Through the lens of science

Insights into molecular mechanisms

16th annual graduate school meeting

Register at
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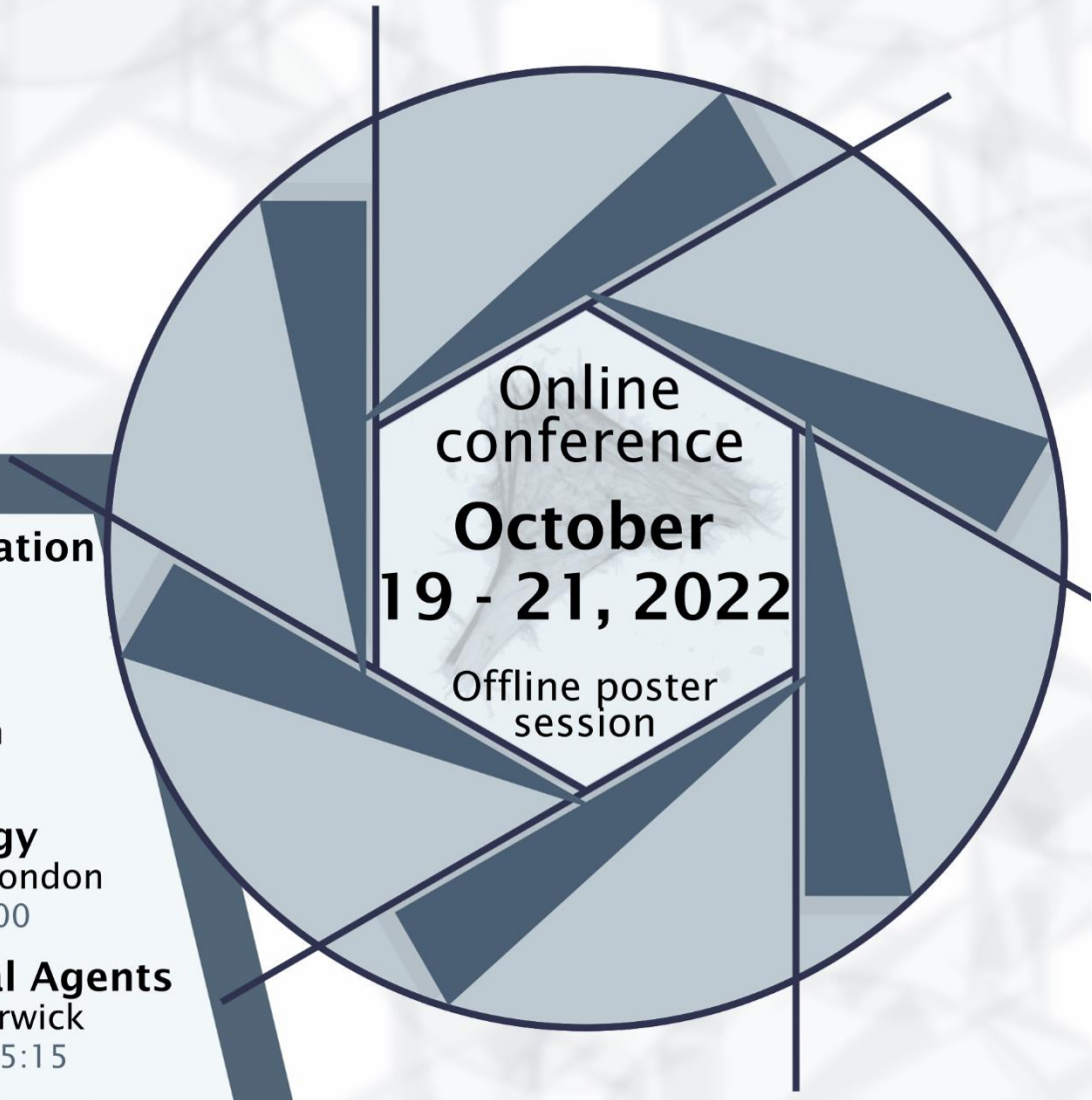
Sept. 30, 2022

registration & abstract submission deadline



Development & Regeneration
Yanlan Mao, London
Oct. 19, 09:30-10:30

Neurobiology
Karl Friston, London
Oct. 19, 13:30-14:30

Vascular Biology
Sussan Nourshargh, London
Oct. 20, 09:00-10:00

Viruses & Anti-viral Agents
Peter Sadler, Warwick
Oct. 20, 14:15-15:15

Computational Biology
Alexandre Bonvin, Utrecht
Oct. 21, 10:00-10:30

Light for Life
Christian Eggeling, Jena
Oct. 21, 14:00-15:00

Lipid Metabolism
Mike Henne, Dallas
Oct. 21, 16:30-17:00

Career
Oct. 19, 17:15-18:45

CiM IMPRS Graduate Program

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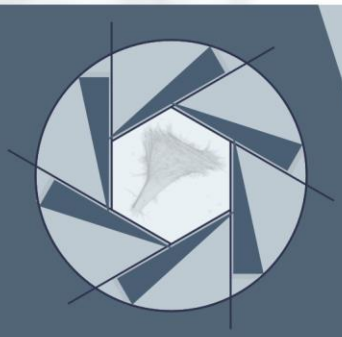
gsm_cim_2022

Westfälische Wilhelms-Universität Münster

Max Planck Institute for Molecular Biomedicine

Through the lens of science



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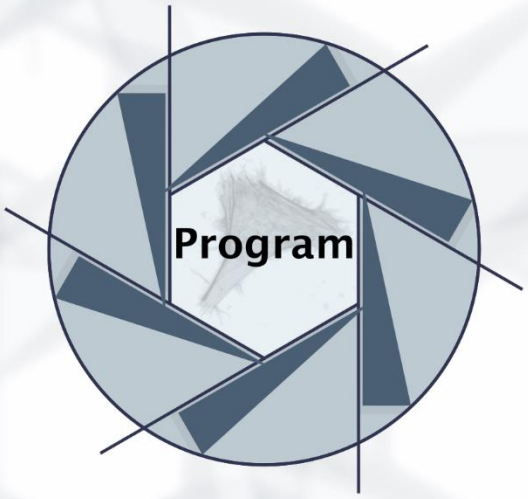


Wednesday, 19.10.2022

09:00 - 09:30 Welcome talk

Session 1: **Development & Regeneration**

09:30 - 10:30 Yanlan Mao (KS)
10:30 - 11:00 Patrick Müller
11:00 - 11:30 QA & Coffee
11:30 - 12:00 Nikita Raj (YI)
12:00 - 12:30 Nuria Monteserrat
12:30 - 12:45 QA & Coffee

12:45 - 13:30 Lunch

Session 2: **Neurobiology**

13:30 - 14:30 Karl Friston (KS)
14:30 - 15:00 Frank Bradke
15:00 - 15:30 QA & Coffee
15:30 - 16:00 Aleksandra Polishchuk (YI)
16:00 - 16:30 Marc Hammarlund
16:30 - 16:45 QA & Coffee

Session 3: **Career**

17:45 - 18:15 Julia Eckhoff
18:15 - 18:45 Michael Spiegelmacher

Thursday, 20.10.2022

Session 4: **Vascular Biology**

09:00 - 10:00 Sussan Nourshargh (KS)
10:00 - 10:30 Jaap van Buul
10:30 - 11:00 QA & Coffee
11:00 - 11:30 Parisa Ghanbari (YI)
11:30 - 12:00 Margarita Shuvalova (YI)
12:00 - 12:15 QA & Coffee

12:15 - 14:15 Poster Session & Lunch

Session 5: **Viruses & Anti-viral Agents**

14:15 - 15:15 Peter Sadler (KS)
15:15 - 15:45 Kenneth Witwer
15:45 - 16:15 QA & Coffee
16:15 - 16:45 Stephan Huveneers
16:45 - 17:15 Raul Andino
17:15 - 17:30 QA & Coffee

Friday, 21.10.2022

Session 6: **Computational Biology**

09:00 - 09:30 Adeel Razi
09:30 - 10:00 Patrick Aloy
10:00 - 10:30 Alexandre Bonvin
10:30 - 11:00 QA & Coffee

Session 7: **Light for Life**

11:00 - 11:30 Pau Gorostiza
11:30 - 12:00 Elisabeth Kugler
12:00 - 12:15 QA & Coffee

12:15 - 14:00 Lunch

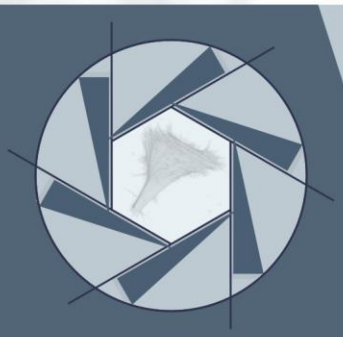
14:00 - 15:00 Christian Eggeling (KS)
15:00 - 15:30 Maximilian Rüttermann (YI)
15:30 - 16:00 QA & Coffee

Session 8: **Lipid Metabolism**

16:00 - 16:30 William Prinz
16:30 - 17:00 Mike Henne
17:00 - 17:15 QA & Coffee

17:15 - 17:30 Closing ceremony

Through the lens of science



WELCOME MESSAGE

Dear Colleagues,

The PhD students of the GSM 2022 Organizing Committee are honored and delighted to welcome you to the 16th Interdisciplinary Graduate School Meeting. Due to the uncertainty of the ongoing Corona pandemic, this year's talks will be online while the poster session will be held offline. By adapting to a virtual delivery of the meeting, we have enabled ourselves to face the ongoing challenges and grow together. This hybrid meeting will give colleagues across the globe the opportunity to participate at the talks and discussions, while local students have the opportunity to present their data and network during the offline poster session.

Additionally, we have organized interesting career talks, giving students the chance to learn more about possible future careers after their PhD. Therefore, we are looking forward to gather inspiring career ideas through this meeting.

It is our sincere wish that many colleagues from different fields of science participate in this meeting to share their meaningful work through exchange of valuable interdisciplinary scientific experience.

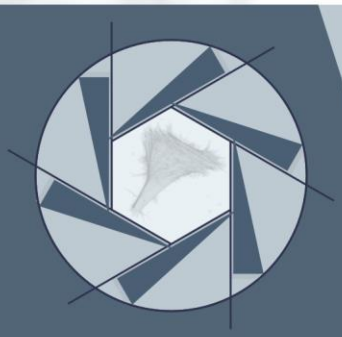
We would like to thank our CiM-IMPRS graduate program to give us the opportunity to organize this meeting. Additionally, we would like to thank our speakers, presenters and sponsors without whose support this meeting would not be possible.

Finally, we thank you all for your participation and hope to have an inspiring event!

Sincerely,

GSM 2022 Organizing Committee

Through the lens of science



HOW TO JOIN THE MEETING

The conference will be held via **Zoom Webinar** from 19th of October 2022 until 21st of October 2022. The meeting link will be shared via e-mail one week before the conference starts.

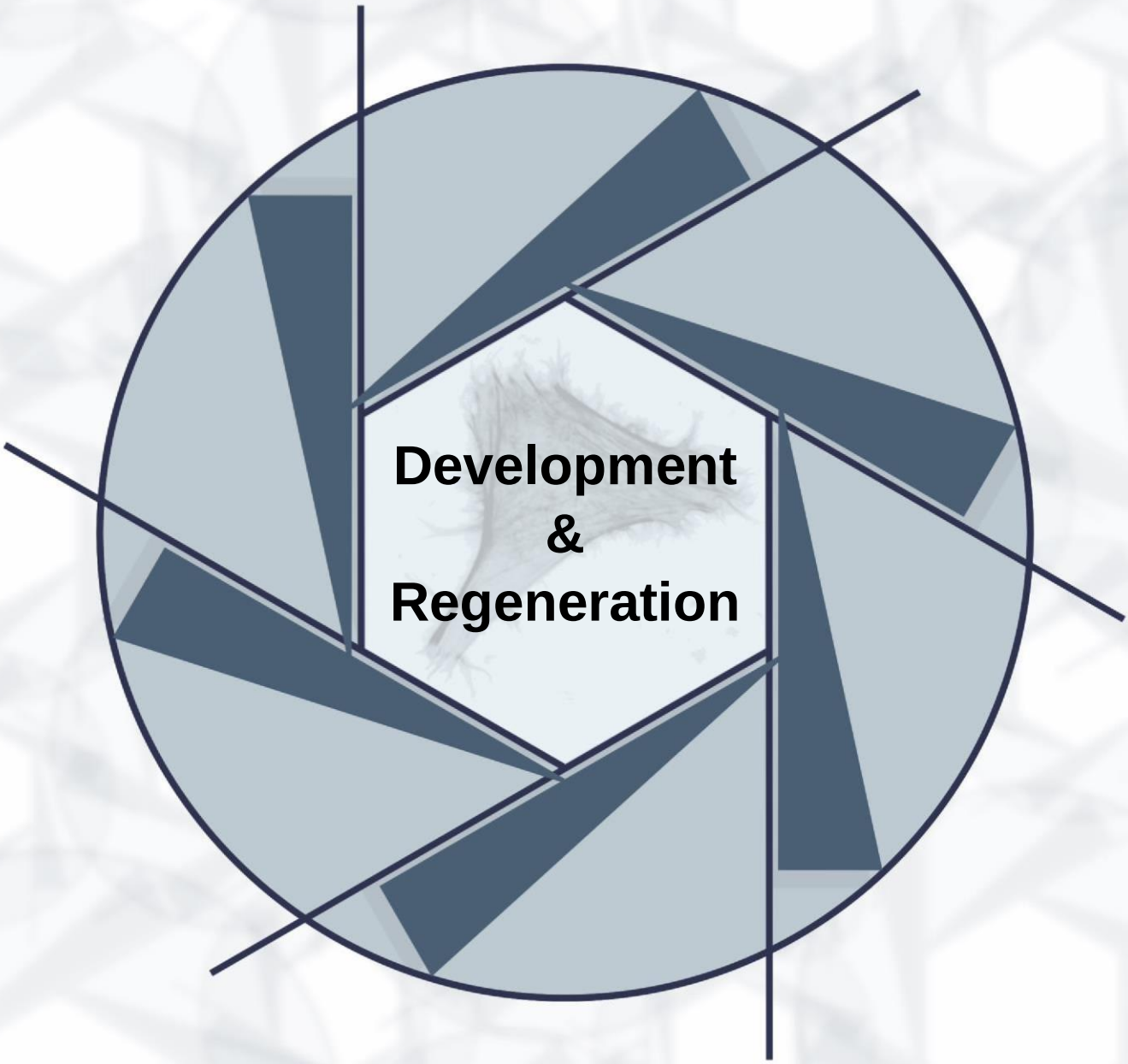
Information on how to join the online talks:

Once you join as a participant, you will be muted and unable to share your screen or video. For questions regarding the talks, Zoom Webinar offers the Q&A functionality. There, you can ask your own questions or vote those of others up, making it more likely for us to ask them to the speakers live during the conference. Ideally, your question should mention the speaker you are addressing. After each talk, the moderators will forward your questions to the respective speaker for 5 min before continuing to the next talk. After all talks of a session, we will have an extended Q&A session during which questions to all speakers of the session that were not answered so far will be forwarded to the speakers. These Q&A sessions also offer the opportunity to talk and discuss with the speakers directly! Just use the "Raise hand" option in Zoom and we will unmute you, so you can ask your questions yourself.

Information on how to join the offline poster session:

The poster session will be held in person at the **Entrance Hall of the Max Planck Institute for Molecular Biomedicine** (Röntgenstrasse 20, 48149 Münster) on **20th of October, 12:15-14:15 CEST**. Please note that we will also serve some food and drinks for you during the poster session.

Through the lens of science



Yanlan Mao (KS)

Laboratory for Molecular Cell Biology,
University College London, United Kingdom

Coping with mechanical stress: Tissue dynamics during development, homeostasis & repair

During growth and development, tissue dynamics, such as tissue folding, cell intercalations and oriented cell divisions, are critical for shaping tissues and organs. However, less is known about how tissues regulate their dynamics during tissue homeostasis and repair, to maintain their shape after development. In this talk, we will discuss how differential growth rates can generate precise folds in tissues. We will also discuss how tissues respond to mechanical perturbations, such as stretching or wounding, by altering their actomyosin contractile structures, to change tissue dynamics, and thus preserve tissue shape and patterning. We combine genetics, biophysics and computational modelling to study these processes.

Key words: tissue dynamics · patterning · regeneration · genetics · biophysics · computer modelling

Yanlan Mao (KS)

Laboratory for Molecular Cell Biology,
University College London, United Kingdom

Biography

Yanlan Mao is a Group Leader at the Laboratory for Molecular Cell Biology, University College London. After receiving her BA in Natural Sciences at Cambridge University, she completed her PhD with Matthew Freeman at the MRC LMB in Cambridge on Drosophila cell signaling and epithelial patterning. During her postdoc with Nic Tapon at the CRUK London Research Institute (now Francis Crick Institute), she became interested in tissue mechanics and computational modeling approaches, and studied the role of mechanical forces in orienting cell divisions and controlling tissue shape. In 2014, when Yanlan started her independent research group, she has continued to investigate the role of mechanical forces in tissue development, homeostasis and repair. She now holds a MRC Senior Fellowship, a Lister Institute Prize and an L'Oreal UNESCO Women in Science Fellowship. She was selected to join the EMBO Young Investigator Programme in 2018, was recently awarded the Early Career Prize in Mechanobiology by the Biophysical Society, and the BSCB Women in Cell Biology Early Career Medal.

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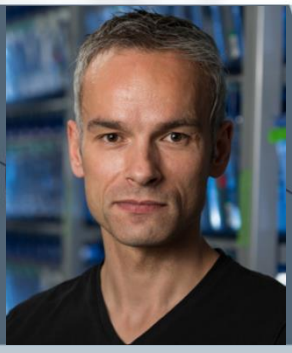
Patrick Müller

Chair of Developmental Biology,
University of Konstanz, Germany



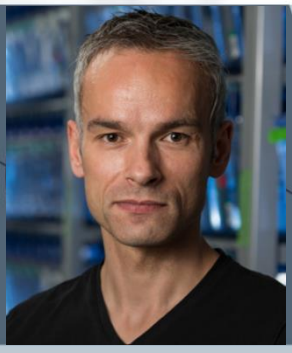
Deep learning in developmental biology

Signaling pathways are essential for embryogenesis, and abolishing their activity leads to characteristic developmental defects. Classification of these defects can identify the underlying signaling mechanisms, but this requires hard-to-obtain expert knowledge. We developed a machine-learning approach for automated phenotyping to identify zebrafish signaling mutants in an unbiased manner. This approach accurately classifies phenotypic defects caused by loss-of-function of the seven major signaling pathways relevant for vertebrate development. Our classification algorithms have wide applications: The approach can be used to robustly identify signaling defects in evolutionarily distant species and to even resolve the mechanism-of-action of pharmaceutical substances.

Key words: development · signaling · machine-learning · disease · zebrafish

Patrick Müller

Chair of Developmental Biology,
University of Konstanz, Germany



Biography

Patrick Müller is a principal investigator at the University of Konstanz. Research in his lab combines genetics, embryology, biophysics, and theoretical approaches to understand how extracellular signaling molecules pattern developing embryos and tissues. From 1999 to 2004, he studied in Göttingen, Berkeley, and New York. He received his Ph.D. from the Max Planck Institute for Biophysical Chemistry in 2007 and did his postdoc in Alex Schier's lab at Harvard University. In 2013, Patrick Müller joined the Max Planck Institute for Developmental Biology in Tübingen and one year later was appointed as a Max Planck Research Group Leader at the Friedrich Miescher Laboratory of the Max Planck Society. Since 2021, he is the Chair of Developmental Biology at the University of Konstanz. His lab works on understanding the mechanisms of morphogen transport, pattern formation in differently sized embryos, and self-organized patterning during vertebrate development. Among the honors he received are the Otto Hahn Medal of the Max Planck Society, the HFSP Career Development Award, ERC Starting and Consolidator Grants, and the EMBO Young Investigator Award.

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Nikita Raj

Institute of Medical Biochemistry, ZMBE,
WWU Münster, Germany

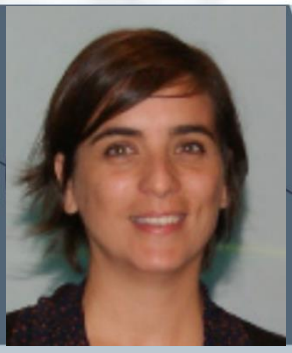
Early endosomes act as local exocytosis hubs to repair endothelial membrane damage

The plasma membrane of a cell is subject to stresses such as mechanical forces resulting from shear stress and mechanical stretch, causing ruptures that must be repaired immediately to preserve membrane integrity and ensure cell survival. Defective plasma membrane repair can lead to eventual tissue damage and has been linked to numerous disease pathologies. Yet, the spatio-temporal membrane dynamics at the wound site 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 human endothelial cells. 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 the Ca^{2+} entering through the wound. This exocytosis is spatially restricted to the vicinity of the wound site, occurs immediately upon wounding, and is mediated by the endosomal SNARE VAMP2, which is crucial for efficient membrane repair. Thus, the specialized Ca^{2+}-triggered and localized exocytosis of early endosomes is an emergency-based process that supplies the membrane material needed for rapid wound closure in endothelial cells. This is essential to prevent wound-induced endothelial leakage and a resulting inflammatory reaction in a mechanically stressed environment.

Key words: membrane damage · Ca2+ entry · early endosome exocytosis · SNARE VAMP2 · plasma membrane repair

Nuria Monteserrat Pulido

Institute of Bioengineering of Catalonia,
Barcelona, Spain



How to engineer human pluripotent stem cells to understand human development & disease

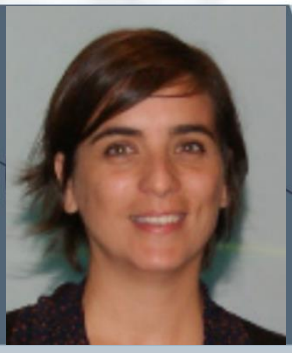
In recent years considerable progress has been made in the development of faithful procedures for the differentiation of human pluripotent stem cells (hPSCs). An important step in this direction has also been the derivation of three-dimensional cell cultures that represent micrometer to centimeter size versions of human organs, the so-called organoids. The convergence of stem cell biology and bioengineering now offers the possibility to provide physiologically relevant stimuli in a controlled fashion, resulting in the development of naturally inspired approaches to overcome major limitations of the organoid field.

Here we will discuss current developments in the kidney organoid field and emphasize the achievements and ongoing challenges of bringing together hPSC organoid differentiation, bioengineering and disease modelling with a particular focus on genetic and systemic disorders as well as COVID19 research.

Key words: stem cells · 3D culture · differentiation · organoids · bioengineering · disease modelling · COVID19

Nuria Monteserrat Pulido

Institute of Bioengineering of Catalonia,
Barcelona, Spain



Biography

Nuria Montserrat became interested in organ regeneration and stem cells during my master and PhD training that finished in 2006. The same year she got a Postdoctoral fellowship from the Fundaçao para a Ciência e Tecnología (Portugal). In 2007 she moved as a post-doctoral researcher at the Hospital of Santa Creu i Sant Pau in Barcelona.

In 2008 she joined the Center of Regenerative Medicine of Barcelona (CMRB) thanks to the support of a Juan de la Cierva fellowship under the direction of Dr. Izpisúa Belmonte. In 2010 she first co-authored how to reprogram cord blood stem cells for the first time (Nature Protocols, 2010). Then she first-coauthor the first work deriving iPSCs with new factors (Cell Stem Cell, 2013). She also collaborated in projects aimed to characterize the genomic integrity of human iPSCs as well as in the differentiation of iPSCs towards different lineages for disease modeling (Stem Cells 2011; Nature 2012; Nature Methods 2012, Nature 2012, Nature Communications 2014). She has first co-authored how the reactivation of endogenous pathways can be artificially reactivated and promote heart regeneration in mammals (Cell Stem Cell, 2014).

Her expertise in the fields of somatic reprogramming and organ regeneration helped her to be awarded with an ERC Starting Grant in 2014 that allowed her to become a Junior group leader at the Institute of Bioengineering of Catalonia (IBEC).

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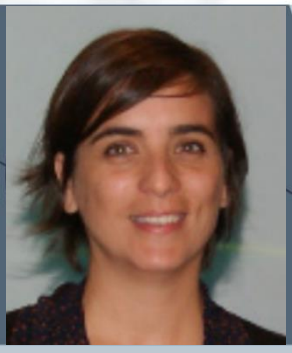
Nuria Monteserrat Pulido

Institute of Bioengineering of Catalonia,
Barcelona, Spain



In January 2015 she got a Ramon y Cajal fellowship and from 2019 she is an ICREA Research Professor and Senior Group Leader. During these years her findings in the field of Regenerative Medicine led to the derivation, for the first time, of cardiac grafts from human pluripotent stem cells and decellularized cardiac myocardium (Biomaterials 2016), and the derivation of renal analogues with 3D bioprinting (Materials Today 2017).

Recently, Nuria Montserrat led the derivation of vascularized kidney organoids (Nature Materials, 2019) and co-led on the application of kidney organoid technology to model SARS-CoV-2 infections (Cell, 2020) identifying a therapeutic compound that nowadays is under clinical trial in COVID19 patients (The Lancet Respiratory Medicine, 2020; EMBO Molecular Medicine 2020). She has recently led the first work on the identification of metabolic regulators protecting the renal tubule from acute injury exploiting kidney organoid technology (Cell Metabolism, 2020), among others.

In December 2020 the ERC has recognized all these efforts and Nuria Montserrat has been awarded with the prestigious ERC-Consolidator Grant to study the interplay between mechanobiology and metabolism during kidney development and disease.

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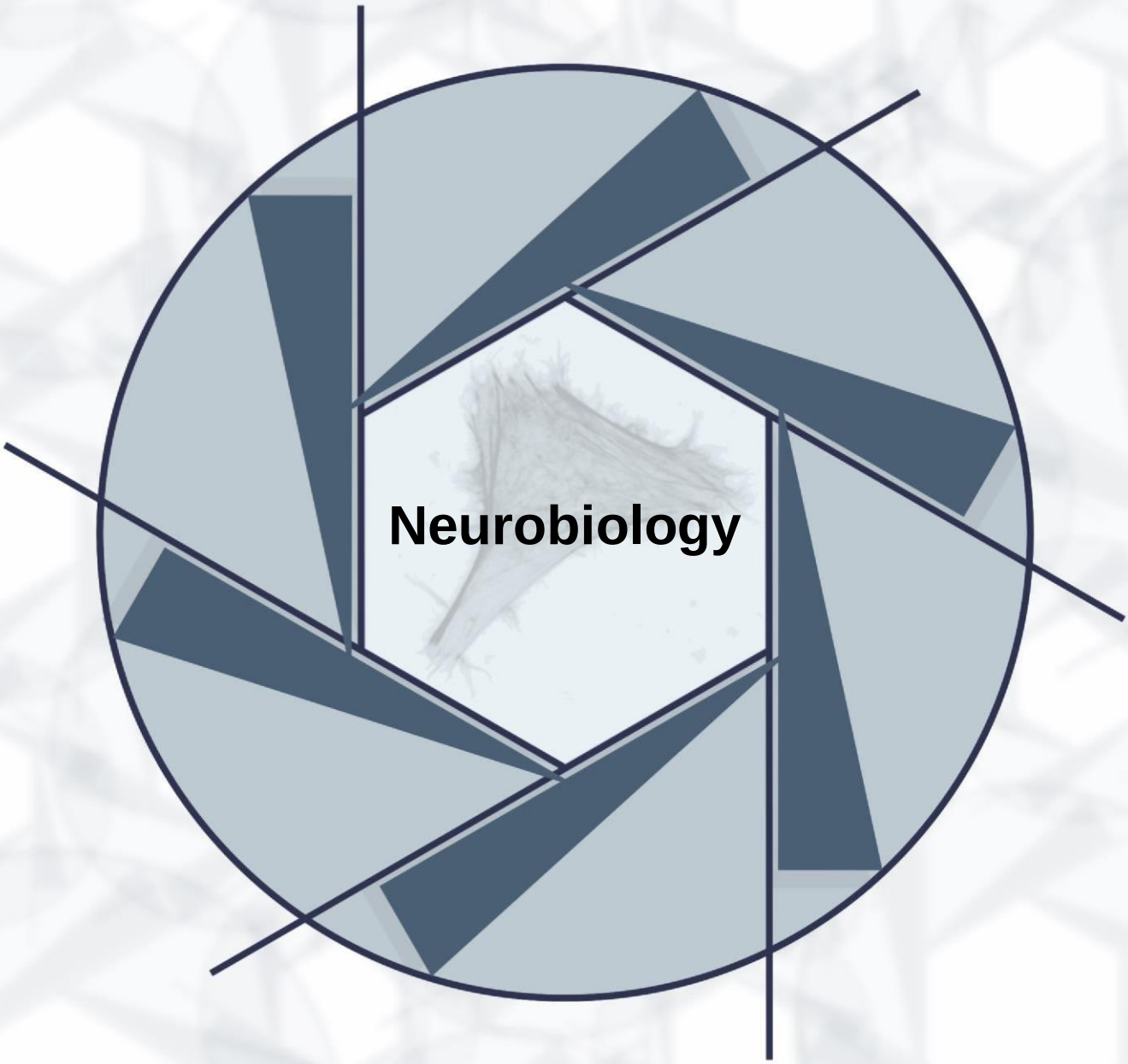
Through the lens of science



Karl Friston (KS)

Institute of Neurology,
University College London, UK

Me and my Markov blanket: Sentience and the Free Energy Principle

How can we understand ourselves as sentient creatures? And what are the principles that underwrite sentient behaviour? This presentation uses the free energy principle to furnish an account in terms of active inference. First, we will try to understand sentience from the point of view of physics; in particular, the properties that self-organising systems - that distinguish themselves from their lived world - must possess. We then rehearse the same story from the point of view of a neurobiologist, trying to understand functional brain architectures. The narrative starts with a heuristic proof (and simulations of a primordial soup) suggesting that life - or biological self-organization - is an inevitable and emergent property of any dynamical system that possesses a Markov blanket.

This conclusion is based on the following arguments: if a system can be differentiated from its external milieu, then its internal and external states must be conditionally independent. These independencies induce a Markov blanket that separates internal and external states. Crucially, this equips internal states with an information geometry, pertaining to probabilistic beliefs about something; namely external states. This free energy is the same quantity that is optimized in Bayesian inference and machine learning (where it is known as an evidence lower bound). In short, internal states will appear to infer - and act on - their world to preserve their integrity. This leads to a Bayesian mechanics, which can be neatly summarised as self-evidencing. In the second half of the talk, we will unpack these ideas using simulations of Bayesian belief updating in the brain and relate them to predictive processing and sentient behaviour.

Key words: active inference · autopoiesis · cognitive · dynamics · free energy · epistemic value · self-organization.

Karl Friston (KS)

Institute of Neurology,
University College London, UK

Biography

Karl Friston is a theoretical neuroscientist and authority on brain imaging. He invented statistical parametric mapping (SPM), voxel-based morphometry (VBM) and dynamic causal modelling (DCM). These contributions were motivated by schizophrenia research and theoretical studies of value-learning, formulated as the disconnection hypothesis of schizophrenia. Mathematical contributions include variational Laplacian procedures and generalized filtering for hierarchical Bayesian model inversion. Friston currently works on models of functional integration in the human brain and the principles that underlie neuronal interactions. His main contribution to theoretical neurobiology is a free-energy principle for action and perception (active inference). Friston received the first Young Investigators Award in Human Brain Mapping (1996) and was elected a Fellow of the Academy of Medical Sciences (1999). In 2000 he was President of the international Organization of Human Brain Mapping. In 2003 he was awarded the Minerva Golden Brain Award and was elected a Fellow of the Royal Society in 2006. In 2008 he received a Medal, College de France and an Honorary Doctorate from the University of York in 2011. He became of Fellow of the Royal Society of Biology in 2012, received the Weldon Memorial prize and Medal in 2013 for contributions to mathematical biology and was elected as a member of EMBO (excellence in the life sciences) in 2014 and the Academia Europaea in (2015). He was the 2016 recipient of the Charles Branch Award for unparalleled breakthroughs in Brain Research and the Glass Brain Award, a lifetime achievement award in the field of human brain mapping. He holds Honorary Doctorates from the University of Zurich and Radboud University.

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Frank Bradke

German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

Mechanisms of Axon Growth and Regeneration

Almost everybody who has seen neurons under a microscope for the first time is fascinated by their beauty and their complex shape. Early on during development, however, neurons look round and simple without signs of their future complexity. How do neurons develop their sophisticated structure? How do they initially generate domains that later have distinct functions within neuronal circuits, such as the axon? And, can a better understanding of the underlying developmental mechanisms help us in pathological conditions, such as a spinal cord injury, to induce axons to regenerate?

Here, I will talk about the cytoskeleton as a driving force for initial neuronal polarization and axon growth. I will then explore how cytoskeletal changes help to reactivate the growth program of injured CNS axons to elicit axon regeneration after a spinal cord injury. Finally, I will discuss whether axon growth and synapse formation could represent mutually excluding processes. Following this developmental hypothesis helps us to generate a novel perspective on regeneration failure in the adult CNS and to envisage new paths to overcome it. Thus, this talk will describe how we can exploit developmental mechanisms to induce axon regeneration in the adult after a spinal cord injury.

Key words: neuronal polarization · cytoskeleton · axon growth · CNS · spinal cord injury · axon regeneration

Frank Bradke

German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

Biography

After studying at the Freie Universität Berlin and University College London, Bradke carried out research at the European Molecular Biology Laboratory (EMBL) in Heidelberg as part of his doctoral thesis. As a postdoctoral researcher, he moved to the University of California in San Francisco and Stanford University in 2000. In 2003, he was appointed a group leader at the Max Planck Institute of Neurobiology in Martinsried. In 2011, he was awarded the IRP Schellenberg Prize, one of the most prestigious awards in the field of regeneration research. In the same year he became full professor at the University of Bonn, and was appointed head of the Axon Growth and Regeneration research group at the DZNE. Bradke is an elected a member of the Leopoldina (the German National Academy of Sciences), the Academia Europaea, and the European Molecular Biology Organization (EMBO). In 2016, he was awarded the Leibniz Prize, which is the most important research award in Germany. In 2018, he received the Roger de Spoelberch Prize and in 2021 he was selected for the Carl Zeiss Lecture.

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Aleksandra Polishchuk

Rovira i Virgili University,
Tarragona, Spain

Synaptic retrograde regulation of the PKA-induced SNAP-25 & Synapsin-1 phosphorylation

At the neuromuscular junction (NMJ), PKA enhances ACh release maybe phosphorylating targets from the synaptic vesicle (SV) exocytotic cycle, although this is unknown. Synaptosomal associated protein (SNAP-25), which is part of the SNARE complex, and Synapsin-1 (Syn-1), which controls the release of the SV from the cytoskeleton to promote their docking, are PKA targets that highly influence the SV exocytosis. Although ACh release mechanism is regulated by presynaptic stimulus and retrogradely by the resulting muscle contraction, PKA regulation by the pre- and postsynaptic activities had not been studied until now. To separate the effect of presynaptic activity from that of the resulting muscle contraction on PKA subunits and its activity, the rat phrenic nerve was stimulated (1 Hz, 30 min) with and without contraction (abolished by μ-conotoxin GIIIB). PKA was pharmacologically inhibited (H-89) to assess the interactions of PKA and its targets (SNAP-25 and Syn-1). We used Western blotting and cytosol/membrane translocation by subcellular fractionation.

We demonstrate that the pre- and postsynaptic activities differentially regulate the PKA subunit dynamics to be catalytic active at the NMJ and to phosphorylate SNAP-25 and Syn-1. Synaptic Cβ subunit regulated by RIIβ or RIIα subunits controls activity-dependent phosphorylation of SNAP-25 and Syn-1 respectively. Muscle contraction retrogradely downregulates presynaptic activity-induced pSyn-1 while that enhances pSNAP-25 T138. We hypothesize that both actions could coordinately contribute to decrease the neurotransmitter release at the NMJ. These results provide a molecular mechanism of the bidirectional communication between nerve terminals and muscle cell to balance the optimal process of ACh release.

Key words: neuromuscular junction · ACh release · SNAP-25 phosphorylation · SNARE · Syn-1 · PKA

Marc Hammarlund

Yale School of Medicine,
New Haven, USA

The CeNGEN project: Neurogenomics at single-cell resolution across an entire nervous system

The remarkable diversity of neurons in the brain arises from differential gene expression that begins during development and continues in the adult. In addition to mechanisms that control transcript abundance, neuronal diversity also depends on precise regulation of alternative RNA processing to produce biochemically distinct gene products.

The CeNGEN project is using a variety of approaches to generate a deep and high-resolution understanding of gene expression and alternative splicing across an entire nervous system, through time.

Key words: neurogenomics · CeNGEN

Marc Hammarlund

Yale School of Medicine,
New Haven, USA

Biography

Marc did his PhD at the University of Utah with Erik Jorgensen, and stayed in Utah to do a postdoc with Mike Bastiani. He started his lab at Yale in 2008, and he is now a Professor with joint appointments in the Departments of Neuroscience and Genetics. Marc's work focuses on the cell biology of axon degeneration and regeneration, and more recently he initiated Cengen, a large-scale neurogenomics project aimed at a complete description of gene expression in the C. elegans nervous system. Marc has received numerous awards, including from the Beckman and the Ellison foundations. Marc is also a co-director of the Neurobiology Course at the Marine Biological Laboratories, and is the Chair of the DEI Committee in the Department of Neuroscience at Yale. A fun fact about Marc is that he worked on Capitol Hill as a legislative aide before grad school.

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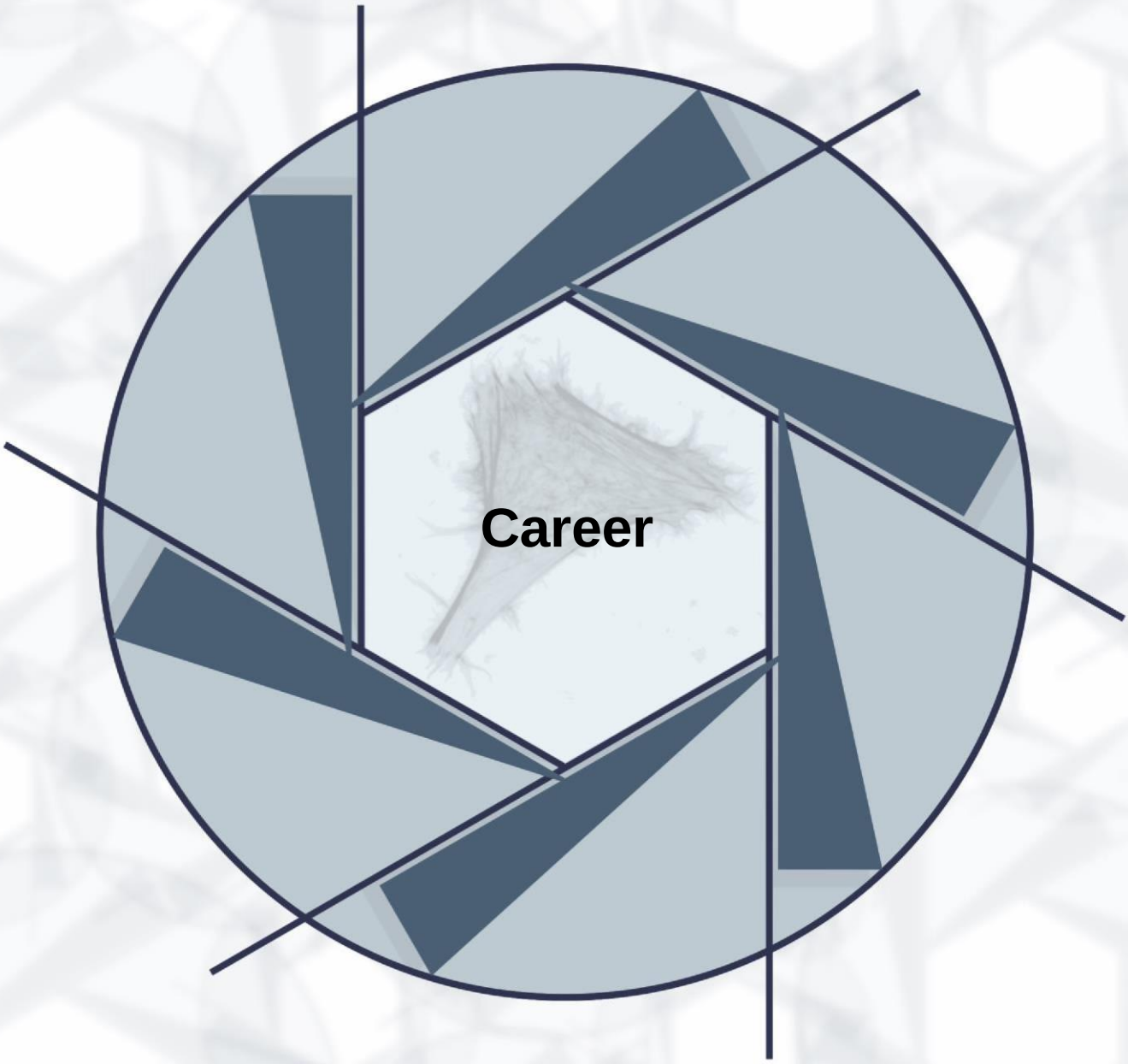
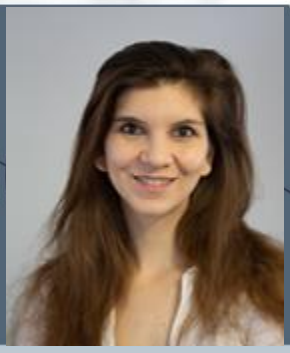
Through the lens of science



Julia Eckhoff

Freelance medical writer, Eckcomms Ltd,
London, United Kingdom



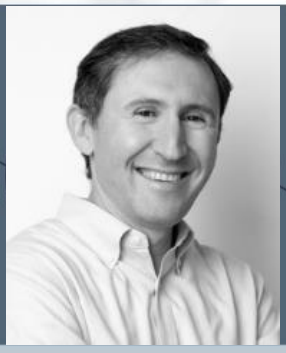
Biography

After obtaining her PhD degree, Julia Eckhoff fully committed to science communication as a career path by joining a medical writing agency in Hamburg, Germany. In 2017 she moved to London (UK), where she became a scientific editor with *Nature Communications*, which is part of the *Nature* group of journals. After a few years of working for *Springer Nature*, Julia Eckhoff joined one of the leading medical communications agencies in the United Kingdom. Taking all her work experience together, in December 2020 she founded Eckcomms Ltd.

Contact: https://yoursciencewriter.com/

Michael Spiegelmacher

CEO and co-founder of BionautLabs,
Los Angeles, CA, USA

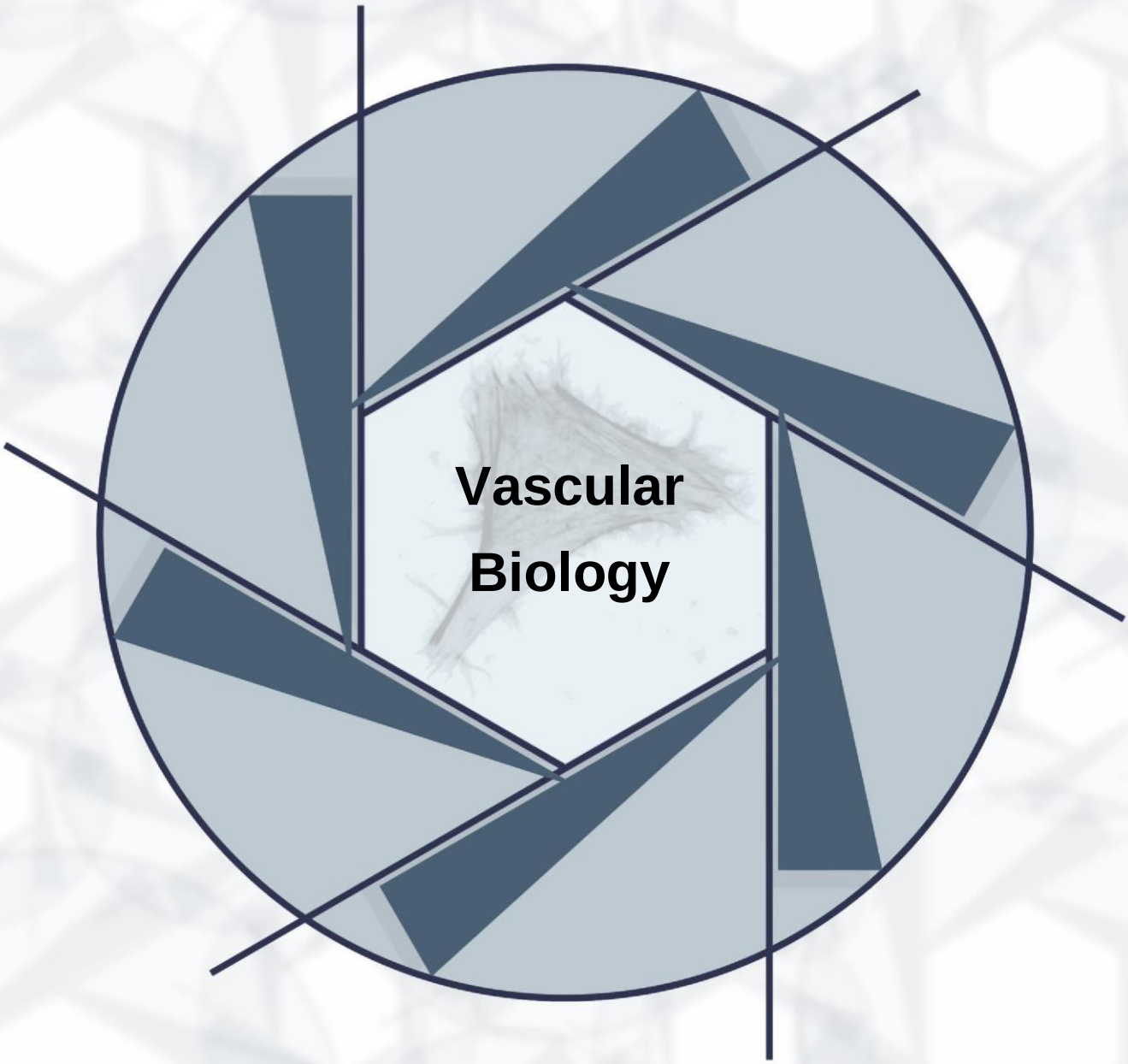


Biography

Michael Spiegelmacher is an expert in Robotics and Artificial Intelligence with specialty in R&D moonshot projects. He has two decades of experience in innovation leadership at the Government of Israel, PrimeSense (acquired by Apple), McKinsey, and Morgan Stanley.

Contact: https://virusure.com/
https://bionautlabs.com/

Through the lens of science



Sussan Nourshargh (KS)

Queen Mary University of London,
London, UK

Neutrophil breaching of venular walls: Novel concepts and pathophysiological regulation

Neutrophil migration into tissues forms a fundamental arm of innate immunity but is also a key instigator of numerous acute and chronic inflammatory disorders. Despite our increased knowledge of this process, there remain many open questions. Their group aims to decipher the mechanisms through which neutrophils breach inflamed venular walls as investigated via high resolution confocal intravital microscopy. With this strategy, as well shedding light on molecular mechanisms of physiological neutrophil trafficking, they have identified numerous aberrant forms of neutrophil-venular wall interactions such as neutrophil reverse transendothelial cell migration (rTEM). Importantly, they have directly aligned the latter response with remote organ injury, most notably following local induction of hyper-permeability reactions and in ageing. Collectively, the talk will discuss how molecular changes in vascular endothelial cells drive dysregulated neutrophil trafficking with pathological consequences.

Key words: inflammation · neutrophil trafficking · rTEM · rTEM in ageing · hyperpermeability reaction · intravital microscopy

Sussan Nourshargh (KS)

Queen Mary University of London,
London, UK

Biography

Sussan Nourshargh is a pharmacologist who studied at University College London (BSc) and King's College London (PhD) and became Professor of Immunopharmacology at Imperial College London in 2006. In 2007 she joined Barts and The London Medical School, Queen Mary, University of London, UK, to establish and head a new Centre focusing on Microvascular Research. Her research, largely funded by the Wellcome Trust and MRC, aims to unravel the molecular and cellular events involved in leukocyte trafficking. Specifically, through the application of high-resolution *in vivo* microscopy she has made seminal contributions to the field of neutrophil transmigration for which her group is internationally rated.

Sussan Nourshargh is Fellow of the UK Academy of Medical Sciences and British Pharmacological Society. She has acted as committee member for numerous national and international funding bodies, scientific societies, editorials and advisory boards and has been recipient of multiple prestigious awards.

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Jaap van Buul

Swammerdam Institute for Life Sciences (SILS),
University of Amsterdam, the Netherlands

How leukocytes breach the vessel wall: Searching for the ideal transmigration hotspot

It is my long-lasting interest to understand the process of leukocyte transendothelial migration (TEM) and how the vessel wall manages to maintain its integrity during this event. TEM is crucial during several (patho) physiological conditions, such as inflammation, atherosclerosis, but also during homing of hematopoietic stem cells after chemo- /radiotherapy and for cancer cell metastasis.

Key words: transendothelial migration (TEM) · TEM hotspot · endothelial integrity

Jaap van Buul

Swammerdam Institute for Life Sciences (SILS),
University of Amsterdam, the Netherlands

Biography

Jaap D. van Buul is professor of Vascular Cell Biology by special appointment at the Swammerdam Institute for Life Sciences (SILS) at University of Amsterdam. He did his postdoc in the lab of Prof. Dr. Keith Burridge at the department of Developmental and Cell Biology at the University of North Carolina in Chapel Hill, USA. This was a wonderful period and showed for the first time a functional role for the small GTPase RhoG. In 2009, he co-founded the Dutch Endothelial Biology Society (DEBS), a society that gives a platform to young investigators in the vascular biology field to present their work to a larger audience and currently holds the chair. In addition, Prof. Van Buul is the president of the Dutch Society for Cell Biology (DSCB), formally known as the NVvC. In 2016, he joined the board of the European Vascular Biology Organization (EVBO) and in 2018 became a member of the F1000. Currently, Prof. Van Buul runs the Vascular Cell Biology lab at Sanquin Research in Amsterdam. Several graduate students who trained in the Van Buul laboratory have gone on to independent academic faculty positions, while others have taken leadership roles in private research institutes or biotechnology companies.

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Jaap van Buul

Swammerdam Institute for Life Sciences (SILS),
University of Amsterdam, the Netherlands

The Van Buul lab uses several cellular and molecular biology techniques to visualize the actual adhesion and transmigration of immune cells across the endothelium. The lab uses transmigration assay under physiological flow in combination with advanced confocal laser scanning microscopy and in collaboration with Airyscan and Lattice Light Sheet microscopy imaging. This allows following transendothelial migration with the highest possible resolution in 3D in time with minimal phototoxicity, enabling to draw conclusions on the spatial and temporal regulation of all different steps of the transendothelial migration cascade. Additionally, they entered the field of optogenetics using FRET-based and light-sensitive and membrane-targeting biosensors: a technique that reveals the localization of protein activation through the transmission of or activation by fluorescent signals. Recently, the lab started generating vessel-on-a-chip that allow us to follow leukocyte transmigration in time leaving the vascular lumen and entering the peri-vascular space using long distance objectives. With the use of all described tools, it is the goal to understand the molecular details of leukocyte Biosketch Jaap D. van Buul 2021 transendothelial migration to ultimately develop therapies that either promote or inhibit leukocyte transendothelial migration and vascular permeability in an organ-specific manner.

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Parisa Ghanbari

Max Planck Institute for Molecular Biology
Münster, Germany

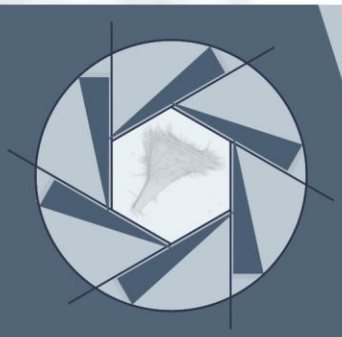
The role of nuclear envelope structure in response to shear stress in Endothelial cells

Endothelial cells (ECs) are constantly exposed to shear stress (SS) induced by blood flow. Mechano-transduction pathways play a major role in adaptation of endothelial cells to SS, however deviations from normal physiological conditions lead to diseases. In these pathways, not only do actin-myosin cytoskeleton and focal adhesions play crucial roles in resisting and adapting to mechanical forces but also nuclear structure and the LINC complex (Linker of Nucleus to Cytoskeleton) act as parts of this pathway and facilitate the appropriate transmission of mechanical stimuli to the nucleus as the biggest and stiffest intracellular organelle accommodating the cellular genome. Therefore, components and structures involved in maintenance and integrity of the nucleus play a crucial role in adaptation, orientation and movements of nucleus. Any disorganization in nuclear architecture could potentially result in disease development.

Emerin, a member of the LEM-domain (LEMD) family, is located at the nuclear envelope and acts as a mechanosensor protein together with the LINC complex and actin to alleviate the mechanical forces exerted to the nucleus. The role of nuclear envelope components including lamins, SUN proteins and myosin in ECs exposed to shear stress is studied to some extent, however, the potential role of Emerin in this concept is poorly understood. Therefore, this project aims to understand the role of Emerin in cooperation with cytoskeletal proteins in ECs to transmit mechanical load in a proper manner and protect the nuclear structure and genomic material from the forces induced by blood flow.

Parisa Ghanbari

Max Planck Institute for Molecular Biomedicine
Münster, Germany



Preliminary data from in vitro studies showed induction of SS (18 dyn) on HUVECs induces morpho dynamic changes and flow-directed orientation of the nucleus after 24h. In addition, actin cap structures covering the nucleus were formed in a timely manner which are abolished in emerin siRNA-KD cells. This is associated with less aligned nuclei toward the flow direction in emerin depleted cells. Thus, there might be a crucial role for emerin in the establishment of actin cap structures to induce nuclear orientation and movements in response to SS in ECs which requires further studies.

Key words: endothelial cells · shear stress · mechanotransduction · nuclear envelope · emerin.

Margarita Shuvalova

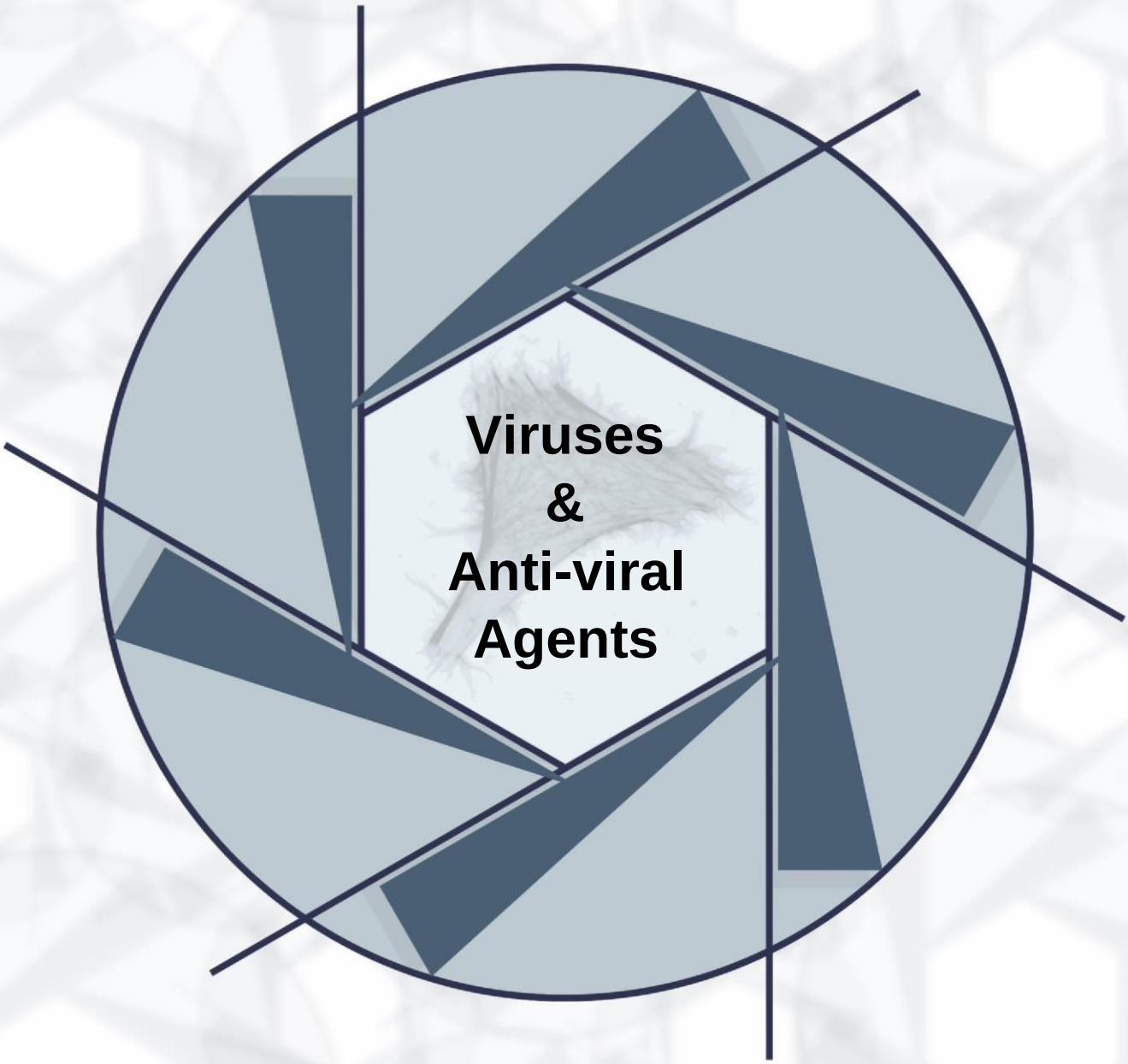
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia

The effect of hydrogen peroxide on the permeability of the blood-brain barrier in vitro

The role of hydrogen peroxide and redox signaling was demonstrated in the regulation of peripheral vascular permeability, but it has been poorly studied for BBB. In our study we demonstrated the effect of endogenous hydrogen peroxide on the permeability of the in vitro blood-brain barrier (BBB) model. Endogenous hydrogen peroxide was generated using a chemogenetic generator - D-amino acid oxidase (DAAO). The DAAO was delivered to endotheliocytes and astrocytes. It was demonstrated for the first time that generation of hydrogen peroxide in individual cellular components of the BBB affects its permeability as a whole. The permeability of the model was determined by the diffusion rate of the fluorescent dye Lucifer yellow. It has been shown that the generation of endogenous hydrogen peroxide in both endotheliocytes and astrocytes increases the permeability of the model. After 24 hours of incubation with 5 mM D-alanine, in the case of peroxide generation in endotheliocytes, the permeability increased by 18%, and in the case of generation in astrocytes - by 36% compared to the initial value. After 48 hours of incubation with 5 mM D-alanine, the permeability was increased by 20% compared to the initial one both in the case of hydrogen peroxide generation in endotheliocytes and astrocytes.

Key words: blood-brain barrier · BBB model · multiple sclerosis.

Through the lens of science



Peter Sadler (KS)

Department of Chemistry, University of Warwick, Coventry, UK

Synthetic metal complexes for in-cell catalysis

We are exploring the use of synthetic transition metal catalysts that might carry out unusual reactions in cells and provide platforms for the design of novel drugs. Half-sandwich Ru(II) arene sulfonyldiamine complexes can reduce coenzyme NAD+ to NADH in cancer cells using non-toxic formate as a hydride source. We have introduced Os(II) analogues that are readily synthesized, more stable, and often more efficient 16-electron catalysts than Ru(II) analogues. DFT calculations for complexes with 5 chiral centres (2 chiral carbons on the N,N-chelated sulfonyldiamine ligand, the N,N-chelate ring, axial chirality of πbonded p-cymene, and metal chirality), have revealed the sequential transfer of hydride and then a proton to an acetophenone substrate (Fig.). These chiral Os(II) complexes catalyse conversion of pyruvate to unnatural D-lactate in cancer cells using formate as hydride source. Synchrotron x-ray fluorescence methods allow mapping of the catalyst in intact cancer cells. Half-sandwich Ir(III) Cp* catalysts can create oxidative stress in cells by converting NADH to NAD+, and octahedral Ir(III) photocatalysts can convert NADH to NAD radicals with high efficiency.

Key words: synthetic transition metal catalysts · NAD+ reduction · cancer therapy· Synchrotron x-ray fluorescence

Peter Sadler (KS)

Department of Chemistry, University of Warwick, Coventry, UK

Biography

Peter Sadler obtained his BA, MA and DPhil at the University of Oxford. Subsequently he was a Medical Research Council Research Fellow at the University of Cambridge and National Institute of Medical Research. From 1973-96 he was Lecturer, Reader and Professor at Birkbeck College, University of London, and from 1996-2007 held the Crum Brown Chair of Chemistry at the University of Edinburgh, and was also Director of the Edinburgh Protein Interaction Centre and EastChem Cancer Research UK Medicinal Chemistry Centre. In June 2007, he took up a Chair in Chemistry at the University of Warwick as Head of Department, where he is now a Professor. From 2010-15 he was a European Research Council Advanced Investigator, and from 2012-2015 Mok Hing Yiu Distinguished Visiting Professor in Chemistry at the University of Hong Kong. Peter is a Fellow of the Royal Society of Chemistry, the Royal Society of Edinburgh, and the Royal Society of London, and an EPSRC RISE Fellow (Recognising Inspirational Scientists and Engineers). He is also an Honorary Fellow of the Chemical Research Society of India, an Honorary Fellow of the Chinese Chemical Society, and a Fellow of the European Academy of Sciences

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Kenneth Witwer

John Hopkins University School of Medicine,
Baltimore, USA

Learning from viruses for EV functionalization

Cells release membrane-delimited particles into the environment. These particles are called “extracellular vesicles” (EVs), and EVs are present in fluids contacting cells, including body fluids and conditioned culture media. Because EVs change and contribute to health and disease, EVs have become a hot topic. From the thousands of papers now published on EVs annually, one easily gets the impression that EVs provide biomarkers for all diseases, and that EVs are carriers of all relevant biomolecules and are omnipotent therapeutics. At the same time, EVs are heterogeneous, elusive and difficult to study due to their physical properties and the complex composition of their environment.

This overview addresses the current challenges encountered when working with EVs, and how we envision that most of these challenges will be overcome in the near future. Right now, an infrastructure is being developed to improve the reproducibility of EV measurement results. This infrastructure comprises expert task forces of the International Society of Extracellular Vesicles (ISEV) developing guidelines and recommendations, instrument calibration, standardized and transparent reporting, and education. Altogether, these developments will support the credibility of EV research by introducing robust reproducibility, which is a prerequisite for understanding their biological significance and biomarker potential.

Key words: extracellular vesicles · disease biomarkers · omnipotent therapeutics

Kenneth Witwer

John Hopkins University School of Medicine,
Baltimore, USA

Biography

Kenneth Witwer's PhD dissertation research was on retroviruses and the innate immune system responses to pathogens such as Visna virus and simian immunodeficiency virus (SIV) as models of human immunodeficiency virus (HIV), specifically regulation of microRNAs, cytokines, and the promyelocytic leukemia protein (TRIM19). He then completed a postdoctoral research project on miRNAs as biomarkers of HIV disease. In 2011, Witwer joined the faculty at Johns Hopkins, and he assumed a tenure-track position in 2012. His primary appointment is in the Department of Molecular and Comparative Pathobiology. He has a secondary appointment in Neurology and Neurosurgery. He is a member of the Cellular and Molecular Medicine program and the Richman Family Precision Medicine Center of Excellence in Alzheimer's Disease at Johns Hopkins.

The Witwer laboratory studies the roles of EVs, exRNA, and ncRNA in HIV disease of the central nervous system and in other neurodegenerative diseases, such as Alzheimer's and Parkinson's. Another focus of the group is on how inflammatory insults like cigarette smoking affect progression of disease. Beginning in 2013, Witwer examined the hypothesis that RNAs such as miRNAs in dietary substances could regulate endogenous genes in mammals. These studies led him and others to the conclusion that this type of regulation is unlikely to occur in normal physiology. He subsequently served on two Scientific Advisory Panels of the US EPA and addressed the European Food Safety Authority on related questions of environmental exposure to RNA.

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Stephan Huveneers

Amsterdam UMC,
University of Amsterdam, the Netherlands

Junctional mechanotransduction in angiogenesis

Angiogenic sprouting depends on collective migration and coordinated rearrangements of endothelial leader and follower cells. VE-cadherin-based adherens junctions have emerged as key cell-cell contacts that transmit forces between endothelial cells and trigger signals during collective cell migration in angiogenesis. Previously, they have described dedicated molecular events that occur at VE-cadherin-based junctions during their force-dependent remodeling. These findings shed light on the role of cytoskeletal adapter proteins such as Vinculin and the membrane curvature-sensing F-BAR proteins in endothelial cell dynamics. Using live cell microscopy, they unraveled how such molecular signals guide the endothelial cells during collective migration and angiogenesis. Their recent findings substantiate the importance of Vinculin in strengthening of the endothelial barrier during vascular development. Moreover, they find that tensional forces propagate directional cues by polarized VE-cadherin trafficking to guide vascular development.

Key words: angiogenesis · sprouting · cell migration · endothelial barrier · tensile forces · vascular development.

Stephan Huveneers

Amsterdam UMC,
University of Amsterdam, the Netherlands

Biography

Dr. Stephan Huveneers studied at Leiden University, NL (PhD) and Netherlands Cancer Institute, NL and later at Hubrecht Institute, NL (Postdoc). In 2012 he became a Professor at Sanquin Research, NL and from 2016 to date, is Associate Professor of the Vascular Microenvironment and Integrity lab at Amsterdam UMC, University of Amsterdam.

The Huveneers lab investigates the role of molecular events that take place at endothelial adhesions within the context of the blood vessel wall. The response of the vascular endothelium to mechanical forces is a research area that is only at the start of its elucidation. Failure to respond to forces has direct consequences for blood vessel development and barrier function and underlies stiffness-related cardiovascular disease. The Huveneers lab studies the molecular and cellular mechanisms that are responsible for endothelial mechanotransduction responses through cell adhesion. Their recent studies have resulted in the identification and unraveling of at least two novel molecular systems in stiffness-related vascular function: these findings place DLC1 as a crucial and prominent direct target of the mechanotransducers YAP/TAZ in angiogenesis (van der Stoel et al, J. Cell Sci 2020) and vascular inflammation (Schimmel et al, Cell Rep 2018). In addition, they discovered a novel force-dependent Pacsin2-EHD4 junction complex which controls trafficking of VE-cadherin for endothelial barrier function and guides new vessels during angiogenesis (Dorland, Malinova et al, Nat. Commun., 2016, Malinova & Huveneers,Trends Cell Biol, 2018 and Malinova, Angulo-Urarte et al, Nat. Commun. in press).

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Raul Andino

University of California,
San Francisco, USA

SARS-CoV-2 adaptation to effective transmission and its replication in airway epithelia

Establishment of infection at the portal of entry is critical for virus replication, transmission and survival. Examining how viruses adapt to different host and physical environments could uncover principles of virus transmission and emergence. How SARS-CoV-2 penetrates the airway barrier of mucus and periciliary mucins to infect nasal epithelium remains unclear.

Using infection of primary nasal epithelial organoid cultures, we show that the virus attaches to motile cilia via the ACE2 receptor. SARS-CoV-2 traverses the mucus layer, using motile cilia as tracks to access the cell body. Depletion of cilia blocks infection for SARS-CoV-2 and other respiratory viruses. Using electron and immunofluorescence microscopy, we observe that SARS-CoV-2 progeny virions attach to airway microvilli 24 hours post infection and trigger formation of apically extended and highly branched microvilli that organize viral egress from the microvilli back into the mucus layer, supporting a model of virus dispersion throughout airway tissue via mucociliary transport. Phosphoproteomics and kinase inhibition reveals microvillar remodeling is regulated by PAK kinases. Importantly, Omicron variants bind with higher affinity to motile cilia and show accelerated viral spread in the epithelia.

Our work implicates motile cilia, microvilli, and mucociliary-dependent mucus flow are critical for efficient virus replication in nasal epithelia. Mathematical modelling explains why Omicron spread faster and has become the dominant global variant.

Key words: SARS-CoV-2 · nasal epithelium infection · motile cilia · ACE2 receptor · virus dispersion model.

Raul Andino

University of California,
San Francisco, USA

Biography

Raul Andino completed his master's degree in Biology in 1980 and his Ph.D. in Chemistry in 1986, both at the University of Buenos Aires. He then went on to work as a postdoctoral researcher first at the Whitehead Institute for Biomedical Research from 1986 to 1991, then at Rockefeller University in the lab of David Baltimore from 1991 to 1992. He then joined the faculty of the University of California, San Francisco as an assistant professor. He was promoted to associate professor in 1999, then full professor in 2003. Raul Andino's research has long focused on poliovirus. Together with Andrew Macadam, Andino redesigned the polio vaccine so it can stop the virus from re-evolving. His research has expanded to other enteroviruses and host defenses against other RNA viruses. His group has also had a long-standing interest in RNA interference as an antiviral defense, and in the dynamics of viral evolution during infection and transmission.

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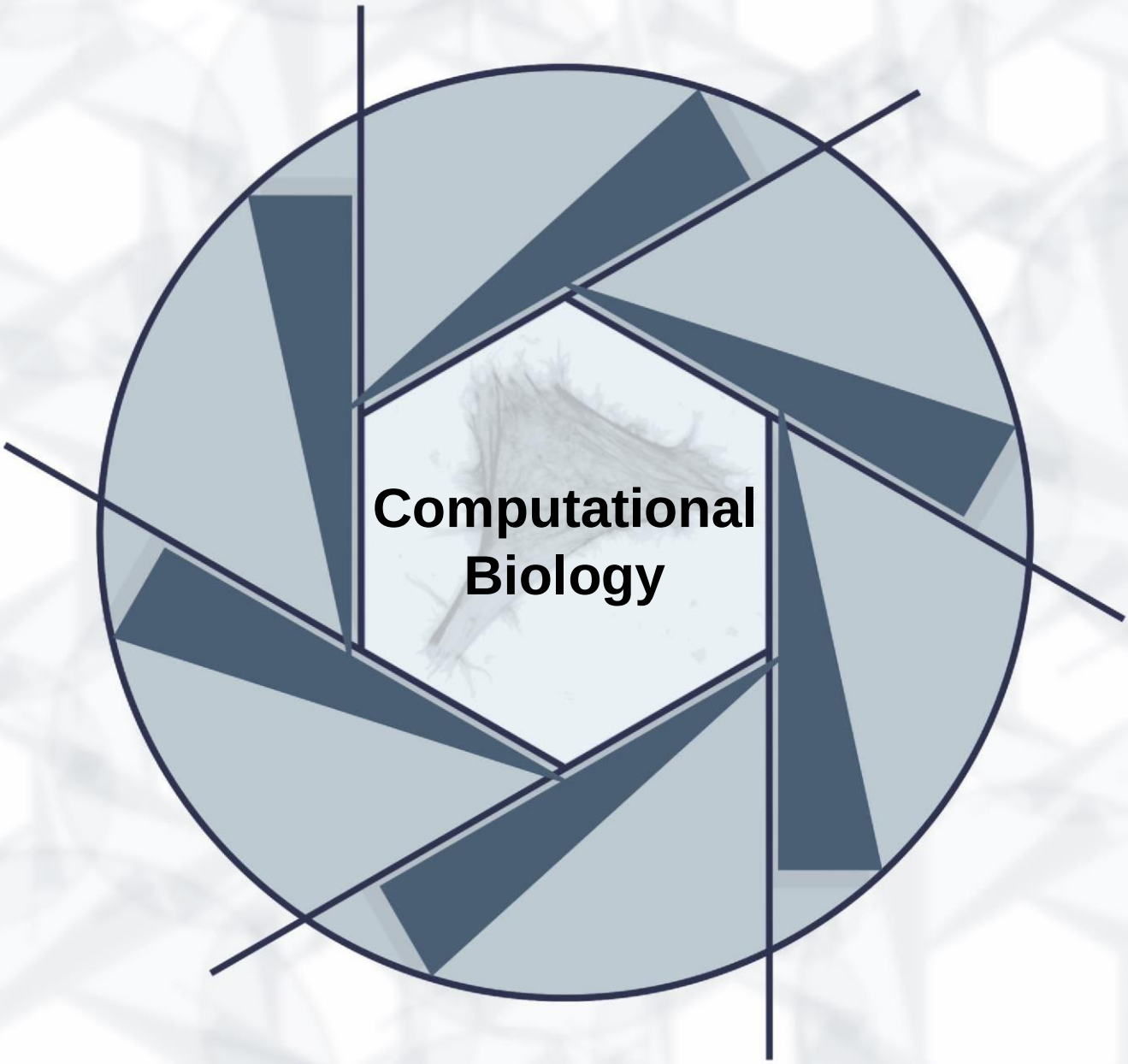
Through the lens of science



Adeel Razi

Institute for Brain and Mental Health
Monash University, Melbourne, Australia

Generative models of brain function: Inference, mechanisms, and psychedelics

The use of modelling to infer brain connectivity has become one of the most important themes in neuroimaging. Over the past decades generative models have superseded other modelling approaches to brain structure and function and represent one of the most promising avenues, offered by computational neuroscience. This talk will focus on the generative modelling of resting state time series or endogenous neuronal activity. I will survey developments in modelling distributed neuronal fluctuations – spectral dynamic causal modelling (DCM) for functional MRI – and how this modelling rests upon functional connectivity. In the second part of this talk, I will use DCM to test hypotheses of brain's functional reorganisation under psychedelics, informed by the accounts of hierarchical predictive processing. I will showcase a series of previous and new findings of how changes to synaptic mechanisms, under the control of serotonin receptors, across the brain hierarchy influence sensory and associative brain connectivity. Understanding these neural mechanisms of subjective and therapeutic effects of psychedelics is critical for rational development of novel treatments and for the design and success of future clinical trials.

Key words: brain · DCM · functional reorganisation · inference · psychedelics

Adeel Razi

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Monash University, Melbourne, Australia

Biography

Dr Adeel Razi is an Associate Professor at the Turner Institute for Brain and Mental Health, in the School of Psychological Sciences, Monash University, Australia. He joined Monash, after finishing his postdoctoral studies (2012-2018) at the Wellcome Centre for Human Neuroimaging, UCL, UK. His research is cross-disciplinary, combining engineering, physics, and machine-learning approaches, to model complex, multi-scale, network dynamics of brain structure and function using neuroimaging. He is currently an NHMRC Investigator (Emerging Leadership, 2021-2025), CIFAR Azrieli Global Scholar (2021-2023) in their Brain, Mind and Consciousness Program and was an ARC DECRA Fellow (2018-2021). He received the B.E. degree in Electrical Engineering (with a Gold Medal) from the N.E.D. University of Engineering & Technology in Pakistan, the M.Sc. degree in Communications Engineering from the University of Technology Aachen (RWTH), Germany, and the Ph.D. degree in Electrical Engineering from the University of New South Wales, Australia in 2012.

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Patrick Aloy

Structural Bioinformatics and Network Biology
IRB, Barcelona, Spain

Formatting biological big data to enable (personalized) systems pharmacology

Big Data analytical techniques and AI have the potential to transform drug discovery, as they are reshaping other areas of science and technology, but we need to blend biology and chemistry in a format that is amenable for modern machine learning. In this talk, I will present the Chemical Checker (CC), a resource that provides processed, harmonized and integrated bioactivity data on small molecules. The CC divides data into five levels of increasing complexity, ranging from the chemical properties of compounds to their clinical outcomes. In between, it considers targets, off-targets, perturbed biological networks and several cell-based assays such as gene expression, growth inhibition and morphological profiles. In the CC, bioactivity data are expressed in a vector format, which naturally extends the notion of chemical similarity between compounds to similarities between bioactivity signatures of different kinds.

We show how CC signatures can boost the performance of drug discovery tasks that typically capitalize on chemical descriptors, including compound library optimization, target identification and anticipation of failures in clinical trials. Moreover, we demonstrate and experimentally validate that CC signatures can be used to reverse and mimic biological signatures of disease models and genetic perturbations, options that are otherwise impossible using chemical information alone. Indeed, using bioactivity signatures we have identified small molecules able to revert transcriptional signatures related to Alzheimer´s disease in vitro and in vivo, as well as compounds against Snail1, a transcription factor with an essential role in the epithelial-to-mesenchymal transition, showing that our approach might offer a new perspective to find small molecules able to modulate the activity of undruggable proteins.

Key words: Big Data · systems pharmacology · chemical checker · Alzheimer's disease · Snail1

Patrick Aloy

Structural Bioinformatics and Network Biology
IRB, Barcelona, Spain

Biography

Dr Patrick Aloy is an ICREA Research Professor and Principal Investigator of the Structural Systems Biology lab at the IRB. He has a BSc in Biochemistry and a MSc in Biotechnology from the Universitat Autònoma de Barcelona, Spain, and spent six years as postdoctoral researcher and staff scientist at the European Molecular Biology Laboratory, Heidelberg, Germany. For twenty years, Dr Aloy has been developing and implementing new technologies and algorithms, applying state-of-the-art methods to specific problems and bridging the gap between theoretical models and experiments in different disciplines. The main goal of his lab is to combine molecular, cell and computational biology to unveil the basic wiring architecture and dynamics of physio-pathological pathways to increase our understanding of how biological systems change from the healthy state to disease. In the last years he has been developing resources to process, harmonize and integrate bioactivity data on small molecules, providing compound bioactivity descriptors that push the similarity principle beyond chemical properties. Currently, the main research line in the lab is to collect heterogeneous datasets and develop novel methodologies to integrate different layers of regulation to unveil disease signatures. Moreover, they are convinced that artificial intelligence (AI) will transform drug discovery, as it is reshaping other areas of science and technology, and biological signatures are the key to guide the (semi) automated design of chemical compounds to globally revert disease states, beyond individual targets.

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Alexandre Bonvin

Bijvoet Center for Biomolecular Research,
Utrecht University, Netherlands

Solving 3D puzzles of biomolecular assemblies by integrative modelling

The prediction of the quaternary structure of biomolecular macromolecules is of paramount importance for fundamental understanding of cellular processes and drug design. In the era of integrative structural biology, one way of increasing the accuracy of modelling methods is to include as much experimental or predictive information as possible in the process. We have developed for this purpose a versatile information-driven docking approach HADDOCK that can integrate information derived from a variety of biochemical, biophysical or bioinformatics methods to guide the modelling process. In my talk I will introduce this topic and illustrate it with various examples.

Key words: HADDOCK · integrative structural biology · bimolecular assemblies

Alexandre Bonvin

Bijvoet Center for Biomolecular Research,
Utrecht University, Netherlands

Biography

Alexandre Bonvin (1964) studied Chemistry at Lausanne University, Switzerland and obtained his PhD at Utrecht University in the Netherlands (1993). After two post-doc periods at Yale University (USA) and the ETHZ (CH) he joined Utrecht University in 1998 where he was appointed full professor of computational structural biology in 2009. In 2006, he received a prestigious VICI grant from the Dutch Research Council. He was director of chemical education from February 2009 until February 2012, vice head of the Chemistry Department from 2010 until April 2012 and since September 2019 Scientific Director of the Bijvoet Centre for Biomolecular Research. He is participating to several EU projects including the BioExcel Center of Excellence in Biomolecular Simulations and the European Open Science Cloud Hub and EGI-ACE projects. His work has resulted in over 250 peer-reviewed publications.

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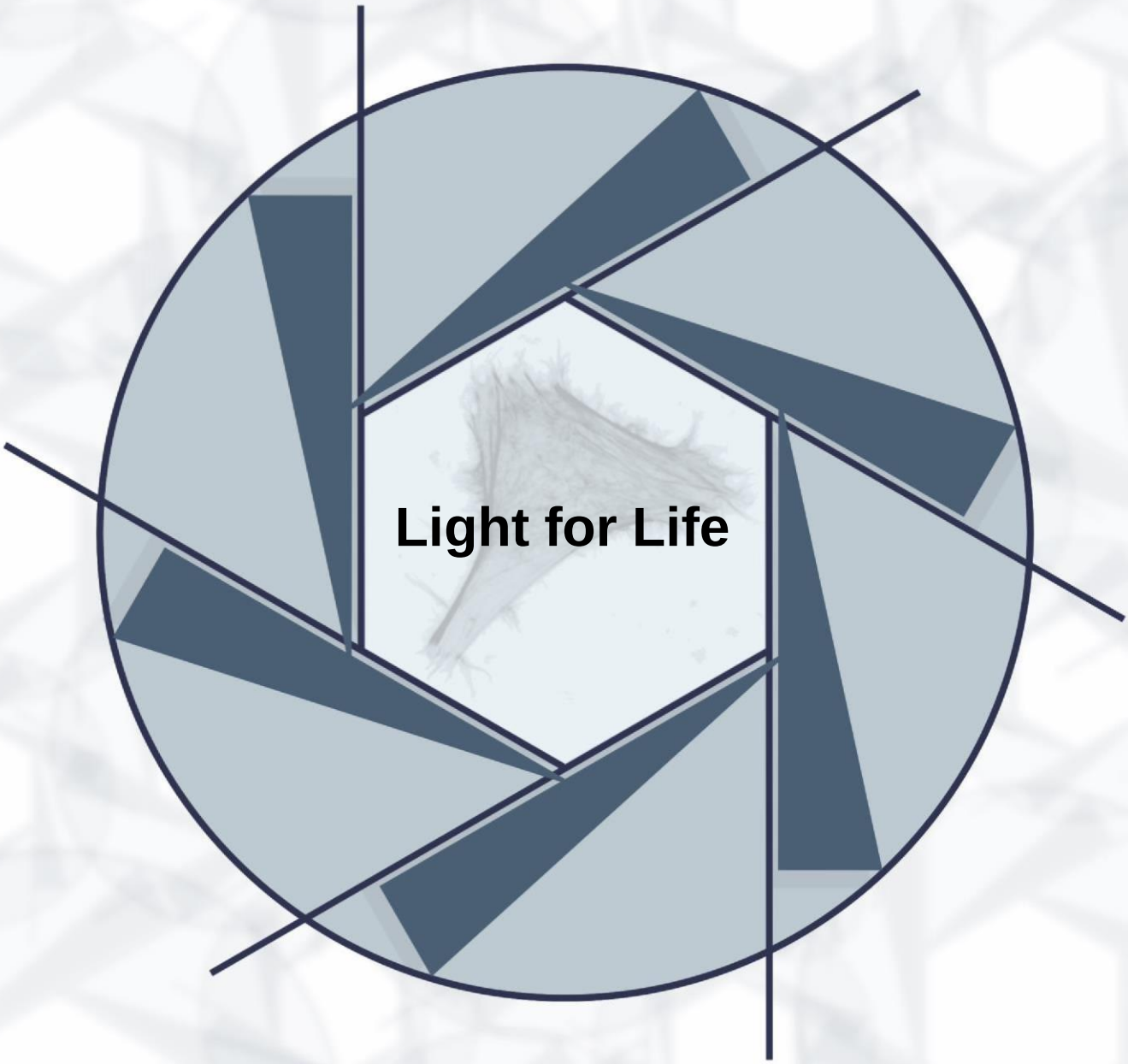
Through the lens of science



Pau Gorostiza

Nanoprobes and Nanoswitches Research Group,
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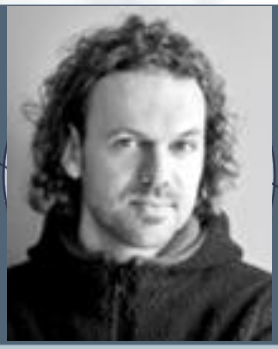

Controlling receptor activity with photoswitchable drugs: basic research and future therapies

The large number of photo switchable biomolecules discovered and developed in recent years covers a great variety of cellular functions like catalysis of metabolic processes, cytoskeletal polymerization and motors, nucleic acids dynamics, intracellular signaling and perhaps most dazzlingly membrane excitability, which has been at the focus of optogenetics and photo pharmacology. The dream of precisely and remotely photo controlling every aspect of the cell's workings in intact tissue appears within reach and offers the promise of interrogating complex cellular processes to discover their molecular mechanisms. Recent and ongoing projects at IBEC focused on photo pharmacology will be reviewed, including the development and applications of freely diffusible and tethered photo switchable ligands of ionotropic and G protein-coupled receptors. These molecular tools allow spatiotemporal control of endogenous proteins in single neurons, and of emerging activity in the brain, including cortical waves

Key words: scanning tunneling microscopy and spectroscopy · photoswitch · optogenetics · phototherapy.

Pau Gorostiza

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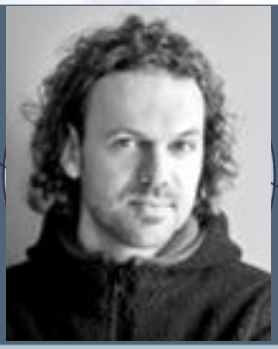

Biography

Pau Gorostiza graduated in physics at the University of Barcelona (UB), where he obtained his PhD (European Doctorate) in the field of semiconductor electrochemistry. He worked at the UB microscopy facility in AFM and STM of biological samples, and in nanotechnology for materials science. He visited the CNRS - Université Pierre et Marie Curie (France), and the University of California at Berkeley (USA). He is currently ICREA Research Professor at the Institute for Bioengineering of Catalonia, where he develops photoswitchable ligands of neuronal proteins and studies charge transport in redox proteins and photosynthetic complexes using EC-STM/AFM. He obtained a Human Frontier Science Program (HFSP) Career Development Award and two European Research Council (ERC) grants. He published more than 120 articles (4200 citations, h-index 33) and holds seven patents (five licensed). He has supervised 11 postdoctoral fellows and 13 PhDs.

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Elisabeth Kugler

Institute of Ophthalmology,
University College London, United Kingdom

How to Quantify A Zebrafish: Research Software for Image-Driven Biomedical Studies

With the advances in modern microscopy, imaging data are ever-increasing in spatial and temporal resolution. As a result, advanced microscopy methods produce large-sized datasets that are beyond subjective visual assessment. Additionally, image analysis is needed to convert images, ideally automatically and objectively, into reproducible knowledge.

As a showcase, I will talk about my PhD work from The University of Sheffield on light sheet fluorescence microscopy (LSFM) data of the zebrafish brain vasculature development. I will cover how the establishment of biomedical image analysis approaches requires cross-disciplinary approaches including data acquisition, understanding, processing, and interpretation. Studying the zebrafish brain using LSFM allowed novel insights into compounds affecting angiogenesis (https://doi.org/10.1242/dev.199720) as well as the discovery of a previously undescribed endothelial cell membrane behaviour.

Together, I will cover state-of-the-art biomedical image analysis approaches to visualize, quantify, and understand the biological processes.

Key words: light sheet fluorescence microscopy · computational modelling · zebrafish · cerebro-vascular Development.

Elisabeth Kugler

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University College London, United Kingdom

Biography

Dr Elisabeth Kugler is an award-winning scientist at the interface of biology, advanced microscopy, and biomedical image analysis. Her interests are to understand the basic cellular processes in neuroscience, with a particular focus on shape-associated functionality of cells. As University College London Research Fellow, she developed the 3D glia analysis tool GliaMorph and established an image-based computational model of retinal neurovascular unit development using advanced in vivo imaging data. For her PhD at the University of Sheffield, she developed ZVQ – an image analysis pipeline for the zebrafish brain vasculature. In addition to this, she characterised a previously undescribed cell membrane behaviour in brain vessels, termed kugeln. She is passionate about science communication, EDI (equality, diversity, and inclusion), and open science

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Christian Eggeling (KS)

Institute for Applied Optics and Biophysics,
Friedrich Schiller University Jena, Germany

Advanced optical microscopy studies of molecular membrane organizations

Molecular interactions are key in cellular signaling. They are usually ruled by the organization and mobility of the involved molecules. For example, the direct and non-invasive observation of the interactions in the living cell membrane is often impeded by principle limitations of conventional far-field optical microscopes, for example with respect to limited spatio-temporal resolution. Here, we present an advanced optical microscopy study involving tools such super-resolution STED microscopy in combination with spectral imaging and fluorescence correlation spectroscopy or single-molecule tracking on a MINFLUX and interferometric Scattering (iSCAT) microscope. We highlight how these approaches can reveal novel aspects of membrane bioactivity such as of the existence and function of potential lipid rafts.

Key words: Superresolution microscopy · single particle diffusion · membrane dynamics · protein-membrane interactions.

Christian Eggeling (KS)

Institute for Applied Optics and Biophysics,
Friedrich Schiller University Jena, Germany

Biography

Dr. Eggeling holds a PhD in Physics from the University of Göttingen, Germany, where he optimized single-molecule fluorescence detection. From 2000 to 2003 he was a research scientist at the biotech company Evotec, Hamburg, Germany, developing advanced fluorescence microscopy techniques for high-throughput drug screening. In 2003, Christian Eggeling joined the Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany as a senior scientist in the department of Professor Stefan Hell, the 2014 Nobel Laureate in Chemistry). Here, he was focused on the field of optical super-resolution microscopy, specifically the biological applicability of stimulated emission depletion (STED) microscopy.

Since 2012, Christian Eggeling has been a principal investigator in the Human Immunology Unit and the scientific director of the newly established Wolfson Imaging Centre Oxford at the Weatherall Institute of Molecular Medicine, University of Oxford, United Kingdom, and in 2014 he has been appointed Professor of Molecular Immunology. From December 2017 on he in addition started as a Professor of Super-Resolution Microscopy and director of the Institute of Applied Optics and Biophysics (IAOB) at the Friedrich Schiller-University Jena, and as the Head of the Department of Biophysical Imaging at the Leibniz Institute of Photonic Technologies in Jena, Germany. Christian Eggeling's research is focused on the development of advanced microscopy for the investigation of molecular organization and dynamics in cells, especially on the cellular plasma membrane, e.g. after infection or immune responses.

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Maximilian Rüttermann

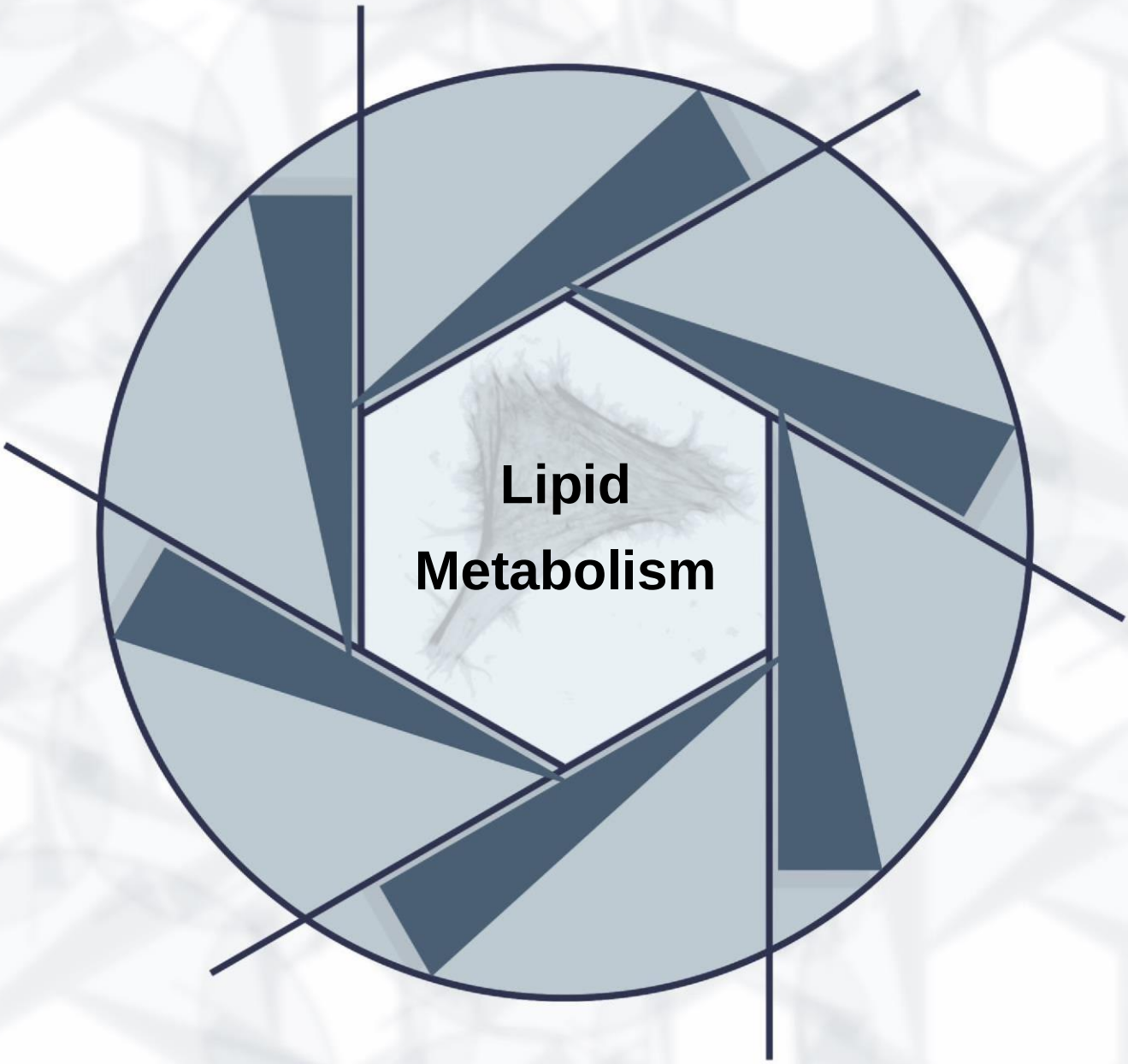
Institute for Medical Physics and Biophysics,
WWU Münster, Germany

CryoEM structure of the peroxisomal AAA-complex Pex1/Pex6 processing a substrate

The peroxisomal type II AAA+ complex, consisting of Pex1 and Pex6, is the central player of the peroxisomal exportomer and responsible for the ATP-dependent unfolding and processing of the peroxisomal receptor Pex5. Because recycling and membrane extraction of Pex5 is the only energy-consuming step and a prerequisite for stable and sustained import of peroxisomal enzymes into the matrix, it has been suggested that the import pathway might be driven by an ATP-dependent receptor export mechanism. Moreover, most peroxisomal biogenesis disorders, such as Zellweger syndrome, are associated with defects or mutations of the Pex1/Pex6 AAA complex. Despite its pathological importance and critical actions, high resolution structural and mechanistic insights are still lacking. Here we present the cryoEM structure of the peroxisomal AAA complex Pex1/Pex6 at 3.9 Å resolution in complex with an endogenous substrate. The structure deciphers the mechanism of substrate processing through the central pore via a staircase-like arrangement of the D2 pore loops in an ATP-dependent manner. Further interdomain communication of Pex1(D2) with Pex6(N1, D1) and Pex6(D2) with Pex1(D1) reveals a potentially unique mechanism for transferring mechanical forces from an ATPase-active D2 ring to the inactive D1 ring and a complex interplay between Pex1 and Pex6.

Key words: structural biology · cryoEM · Pex1/Pex6 · mechanical forces.

Through the lens of science



William Prinz

National Institute of Diabetes and Digestive and Kidney Diseases (NIH), Bethesda, Maryland, USA

Lipid transport at contact sites by tube-forming lipid transport proteins

Lipid transport proteins facilitate lipid exchange between organelles, often at sites where organelles are closely apposed called membrane contact sites. It had been thought that all lipid transport proteins in eukaryotes functioned as shuttles that bind lipids in one membrane and bring them to a second. However, recent studies suggest that some lipid transport proteins use an entirely different mechanism: they bind two membranes simultaneously and allow lipids to flow between membranes. We have been investigating the role of this type of lipid transport protein in lipid metabolism and homeostasis. We find a role for them in modulating glycerophospholipid metabolism in response to stress and in supporting synthesis of glycosylphosphatidylinositol (GPI) anchors in the ER. I will discuss recent insights into how tube-like lipid transport proteins regulate lipid metabolism and organelle biogenesis.

Key words: lipid transport · contact sites · organelle biogenesis · lipid metabolism

William Prinz

National Institute of Diabetes and Digestive and Kidney Diseases (NIH), Bethesda, Maryland, USA

Biography

- Editorial Boards: The Journal of Cell Biology, Developmental Cell, The Journal of Biological Chemistry, Contact
- Senior Investigator, 2008-present
- Tenure-Track Investigator, 2001-2008
- Postdoctoral Fellow, Harvard University, advisor, Tom Rapoport, 1996-2001
- Ph.D., Harvard University, 1996

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Mike Henne

Department of Cell Biology,
UTSW Medical Center University, Dallas, USA

Dissecting the biophysical phase properties of cellular lipid storage

To persist in a constantly changing environment, cells adapt by storing excess energy in the form of neutral lipids within unique cytoplasmic organelles termed lipid droplets (LDs). Observed for over a century but generally ignored, LDs are now appreciated as centers of metabolic signaling and key facilitators of cellular homeostasis. LDs generally contain triglycerides (TG) as well as sterol-esters (SEs), but how each of these neutral lipids is delivered to and organized within LDs remains poorly understood. During my seminar, I will discuss the biophysical properties of LDs, and dissect how cellular metabolic stress can drive lipid phase transitions within LDs, creating smectic liquid-crystalline LDs. I will discuss the mechanisms governing this liquid-crystalline phase transition, as well as how alterations in the lipid phase properties of LDs influence organelle protein targeting and the LD proteome. Lastly, I'll discuss how proper LD storage and adipocyte cell size influence whole-organism development, using Drosophila as a genetic model system.

Key words: lipid droplet · organelles · lipid storage · lipid phase transitions · drosophila

Mike Henne

Department of Cell Biology,
UTSW Medical Center University, Dallas, USA

Biography

I am an Associate Professor and Endowed Scholar of Cell Biology in the Dept of Cell Biology, UTSW. I attended Cambridge University, UK for my graduate training where I dissected the roles of membrane sculpting F-BAR proteins in endocytosis. During my post-doc with Scott Emr (Cornell University, US) I dissected how the ESCRT pathway generates multi-vesicular bodies through membrane remodeling. My lab at UTSW is interested in how cells spatially organize their metabolism. We are also interested in how cells make and organize fat, and how lipid droplets enable cells to adapt to metabolic challenges such as nutrient stress or development.

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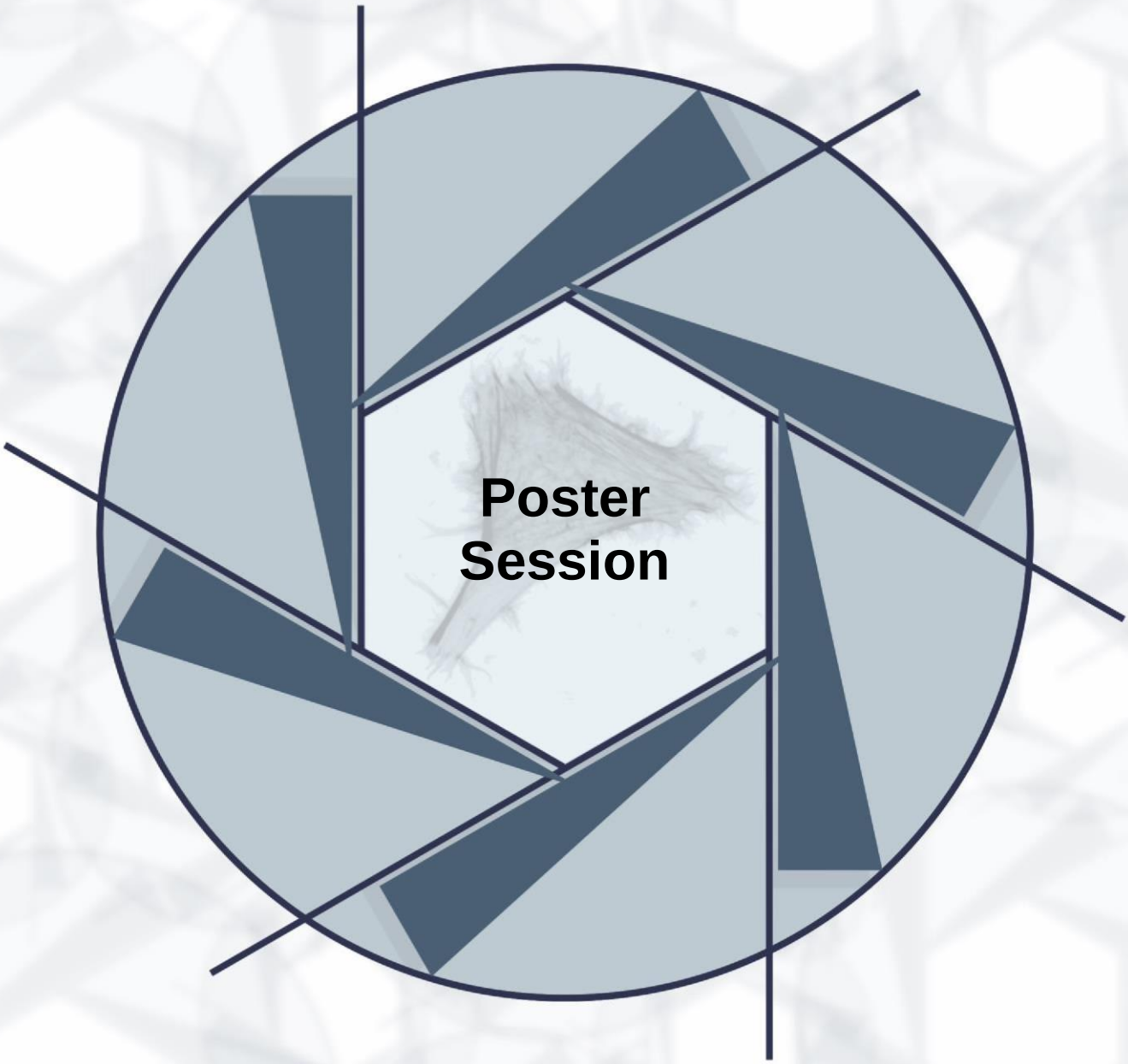
Through the lens of science



Aniket Bandyopadhyay[1], Sarah Weischer[2], Annegret Elting[1], Christian Schuberth[1], Roland Wedlich-Söldner[1]

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[2] Imaging Network, University of Münster, Von-Esmarch-Str. 56, 48149 Münster, Germany

Understanding the functional relevance of lateral segregation and distribution of Pma1 on yeast plasma membrane

Functional significance of compartmentalization, distribution, lateral mobility and abundance of proteins on the plasma membrane is not completely understood. Here, in my project we are dealing with a physiologically vital and relatively unexplored sub domain on the yeast plasma membrane i.e., Membrane Compartment occupied by Plasma Membrane ATPase1 or Pma1 (MCP) with an overarching aim to understand whether membrane localization of Pma1 has any impact on its function. Pma1 is the major proton pump on the yeast plasma membrane and is responsible for maintenance of electrochemical gradient across the plasma membrane. With the help of TIRFM as an imaging modality, we have identified another candidate membrane protein, Mrh1, which has been found to greatly impact the distribution of Pma1 on yeast plasma membrane upon prolonged glucose starvation. Both the proteins also have the tendency to colocalize both in normal as well as in starved condition. Our present investigation is mainly focused on whether this change in distribution of Pma1 has any physiological significance. To this end, with the help of cytosolic pH sensitive fluorescent probes, we are trying to decipher whether this phenomenon has any role to play in maintaining cytosolic pH homeostasis. Additionally, our preliminary results have suggested that there is a physical interaction between C terminals of both the proteins. We are presently working to further characterize this interaction with the help of molecular biology techniques and also live cell super-resolution imaging techniques.

Elena Bekker[1], Dustin Dzikonski[1], Riccardo Zamboni[1], Cornelia Denz[1]

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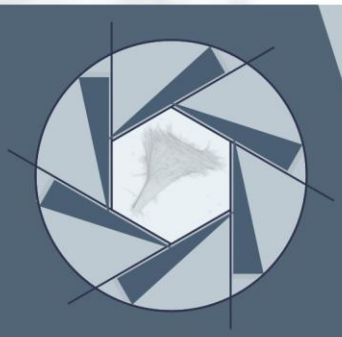


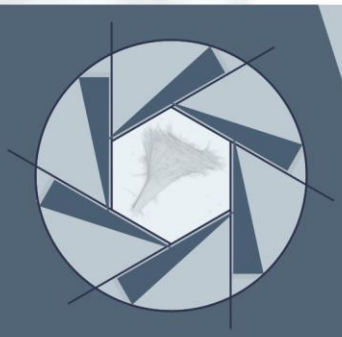
Two-photon polymerization inside microfluidic devices for confined cell migration investigation

Cell and tissue cultures in 2D microenvironments often fail to accurately demonstrate the mechanics, activities, and physiological response of in vivo tissue. Therefore, a remaining challenge in tissue engineering is the simulation of the 3D microenvironment of cells containing a mixture of soft and rigid materials that are flexible and dynamic. Microfluidic platforms provide these conditions to some extent, as they offer a 3D culture environment with the ability of exposing the cells to fluid flow but lack complex architectures and varying mechanical properties. By combining additive manufacturing of soft biomaterials such as hydrogels with microfluidics, this limitation can be overcome. In this project, an integrated platform of two- photon polymerization inside microfluidic devices is realized, to investigate the biomechanical aspects of confined cell migration during cancer metastasis and immune response.

Fei Chen[1], Jan Bruder[1], Jie Wu[2], Manuel Koesters[2], Martin Stehling[1], Hannes Drexler[1], Rui Fan[1], Sebastian Leidel[2], Hans Schöler[1] and Ivan Bedzhov[1]

[1] Max Planck Institute for Molecular Biomedicine

[2] University of Bern

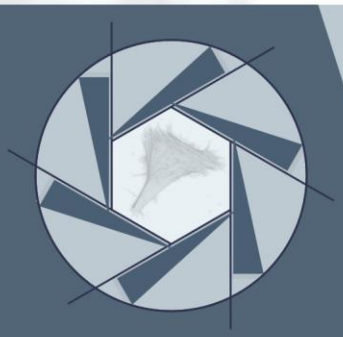


Identification of novel factors required for pluripotent epiblast cells transition during implantation via large scale functional screening

At blastocyst stage the mouse embryo reaches the uterus and initiates implantation. Within 24 h the hollow shaped embryo radically changes to a tube-like conceptus (egg cylinder). Meanwhile, the transiently generated naïve pluripotency is dismantled and the epiblast cells transform into a more developmentally advanced post-implantation pluripotent state. The implantation process is critical for following embryonic development. However, the mechanisms mediating the reorganization of morphology and transcriptional network remain largely unexplored as the maternal tissues conceal the embryo. Here we established an in vitro model by culturing embryonic stem cells in matrigel. By combining transcriptomics and quantitative proteomics, we revealed the transition of epiblast cells can be recapitulated faithfully. Using this model, we performed a functional screening of over 1800 chemicals to identify factors required for pre- to post-implantation embryogenesis shift. We discovered inhibiting protein kinase (PK) X delay cells exit of naïve pluripotency and this effect is even stronger in combination with Mek1/2 inhibitor PD032. Combined inhibitors treated cells are more similar to naïve pluripotent cells compared with single inhibitor treated cells.

Furthermore, the compromised cell survival caused by PK X inhibition can be compensated by PD032. Newly ES cell lines can be derived from embryo successfully and be cultured for > 20 passages with the two inhibitors, even though less efficiently than 2iLif. To unravel the mechanism, we found PK X and Mek1/2 substrate, p-ERK1/2 co-localize which provides a platform for their interaction. By conducting Co-IP, more PK X and ERK1/2 form a complex in naïve pluripotent cells. This hits PK X may compete with Mek1/2 in binding with Erk1/2 in different pluripotency state thus playing a role in regulating pluripotency via repressing MAPK signaling pathway. Together, our findings demonstrate PK X-Erk signaling in the regulation of cell proliferation and pluripotency transition during implantation.

Katharina Uphoff, Irina Suárez, Yvonne Huisman,
Andreas van Impel, Stefan Schulte-Merker



Dab2 is required for the scavenging function of brain lymphatic endothelial cells

The lymphatic system is essential for maintaining fluid homeostasis, transporting immune cells and absorbing dietary fats in the intestine (González-Loyola & Petrova, 2021). To maintain fluid homeostasis, the lymphatic network takes up interstitial fluid from tissues and directs it back to the venous blood circulation and therefore lymphatic vessels also contribute to waste removal. How the brain is cleared from waste generated by its high metabolic activity is, however, still under investigation and is particularly important for the understanding of neurodegenerative disease such as Alzheimer's disease.

Recently, the topic has risen lots of interest due to the discovery of lymphatic vessels in the meninges of mice, human and zebrafish (Aspelund et al., 2015; Castranova et al., 2021; Louveau et al., 2015) and furthermore, due to the description of lymphatic endothelial cells (LECs) with scavenging function in the zebrafish meninges, known as brain LECs (BLECs), mural LECs (muLECs) and fluorescent granular perithelial cells (FGPs) (Bower et al., 2017; Galanternik et al., 2017; van Lessen et al., 2017). BLECs retain a single cell status with a high endocytic activity and take up substances from cerebrospinal fluid in lysosomal vesicles. Thus, BLCEs contribute, together with microglia, to tissue homeostasis in the central nervous system whereby they are more efficient than microglia (Huisman et al., 2022; van Lessen et al., 2017). It has previously been shown that the adaptor protein Disabled homolog 2 (Dab2) plays an important role in Clathrin-mediated endocytosis (Tao et al., 2016). Furthermore, it was reported that DAB2 binds VEGFR3 upon activation by VEGF-C and that, in Dab2 knocked-down ECs, VEGFR3 internalization is impaired (Nakayama et al., 2013). We have shown that in zebrafish, zygotic dab2 is not required for the survival of embryos and dab2 mutants can therefore be employed to study lymphatic or vascular defects. Furthermore, we found dab2 to be specifically expressed in BLECs and we are currently assessing whether dab2 mutants show altered internalization of specific substances injected into the cerebrospinal fluid. The precise function of BLECs and the question of how they work together with other known clearance mechanisms is still under investigation, and we will present an update of these studies at the meeting.

Lakshmi Penothil Sunil, Sai Krishnan, and Jürgen Klingauf

Institute of Medical Physics and Biophysics, University of Münster

Adaptor protein-mediated release site clearance during compensatory endocytosis

Synaptic vesicles (SVs) are released at active zones (AZ), and SV constituents are retrieved by compensatory endocytosis at distinct sites outside the AZ, the peri-active zone (periAZ). For repeated neurotransmission sorting of SV components from the AZ to the periAZ, so-called release site clearance (RSC), is necessary. Although various mechanisms of RSC have been proposed, involving passive diffusion and active chaperoning of SV components, the small size of vertebrate synaptic boutons near the diffraction limit has hampered direct observation of the spatio-temporal dynamics of RSC.

We used ‘xenapses’, purely presynaptic boutons directly formed on micropatterned and functionalized coverslips, to overcome this limitation. Xenapses provide an advantage over conventional cultures owing to their size, TIRF amenability and the absence of apposed post-synapses, rendering them better suited for super-resolution microscopy. The presence of distinct AZ and periAZ in xenapses was exploited to study sorting of one of the most abundant SV components, Synaptobrevin2 (Syb2) by its cargo-specific adaptor, AP180. Combining super-resolution, TIR-FRAP and live TIRF microscopy, we revealed a population of AP180 stably enriched at the periAZ at rest. Upon stimulation, free AP180 from the cytosol translocates first to the AZ, and later along with exocytosed Syb2 to the periAZ. This AP180-mediated RSC is abolished in AP180 mutants with reduced affinity for Syb2.

These data confirm previous biochemical data and corroborate the notion of AP180 as an important RSC factor. Our xenaptic system provides a platform for visualizing molecular events during RSC and exo-endocytosis coupling.

Marie Hugenroth[1,2], Muriel Mari[3], Maria Bohnert[1,2]

[1] Institute of Cell Dynamics and Imaging, University of Münster, Münster, Germany

[2] Cells in Motion Interfaculty Centre (CiM), University of Münster, Germany

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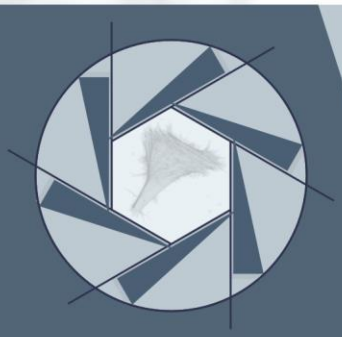

Pex31 – A new player in lipid droplet biogenesis

Cells need to balance membrane expansion with lipid storage in lipid droplet (LDs) to adapt to changing physiological parameters. The lipin Pah1 mediates the conversion of phosphatidic acid to diacylglycerol at the endoplasmic reticulum (ER) membrane, thus acting at the crossroads of phospholipid and triacylglyerol synthesis. Mutants lacking Pah1 have few LDs and an expanded, misshaped ER. We performed a microscopy-based genetic screen for factors implicated in the ∆pah1 phenotype, and found that loss of Pex31, a reticulon homology-domain protein of the ER membrane, restores LD formation, ER morphology, and cell growth.

Intriguingly, LDs of ∆pah1∆pex31 cells store mainly sterol esters, indicating that PEX31 deletion bypasses rather than directly counteracts defects in triacylglycerol storage. By abolishing sterol ester synthesis, rescue of ER morphology can be genetically uncoupled from LD formation, suggesting that ER restoration occurs upstream to LD formation. Beneficial effects of PEX31 deletion depend on the presence of Pex30, an ortholog of Pex31. Our results suggest a functional interplay of two structurally related proteins in modulation of ER membrane properties and lipid storage, opening a door to a better understanding of cellular lipid homeostasis.

Maximilian Rüttermann[1,2], Michelle Koci[3], Pascal Lill[1,2,3], Ralf Erdmann[4], Christos Gatsogiannis[1,2,3]

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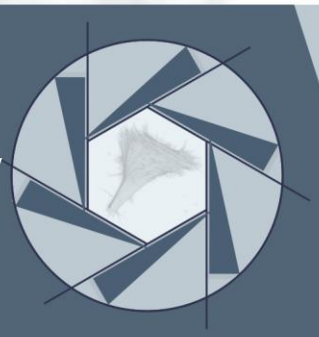
[3] Dept. of Structural Biochemistry, MPI of Molecular Physiology, Germany.
[4] Institute for Biochemistry and Pathobiochemistry, Department of Systems Biochemistry, Ruhr-University Bochum, Germany



CryoEM structure of the peroxisomal AAA-complex Pex1/Pex6 processing a substrate

The peroxisomal type II AAA+ complex, consisting of Pex1 and Pex6, is the central player of the peroxisomal exportomer and responsible for the ATP-dependent unfolding and processing of the peroxisomal receptor Pex5. Because recycling and membrane extraction of Pex5 is the only energy-consuming step and a prerequisite for stable and sustained import of peroxisomal enzymes into the matrix, it has been suggested that the import pathway might be driven by an ATP-dependent receptor export mechanism. Moreover, most peroxisomal biogenesis disorders, such as Zellweger syndrome, are associated with defects or mutations of the Pex1/Pex6 AAA complex. Despite its pathological importance and critical actions, high resolution structural and mechanistic insights are still lacking. Here we present the cryoEM structure of the peroxisomal AAA complex Pex1/Pex6 at 3.9 Å resolution in complex with an endogenous substrate. The structure deciphers the mechanism of substrate processing through the central pore via a staircase-like arrangement of the D2 pore loops in an ATP-dependent manner. Further interdomain communication of Pex1(D2) with Pex6(N1, D1) and Pex6(D2) with Pex1(D1) reveals a potentially unique mechanism for transferring mechanical forces from an ATPase-active D2 ring to the inactive D1 ring and a complex interplay between Pex1 and Pex6.

Mehmet Erguven[1,2], Nicolas V. Cornelissen[1], Aileen Peters[1], Ann-Marie Lawrence[1], Ezgi Karaca[3,4], Andrea Rentmeister[1,2]

[1] Dept. of Chemistry and Pharmacy, Institute of Biochemistry, Münster, Germany
[2] Cells in Motion Interfaculty Center, University of Münster, Germany
[3] Izmir Biomedicine and Genome Center, Izmir, Turkey
[4] Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Turkey

Substrate scope of Methanocaldococcus jannaschii MAT unveils ATP analogues suitable for MAT-MTase cascade applications

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 substrates. However, MTase promiscuity poses a problem for such applications in living systems. For this reason, we have recently focused on nucleoside modifications of the MTase cosubstrate, AdoMet, aiming to achieve selective labeling. In the present study, we further explored this option by expanding our set of ATP analogues, testing a different set of methyltransferases (NovO, RnCOMT-var, and GlaTgs2-var), and by using a thermostable MAT from *Methanocaldococcus jannaschii* (MjMAT-var) that is highly active at 37°C and can accept bulky N^6 base modifications. By using bulky N^6 base-modified ATP analogues as the starting material, we achieved MTase selectivity to a degree in vitro. However, despite the phenomenon of MTase promiscuity for sulfonium-center modifications, to our surprise, use of different transferable groups (methyl, *ortho*-nitrobenzyl, or propargyl) has a remarkable impact on the substrate preference. Importantly, selectivity achieved by N^6 base modifications was greatly affected by the presence of different transferable groups, in an unpredictable manner. We believe that our results will encourage further research aiming to achieve orthogonality in MTase-based biomolecular labeling, by using double-modified AdoMet analogues.

Min Xia, Keisuke Shirakura, Stefan Butz, Dietmar Vestweber

MPI for Molecular Biomedicine, Vascular Cell Biology Department, Röntgenstraße 20, 48149, Münster, Germany

Plasticity of the leukocyte transmigration route in different tissues

Leukocyte transmigration from the blood circulation into tissues is an important step during inflammation. Blood leukocytes transmigrate either directly through individual endothelial cells (ECs), which is known as transcellular route, or between endothelial junctions, which is known as paracellular route. VE-cadherin plays an essential role in controlling endothelial junction integrity. Previously, we observed that stabilizing endothelial junctions through replacement of VE-cadherin with VE-cadherin-α-catenin (VECαC) inhibited leukocyte transmigration in the inflamed cremaster, skin and lung in vivo. However, this inhibitory effect was rather absent in the inflamed peritoneal cavity. Therefore, we aimed to identify the factors which govern different routes for leukocyte transmigration in different organs.

Here, based on intravital video microscopy, we show that the paracellular route is the dominant way for leukocyte extravasation in WT mice, with up to 86% in the cremaster muscle and 87 % in the omentum. However, transcellular migration strongly increased from 13 % to 28 % in the omentum when endothelial junctions were stabilized (VECαC mice), whereas the percentage of transcellular migration did not change in the cremaster of these mice. Furthermore, we noticed changes in the morphology of ECs expressing VECαC in omentum but not in cremaster. Additionally, we investigated the expression of proteins which could play a role in transcellular migration via whole mount staining. We observed that ICAM-1 and ACKR-1 have different expression patterns in cremaster and omentum.

Together, we propose that the higher plasticity of leukocyte transmigration is the reason why stabilizing endothelial junctions does not inhibit leukocyte extravasation in the omentum. Insights into the mechanism behind this greater plasticity would allow us to develop new targets of anti-inflammation in certain organs where transcellular migration might be relevant.

Mirsana Ebrahimkutty[1,2,3], Junxiu Duan[1,2,3], Jürgen Klingauf[1,2], Milos Galic[1,2]

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[2]Institute of Medical Physics and Biophysics, University of Muenster, Germany
[3]CiM-IMPRS Graduate Program, Muenster, Germany

Nanopatterned substrates for parallelized investigation of curvature-dependent signaling

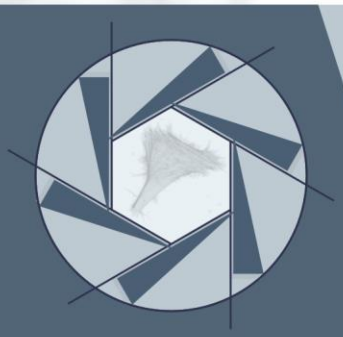
Signaling across the plasma membrane enables cells to sense and respond to changes in their physical environment. This information exchange across the membrane is dictated not only by the transmembrane proteins that traverse it, but recent advances suggest that membrane parameters such as membrane geometry by itself can also play a role. The membrane geometry triggers a local signaling cascade with the recruitment of a group of proteins that, among other roles, also function as actuators of cytoskeletal remodeling.

One such protein family are the I-BAR domain proteins, which share a characteristic crescent shape that can sense outward membrane deformations. Though much is known about the downstream signaling and functional relevance of I-BAR domain proteins, the precise effect of curvature in the recruitment and activation of these proteins at protruding sites stays largely unexplored in living systems. This is partly due to the lack of techniques that allow precise induction of negative curvature in the physiological curvature sensing regimes of these proteins in live cells. Here, we use custom patterned nanostructures to create defined negative curvatures in the regime of protruding structure present in the cells. We show that different I-BAR domains enrich at these curved sites. Furthermore, our data indicates that these sites may serve as active actin-regulatory hubs.

Collectively, our lab-on-a-chip platform for parallelized investigation of curvature dependent recruitment of proteins in cells suggests the actuation of signaling hubs at negatively curved membrane sites.

Sarah Weischer, Jens Wendt, Thomas Zobel

Münster Imaging Network, Cells in Motion Interfaculty Centre



A FAIR place for your image data

In life science, light microscopy has been a valuable tool for various research areas which delivers multidimensional data providing major insights into the biology of molecules, cells, and whole organisms. Bioimaging data should be handled according to the FAIR principles, ensuring the data is findable, accessible, interoperable and reusable. To comply with the FAIR principles image data should be enriched by appropriate metadata. The required metadata concerns different aspects, such as technical metadata about microscope configurations during image acquisition (e.g., laser lines & detector settings), experimental metadata (e.g., tissue or cell type, fluorescent proteins, antibodies & fixation methods), and analysis metadata (e.g., software, analysis tools & analysis parameters).

Several consortia have published guidelines about metadata for biological images to advance quality assessment and reproducibility. Here, we present the minimal annotation standard for microscopy images based on the community-driven recommendations which are summarized in the “Recommended Metadata for Biological Images (REMBI)”. We focus on the practical aspects on how to find and document image metadata during sample preparation, image acquisition and data analysis. To facilitate this documentation in practice, we introduce tools for easy and efficient image data and metadata management using the image database OMERO. OMERO can be interactively accessed by several image analysis programs (Fiji, QuPath, Python) allowing direct injection of images into data analysis pipelines. We present ways for automated data analysis and upload of the image and analysis results. We furthermore showcase how image data can be published directly from OMERO and can be uniquely identified using DOIs. In summary, we would like to give best-practice advice for early career researchers and introduce tools for efficient data management and image analysis that are available at the Münster Imaging Network.

Suganja Sivaloganathan[1,2], Raquel Blazquez[3], Tobias Pukrop[3], Darius Wlochowitz[4], Tim Beißbarth[4], Simone König[2,5], Uwe Hansen[6], Matthias Schulz[7], Claudia Binder[7], Kerstin Menck[1,2], Annalen Bleckmann[1,2,7]

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[3]Dept. of Internal Medicine III, Hematology and Medical Oncology, University Hospital Regensburg, Regensburg, Germany
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[5]IZKF, Core Unit Proteomics, University of Münster, Germany
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Tumor-derived microvesicles are a critical determinant for successful metastatic colonization in colorectal cancer

The formation of distant metastases is the major obstacle in treating cancer. Metastatic spread requires a cancer-induced modulation of the surrounding environment, which can be mediated via the release of tumor-derived extracellular vesicles (T-EV). Using a syngeneic mouse model for colorectal cancer (CRC) metastasis, we identified two variants of the murine CRC cell line CMT93, which differed significantly in their metastatic colonization in vivo. The aim of this study was to identify the molecular mechanisms responsible for the observed effect. The two CMT93 variants were characterized regarding their basic characteristics using proliferation, invasion, migration and adhesion assays. T-EV released by the cells were collected via differential ultracentrifugation at 17,000 g for larger microvesicles (MV) and 143,000 g for small EVs. The obtained EV were characterized by immunoblotting, nanoparticle tracking analysis, transmission electron microscopy and proteomics.

Analysing the cell-intrinsic basic characteristics of the cells revealed no major difference between the two CMT93 variants. Thus, we extended our analysis to the released secretome, which revealed a major difference in the amount and composition of released T-EV. While T-EV released by the malignant CMT93 variant were able to induce tumor invasion, this effect was not observed with T-EV from the less aggressive CMT93 variant. Intriguingly, this functional difference was more prominent with the plasma membrane-derived MV than with small EV. To identify the molecular characteristics responsible for this difference, the MV released by both CMT93 variants were compared by proteomic analysis. The results revealed that the MV released by the malignant CMT93 variant were enriched in proteins associated with adhesion. Using immunoblotting, we were able to validate an upregulation of the proteins Itga3 and Fascin1 on MV released by the malignant variant. Analysing microarray data, we identified that these proteins were upregulated in human primary and metastatic CRC in contrast to normal colon tissue, suggesting the translational relevance of our findings.

Conclusively, we identified MV as the critical determinants for successful tumor invasion in CRC and attributed this effect to the enrichment of tumor-supporting adhesion proteins on the vesicles. These results shed further light on the molecular mechanisms underlying EV-mediated metastatic colonization and open new options for targeted therapy.

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