to produce restriction enzymes on a commercial scale and continues to specialize in DNA modification enzymes and polymer ases, where it consistently maintains a position at the forefront of these fields. NEB's reagents have gained a worldwide reputation for setting the highest of standards for quality and value. Since 1999 New England Biolabs GmbH is also the exclusive source for reagents from Cell Signaling Technology (CST). Originally founded by scientists from NEB, Cell Signaling Technology is dedicated to providing innovative research tools that are used to help define mechanisms underlying cell function and disease. CST has become the world leader in the production of the highest quality antibodies utilized to expand knowledge of cell signaling pathways. E New England Biolabs GmbH - NEB's subsidiary in Germany - represents its service hub for Central Europe. From our location in Frankfurt, we serve scientists and industry customers in Germany, Austria and Eastern Europe.

Perkin Elmer - *Imaging of disease biology and therapeutic response in vivo by Fluorescence Molecular Tomography (FMT) utilizing Pre-clinical Imaging Agents. Applications in Oncology, Cardiovascular, Pulmonary, Bone and Inflammatory Diseases and many other pathological disease states in small animal models.*

Doctoral candidates are at the heart of the research culture here at the Technische Universität München. The **TUM Graduate School** *particularly promotes interdisciplinary and international qualification programs so that at the end of the doctoral program, candidates will not only have team leadership and project management skills, but also the entrepreneur-*

ial spirit for leading roles in industry, academia and science. For a quick and successful completion of your doctorate, we ensure attentive supervision through both an academic supervisor and an independent mentor. Our networking events offer our doctoral candidates points of contact for the latest research projects across all disciplines. Our 13 Faculty Graduate Centers and 10 interdisciplinary Thematic Graduate Centers provide doctoral candidates with courses designed to complement their program in addition to individual guidance. At our desk at the <interact> you can meet representatives of the TUM Graduate School and also of the Faculty Graduate Center of the Centre of Food and Life Sciences Weihenstephan.

The **Helmholtz Graduate School Environmental Health (HELENA)** *is a joint initiative for the promotion of graduate students of the Helmholtz Zentrum München – German Research Center for Environmental Health, the Ludwig-Maximilians-Universität München (LMU) and the Technische Universität München (TUM). At the <interact>, HELENA informs about its' graduate program and about research activities in the field of Environmental Health. HELENA is currently also in the process of recruiting new doctoral students. Interested master students are invited to inform themselves on the open positions and career opportunities within HELENA. Students have also the opportunity to take part in a "wheel of fortune"-game.*

Internet Access

We provide WLAN (802.11b/g) internet access in the following rooms:

Grosse Aula

Lichthof

Dekanatsgang

Speerträger

Senatssaal

... open access from 7:00 to 21:00!

... no validation necessary!

SSID	con
Network authentication	open
Data encryption	no encryption
TCP/IP	obtain an IP address automatically

Please configure your WLAN adapter with SSID „**con**“. This network name is not always broadcasted. If necessary, enter it manually.

Do not specify network authentication or any encryption. For TCP/IP properties choose “**Obtain an IP address automatically**“. You get a worldwide valid IP address, no proxy server is needed.

All internet applications except microsoft file and printer sharing should work.

Our Great Prizes for Talks & Posters

Awards

- You can vote for the best poster and for the best talk. We need your voice even if you haven't seen all posters or heard all the talks.
- You'll find the voting sheets in your booklet.
- Please drop off your vote at the registration desk before the key-note talk II at 5.00 pm.
- Winners will receive great prices and are announced at the end of the dinner at 7.30 pm.

Best Talks

First prize

XZ-1 digital camera, Olympus

Second prize
150 € Hugendubel voucher

Third prize
100 € Hugendubel voucher

Best Posters

First prize

SZ-30MR digital camera, Olympus

Second prize
150 € Hugendubel voucher

Third prize
100 € Hugendubel voucher

First prizes donated by Olympus, second and third prizes donated by the TUM Graduate Center Weihenstephan.

Acknowledgements

The <interact> 2012 organizing team would like to thank all our supporters. Without your help, the symposium would not have been possible!

Our special thanks go to

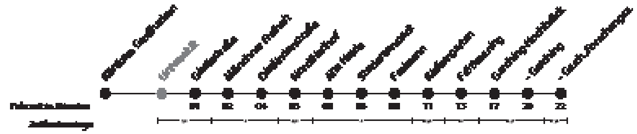
- Our keynote speakers Prof. Ada Yonath, Prof. Herbert Jäckle and Prof. Gero Miesenböck
- The members of our advisory board
- All our generous donors
- Hans-Jörg Schäffer (IMPRS)
- Christian Ude (Major of Munich)
- Prof. Bernd Huber (President of the LMU Munich)
- All the participating institutes
- All helpers

Public Transport

U6

Klinikum Großhadern - Holzapfelkreuth - Harras - Marienplatz - Fröttmaning - Garching, Forschungszentrum

Universität



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= bis Kieferngarten
= bis Fröttmaning
V93 = nicht Nächte vor Feiertagen
V97 = Nächte vor Feiertagen, auch 19./20. und 20./21.02.2012
⊠ = Fröttmaning - Gleisfinderstelle nur an Schultagen
HUM = Linie U3 Richtung Moosach; U6 fährt ab Münchner Freiheit

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U6

Garching, Forschungszentrum - Fröttmaning - Marienplatz - Harras - Holzapfelkreuth - Klinikum Großhadern

Universität

Haltestelle	Garch.-Forschungsz.	Universität	Odeonsplatz	Marienplatz	Sendlinger Tor	Goetheplatz	Poccistraße	Implerstraße	Harras	Partnachplatz	Westpark	Holzapfelkreuth	Haderner Stern	Großhadern	Klinikum Großhadern
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■ = bis Implerstraße
▮ = bis Harras
V11 = Nicht in der Nacht vom 31.12.2011/1.1.2012 (siehe Sonderfahrplan)
V82 = nicht 24. und 31. Dezember
V93 = nicht Nächte vor Feiertagen
V97 = Nächte vor Feiertagen, auch 19./20. und 20./21.02.2012
⊠ = Fröttmaning - Dietlindenstraße nur an Schultagen

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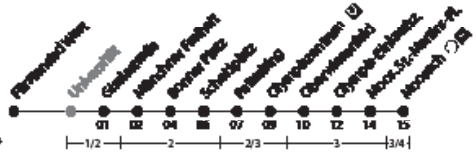
SWM/MVG Tel.: 01803/442266 9Ct./Min

U3

Förstenried West - Thalkirchen - Marienplatz ⓘ - MÜnchner Freiheit - Scheidplatz - Olympia-Einkaufszentrum - Moosach ⓘ

Universität



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18	01 ▮ 06 11 ▮ 16 21 ▮ 26 31 ▮ 36 41 ▮ 46 51 ▮ 56	06 16 26 36 46 56	06 16 26 36 46 56	18
19	01 ▮ 06 11 ▮ 16 21 ▮ 26 31 ▮ 36 46 56	06 16 26 36 46 56	06 16 26 36 46 56	19
20	06 16 26 36 46 56	06 16 26 36 46 56	06 16 26 36 46 56	20
21	06 16 26 36 46 56	06 16 26 36 46 56	06 16 26 36 46 56	21
22	06 16 26 36 46 56	06 16 26 36 46 56	06 16 26 36 46 56	22
23	06 16 26 36 46 56	06 16 26 36 46 56	06 16 26 36 46 56	23
0	06 16 26 V97 36 56	06 16 26 36 56	06 16 V11 26 V11 36 V11 56 V11	0
1	21 51 V97	21 51	21 V11 51 V11	1
2	21 V97	21	21 V11	2

▮ – bis MÜnchner Freiheit
▮ – bis Olympiazentrum ⓘ

V11 – Nicht in der Nacht vom 31.12.2011/1.1.2012 (siehe Sonderfahrplan) V97 – Nicht vor Feiertagen, auch 19./20. und 20./21.02.2012

U3

Moosach - Olympia-Einkaufszentrum - Scheidplatz - Münchner Freiheit - Marienplatz - Thalkirchen - Fürstenried West

Universität

	Moosach	Universität	Odeonsplatz	Marienplatz	Sendlinger Tor	Goetheplatz	Poccistraße	Implerstraße	Brudermühlstraße	Thalkirchen (Tierpark)	Obersendling	Aidenbachstraße	Machtlfinger Straße	Forstenrieder Allee	Basler Straße	Fürstenried West
Fahrzeit in Minuten			01	03	04	06	07	08	10	12	13	15	16	18	19	20

Zeitkartenringe: 1/2 – 1 – 1/2 – 2 – 2/3 – 3 – 3/4 – 4

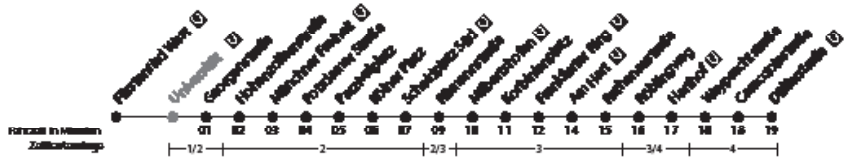
Uhr	Montag - Donnerstag	Freitag	Samstag	Uhr
4	28 52	28 52	28	4
5	09 29 39 49 59	09 29 39 49 59	09 29 49	5
6	09 19 29 39 44 49 54 59	09 19 29 39 44 49 54 59	09 29 49	6
7	04 09 14 19 24 29 34 39 44 49 54 59	04 09 14 19 24 29 34 39 44 49 54 59	09 29 39 49 59	7
8	04 09 14 19 24 29 34 39 44 49 54 59	04 09 14 19 24 29 34 39 44 49 54 59	09 19 29 39 49 59	8
9	04 ■ 09 14 ■ 19 29 39 49 59	04 ■ 09 14 ■ 19 29 39 49 59	09 19 29 39 49 59	9
10	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	10
11	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	11
12	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	12
13	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	13
14	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	14
15	09 19 24 29 34 39 44 49 54 59	09 19 29 39 49 59	09 19 29 39 49 59	15
16	04 09 14 19 24 29 34 39 44 49 54 59	09 19 29 39 49 59	09 19 29 39 49 59	16
17	04 09 14 19 24 29 34 39 44 49 54 59	09 19 29 39 49 59	09 19 29 39 49 59	17
18	04 09 14 19 24 29 34 39 44 49 54 59	09 19 29 39 49 59	09 19 29 39 49 59	18
19	04 ■ 09 14 ■ 19 24 ■ 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	19
20	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	20
21	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	21
22	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	22
23	09 19 29 39 ■ 49 59 ■	09 19 29 39 49 59 ■	09 19 29 39 49 59 ■	23
0	09 19 ■ 29 39 ■ 49 59 ■ V97	09 19 ■ 29 39 ■ 49 59 ■	09 19 ■ V11 29 V11 39 ■ V11 49 V11 59 ■ V11	0
1	10 40 V97	10 40	10 V11 40 V11	1
2	10 V97	10	10 V11	2

■ – bis Sendlinger Tor
■ – bis Thalkirchen (Tierpark)
V11 – Nicht in der Nacht vom 31.12.2011/1.1.2012 (siehe Sonderfahrplan)
V97 – Nächte vor Feiertagen, auch 19./20. und 20./21.02.2012

BUS N41

Förstenried West U - Harras U - Karlsplatz (Stachus) U - Münchner Freiheit U - Dülferstraße U

Universität U



Uhr	Montag - Freitag	Samstag, Sonn- und Feiertag	Uhr
1	41	41 56 A01	1
2	11 jm3_ 41	11 26 A01 41 56 A01	2
3	11 jm3_ 41	11 26 A01 41 56 A01	3
4	11 jm3_ 41	11 26 A01 41	4
5			5
6			6
7			7
8			8
9			9
10			10
11			11
12			12
13			13
14			14
15			15
16			16
17			17
18			18
19			19
20			20
21			21
22			22
23			23

A01 = nur 1.1. jm3_ = Faschingsmontag+Dienstag

Montag - Freitag = Nächte So/Mo bis Nächte Do/Fr, Samstag, Sonntag und Feiertag = Nächte Fr/Sa, Sa/So und vor Feiertagen
In den Nächten 23./24.12 und 30./31.12 Betrieb wie Samstag

www.mvv-muenchen.de

Änderungen vorbehalten SMS-Auskunft an 01 75 4321 409 SWM/MVG Tel: 01805/442266 (0,14/Min)

Gültig ab 11. Dezember 2011

BUS N41

Dülferstraße U - Münchner Freiheit U - Karlsplatz (Stachus) U S - Harras U S - Fürstenried West U

Universität U

Dülferstraße U – Universität U – …

Fahrzeit in Minuten: 02 03 06 08 15 16 18 20 21 22 23 24 25 26 27 28 30 30 31 32 33 33 34 35 35 36 36 37 38 39

Zonen: 1/2 | 1 | 1/2 | 2 | 2/3 | 3 | 4

Uhr	Montag - Freitag	Samstag, Sonn- und Feiertag	Uhr
2	22 52 jm3_	22 37 A01 52	2
3	22 52 jm3_	07 A01 22 37 A01 52	3
4	22 52 B jm3_	07 A01 22 37 B A01 52 B	4
5			5
6			6
7			7
8			8
9			9
10			10
11			11
12			12
13			13
14			14
15			15
16			16
17			17
18			18
19			19
20			20
21			21
22			22
23			23

B = bis Karlsplatz (Stachus) U S   A01 = nur 1.1.   jm3_ = Faschingsmontag+Dienstag

Montag - Freitag = Nächte So/Mo bis Nächte Do/Fr, Samstag, Sonntag und Feiertag = Nächte Fr/Sa, Sa/So und vor Feiertagen
In den Nächten 23./24.12 und 30./31.12 Betrieb wie Samstag

Notes

Notes

Notes

Schedule Symposium

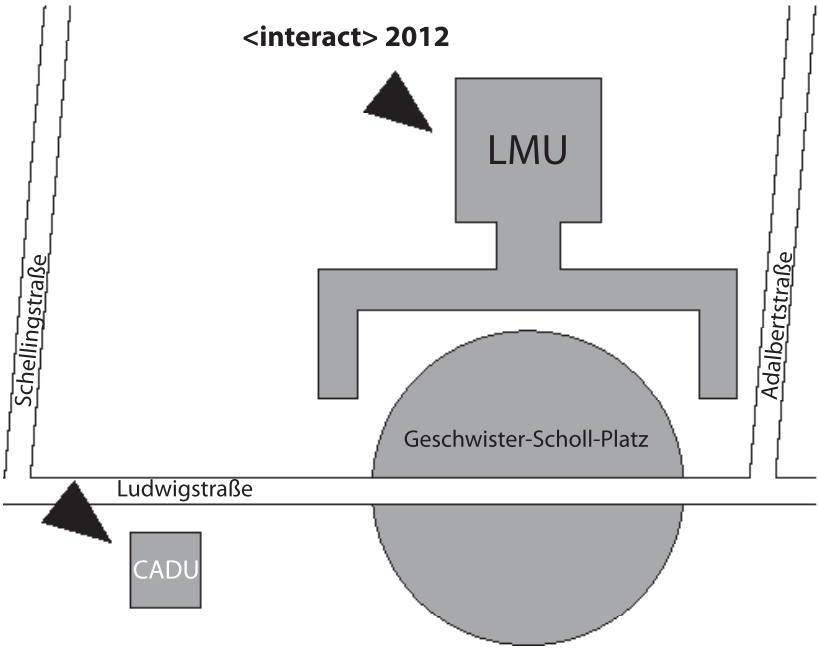
08.00 – 08.45 am	*Check-in*
09.00 – 09:15 am	*Welcome Words*
09.15 – 10.20 am	*Keynote Talk I : Gero Miesenböck*
10.20 – 10.40 am	*Refreshments*
10.40 – 11.45 am	*Morning Parallel Sessions*
11.45– 01.00 am	*Coffee Break and Poster Session I*
01.00 – 02.00 pm	*Lunch*
02.00 – 03.20 pm	*Afternoon Parallel Sessions*
03.30 – 04.00 pm	*Methods Seminar*
04.00 – 05.15 pm	*Coffee Break and Poster Session II*
05.15 – 06.15 pm	*Keynote Talk II: Ada Yonath*
06.15 – 06.30 pm	*Final Words*
06.30 – 07.30 pm	*Dinner*
07.30 - 07.45 pm	*Poster & Talk Awards*
07.45 - open end	*Party*

Let's Party...

After the announcement of the award winners, the party will take place at the CADU (Cafe an der Uni) in the Ludwigsstraße 24.

Everyone is invited. You will find a coin for one free drink (beer, wine or softdrink) in your goodie bag.

We hope you had a great day and see you again at next year's <interact>!



Party

Scientists need food for thought. Coffee and refreshments will be offered during the break (10:20 - 10:40pm) and the poster sessions (11:45 - 1:00pm and 4:00 - 5:15pm). Lunch is served after the first poster session (1:00-2:00pm) and dinner will be at 6:30-7:30pm. Have a look at the menu below, it certainly will make your mouth water!

Menu

Lunch

Herb-Scented Tuscan Roast Pork with Tomatoes, Pepperoncini, and Baked Rosmary Potatoes
Toskanischer Schweinebraten geschmort mit Tomaten, Peperoncini, Oliven und frischen Kräutern, dazu Ofenkartoffeln mit Rosmarin

Lasagne with Ricotta and Spinach, Mozzarella, and Rocket Sauce
Lasagne mit Ricotta und Blattspinat, mit Mozzarella und Rucolarahm

Dinner

Antipasti
Antipasti

****

Chicken Breast filled with Chorizo served with Peperonata, and Broccoli Potatoe Gratin
Hähnchenbrust gefüllt mit Chorizo auf Peperonata, dazu cremiges Kartoffel-Brokkoligratin

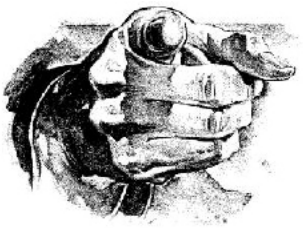
Tilpia with Lime-Saffron Sauce and Basmati Rice
Tilapiafilet in Safran-Limettensauce, dazu Basmati-Wildreis

Gnocchi with roasted vegetables, herbs, and sliced parmesan
Gnocchi mit gebratenem frischen Gemüse und frischen Kräutern, serviert mit gehobeltem Parmesan

****

Dessert
Dessert

We want you…



… for the next <interact> organizing team!

If you already have experience in organizing a big event or want to gain some, you are the right person for our new team. There are a lot of reasons, why joining the organizing team is a good idea: You make new acquaintances, collect credit points for you PhD program, it always looks good in your CV and – most importantly – it is a lot of fun!

You will have the possibility to join a workshop, which is very useful and recommended to all new organizers:

Simon Golin, "The 'do it yourself' doctoral symposium – <interact> 2013: Objective setting – team building – project start", May 30th - 31st, 2012

Interested in joining us? Please send an email to Michael Breckwoldt (Michael.breckwoldt@lrz.tum.de) to get more information.

We are looking forward to welcome you in the next <interact> organizing team!

Support

ACTIVE MOTIF

Basecamp

BayGene
Bayerisches Genomforschungsnetzwerk

BECKMAN COULTER
We're better together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CeNS
Center for NanoScience | LMU


CIPSM


Graduate
Center LMU


Graduate School of
Systemic Neurosciences
LMU Munich


Graduiertenzentrum
Weihenstephan


HELENA
Helmholtz Graduate School Environmental Health


HELMHOLTZ
GEMEINSCHAFT


IMPRS
From Biology
to Medicine


TUM Graduate School


TUM Graduate School
Faculty Graduate Center Chemistry