CELLS IN ACTION

Challenging the tango of life

OCTOBER 2023

25th Online conference

26th 27th **In-person conference**
MPI for Molecular Biomedicine
Münster - Germany

The 17th CiM-IMPRS Interdisciplinary Graduate School Meeting

Universität Münster

MAX PLANCK INSTITUTE FOR MOLECULAR BIOMEDICINE

CiM-IMPRS
Graduate Program | Münster Germany

Welcome Message

Dear attendees,

We're excited to have you join us in exploring cutting-edge research online and engaging with leading experts offline. Our interdisciplinary sessions cover a diverse range of topics, including and throughout the event, doctoral students will showcase their research in interactive poster sessions, followed by career talks offering valuable insights into post-PhD options. Join us to share the excitement and become an integral part of this inspiring conference hosted by CiM-IMPRS PhD students in Münster, Germany.

The GSM2023 team

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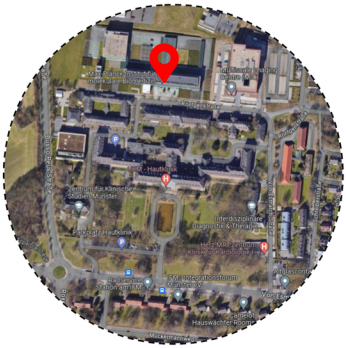
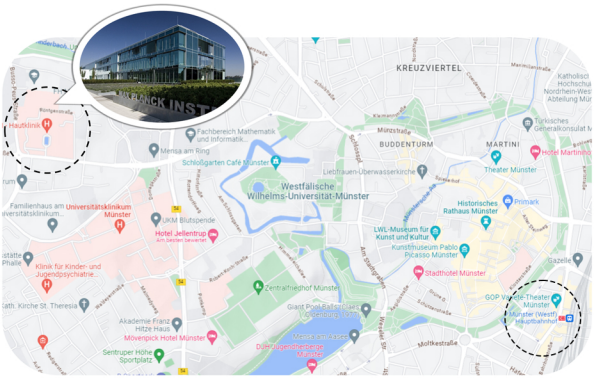




Main Station

Bus line options

Stop Hautklinik 5 11 22

Stop Wilhelm-Klemm-Str. 2

Social Event - Thursday 26.10.2023

Take part in our get together in the MPI Foyer at 18h, food included!

Pub QUIZ!

Day 1 - Online sessions - Morning

09:00 - Opening

9:10 - 12:50
Developmental & Cell Biology I

09:10 - **Impact of lamin-A/C on cancer progression in vivo**

Katarina Wolf
Radboud University Medical Center, Radboud University, Nijmegen - Netherlands

09:50 - **A question of dynamics**

Barbara di Ventura
The BIOSS Centre for Biological Signalling Studies, University of Freiburg, Freiburg im Breisgau - Germany

10:30 - 10:50 **Q&A and Coffee Break**

10:50 -12:50
Vascular Biology

10:50 - **Guiding Mechanotransduction in Blood Vessels**

Ellie Tzima
Radcliffe Department of Medicine - Cardiovascular Medicine, University of Oxford, Oxford - UK

11:30 - **Skeletal stem/progenitor cells: interactions within the bone vascular microenvironment and beyond**

Christa Maes
Skeletal Biology and Engineering Research Center, KU Leuven, Leuven - Belgium

12:10 - **Leukocyte breaching of blood vessel walls: Insights into the role of pericytes**

Tamara Girbl
Rudolf Virchow Center for Integrative and Translational Bioimaging, University of Würzburg, Würzburg - Germany

12:50 - 14:00 **Lunch Break**

AGENDA

Day 1 - Online sessions - Afternoon

14:00 - 15:50
Developmental & Cell Biology II

14:00 - **Membrane repair mechanims and membrane integrity**

Jesper Nylandsted
Cancer and Inflammation Research Unit, Danish Cancer Society Research Centre,
University of Southern Denmark, Copenhagen - Denmark

14:40 - **Cellular roadmaps: Control of cell architecture by dynamic microtubules**

Anna Akhmanova
Cell Biology, Neurobiology and Biophysics, Department of Biology,
Faculty of Science, Utrecht University, Utrecht - Netherlands

15:20 - 15:50 **Q&A and coffee break**

15:50 - 17:50
Immunology & Virology

15:50 - **Unraveling the Molecular Architecture of the Intestinal Barrier: Insights from Spatial Transcriptomics**

Eduardo Villablanca
Department of Medicine, Karolinska Institute, Stockholm - Sweden

16:30 - **Circadian control of enteric virus infection**

Julie Pfeiffer
Microbiology department, University of Texas Southwestern Medical Center,
Dallas - USA

17:10 - **Culturing Hepatitis E Virus - Recent advances for basic science and drug discovery**

Eike Steinmann
Department of Molecular & Medical Virology, University Bochum
Bochum - Germany

Day 2 - In-person sessions - Morning

08:00 - Registration
08:50 - Welcome Talk

09:10 - 10:30
Young Investigator Talks

09:00 - **Double-modified AdoMet analogues for selective targeting of methyltransferases**

Mehmet Ergüven | mergueve@uni-muenster.de
Institute of Biochemistry, University of Münster

09:20 - **Two-way Dispatched function in Sonic Hedgehog shedding and transfer to high-density lipoproteins**

Janna Puschmann | janna.puschmann@uni-muenster.de
Institute for Physiological Chemistry and Pathobiochemistry, University of Münster

09:40 - **Unexpected function of the antioxidative xCT system/Slc7a11 in systemic sclerosis.**

Jessica Tiemann | jessica.tiemann@ukmuenster.de
Department of Dermatology, University of Münster

10:00 - 10:30 **Poster Flash Talks**

10:30 - 10:50 **Q&A and Coffee Break**

10:50 Non-academic Session

Career Seminar - Aishwarya Sundaram
Nature Communications Editor
Berlin - Germany

Dr. Aishwarya Sundaram is a Senior Editor at Nature Communications, handling submissions related to cancer metastasis and therapy. After obtaining her PhD from Heidelberg University, she worked for several years in the pharmaceutical industry with a focus on drug discovery.

12:30 - 13:30 - Lunch Break and Poster Session

AGENDA

Day 2 - In-person sessions - Afternoon

13:00 - 16:30
Systems Biology

13:00 - **Mitotic rewiring on evolutionary timescales**

Gautam Dey
Cell Biology and Biophysics Unit, EMBL, Heidelberg - Germany

14:10 - **Cellular regulation in context – Integration of mathematical modeling in time and space with dedicated experiments on different scales**

Edda Klipp
Institute of Biology, Humboldt University of Berlin, Berlin - Germany

14:50 - **The EGFR/RPTPg sensory morphogenic system: a loop onto itself**

Philippe Bastiaens
Systemic Cell Biology, Max Planck Institute of Molecular Physiology, Dortmund - Germany

15:30 - **From molecules to life: building living systems from scratch**

Dora Tang
Department of Life Sciences, Saarland University, Saarbrücken - Germany

16:10-16:30 - **Q&A and Coffee Break**

16:30 -17:50
Biophysics Part I

16:30 - **Liquid–liquid phase separation? Ask the water!**

Martina Havenith
Center of Molecular Spectroscopy and Simulation of Solvent Controlled Processes,
Ruhr University Bochum, Bochum - Germany

17:10 - **Building blocks of life: protein patterns on model membranesinfection**

Katja Zieske
Max Planck Institute for the Science of Light, Friedrich-Alexander University Erlangen-Nürnberg,
Erlangen - Germany

18h - **Social Event**

Day 3 - In-person sessions - Morning

09:45 - Registration
10:00 - Welcome Talk

10:10 - 14:40
Neuroscience

10:10 - **Interactions of Tau protein condensates**

Susanne Wegmann
Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Berlin - Germany

10:50 - **Programming cell fate and age for neuronal disease modeling and repair**

Oliver Brüstle
Institut für Rekonstruktive Neurobiologie, Universitätsklinikum Bonn, Bonn - Germany

11:30 - 12:30 KEYNOTE

Synaptic vesicles – key organelles in synaptic transmission

Reinhard Jahn

Max Planck Institute for Multidisciplinary Sciences, University of Göttingen, Göttingen - Germany

12:30 -14h - **Poster Sessions and Lunch Break**

14:00 - **Single-cell delineation of lineage and genetic identity in the mouse brain**

Christian Mayer
Max Planck Institute for Biological Intelligence, LMU Munich, Martinsried, Munich - Germany

AGENDA

Day 3 - In-person sessions - Afternoon

14:40 - 16:00
Biophysics II

14:40 - **Cytoskeletal fibres as building blocks for life**

Franziska Lautenschläger
Center for Biophysics, Saarland University, Saarbrücken - Germany

15:20 - **Cellular cartography: mapping cytoskeletal organization and intracellular transport in neurons**

Lukas Kapitein
Cell Biology, Neurobiology and Biophysics, Department of Biology, Faculty of Science
Utrecht University, Utrecht - Netherlands

16:00-16:30 - **Awards & Closing Ceremony**

Speaker

Cellular roadmaps: Control of cell architecture by dynamic microtubules

Anna Akhmanova
Cell Biology, Neurobiology and Biophysics, Department of Biology, Faculty of Science, Utrecht University, Utrecht, The Netherlands

Microtubules are dynamic cytoskeletal filaments that control different aspects of cell architecture. Microtubules are intrinsically asymmetric polymers, with fast-growing plus ends, which in cells serve as major sites of microtubule assembly and disassembly, and slow-growing minus ends, which are often stabilized and attached to different cellular structures. In my lab, we use in vitro assays combined with single molecule imaging to dissect how the proteins that bind to microtubule plus- and minus ends control microtubule nucleation and dynamics. In parallel, we employ live cell imaging to study how microtubules contribute to cell polarity, migration, division and differentiation. The combination of in vitro reconstitution assays with experiments in cells allows us to decipher how the specific molecular properties of microtubule regulators contribute to cellular function and how microtubule-targeting anti-cancer drugs affect the cytoskeleton.

Speaker

The EGFR/RPTPg sensory morphogenic system: a loop onto itself

Philippe Bastiaens
Systemic Cell Biology, Max Planck Institute of Molecular Physiology,
Dortmund - Germany

to be announced

Speaker

Programming cell fate and age for neuronal disease modeling and repair

Oliver Brüstle
Institut für Rekonstruktive Neurobiologie, Universitätsklinikum Bonn, Bonn - Germany

to be announced

Speaker

Mitotic rewiring on evolutionary timescales

Gautam Dey
Cell Biology and Biophysics Unit, EMBL, Heidelberg - Germany

Despite the fundamental role of cell division in the propagation of cellular life, eukaryotes have evolved a diverse range of strategies to remodel and partition organelles and cellular contents in mitosis. What drives the evolution of mitotic mechanisms? Bridging lab and field expeditions, we use a range of protist and fungal model systems, comparative genomics, imaging and experimental evolution to probe mitotic diversity on short and long evolutionary timescales. I will present our recent work on karyotype evolution in budding yeast and on mitotic mechanisms in close holozoan relatives of animals and fungi, the Ichthyosporea.

Speaker

A question of dynamics

Enoch B. Antwi[1,2*], Yassine Marrakchi[2,3], Özgün Çiçek3, Thomas Brox[1,3,4], Barbara Di Ventura[1,2]

[1] Centers for Biological Signalling Studies BIOSS and CIBSS, Albert-Ludwigs-University, Freiburg, Germany
[2] Faculty of Biology, Institute of Biology II, Albert-Ludwigs-University, Freiburg, Germany
[3] Department of Computer Science, Albert-Ludwigs-University, Freiburg, Germany
[4] BrainLinks-BrainTools, Albert-Ludwigs-University, Freiburg, Germany

In response to different stimuli many transcription factors (TFs) display different activation dynamics that trigger the expression of specific sets of target genes, suggesting that promoters have a way to decode dynamics. In order to understand how mammalian promoters decode TF dynamics we needed a way to generate different TF dynamics at will. For this purpose, we constructed a synthetic TF based on the blue light-dependent nuclear localization signal LINuS previously developed in our lab. This way we could directly manipulate the nuclear localization of the synthetic TF in mammalian cells without affecting other processes. We then generated two different pulsatile and a sustained TF dynamics and employed live cell microscopy and mathematical modelling to analyse the behaviour of a library of reporter constructs. We found that decoding of TF dynamics occurs only when the coupling between TF binding and transcription pre-initiation complex formation is inefficient and that the ability of a promoter to decode TF dynamics gets amplified by inefficient translation initiation. Using the knowledge acquired, we built a synthetic circuit that allows obtaining two gene expression programs depending solely on TF dynamics. Finally, we translated our findings to two natural TFs: p65 and p53. These results help elucidate how gene expression is regulated in mammalian cells and open up the possibility to build complex synthetic circuits steered by TF dynamics.

Speaker

Leukocyte breaching of blood vessel walls: Insights into the role of pericytes

Tamara Girbl
Rudolf Virchow Center for Integrative and Translational Bioimaging,
University of Würzburg, Würzburg - Germany

The efficient recruitment of leukocytes to sites of inflammation is a prerequisite for the establishment of successful immune responses. In order to enter extravascular tissues, circulating immune cells need to breach two distinct cellular barriers of venular walls: i) the endothelium and ii) the surrounding pericyte layer. While the cascade of adhesive events mediating leukocyte interactions with endothelial cells have been investigated over the past decades, very little is still known about how leukocytes subsequently overcome the pericyte layer. We have previously shown that pericytes provide important chemotactic cues for transmigrating neutrophils and play a decisive role in neutrophil entry into inflamed sites during innate immune responses.

The main goal of our current research is to gain a better understanding of the little investigated role of pericytes during adaptive immune responses. For this purpose, we use models of acute dermal inflammation in the mouse ear skin and a combination of whole mount immunofluorescence stainings, confocal and 2-photon (intravital) microscopy and primary pericyte analyses. Our results show that the venular pericyte sheath is extensively modulated during dermal inflammation. This is related to the retraction of pericyte processes and a reduced pericyte coverage of the venular endothelium. Despite these modifications, transmigrating effector T cells engage in substantial physical interactions with pericytes within venular walls. Further analyses showed that TNF and IFN-gamma strongly induce the expression of key T cell adhesion molecules and chemokines in dermal pericytes. We currently aim to unravel the contribution of the specific pericyte-associated molecules in mediating effector T cell breaching of venular walls in vivo and thereby contribute to our basic understanding of T cell recruitment to extravascular sites of inflammation.

Speaker

Liquid–liquid phase separation? Ask the water!

Martina Havenith
Physical Chemistry II, Ruhr University Bochum - Germany

Liquid–liquid phase separation (LLPS) describes the reversible compartmentalization of protein solutions into a protein-rich and a protein-poor phase. It is assumed that the protein-rich droplets promote the formation of neurotoxic protein aggregates in case of Alzheimer or Huntington disease – a hot spot for neurodegenerative diseases. Tuning LLPS is a major goal for biological and medical applications. Using THz calorimetry, we can map the formation of these protein-rich droplets on a molecular level. We observe how LLPS is dictated by changes in hydration entropy and enthalpy. This allows to unravel the two underlying molecular mechanism, which drive this process. "Cavity-wrap" water hydrating hydrophobic patches is released during LLPS leading to an increase in entropy. "Bound" water hydrating hydrophilic patches is retained, which is enthalpically favorable. We introduce a THz-phase diagram mapping these spectroscopic/thermodynamic changes. This provides not only a precise understanding of hydrophobic and hydrophilic hydration driving forces as a function of temperature and concentration but also a rational means to tune LLPS.

References:
1. S. Pezzotti, B. König, S. Ramos, G. Schwaab, M. Havenith, J. Phys. Chem. Lett. 14, 1556 (2023).

Speaker

Synaptic vesicles – key organelles in synaptic transmission

Reinhard Jahn
Laboratory of Neurobiology, Max Planck Institute for Multidisciplinary Sciences, Göttingen - Germany

Neurons receive and process signals and transmit them to recipient cells such as muscle cells or other neurons. Transmission occurs at specialized contact sites, termed synapses where electrical signals are re-coded into chemical signals composed of specialized signaling molecules, the neurotransmitters. In presynaptic nerve endings, neurotransmitters are stored in synaptic vesicles. Upon arrival of a depolarizing signal, voltage-gated calcium channels open, and some of the synaptic vesicles fuse with the cell membrane. The released neurotransmitters are recognized by receptors in the postsynaptic membrane. After exocytosis, synaptic vesicles are rapidly recycled within nerve terminals and re-filled with transmitters from cytoplasmic pools.

Synaptic vesicles are specialized trafficking organelles that can be viewed as nanoparticles of the brain, and their membrane composition is better understood than that of most other trafficking membranes. They possess proteins functioning in membrane trafficking such as the SNAREs and the calcium sensor synaptotagmin, as well as proteins functioning in the loading and storage of neurotransmitters. We presently focus on the mechanisms underlying filling of synaptic vesicles with neurotransmitters, which is mediated by a set of specific vesicular neurotransmitter transporters drawing on an electrochemical proton gradient generated by a vacuolar ATPase. Unlike transporters at the plasma membrane, the ion and solute content of the vesicle interior changes dramatically during transport. Furthermore, charge and osmotic balance need to be maintained during transport, particularly when considering that thousands of uncharged (GABA, glycine) or charged (glutamate, monoamines) transmitter molecules must be translocated within seconds. In our own work, we use reconstitution of purified transporters in artificial vesicles, newly developed hybrid vesicles in which native synaptic vesicles were fused with liposomes of defined content, and single vesicle imaging to shed light on the transport mechanisms.

Speaker

Cellular cartography: mapping cytoskeletal organization and intracellular transport in neurons

Lukas Kapitein
Cell Biology, Neurobiology and Biophysics, Department of Biology, Faculty of Science, Utrecht University, Utrecht - Netherlands

The polarized organization of neurons into axons and dendrites depends on the selective targeting of cargoes to either axons or dendrites, driven by motor proteins that move selectively towards either the plus or minus end of microtubules. Our goal is to unravel the interplay between the complex organization of the neuronal cytoskeleton and the motor-based transport pathways in neurons. To this end, we have developed several novel techniques that enable 1/ exploring the activity of specific motors and combinations of motors inside neurons, as well as 2/ mapping the microtubule cytoskeleton, including different subsets and their orientations. I will highlight new developments and the novel insights obtained using these approaches.

Speaker

Cellular regulation in context – Integration of mathematical modeling in time and space with dedicated experiments on different scales

Edda Klipp
Humboldt-Universität zu Berlin, Theoretical Biophysics, Berlin - Germany

The yeast Saccharomyces cerevisiae is frequently used as the model organism for eukaryotic cells allowing to comprehensively analyzing regulatory networks and to collect massive amounts of data of different types. Here, we consider that yeast cells have to respond reliably to changes in the environments irrespective of their current state. They must integrate stress response with ongoing processes such as cell cycle. Different well-organized and highly regulated processes have to contribute to successful survival. Understanding the complex relationship between regulatory networks and cell growth is still a challenging task.

It is of interest that baker's yeast can engage in a mating process where haploid mating types MAT**a** and MAT**α** cells can also mate to form diploid cells again. To this end, they secrete the pheromones **a**-factor and **α**-factor, sense the opposite pheromone and form protrusions in the direction of a potential mating partner. Importantly, they cannot move towards their mating partner, thus, the formation of the mating shape called shmoo is a significant growth investment.

Combining experimental studies of the cellular responses to mating factor and the resulting shape changes with spatial mathematical modeling, we investigated major steps in the mating process.We combine different modeling techniques to understand stress response, cell cycle regulation, and mating in changing environments, with specific focus on the interplay of different regulatory networks. The results indicate that yeast cells have developed different mechanisms for coping with external stress during different periods of their life time.

Speaker

Cytoskeletal fibres as building blocks for life

Franziska Lautenschläger
Center for Biophysics, Saarland University, Saarbrücken - Germany

The cytoskeleton is a fibrous network of biopolymers with incredible functions in living cells. In my lab, we study the role of the cytoskeleton in the structure, the properties, the state and the movement of living cells. For example, we investigate how the cytoskeleton defines the shape of cells, their mechanical properties, their ability to adhere or to migrate.

The cytoskeleton consists of three different subtypes, namely actin, microtubules, and intermediate filaments. While some projects in my lab study the effect of one subtype on a particular cellular function and how we can use this cytoskeletal fibre to control this function, others investigate how actin, microtubules and the intermediate filament vimentin collaborate with each other. In this talk, I will discuss the following projects in details:

- How to observe, quantify and control the migration of immune cells.

- What is the universal correlation of speed and persistence in migrating cells, its meaning for life and how we can perturb it.

Speaker

Skeletal stem/progenitor cells: interactions within the bone vascular microenvironment and beyond

Christa Maes
Laboratory of Skeletal Cell Biology and Physiology (SCEBP) (https://bit.ly/2TUXygz)
Skeletal Biology and Engineering Research Center (SBE),
Department of Development and Regeneration, KU Leuven, Leuven, Belgium
Twitter/X: @SCEBP_Lab

Metabolic bone diseases, such as osteoporosis, and failed fracture repair, represent an important clinical problem that continues to grow in parallel with our increasing life expectancy. More than 200 million people worldwide have osteoporosis, and 1 in 3 women over the age of 50 years and 1 in 5 men will experience osteoporotic fractures in their lifetime. Up to 10% of all bone fractures show compromised repair. Many basic bone research efforts are currently focused on finding leads for the development of osteoanabolic therapies, to promote the production of mineralized bone matrix in patients with low bone mass, and to stimulate fracture repair in the event of a non-healing fracture.

Bone formation always requires osteoblasts, during skeletal development, growth, and physiological bone remodeling for maintaining bone homeostasis, as well as in fracture repair and bone regeneration settings. Osteoblasts are short-lived cells, meaning that the active population needs to be replenished continuously from the precursor cells. Osteoblasts arise from populations of skeletal stem cells (SSCs) or more broadly defined skeletal stem/progenitor cells (SSPCs). A better understanding of the maintenance of these cells, the niches they reside in, and the molecular control of their fates and differentiation processes, will help toward improved and novel bone osteoanabolic and regenerative therapies.

In recent years, methodological advances and new technologies have expedited research into the sources of osteoblasts and the biology of SSPC. In vivo lineage tracing strategies and sophisticated genetic targeting in mice, advanced imaging methodologies, flowcytometry, and high-resolution bulk/single-cell RNA-sequencing technologies are providing increased insights in the relationships among skeletal cell lineages and in the origin and fate of bone cells. Although many outstanding questions remain to be answered, much progress has been made in the identification and characterization of endogenous SSPC populations with osteogenic, chondrogenic, and/or adipogenic capacity, residing in the growth plate, the periosteum, and the bone marrow environment.

The SCEBP Lab team studies the cellular and molecular control of bone growth, homeostasis, and repair, including the impact of ageing and disease, with a key focus on SSPCs and their interplay with the local vasculature. We are also interested in the impact that these local interactions and bone cell functioning can have on the global (patho-)physiological context of the organism, particularly looking at the regulation of hematopoiesis and the interplay between the skeleton and systemic energy metabolism. Recent results from studies in our lab will be presented.

Speaker

Single-cell delineation of lineage and genetic identity in the mouse brain

Christian Mayer
Max Planck Institute for Biological Intelligence, LMU Munich, Martinsried, Munich - Germany

During neurogenesis, mitotic progenitor cells lining the ventricles of the embryonic mouse brain undergo their final rounds of cell division, giving rise to a wide spectrum of postmitotic neurons and glia. The talk discusses the link between developmental lineage and cell-type diversity, which is still an open question in the field. We used massively parallel tagging of progenitors to track clonal relationships and transcriptomic signatures during mouse forebrain development. We quantified clonal divergence and convergence across all major cell classes postnatally, and found diverse types of GABAergic neurons that share a common lineage. Divergence of GABAergic clones occurred during embryogenesis upon cell-cycle exit, suggesting that differentiation into subtypes is initiated as a lineage-dependent process at the progenitor cell level.

Speaker

Cellular Self-Healing: Exploring Plasma Membrane Repair Mechanisms

Jesper Nylandsted

Danish Cancer Institute, Membrane Integrity, Strandboulevarden 49, DK-2100 Copenhagen, Denmark / University of Southern Denmark, Department of Molecular Medicine, J.B. Winsløws Vej 21-25Odense C - DK-5000, Denmark

The plasma membrane of eukaryotic cells defines the essential boundary to the extracellular environment and, thus injuries to the cell membrane pose a lethal threat to cells. Cells cope by activating their plasma membrane repair system, which includes mechanisms to reseal and remove damaged membrane. Inadequate repair responses can tip the balance between physiology and pathology, highlighting the significance of plasma membrane integrity. For example, an over-activated repair response can promote cancer invasion, while the inability to efficiently repair membrane can drive neurodegeneration and muscular dystrophies [1]. However, the underlying molecular and biophysical mechanisms used to repair membrane lesions during physiological and pathological conditions are not well characterized. The family of annexins are Ca2+-triggered proteins involved in various steps of the plasma membrane repair response. Our recent results, based on interdisciplinary research synergy across molecular cell biology, experimental membrane physics, and computational simulations show that annexins have additional biophysical functions in the repair response besides enabling membrane fusion. Our data suggest that annexins possess different membrane-shaping properties, allowing for a tailored response that involves rapid bending, constriction, and fusion of membrane edges for resealing [2]. Moreover, some annexins have high affinity for highly curved membranes that appear at free edges near rupture sites, a property that might accelerate their recruitment for rapid repair. To this end, cancer cells are more dependent on efficient plasma membrane repair to counteract stress-induced membrane injuries, which opens novel avenues to target cancer cells through their repair system [3]. Here, novel aspects of plasma membrane repair and regeneration [4] will be presented.

REFERENCES
[1] Dias C, Nylandsted J. Plasma membrane integrity in health and disease: significance and therapeutic potential. Cell Discovery. 2021; 19;7(1):4.
[2] Boye TL, Maeda K, Pezeshkian W, Lauritzen SP, Hager SC, Gerke V, Simonsen AC,and Nylandsted J. Annexin A4 and A6 induce membrane curvature and constriction during cell membrane repair. Nature Communications. 2017; 8(1):1623.
[3] Jaiswal JK, Lauritzen SP, Scheffer L, Sakaguchi M, Bunkenborg J, Simon SM, Kallunki T, Jäättelä M, Nylandsted J. S100A11 is required for efficient plasma membrane repair and survival of invasive cancer cells. Nature Communications. 2014; 8;5:3795.
[3] Sønder SL, Häger SC, Heitmann ASB, Frankel LB, Dias C, Simonsen AC, Nylandsted J. Restructuring of the plasma membrane upon damage by LC3-associated macropinocytosis. Science Advances. 2021; 7(27):eabg1969.

Speaker

Circadian control of enteric virus infection

John F. Brooks*, Robert W. Maples*, Mikal A. Woods Acevedo*, Broc T. McCune, Chaitanya Dende, Joseph S. Takahashi, Lora V. Hooper, Julie K. Pfeiffer
Microbiology department, University of Texas Southwestern Medical Center, Dallas - USA

Circadian rhythms are 24h oscillations in a variety of processes that are entrained by environmental cues including light. Molecularly, this "clock" is driven by key transcription factors and feedback loops that generate rhythmic expression of thousands of mammalian genes in a variety of tissues. Past work has revealed the impact of circadian rhythms on metabolism and immunity. However, the impact of circadian rhythms on infection, particularly enteric virus infection, is understudied. Using the enteric virus coxsackievirus B3 (CVB3), we found a profound circadian effect on infection: Mice orally inoculated with CVB3 in the morning had viral titers 10-100 fold lower than mice inoculated in the evening. Inhibition of viral replication in the morning correlated with increased expression of antiviral proteins at this time in uninfected animals. Importantly, the circadian effect on infection was lost in mice lacking specific innate immune genes or key clock transcription factors. Overall, these results suggest that circadian-driven rhythmicity of innate immune responses influences viral infection efficiency.

Speaker

Culturing Hepatitis E Virus– recent advances for basic science and drug discovery

Eike Steinmann
Department Of Molecular & Medical Virology, University Bochum, Bochum - Germany

With an estimated 20 million infections per year, HEV is one of the most common causes of acute viral hepatitis worldwide. Chronic HEV infections pose a significant clinical problem in immunocompromised individuals. The lack of an efficient cell culture system has severely limited investigation of the HEV life cycle and the development of effective antivirals. I will report about the establishment of a robust HEV cell culture system in human hepatocytes. These produced intracellular-derived HEVcc particles demonstrated replication to high viral loads in human liver chimeric mice and were able to efficiently infect primary human as well as porcine hepatocytes. This unique infectious cell culture model provides a powerful tool for the analysis of host–virus interactions that should facilitate the discovery of antiviral drugs for this important zoonotic pathogen, where I will touch on the most recent developments.

Speaker

From molecules to life: building living systems from scratch

T-Y Dora Tang
Max Planck Institute for Cellular Molecular Biology and Genetics
Pfotenhauer Strasse 108, 01307 Dresden - Germany

One of the goals of bottom-up synthetic biology is to build living cells from scratch. Biology is well equipped in exploiting a large number of out-of-equilibrium processes to support life. A complete understanding of these mechanisms is still in its infancy due to the complexity and number of the individual components involved in the reactions. However, a bottom-up approach allows us to replicate key biological processes using a small number of basic building blocks. Moreover, this methodology has the added advantage that properties and characteristics of the artificial cell can be readily tuned and adapted.

In this talk, I will provide an overview of the strategies we adopt in our lab to build living systems from scratch that rely on compartmentalisation as the defining feature to support out-of-equilibrium behaviour. Specifically, I will talk about the design and synthesis of artificial cells based on liquid-liquid phase separation (coacervation) and hydrophobic effects such as lipid vesicles and proteinosomes and describe how these compartments may be used as platforms for implementing dynamic biological behaviours including: RNA catalysis and communication. I propose that our bottom-up approaches are effective in establishing living systems from scratch and in doing so provide unique model systems that can help to unravel the physico-chemical principles of living systems.

Speaker

Guiding Mechanotransduction in Blood Vessels

Ellie Tzima
Radcliffe Department of Medicine - Cardiovascular Medicine, University of Oxford, Oxford - UK

Ellie Tzima has been a Wellcome Senior Fellow in Biomedical Sciences and Professor of Cardiovascular Science at the Radcliffe Department of Medicine and the Wellcome Centre for Human Genetics at the University of Oxford since 2015. Ellie was Assistant and Associate Professor at the University of North Carolina at Chapel Hill from 2005-2015. Ellie was an AHA-postdoctoral fellow in Professor Martin Schwartz's laboratory at the Scripps Research Institute (La Jolla) from 2000-2005, where she discovered the junctional mechanosensory complex. Ellie held NIH R01 grants, served as Director of Graduate Studies, and was on the Editorial Board of Circulation Research and ATVB. She was a recipient of an American Heart Association Established Investigator Award, Ellison Medical Foundation Scholar in Aging and a Charter member of the NIH Study section Vascular Cell Molecular Biology.

The Tzima lab investigates the role of mechanotransduction in regulating cardiovascular function in health and disease. The group has made significant conceptual advances in our understanding of flow sensing and systematically characterised one of the most comprehensive models of endothelial mechanotransduction available to date. This talk will focus on the recent discovery of a new class of mechanosensors which determine the site-specific distribution of atherosclerosis.

Speaker

Unraveling the Molecular Architecture of the Intestinal Barrier: Insights from Spatial Transcriptomics

Eduardo Villablanca
Department of Medicine, Karolinska Institute, Stockholm - Sweden

The complex cellular network that constitutes the intestinal barrier is crucial for maintaining health and preventing diseases. In this talk, I will present the remarkable capabilities of spatial transcriptomics (ST) in unveiling the molecular organization of the entire colonic tissue during mucosal healing and tumorigenesis. By leveraging ST, we revealed a previously undiscovered regionalization of the colon's transcriptomic landscape under steady state conditions, which undergoes dramatic changes during mucosal healing. We identified spatially organized transcriptional programs that define compartmentalized mucosal healing, including regions exhibiting dominant wired pathways. Furthermore, I will discuss the translational potential of our findings by mapping transcriptomic modules associated with human diseases.

Speaker

Interactions of Tau protein condensates

Susanne Wegmann
Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Berlin, Germany

The microtubule associated protein Tau is an abundant intrinsically disordered protein in neurons of CNS. It is mostly known for its aggregation and toxicity in neurodegenerative diseases, like Alzheimer's disease and frontotemporal dementia. Tau's canonical function of microtubule binding and bundling is complemented by a number of poorly understood interactions with proteins, RNA, and organelles that are associated with additional normal functions and/or disease-associated dysfunctions of Tau. Recent data from our lab suggest that a number of these enigmatic Tau interactions, including microtubule and synaptic interactions, involve Tau protein condensation. Our data provide insides into the role of liquid-liquid and liquid-solid phase transitions in canoninal and alternative Tau functions and dysfunctions in neurodegenerative diseases.

Speaker

Impact of lamin-A/C on cancer progression in vivo

Katarina Wolf
Department of Medical BioSciences, Radboud University Medical Center,
Nijmegen - Netherlands

Nuclear A-type lamins contribute to the rigidity of the cell nucleus, scaling in normal cells with tissue stiffness. In tumor cells lamis have been found to be deregulated, however, the biomechanical roles of lamins in cancer cell invasion, survival and metastatic rates in vivo remain unclear. To examine the impact of lamin-A/C expression on cancer progession in a preclinical mouse model, human fibrosarcoma and melanoma cell lines with three different lamin-A/C levels were generated using CRISPR/Cas9 and clonal selection. Cells were injected into the tail vein, or implanted into the dermis of living mice carrying a dorsal imaging window to monitor cell invasion by intravital multiphoton microscopy, followed by detection of circulating tumor cells and metastasis to distant organs. We find a higher adaptability of lamin-depleted cells migrating in confining tumor environments together with, however, reduced rates of circulating tumor cells, as well as greatly diminshed metastatic rates in completely- but not partly lamin A-depleted cells. Our results point to a role of lamin A/C as a critial, rate-limiting regulator of metastatic organ colonization, connecting the biology of A-type lamins in vitro with their impact on tumor cell integrity and metastasic dissemination in the living organism.

Speaker

Building Blocks of Life: Protein Patterns on Model Membranes

Katja Zieske
Max Planck Institute for the Science of Light, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen - Germany

Understanding the intricate interplay of biomolecular self-assembly is essential for unraveling the mysteries of life at its most fundamental level. In this presentation, I will present cell-free approaches to reconstitute purified proteins on model membranes. The characterization of biomolecular condensates, dynamic wave-like and snowflake-like patterns on lipid membranes formed by purified proteins underscores the elegance of natures design principles with potential implications in various biological context.

Poster

Nucleomechanical regulation of the cellular identity – from stem cells to cancer

Mirjam Isabel Binner
Max Planck Institute for Molecular Biomedicine, Münsster - Germany

Oncogenic driver mutations have been documented in various cancer, yet they may not be sufficient to explain cancer onset and progression. Other factors – such as microenvironmental and epigenetic factors - may therefore be involved in the development of the disease. Nuclear architecture, chromatin organization, and more recently nuclear mechanics have been shown to control cell states and behavior, and also to be altered in diverse cancers and may play a role in the evolution of cancer. Nuclear morphology and mechanics are influenced by the cellular microenvironment and shape the spatial organization of chromosomes and genes. Thus, abnormalities in cancer cells and in the tumor microenvironment lead to impaired nuclear mechanics and altered gene expression, which ultimately may benefit cancer progression. Yet the underlying genetic alterations that drive these morphological and mechanical changes and their implications still remain to be investigated. Here we generate oral epithelial stem cells harboring the most frequent head and neck squamous cell carcinoma (HNSCC) mutations – TP53, NOTCH1 and CDKN2A – to investigate their effect(s) on nuclear morphology and mechanics as well as on the subsequent feedback with the epigenetic landscape to elucidate their contribution to cancer evolution and heterogeneity. Through comparative analyses with HNSCC patient samples and patient-derived organoids we will establish the clinical relevance of these findings and impact on patient prognosis and cancer aggression. Collectively this work will reveal the interplay between single oncogenic mutations, nuclear architecture and mechanics and cancer progression.

Young Investigator Talk

Double-modified AdoMet analogues for selective targeting of methyltransferases

M. Erguven, N. V. Cornelissen, A. Peters, A. Lawrence, E. Karaca, A. Rentmeister

Department of Chemistry and Pharmacy, Institute of Biochemistry, University of Münster, Correnßtrasse 36, 48149 Münster,
Cells in Motion Interfaculty Center, University of Münster, Waldeyerstraße 15, 48149 Münster

Methyltransferase-based bioalkylation has been an important tool in the field of biomolecular labeling. We have previously deployed one-pot biocatalytic cascades by combining methionine adenosyltransferases (MATs) and methyltransferases (MTases) together, as a means to transfer clickable or photocaging groups to various substrates1–4. However, MTase promiscuity poses a problem for such applications in living systems. For this reason, we have recently focused on base modifications of the MTase cosubstrate, AdoMet, aiming to achieve selective labeling5. In the present study, we further explored this option by expanding our set of ATP analogues. We used a thermostable MAT from Methanocaldococcus jannaschii (PC-MjMAT) that is highly active at 37oC and can accept bulky N6 base modifications, effectively producing the substrate AdoMet analogues for the MTases. These MAT reactions were combined in cascades with three different MTases, named NovO, RnCOMT, and GlaTgs2. By using bulky N6 base-modified ATP analogues as the starting material, we achieved MTase selectivity to a degree in vitro. We believe that our results will encourage further research aiming to achieve orthogonality in MTase-based biomolecular labeling.

Poster

Neutrophils promote remote organ damage after local ischemia

Merieme Farjia[1,2], Lina Vöcking[1], Collins Osei-Sarpong[1], Ali Hageb[1], Bartolo Ferraro[3] , Oliver Söhnlein[1], Carlos Silvestre-Roig[1]

[1] Institute of Experimental Pathology, ZMBE, University of Münster, Münster, Germany.
[2] CiM-IMPRS PhD program, Münster, Germany.
[3] Biomedical Center LMU Munich, Munich, Germany.

Local ischemia, also known as tissue- or organ-specific ischemia, occurs when a specific organ experiences reduced blood supply, resulting in insufficient oxygen and nutrients. This condition's impact extends beyond the affected organ, triggering systemic inflammation and damage to secondary organs. This multi-organ dysfunction often leads to organ failure, causing mortality or long-term complications in survivors. However, despite its clinical significance, the immune processes driving secondary organ damage are not entirely understood.

Neutrophils play an essential role as body's first line of defense. They combat threats by generating reactive oxygen species, cytotoxic granule proteins, and Neutrophil Extracellular Traps (NETs). However, excessive neutrophil activation can contribute to host tissue damage and inflammation.

Our hypothesis proposes that systemic neutrophil activation following local ischemia induces their infiltration into distant organs, releasing harmful molecules that cause organ inflammation and dysfunction. To investigate this, we're studying neutrophil function in mouse models of Myocardial Infarction (MI) and Kidney Ischemia/Reperfusion, after modulating neutrophil numbers and NETs release.

Our findings demonstrate that MI triggers neutrophil recruitment to the liver, kidney, and lungs, causing tissue damage 4-hours post-ischemia. Moreover, depleting neutrophils during MI improves this damage. Additionally, kidney ischemia/reperfusion results in two distinct peaks of systemic neutrophil activation. These insights shed light on the complex interplay between local ischemia, neutrophils, and secondary organ damage.

Subsequent steps involve analyzing neutrophil recruitment dynamics to secondary organs post-ischemia, the underlying mechanisms of this, and their long-term impact on the host immune response.

Poster

Leukocyte-driven endocytosis targets the non-actin bound pool of VE-cadherin

Vallari Ghanekar, Dietmar Vestweber

MPI for Molecular Biomedicine, Vascular Cell Biology Department,
Röntgenstraße 20, 48149, Münster, Germany
vallari.ghanekar@mpi-muenster.mpg.de

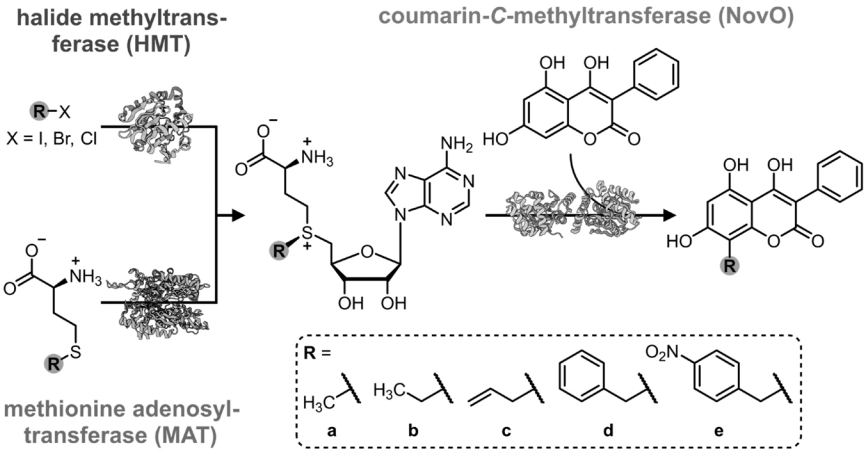
VE-cadherin is instrumental in maintaining endothelial barrier integrity via its interaction with associated catenins and actin cytoskeleton. Its regulation by endocytosis is important for the control of vascular permeability and leukocyte diapedesis and occurs both constitutively and under inflammatory conditions. Leukocyte docking to endothelium triggers internalization of VE-cadherin and opening of junctions.
Previously, we observed that stabilizing endothelial junctions through replacement of VE-cadherin with a VE-cadherin-α-catenin (VECαC) fusion-construct led to reduced leukocyte diapedesis. We thus wanted to test if there was an effect on leukocyte-induced internalization of VE-cadherin. We first compared HUVECs expressing VECαC to WT condition, and saw 50% reduction in the constitutive endocytosis of VE-cadherin. Interestingly, on addition of HL-60 derived neutrophils, VE-cadherin internalisation was enhanced 1.5 folds in WT condition but this increase was dampened in VECαC condition. Thus, we hypothesized that the non-actin bound pool of VE-cadherin undergoes endocytosis more readily upon leukocyte adhesion. Next, we analysed VE-cadherin internalization in a construct that cannot bind actin and saw a 1.5 fold increase in the constitutive endocytosis as compared to WT. Upon addition of HL-60 derived neutrophils an increase in internalization was still observed but was less significant than for WT-VE-cadherin; hence reinforcing our hypothesis.

Poster

Comparative S-adenosyl-L-methionine analogue generation for selective

Arne Hoffmann, Kai H. Schülke, Stephan C. Hammer, Andrea Rentmeister and Nicolas V. Cornelissen

Methyltransferases provide excellent specificity in late-stage alkylation of biomolecules. Their dependence on S-adenosyl-L-methionine (SAM) mandates efficient access to SAM analogues for biocatalytic applications. We directly compared halide methyltransferase (HMT) and methionine adenosyltransferase (MAT) to access SAM analogues and explored their utility in cascade reactions with NovO for regioselective, late-stage Friedel-Crafts alkylation of a coumarin. The HMT cascade efficiently provided SAM for methylation, while the MAT cascade also supplied high levels of SAM analogues for alkylation reactions.



Poster

FLIM based analysis of mechanical tension across VE-cadherin during leukocyte extravasation

Pragya Kaul, Dietmar Vestweber
Max Planck Institute for Molecular Biomedicine, Münster, Germany

Leukocyte transmigration is a hallmark of inflammation. It predominantly occurs through endothelial cell-cell junctions and requires regulation of VE-cadherin.

Our group previously established that "opening" junctions requires tension on VE-cadherin-catenin complexes via acto-myosin-mediated contraction. However, the signaling mechanism inducing tension remains elusive. Here, we focused on the role of Calcium and ROCK signaling as promising candidates in driving acto-myosin pulling during various cellular processes.

We have expressed a VE-cadherin FRET sensor in HUVECs which allows to measure tension across VE-cadherin by fluorescence lifetime imaging (FLIM). Interestingly, we found that leukocyte driven tension across VE-cadherin is completely abolished in MAPTAM (calcium chelator) treated HUVECs. Likewise, we could demonstrate a reduction in the rate of leukocyte transmigration under flow through MAPTAM treated HUVECs. Additionally, we showed that inhibition of ROCK signaling using Y-27632 partially inhibited leukocyte induced tension across VE-cadherin.

Thus, our results demonstrate that calcium signaling is important to drive mechanical tension across VE-cadherin during leukocyte transmigration. Similarly, ROCK signaling has a partial role in inducing force on VE-cadherin, which leaves room for further signaling molecules (e.g. MLCK) in this pathway.

Poster

In-vitro ciliary transport assay to diagnose Primary Ciliary Dyskinesia

Julia Koenig, Kai Wohlgemuth, Sebastian George, Niki Tomas Loges, Johanna Raidt, Sandra Cindrić, Gerard W. Dougherty, Heike Olbrich, Petra Pennekamp, Bernd Dworniczak, Heymut Omran

Department of General Paediatrics, University Children's Hospital Muenster, Muenster,

Primary Ciliary Dyskinesia (PCD) is a rare genetic disorder characterized by recurrent respiratory tract infections. The respiratory disease phenotype is caused by defects of motile cilia, resulting in impaired mucociliary clearance (MCC). MCC is an innate defense mechanism of the respiratory tract to clear the airways from debris, inhaled particles, microorganisms, and pollutants by coordinated ciliary beating. To date, PCD cannot be confirmed or excluded using a diagnostic test, and appropriate methods to measure ciliary clearance in vitro are lacking. Additionally, the diagnosis can be complicated by secondary effects such as damage during sampling, local inflammation, or recent infection. Therefore, culture of human respiratory epithelial cells (hRECs) on Air-Liquid Interface (ALI) is a robust and informative tool to support the diagnosis.

This study aimed to establish an objective test to evaluate the efficiency of the ciliary clearance in genetically confirmed PCD individuals in vitro. For this purpose, the transport of fluorescent microbeads placed on the apical surface of ALI cultures of nasal epithelial cells were analysed. Fluorescent particles were used as markers of the fluid flow generated by cilia activity and offered an objective assessment of ciliary transport capacity and efficiency, respectively. In respiratory cell cultures from healthy control individuals, fluorescent particles were transported at high velocities. In contrast, respiratory cell cultures from genetically confirmed PCD individuals showed a severely impaired transport of fluorescent particles.

In summary, this method enables evaluation of ciliary transport capacity and efficiency to facilitate the diagnosis of motile ciliopathies.

Poster

The role of nuclear transport and mechanical signaling in the regulation of genome integrity

Anja Michelbach

Genomes are vulnerable to damage from intra- and extracellular sources such as radiation, reactive oxygen species, genotoxic chemicals, replication or mechanical stress. Mechanical stress is ubiquitous, particularly in tissues such as skin that is subjected to constant stretch and compression during movement, touch or from forces arising during growth from the underlying tissues, but whether these stresses are capable of damaging the genome and the physiological relevance of such damage is unclear. Mechanical stress and calcium signaling regulate the level and form of nuclear and cytoplasmic pools of actin, which among other effects, mediates transcription and chromatin organization as well as DNA damage repair. This project aims to unravel the role of nuclear actin transport, as well as the relevance of other yet unidentified cargo, in genome mechanoprotection. Using a tissue-specific mouse knockout model of the nuclear actin transporter Exportin-6 to control nuclear actin transport in the skin epidermis, together with various cell biological and biophysical experiments, we try to unravel the role of mechanosensitive nuclear transport in morphogenesis and homeostasis, as well as in critical nuclear processes such as the coordination of transcription and replication and the maintenance of genome integrity.

Young Investigator Talk

Two-way Dispatched function in Sonic Hedgehog shedding and transfer to high-density lipoproteins

K. Ehring, S.F. Ehlers, J. Froese, F. Gude, J. Puschmann, K. Grobe
Institute for Physiological Chemistry and Pathobiochemistry, University of Münster, Münster

Introduction:
The Sonic Hedgehog (Shh) morphogen is an essential regulator of embryonic development and tissue homeostasis after birth. One intensely investigated question is how the solubilization of dual-lipidated (N-palmitoylated and C-cholesteroylated), plasma-membrane associated Shh is regulated. So far, it is known that the transmembrane protein Dispatched (Disp) and its sterol-sensing domain play an important role in this process, but it remains unclear how lipidated Shh solubilization from plasma membrane is achieved mechanistically. One possibility is that Disp indirectly modulates the proteolytic removal (shedding) of Shh from its lipidated terminal peptide anchors. Another possible mechanism is that Shh is directly transferred to high-density lipoprotein (HDL) that act as soluble Shh carriers.

Results:
We show that Disp-mediated Shh release is enhanced by a serum factor that we identify as HDL. HDLs serve as soluble sinks for free membrane cholesterol, but also accept the cholesterol-modified Shh peptide from Disp. The cholesteroylated Shh peptide is required and sufficient for Disp-mediated transfer because mCherry linked to cholesteroylated peptides associates with HDL in a Disp-dependent manner, but a Shh variant lacking C-cholesterol does not. Disp-regulated proteolytic processing of the palmitoylated N-terminal membrane anchor completes the transfer of Shh to HDL.

Discussion:
It is established that Hh signaling depends on Disp co-expression in source cells. Disp has two functions: First, it functions as a cholesterol pump and increases Shh shedding from the cell surface. Second, we showed that the cholesteroylated C-peptide of Shh can be transferred to HDL, and that Disp is necessary for this process. We also identified HDL as a soluble acceptor of cholesterol pumped by Disp and of Shh C-peptide. These findings support previous results that Hh are transferred to lipoproteins in the fly.

References:
Ehring K. et al. (2022) Conserved cholesterol-related activities of Dispatched 1 drive Sonic hedgehog shedding from the cell membrane J Cell Sci 135
K. Ehring S.F. Ehlers J. Froese F. Gude J. Puschmann K. Grobe (2023) Two-way Dispatched function in Sonic hedgehog shedding and transfer to high-density lipoproteins eLife 12:RP86920

Poster

Endothelial membrane is protected from mechanical damage by early

Nikita Raj[1,2], Lilo Greune[3], Chang Pan[4], Martin Kahms[5], Karina Mildner[6], Ann Marleen Starke[1], Rico Franzkoch[7], Olympia Ekaterini Psathaki[7], Anna Matos[4], Thomas Zobel[8], Dagmar Zeuschner[6], Jürgen Klingauf[5], Oliver Söhnlein[4], Volker Gerke[1,2]

[1]Institute of Medical Biochemistry, Centre for Molecular Biology of Inflammation (ZMBE),University of Muenster; Münster, Germany.
[2]Multiscale Imaging Centre, Cells in Motion Interfaculty Center, University of Münster; Münster, Germany.
[3]Institute for Infectiology, Center for Molecular Biology of Inflammation (ZMBE),University of Münster; Münster, Germany.
[4]Institute of Experimental Pathology, Centre for Molecular Biology of Inflammation (ZMBE), University of Muenster; Münster, Germany.
[5]Institute of Medical Physics and Biophysics, University of Münster; Münster, Germany.
[6]Electron Microscopy Facility, Max Planck Institute for Molecular Biomedicine; Münster, Germany.
[7]Department of Biology, integrated Bioimaging Facility (iBiOs), Center of Cellular Nanoanalytics (CellNanO), University of Osnabrück; Osnabrück, Germany.
[8]Imaging Network, Cells in Motion Interfaculty Centre, University of Münster; Münster, Germany.

Disruptions of the eukaryotic plasma membrane due to imposed challenges are frequent and detrimental, and thus need to be repaired efficiently to maintain proper cellular functioning and avoid cell death. These membrane-rupturing challenges can vary from mechanical forces resulting from shear stress and stretch to invasive pathogenic attacks. Unrepaired plasma membrane ruptures can eventually lead to tissue damage and has been linked to numerous disease pathologies. Although membrane repair is crucial for cell life, the spatio-temporal membrane wound dynamics and the source of membrane required for wound repair are poorly understood. Here, we show that early endosomes, previously known to function in the uptake of extracellular material and its endocytic transport, are involved in plasma membrane repair
in primary human vascular endothelial cells and in mouse endothelial cells in vivo. Using live-cell imaging and correlative light and electron microscopy, we demonstrate that membrane injury triggers a previously unknown exocytosis of early endosomes that is induced by Ca2+ entering through the wound. This exocytosis is restricted to the vicinity of the wound site and mediated by the endosomal SNARE VAMP2, which is crucial for efficient membrane repair. Further, we show that alterations in the shear stress of blood flow induce plasma membrane wounds in endothelial cells in vitro and in vivo in the mouse aorta. These shear-wounded cells are also repaired by an activation of early endosomal exocytosis, indicating a conserved mechanism of membrane repair under physiological conditions. Thus, the Ca^{2+}-evoked exocytosis of early endosomes supplies the membrane material required for rapid resealing of damaged plasma membranes to prevent cell leakage and death.

Poster

Multi-scale image registration by combining information theory and deep learning

Robin Rexeisen, Xiaoyi Jiang

University of Münster, Department of Computer Science,
Einsteinstraße 62, 48149, Münster, Germany
r.rexeisen@uni-muenster.de

Working with a variety of images from different modalities is essential for accurate diagnosis. These modalities, ranging from whole-body MR scans to microscopic tissue examinations, are acquired at different scales and need to be aligned to each other to be most informative. Current state-of-the-art approaches based on deep learning for automatic image registration are only specialized for two concrete modalities while also demanding a large amount of infrequent training data. Inourcurrent research, weattempt toaddressthesep-roblemsbycombining the well-studied classical information-theoretic registration approaches with deep learning. Specifically, we impose various constraints to integrate the neural network into the severity index framework that explains the strengths and usefulness of mutual information (a popular information-theoretic registration measure). This allows to abstract away the modality and achieve a lighter architecture, which in turn reduces the amount of necessary training data.

Poster

Fantastic Four: Isoforms of curvature-sensitive protein BAIAP2 and their role in developing neurons

Anastasiia Sokolova[1,2], Milos Galic[1]
[1]Institut of Medical Physics and Biophysics UKM, Münster, Germany
[2]CiM-IMPRS Graduate School, Münster, Germany

Though a vast majority of biological mechanisms are genetically regulated, some of them spring from spontaneous events. For example, cell migration, formation of filopodia or endocytic vesicles could be stimulated by fluctuations of cellular plasma membrane (PM). Integral transmitters of PM signals into the cell are curvature- sensitive proteins (BAR-domain proteins). Depending on the structure of N-terminal BAR-domain they detect either positive (invaginations) or negative (protrusions) membrane curvature. However, regardless of the shape all of them are active only as dimers. Intriguingly, while positive curvature sensors can form heterodimers between group members, growing evidence suggest that it may be not the case of negative curvature sensors. In current work we are addressing the question of negative curvature sensor composition and its impact on protein function on example of BAIAP2.
BAIAP2 is involved into many cellular processes from migration to neuronal plasticity. Though the general mechanisms of its action were thoroughly described, potential role of its splice isoform and resulting dimers was largely neglected. This aspect becomes particularly interesting in the context of neuronal development. Our results demonstrate the important role of BAIAP2 at early stages of neuronal formation and effect of its isoforms on neuronal morphology. What is more important, according to RNA sequencing data, the ratio of isoforms changes as a neuron develops, which highlights their significance.

Poster

Neutrophil-Mediated Transfer of miRNA via Histone H4: Implications for Cellular Modulation

Merle F. Sternschulte[1,2], Anna L. L. Matos[1], Andrea Herrero[1], Jürgen Schmitz[1], Oliver Söhnlein[1]

[1] Institute of Experimental Pathology, Centre of molecular biology of inflammation (ZMBE), University of Münster, Münster, Germany.
[2] CiM-IMPRS PhD program, Münster, Germany.

Neutrophils, the predominant leukocytes in human circulation, constitute the initial defense against invading pathogens through various mechanisms, including the release of neutrophil extracellular traps (NETs). NETs are composed of DNA, proteins including nucleus derived Histones. In addition, NETs harbor microRNAs (miRNAs), pivotal regulators of cellular transcriptional behavior. Notably, cationic Histone H4 released from neutrophils has been shown to form pores in neighboring cells. In addition, cationic antimicrobial peptides such as LL-37 can bind to anionic DNA in biological fluids, preventing them from degradation. We suggest that Histone H4 facilitates miRNA transfer from neutrophils to neighboring cells, mediating miRNA entry into the target cell. Bioinformatic analysis revealed the enrichment of specific miRNAs within NETs. Preliminary transfection experiments using a miRNA mimic showed that Histone H4 enables miRNA transfer. Utilizing Quartz Crystal Microbalance with Dissipation (QCM-D), we observed concentration- and cholesterol-dependent interactions between Histone H4 and cell membranes.

Our hypothesis posits that neutrophils release miRNAs capable of modulating target cell behavior. To elucidate the mechanism, the H4-miRNA complex will be characterized, examining its ZETA-potential, and its binding to lipid bilayers using membranes of varying lipid compositions in Giant Unilamellar Vesicles (GUVs) and QCM-D, alongside Cryo-Electron Microscopy (CRYO-EM). Furthermore, this study aims to identify specific target cells that are susceptible to miRNA-mediated modulation, exploring the following functional consequences in a medical context.

This research contributes to the understanding of the interaction between neutrophils, miRNA, and histone H4, thus providing perspectives on therapeutic applications for cellular modulation in pathological conditions.

Poster

Molecular Mechanisms of Leukocyte Extravasation Across Endothelial

Elisa Terenzani[1], Huiyu Wang[1], Adan Olguin-Olguin[1], Sophie Loismann[1], Timo Betz[2], Joachim Spatz[3], Christoph Frey[3] and Lydia Sorokin[1]

[1]Institute of Physiological Chemistry and Pathobiochemistry, and Cells-in-Motion Interfaculty Centre (CIMIC), University of Muenster, Muenster, Germany.
[2]Physics Department, University of Göttingen, Germany.
[3]Max-Planck Institute for Medical Research, Heidelberg, Germany.

Leukocyte extravasation across postcapillary venules in inflammation is a multistep cascade, in which transmigration across the endothelial basement membrane (BM) is a rate-limiting step [1; 2]. Laminins, BM components, influence this process. Endothelial BMs of postcapillary venules show ubiquitous distribution of laminins 411 and patchy distribution of laminin 511. Previous in vivo studies indicate that sites containing little or no laminin 511 are the preferred sites of leukocyte extravasation [1; 3]. Our data show that laminin 511 is highly adhesive for both neutrophils and T cells, but that neutrophil motility on laminin 511 is higher than T cells, suggesting that endothelial laminins may differentially affect the extravasation of different leukocytes. Ex vivo analyses of BMs isolated from laminin α4 knockout mice (Lama4-/-) and endothelial specific laminin α5 knockout mice (Tie2cre/Lama5-/-) show that laminin 511 decreases BM flexibility. In vitro microchannels experiments aimed at mimicking the physical constraints that leukocytes undergo during migration across BMs reveal fundamental differences between neutrophil and T cell migration. We are investigating the relative contribution of the molecular composition versus the mechanical properties of endothelial BMs to immune cell cytoskeletal arrangements and force generation in T cell versus neutrophil extravasation.

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Young Investigator Talk

Unexpected function of the antioxidative xCT system/Slc7a11 in systemic sclerosis

J. Tiemann[1], S. Mücklich[2], S. Leson1, M. Kneilling[3,4], R. Mehling[4], M. Röcken[3], K. Schwegmann[5], K. Steinbrink[1] and V. K. Raker[1].

[1]Department of Dermatology, University of Münster, Germany; [2]Department of Dermatology, University Medical Center Mainz, Germany; [3]Department of Dermatology, University Tübingen, Germany; [4] Werner Siemens Imaging Center, University Tübingen, Germany; [5]European Institute for Molecular Imaging (EIMI), University of Münster.

Systemic sclerosis (SSc) is a disease of the connective tissue. One important event in SSc pathogenesis is oxidative stress response. Reactive oxidative species (ROS) acuumulate in untreated SSc fibroblasts compared to healthy donors (HD). Antioxidative pathways like xCT/Glutathione (GSH) are under physiological conditions used to regulate ROS levels and prevent cell death. Hereby, soluble carrier 7 a11 (Slc7a11), which is a part of the xCT system, transports cystine into the cells to synthesize GSH for antioxidative defence.

A bulk RNA sequencing analysis of CD45- skin cells revealed a Slc7a11 overexpression in SSc patients compared to HD. Further, cystine uptake and therefore xCT system activity increased in ROS stressed fibroblasts. To address Slc7a11´s effect on fibrosis more functional, we used a hypochlorous acid (HOCl) fibrosis mouse model. Here, intradermal injection of HOCl induce chronic oxidative stress and lead to collagen accumulation. We monitored the potential of [68Ga]Ga-FAPI-46 to detect early fibrotic activity in the skin using PET/CT. We observed less fibrotic activity in HOCl-treated xCT KO dorsal skin compared to WT dorsal skin. Interestingly, xCT deficiency protected mice from myofibroblast activation and dermal collagen acuumulation, while WT mice exhibited strong fibrosis upon HOCl treatment. When we set out to study xCT deficient fibroblasts in vitro, they failed to survive and depend on ROS-scavenging for survival. A finding that could also be observed in vivo, as xCT KO skin grafts showed reduced healing capacity after transplantation to WT and xCT KO mice. However upon HOCl treatment xCT KO grafts kept there fibrosis resistance although transplanted to fibrosis prone WT mice.

Finally, we confirmed xCT as a new therapeutic target for SSc by using Immidazol-Keton-Erastin mediated inhibition in vivo to prevent fibrosis.

Poster

How imidazolium salts belong in the toolbox of every molecular biologist

Nele Van Wyngaerden[1], Tristan Wegner[1], Anna Livia Linard Matos[2], Yanjun Zheng[3], Alexander Dombovski[4], Joachim Jose[4], Seraphine Wegner[3], Volker Gerke[2], Frank Glorius[1]

[1]Institute of Organic Chemistry, University of Münster, Corrensstraße 40, 48149 Münster, Germany
[2]Institute of Medicinal Biochemistry, Center for Molecular Biology of Inflammation, Von-Esmarch-Straße 56, 48149 Münster, Germany
[3]Institute of Physiological Chemistry and Pathobiochemistry, Waldeyerstraße 15, 48149 Münster,Germany
[4]Institute for Pharmaceutical and Medicinal Chemistry, Corrensstraße 48, 48149 Münster, Germany

Biological membranes and their constituents are some of the most important and fundamental building blocks of life. Cholesterol is an important component in most biological membranes which plays a vital role in regulating membrane integrity and cellular processes. However, their exact role in many essential cellular processes as well as in the development of diseases such as cancer or Alzheimer's is still not very well understood. And the tools to probe the cholesterol dynamics in live cells are scarce and limited in their applicability. Our group has previously developed imidazolium-based cholesterol analogues called CHIMs. These can be modified with different chemically functional groups, such as azides, that point towards the extracellular matrix, while maintaining the integral properties of cholesterol. Such azide-containing CHIMs (CHIM-L) were shown to be able to efficiently label cell membranes with fluorophores in vitro.

In this project, more novel CHIMs are further developed as chemical tools to investigate cholesterol-related membrane dynamic as well as to enable the targeted modification and functionalization of biological membranes. X-CHIM is a CHIM with a bifunctional group to probe protein-cholesterol interactions. CHIM-NTA (CHIM-nitrilotriacetic acid) has the ability to interact with the his-tag of proteins to anchor them to the membrane, displaying proteins on the cell surface. Last but not least, Pd-CHIM (lipid mimetic N-heterocyclic carbene-palladium complex) has a catalytic moiety to preform chemical reactions on the cell surface that is able to participate in reaction cascades with membrane enzymes.

Poster

The role of TAP73 in neurodevelopment

Karina Violou[1,2], Yotam Menuchin-Lasowski[2], Thomas Rauen[2], Hans R. Schöler[2], Heymut Omran[1], Julia Wallmeier[1,2]

1 General Pediatrics, University Hospital Münster
2 Department for Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Röntgenstrasse 20, 48149 Münster, Germany

The transcription factor P73 belongs to the p53 protein family, and its gene encodes two main isoforms: the transcriptionally active TAp73 isoform (transcribed from the P1 promoter) and the dominant-negative ΔNp73 (transcribed from the P2 promoter). Both isoforms are involved in maintenance of neural stem/progenitor cell self-renewal and differentiation, thus play a critical role in brain development during neuronal differentiation.

Mutations in TP73 are associated with lissencephaly in humans and severe brain defects in mice. The role of TP73 and its isoforms during neurogenesis in human is still unclear. Aiming to further scrutinize the role of TAP73 in corticogenesis and neurodevelopment in humans, we generated forebrain organoids derived from human induced pluripotent stem cells carrying a TAP73 LOF mutation generated by CRISPR-Cas.

Using immunoblotting and immunofluorescence we analysed forebrain organoids harvested at different developmental stages with antibodies marking neural progenitors such as radial glia cells, intermediate progenitors, as well as neurons. Our data indicate that in contrast to control organoids, TAP73 organoids exhibited less progenitor cells and more differentiated cells at day 40 suggesting an early differentiation pattern. Furthermore, we examined the proliferation in the control and TAP73 organoids by using immunofluorescence with anti-PHH3. Overall, TAP73 organoids showed less proliferative cells and the PHH3 expression pattern was distinct from the control.

We thus conclude that the differentiation/proliferation process is altered in TAP73 organoids than in controls, consistent with an impaired cortical development. Studying the role of TP73 isoforms can help us understand the underlying mechanisms behind TP73-associated neurodevelopmental disorders such as lissencephaly.

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