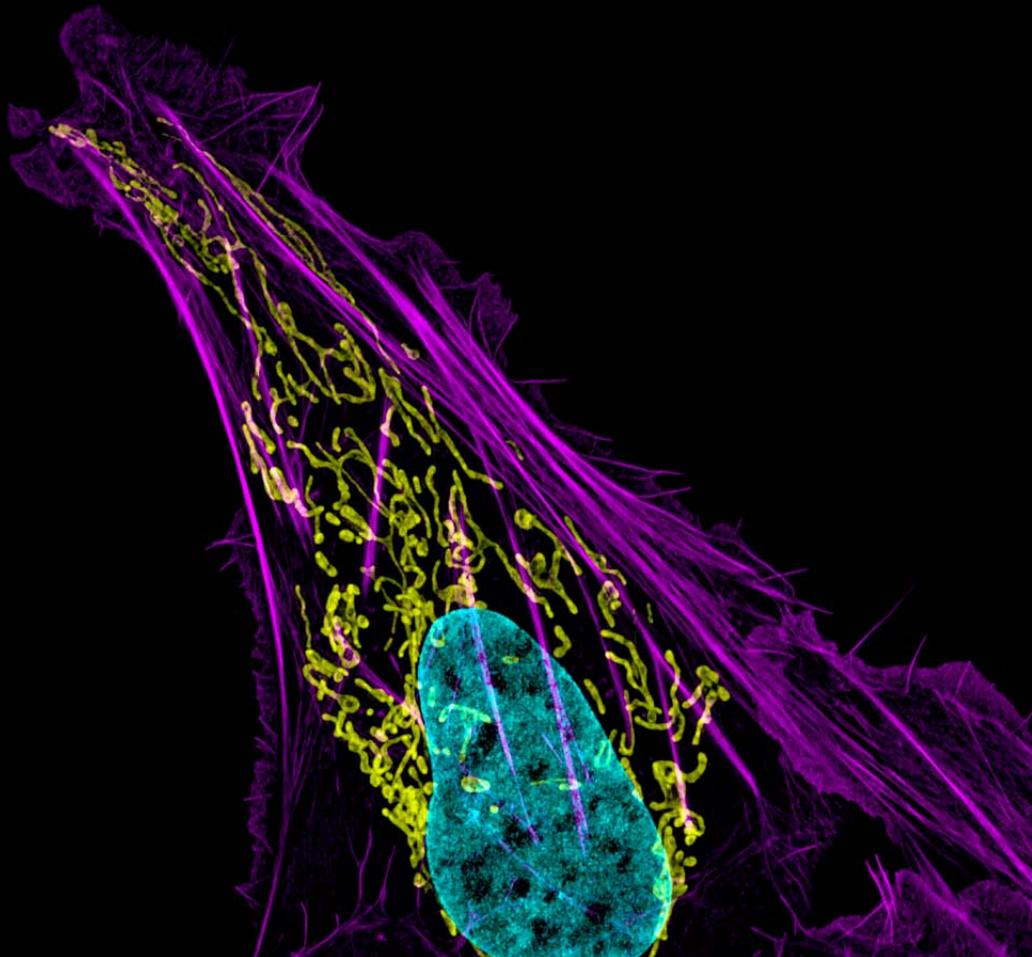


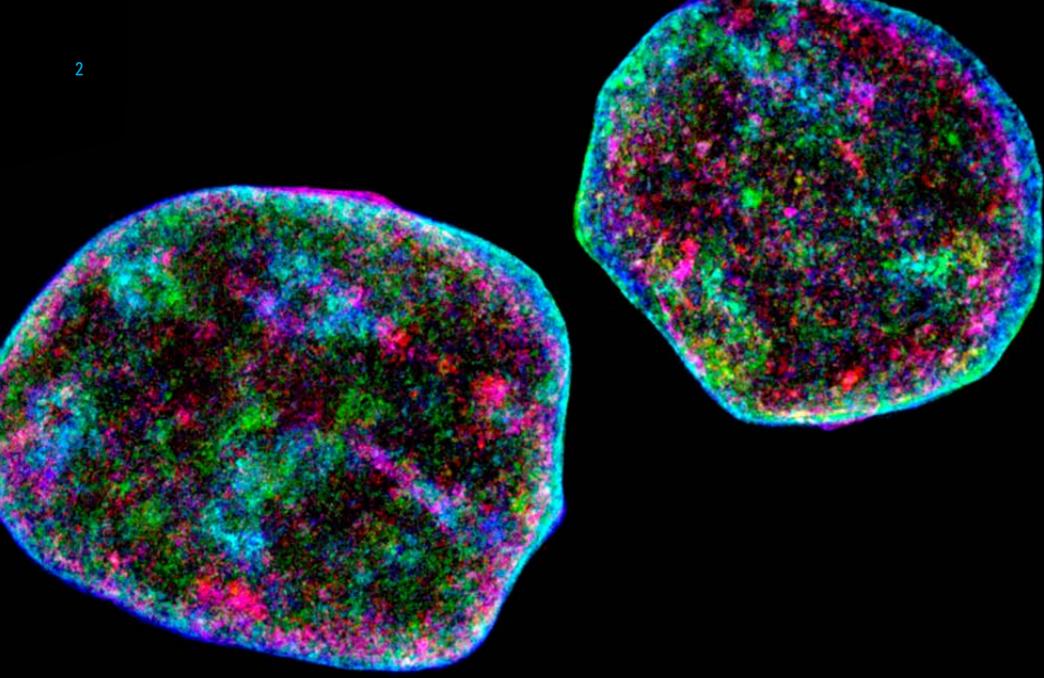
FRIEDRICH-MERZ PROFESSORSHIP 2013

# MEMBRANE KINESIS

SHAPING AND TRANSPORT OF CELL MEMBRANES

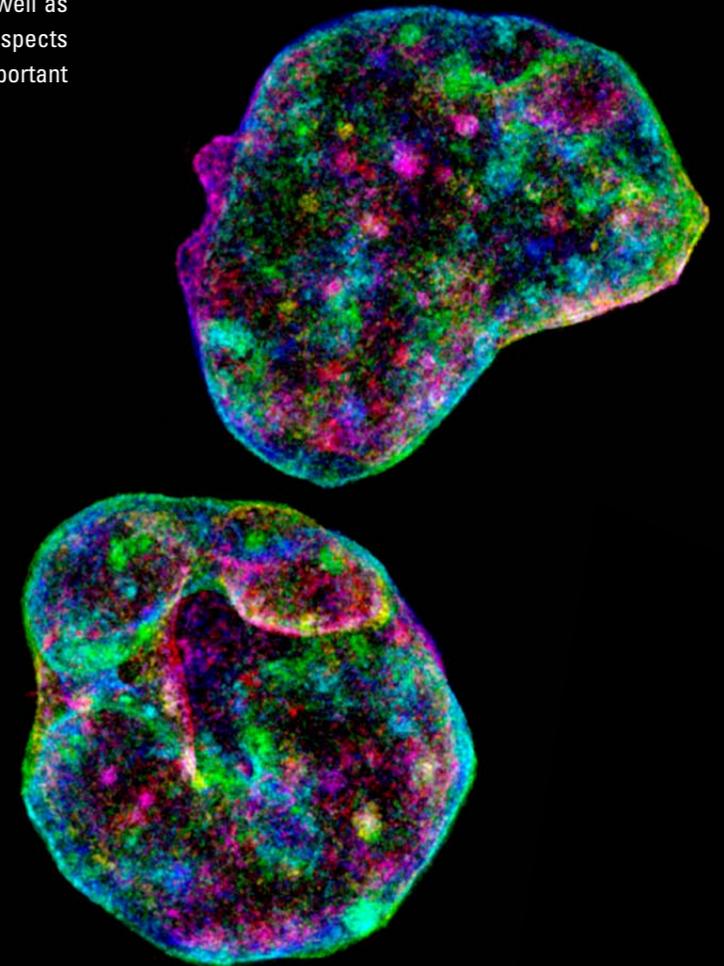


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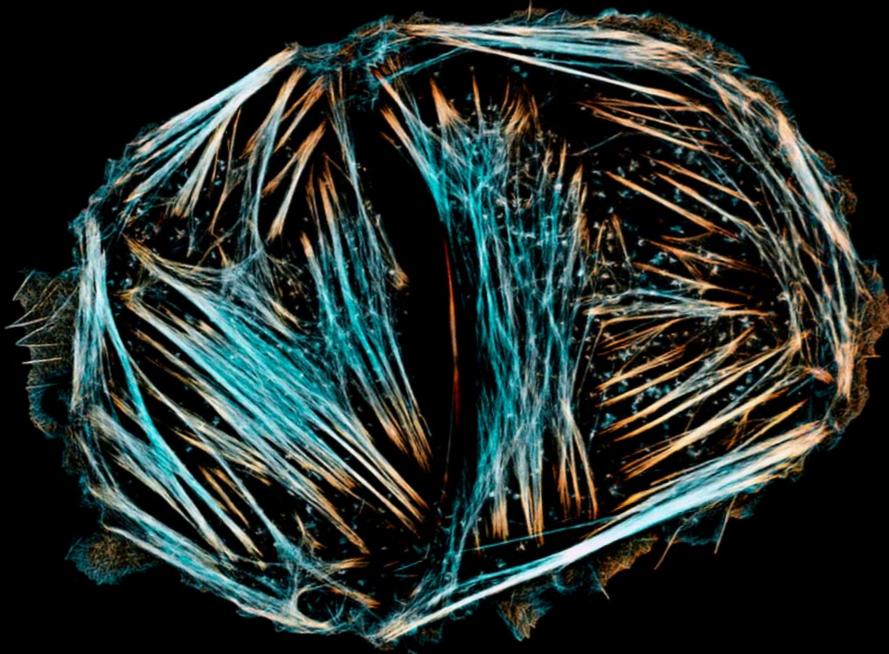
## Dynamic nature of cells and their organelles

The Friedrich Merz Symposium 2013 on »Membrane Kinesis – Shaping and Transport of Cellular Membranes« will focus on exciting new developments in Cell Biology, Biomedicine, Infectious Biology, Chemical Biology and Biophysics, where dynamic nature of cells and their membranes play important roles. These areas include organelle biogenesis, endocytosis and vesicular trafficking, but also novel approaches using model systems as well as chemical or optogenetic tools. This symposium will discuss various aspects by which membrane shaping, transport and remodeling achieve important biological functions and malfunction leading to human diseases.



As an outstanding researcher in describing the dynamic nature of cells and their organelles, Prof. Jennifer Lippincott-Schwartz has been appointed this year visiting professor of the Friedrich Merz Foundation, one of the most salient guest professorships of Johann Wolfgang Goethe University. Dr. Lippincott-Schwartz's research at the *National Institute of Health* (USA) focuses on organelle biogenesis and dynamics membrane processes directly impacting a series of neurodegenerative, infectious, metabolism and cancer diseases.

Guiding idea of the Friedrich Merz Foundation Professorship is above all to promote the international scientific contacts of Goethe University in the fields of medicine and pharmacy. In 1987, Merz & Co. founded its Friedrich Merz Foundation Professorship, which includes the participation in an international interdisciplinary symposium beside research activities and lectures. The symposium is dedicated to the main research focus of the visiting professor.



Lippincott-Schwartz investigates the fundamentals in the organization and dynamic distribution of components of eukaryotic cells. New developments in microscopic techniques with almost molecular resolution allowed her to examine the subcellular localization, mobility, transport pathways and turnover of numerous cell components in a temporal and spatial resolution unknown up to now. Her results led to a key understanding of a series of metabolism diseases, cancer, as well as HIV and showed possible therapeutic approaches.

Lippincott-Schwartz was born in 1952 in Manhattan (Kansas, USA). Her father being a chemistry professor, she was born with a pipette in her hand so to speak. »We had a periodic table hanging in the kitchen«, she recounts. Moving to a farm in Virginia, where her family kept horses and other animals, awakened her interest in biology. After a bachelor degree at Swarthmore College, teaching chemistry, physics, and biology at a girls' school in Kenya, and a M.Sc. degree in biology at Stanford University, she graduated at Johns Hopkins University (Baltimore). She then worked with Richard Klausner at National Institute of Health (NIH). Lippincott-Schwartz leads the section on organelle biology in the field of cell biology and metabolism at NIH. Dr. Lippincott-Schwartz is a member of the National Academy of Sciences and a pioneer in modern cell biology. She also is a distinguished guest professor of the Friedrich Merz Foundation.

Dr. Lippincott-Schwartz provides fascinating insights in the unknown interior of the cell and in its pathological alterations within a series of lectures dedicated to pupils, students, and scholars as well as within the international symposium »*Membrane Kinesis – Shaping and Transport of Cell Membranes*« organized in her honor.

We take this opportunity to welcome you to this outstanding event.

Sincerely,

**Robert Tampé**

PROFESSOR, INSTITUTE OF BIOCHEMISTRY



# MEMBRANE KINESIS

## SHAPING AND TRANSPORT OF CELL MEMBRANES

October 22, 2013 – Biocenter B1, Campus Riedberg, Goethe-University

9:00–9:10 Manfred Schubert-Zsilavecz, Vice President, JWGU  
Welcome Address Goethe-University

9:10–9:20 Alexander Gebauer, Merz-Pharma, CEO  
Welcome Address

CHAIR: ERIN SCHUMAN (FRANKFURT)

9:20–9:50 KEYNOTE OPENING LECTURE  
Jennifer Lippincott-Schwartz (Bethesda)  
Insights into HIV viral assembly and membrane abscission using super resolution microscopy

9:50–10:20 Volker Haucke (Berlin)  
Greasing endocytosis by membrane lipids

10:20–10:40 Andreas Reichert (Frankfurt)  
Molecular mechanisms shaping mitochondrial membranes

10:40–11:00 Robert Ernst (Frankfurt)  
From fatty acid desaturation to membrane stress responses

BREAK

CHAIR: AMPARO ACKER-PALMER (FRANKFURT)

11:30–12:00 Antoine Triller (Paris)  
Receptor kinesis accounting for synapse stability and plasticity

12:00–12:20 Ernst Stelzer (Frankfurt)  
High resolution three-dimensional imaging of cellular spheroids with light sheet-based fluorescence microscopy

12:20–12:40 Alexander Gottschalk (Frankfurt)  
In vivo synaptic recovery following optogenetic hyperstimulation

LUNCH

CHAIR: VOLKER DÖTSCH (FRANKFURT)

14:00–14:30 Petra Schwille (Martinsried)  
Membrane models to investigate protein-induced transformations

14:30–15:00 Horst Vogel (Lausanne)  
Imaging and manipulating cellular biochemical networks: From single cells to single molecules

15:00–15:30 Jacob Piehler (Osnabrück)  
Assembly and dynamics of cytokine receptor complexes

15:30–15:50 Mike Heilemann (Frankfurt)  
Quantitative single-molecule super-resolution microscopy of membrane proteins

BREAK

CHAIR: ANNA STARZINSKI-POWITZ (FRANKFURT)

16:20–16:50 Carsten Schultz (Heidelberg)  
Searching for the perfect fluorescent probe

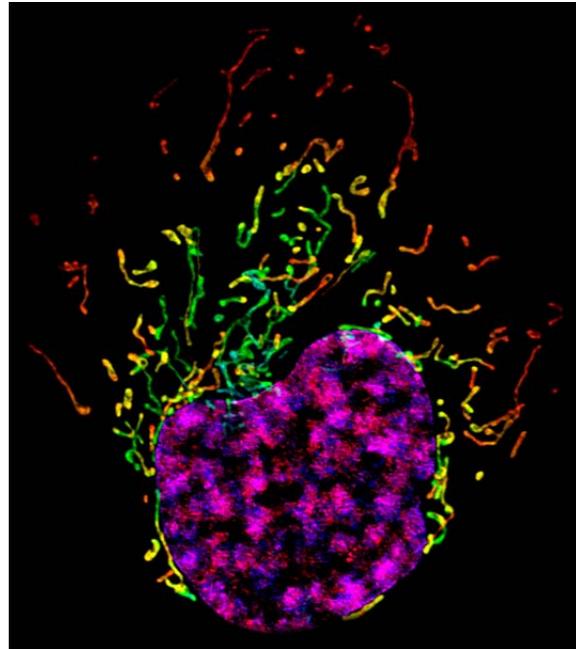
16:50–17:20 Jacques Neefjes (Amsterdam)  
The strange dance of MHC class II molecules: motors, motors and more motors

17:20–17:50 Peter Friedl (Nijmegen)  
Mechanics of cancer cell invasion in vitro and in vivo

17:50–18:00 Robert Tampé (Frankfurt)  
Closing Remarks

Jennifer Lippincott-Schwartz, Prabuddha Sengupta, Antony Chen and Schuyler van Engelenburg

## Insights into HIV viral assembly and membrane abscission using super resolution microscopy

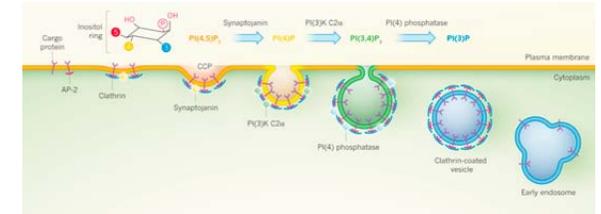


The Human Immunodeficiency virus (HIV-1) life cycle involves several highly choreographed steps during which the virus assembles at the plasma membrane (PM) of an infected cell and buds off the membrane as a viral particle. We have used conventional and super resolution imaging approaches to investigate cell biological mechanisms underpinning three key steps in the viral assembly/budding pathway: clustering of the viral coat in the plasma membrane; recruitment of proteins into the viral bud; and virus budding off the membrane. In the first step involving viral Gag coat assembly, we show it is critically dependent on viral and/or host mRNA, which drives Gag clustering through RNA-Gag electrostatic interactions. In the second step involving protein recruitment into the viral bud, we demonstrate that Env proteins incorporate into viral buds through partitioning into a specialized microenvironment created by multimerization of Gag at the PM. In the final step involving viral abscission from the PM, we examine the 3D molecular organization of ESCRT machinery with respect to HIV bud sites to gain critical insight. We find ESCRT-III proteins assemble within the head of the budding virion, not the base as previously proposed. This later finding prompts a reevaluation of current models for ESCRT-III scaffolding, and suggests that ESCRT abscission initiates from within the head of the budding virion.

York Posor<sup>1</sup>, Marielle Eichhorn-Gruenig<sup>1\*</sup>, Dmytro Puchkov<sup>1\*</sup>, Johannes Schöneberg<sup>2\*</sup>, Alexander Ullrich<sup>2\*</sup>, André Lampe<sup>1</sup>, Rainer Müller<sup>3</sup>, Sirius Zerbakhsh<sup>3</sup>, Federico Gulluni<sup>4</sup>, Emilio Hirsch<sup>4</sup>, Michael Krauss<sup>1</sup>, Carsten Schultz<sup>3</sup>, Jan Schmoranzler<sup>1</sup>, Frank Noé<sup>2</sup> and Volker Haucke<sup>1,5</sup>

## Greasing endocytosis by membrane lipids

Phosphoinositides (PIs) serve crucial roles in cell physiology ranging from cell signalling to membrane traffic. Among the seven eukaryotic PIs the best studied species is phosphatidylinositol (4,5)-bisphosphate [PI(4,5)P<sub>2</sub>], which is concentrated at the plasma membrane where among other functions it is required for the nucleation of endocytic clathrin-coated pits (CCPs). No PI other than PI(4,5)P<sub>2</sub> has been implicated in clathrin-mediated endocytosis (CME), whereas the subsequent endosomal stages of the endocytic pathway are dominated by PI 3-phosphates. How PI conversion from PI(4,5)P<sub>2</sub>-positive endocytic intermediates to PI 3-phosphate [PI(3)P]-containing endosomes is achieved is unclear. Here, we show that formation of phosphatidylinositol-3,4-bisphosphate [PI(3,4)P<sub>2</sub>] by class II phosphatidylinositol 3-kinase C2α (PI3K C2α) spatiotemporally controls CME. Depletion of PI(3,4)P<sub>2</sub> or PI3K C2α impairs the maturation of late-stage CCPs before fission. Timed formation of PI(3,4)P<sub>2</sub> by PI3K C2α is required for selective enrichment of the BAR domain protein SNX9 at late-stage endocytic intermediates. These findings provide a mechanistic framework for the role of PI(3,4)P<sub>2</sub> in endocytosis and unravel a novel discrete function of PI(3,4)P<sub>2</sub> in a central cell physiological process.



(Taken from Mettlen & Schmid, *Nature* 2013)

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\* equal contributions

Andreas S. Reichert<sup>1,2</sup>

## Molecular mechanisms shaping mitochondrial membranes

The morphology of cristae membranes is highly variable and aberrant mitochondrial structures have been associated with numerous severe human diseases. Cristae are connected to the inner boundary membrane by crista junctions (CJs) – highly curved membrane structures with a tubular, ring, or slit-like appearance. CJs have been proposed to act as diffusion barriers for metabolites and membrane proteins and to be fundamentally important in bioenergetics as they may limit the diffusion of ADP/ATP and affect the pH gradient across the inner membrane. Earlier studies indeed showed that the inner membrane is dynamically subcompartmentalized. Cristae and CJ remodeling occurs during apoptosis promoting the efficient release of cytochrome c. The molecular basis for CJ formation is not well understood.

We identified and characterized the first protein component required for the formation of CJs in yeast – Fcj1 (Formation of crista junction protein 1). Mitochondria lacking Fcj1 contained concentric stacks of membrane vesicles in their matrix (Figure 1). We further showed that impairing  $F_1F_0$  ATP synthase oligomerization through the deletion of subunits e or g, led to altered CJ structure and increased internal cristae branching. We proposed that Fcj1 and subunits e/g act antagonistically to control  $F_1F_0$  ATP synthase oligomerization, thereby modulating membrane curvature, and thus the formation of CJs and cristae tips. Newer studies identified Fcj1 as a central subunit of the high molecular weight MINOS complex consisting of at least five other subunits. Several components of the human MINOS complex have been linked to ageing and to human disorders such as Down's syndrome, Parkinson's disease, and cancer [8] underlying the functional importance of CJs and mitochondrial ultrastructure. We recently identified the cardiolipin-binding protein APOOL as a novel subunit of the mammalian MINOS complex. Here I will discuss novel insights how crista junctions are formed and how they are anchored to the outer membrane.

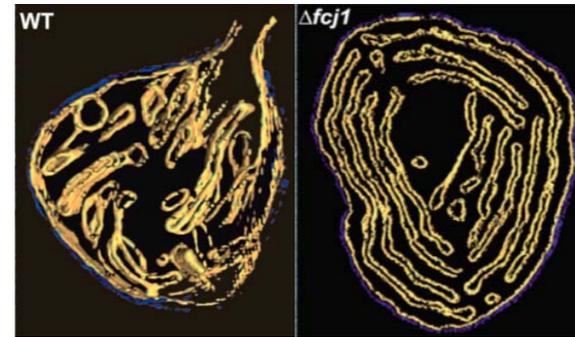


Fig. 1. Fcj1 is essential for crista junction (CJ) formation.

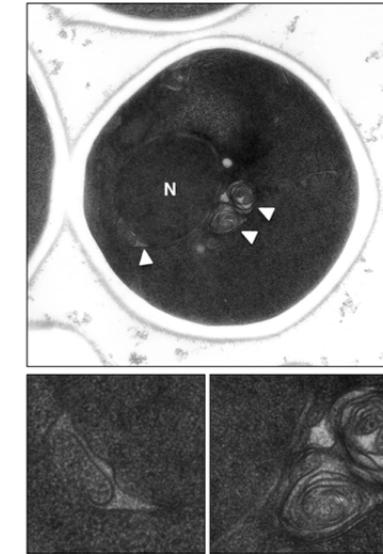
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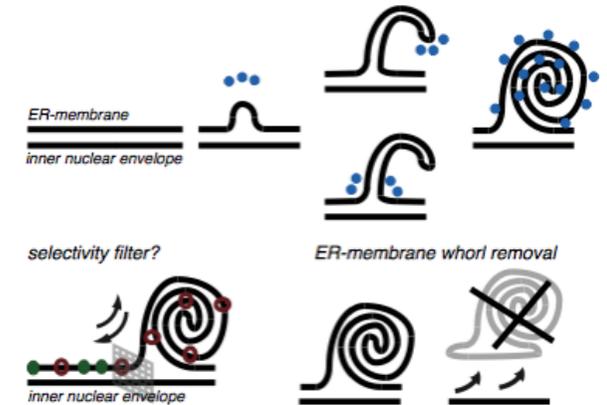
Kristina Puth<sup>1</sup>, Claudius Stordeur<sup>1</sup>, and Robert Ernst<sup>1,2</sup>

## From fatty acid desaturation to membrane stress responses

Biological membranes are complex and the mechanisms underlying their homeostasis are incompletely understood. Here, we present a quantitative genetic interaction map (E-MAP) focused on various aspects of lipid biology including their metabolism, sorting, and trafficking. This E-MAP contains ~250,000 genetic interaction scores, both negative and positive, and identifies a molecular crosstalk of protein quality control pathways with lipid bilayer homeostasis. Ubx2p, a component of the ER-associated degradation (ERAD) pathway, surfaces as a key upstream regulator of the essential fatty acid desaturase Ole1p. Loss of Ubx2p affects the transcriptional control of *OLE1* resulting in impaired fatty acid desaturation and a severe shift towards more saturated membrane lipids. Both the induction of the unfolded protein response and aberrant nuclear membrane morphologies observed in cells lacking *UBX2* are suppressed by supplementation of unsaturated fatty acids. Currently, we are studying the formation and removal of aberrant nuclear membrane whorls by live cell imaging (see figure below). Our results point towards the existence of dedicated bilayer stress responses for membrane homeostasis.



ER-membrane whorl formation and stabilization



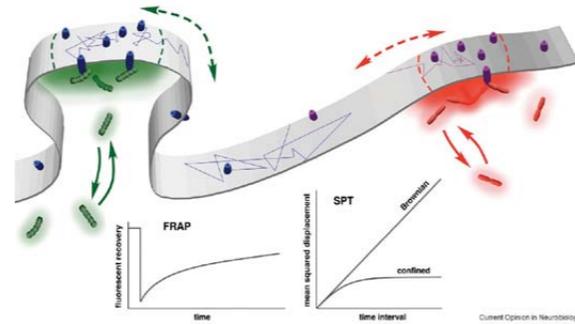
<sup>1</sup> Institute of Biochemistry, Biocenter, Goethe-University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany

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Antoine Triller

## Receptor kinesis accounting for synapse stability and plasticity

The variability of the postsynaptic response following a single action potential arises from two sources: the neurotransmitter release is probabilistic, and the postsynaptic response to neurotransmitter release has variable timing and amplitude. At individual synapses, the number of molecules of a given type that are involved in these processes is small enough that the stochastic (random) properties of molecular events cannot be neglected. How the stochasticity of molecular processes contributes to the variability of synaptic transmission, its sensitivity and its robustness to molecular fluctuations has important implications for our understanding of the mechanistic basis of synaptic transmission and of synaptic plasticity. Using single particle tracking and super-resolution imaging, we will address the issue of postsynaptic receptors dynamic, their interactions with scaffolding protein and regulations implicated in synaptic plasticity. Combination of single particle tracking and super-resolution methods, open access to molecular counting and energy involved in receptor-scaffold interactions as well as on and off rate of molecular interactions. Thus beyond super-resolution methods is chemistry »in cellulo« accounting for the regulation of receptor number and consecutively that of synaptic strength.

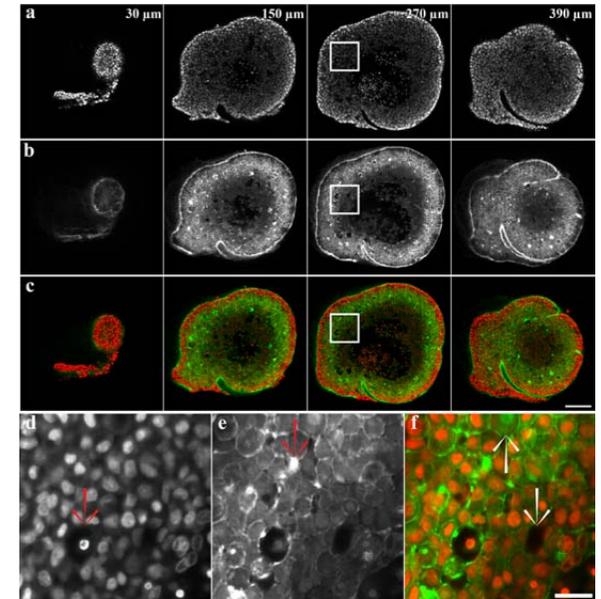


Dynamic behaviour of synaptic proteins. The diffusion of excitatory (blue) and inhibitory receptor complexes (purple) in the plasma membrane is free (Brownian) at extrasynaptic locations and confined within synapses. These patterns of diffusion can be differentiated by SPT of individual receptor complexes (see right graph). At excitatory (left, green) and inhibitory synapses (right, red) receptors are immobilised through interactions with the synaptic scaffold. Finally, receptors can enter and exit synapses (blue and red dashed arrows). Synaptic scaffold proteins such as PSD-95 at excitatory synapses, green) and gephyrin inhibitory, red) also exchange between synaptic and nonsynaptic compartments. Their dynamic exchange is frequently measured by FRAP (see left graph). Dotted lines delineate excitatory (green, left) and inhibitory synaptic regions (red, right). (Renner, M., Specht, C.G., Triller, A. (2008) Molecular dynamics of postsynaptic receptors and scaffold proteins, *Current Opinion in Neurobiology*, **18**: 532-540)

Ernst H.K. Stelzer, Christian Mattheyer, and Francesco Pampaloni

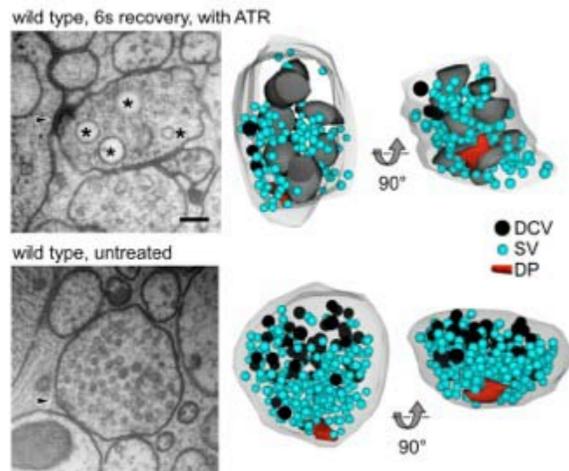
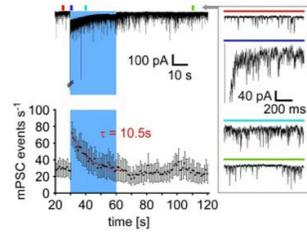
## High resolution three-dimensional imaging of cellular spheroids with light sheet-based fluorescence microscopy

Conventional two-dimensional cell monolayers do not provide the geometrical, biochemical, and mechanical cues found in tissues. In fact, the cells that form tissues interact through chemical and mechanical stimuli with adjacent cells and via the extracellular matrix (ECM). Such a highly interconnected communication network extends along all three dimensions. Many studies have demonstrated that the differences between three-dimensional and two-dimensional cultured cells are striking at the morphological and at the molecular level, and that three-dimensional cell cultures can be employed in order to shrink the gap between tissues and *in vitro* cell models. Fluorescence imaging of three-dimensional cell cultures sets new challenges and imposes specific requirements concerning the choice of a suitable microscopy technique. Deep penetration into the specimen, high imaging speed, and ultra-low intensity of the excitation light are key requirements. Light sheet-based fluorescence microscopy (LSFM) offers a favorable combination of these requirements and is currently established as the technique of choice for the study of three-dimensional cell cultures. The development of LSFM draws on many previous developments, in particular, confocal theta fluorescence microscopy.



Maike Kittelmann<sup>1</sup>, Jana Liewald<sup>2,3</sup>, Jan Hegemann<sup>1</sup>, Christian Schultheis<sup>2,3</sup>, Martin Brauner<sup>2,3</sup>, Wagner Steuer Costa<sup>2,3</sup>, Sebastian Wabnig<sup>2,3</sup>, Stefan Eimer<sup>1</sup>, and Alexander Gottschalk<sup>2,3</sup>

## In vivo synaptic recovery following optogenetic hyperstimulation

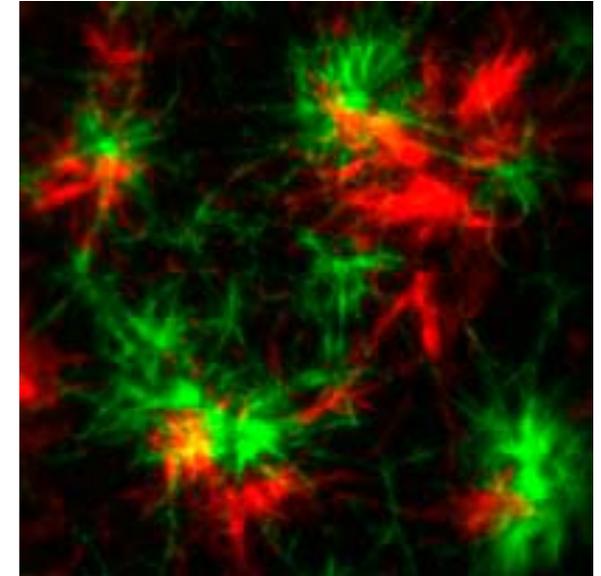


Local recycling of synaptic vesicles (SVs) allows neurons to sustain transmitter release. Extreme activity, e.g. during seizure, may exhaust synaptic transmission and, in vitro, induces bulk endocytosis to recover SV membrane and proteins; how this occurs in animals is unknown. Following optogenetic hyperstimulation of *Caenorhabditis elegans* motoneurons, we analyzed synaptic recovery by time-resolved behavioral, electrophysiological and ultrastructural assays. Recovery of docked SVs and of evoked release amplitudes (indicating RRP-refilling) occurred within ~8–20s, while locomotion recovered only after ~60s. During ~11s stimulation, 50–200nm non-coated vesicles («100nm vesicles») formed, which disappeared ~8s post stimulation, likely representing endocytic intermediates from which SVs may regenerate. In endophilin, synaptojanin and dynamin mutants, affecting endocytosis and vesicle scission, resolving 100nm vesicles was delayed (>20s). In dynamin mutants, 100nm vesicles were abundant and persistent, sometimes continuous with the plasma membrane; incomplete budding of smaller vesicles from 100nm vesicles further implicates dynamin in regenerating SVs from bulk-endocytosed vesicles. Synaptic recovery after exhaustive activity is slow, and different time scales of recovery at ultrastructural, physiological and behavioral levels indicate multiple contributing processes. Similar processes may jointly account for slow recovery from acute seizures also in higher animals.

Petra Schwille

## Membrane models to investigate protein-induced transformations

A notorious dilemma of biophysical methods is that the precision with which they can capture and analyse processes in biological systems is constantly increasing, but at the same time, the reproducibility and predictability of measurements taken in living systems worsens – due to the inherently stochastic nature of living systems on the molecular level. One obvious solution is to reconstitute biological processes of interest in simplified and better controllable cell-free environments, but whether the insights obtained there also apply under physiological conditions has to be constantly scrutinized. In the last decades, supported membranes, as well as Giant Unilamellar Vesicles (GUVs) have become invaluable tools in studying the function of membrane proteins, and their dependence on the actual local membrane environment, in particular of lipid domains. Due to their limited degree of compositional complexity, these model systems allow unprecedented quantitative access to fundamental biological phenomena. In my talk, I will discuss our recent advances in reconstituting membrane sculpting and membrane transforming protein machineries, such as the bacterial divisome protein FtsZ and an artificial actomyosin cortex, on model membranes. Our long-term goal is to identify, in a bottom-up synthetic biology approach, a fundamental canon of functional protein modules of a minimal cell division machinery.



Motor-induced contractions of an artificial actomyosin cortex

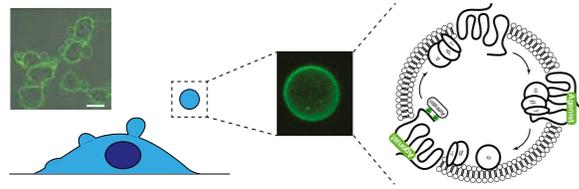
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Horst Vogel

## Imaging and manipulating cellular biochemical networks: From single cells to single molecules



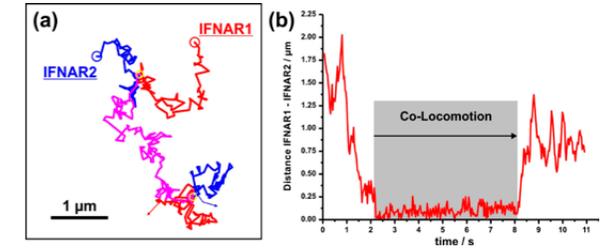
After adding cytochalasin, cultured cells formed within a few minutes blebbing structures on their plasma membranes which can be sheared off as (sub-)micrometer-sized closed plasma membrane vesicles. Inset: Confocal micrograph showing the YFP fluorescence of HEK cells expressing A2AR-YFP after addition of cytochalasin B; scale bars: 10  $\mu\text{m}$ . Inset 2: Confocal micrograph of a blebbing cell expressing a fluorescent membrane receptor (A2A-YFP, green). Adapted from Grasso et al. (2013) *PLoS One* 8, e70929.

Cellular signaling reactions are classically investigated by measuring optical or electrical properties of individual living cells or suspensions of cells in microliter volumes. Here we show how the binding of ligands to cell surface receptors and the subsequent activation of signaling reactions can be monitored both in single cells and in single, sub-micrometer sized native vesicles with single molecule sensitivity. The native vesicles are derived from live cells and represent the smallest autonomous containers capable of performing cellular signaling reactions. They function like minimal artificial cells and thus open the door to downscale the analysis of cellular functions to the sub-micrometer and sub-femtoliter range. We describe a method that allows the parallel isolation of such ultra-small volumes and their incorporation as individuals in ordered arrays on nanopatterned solid surfaces or in solution using multiple optical tweezers. This miniaturization and parallelization opens unique possibilities for multiplexing single-cell analysis. We report on cellular signaling reactions mediated by ligand-gated ion channels and G-protein coupled receptors in native vesicles using combined single molecule optical and electrophysiological techniques.

Stephan Wilmes, Friedrich Roder, Christian Richter, Changjiang You, and Jacob Piehler

## Assembly and dynamics of cytokine receptor complexes

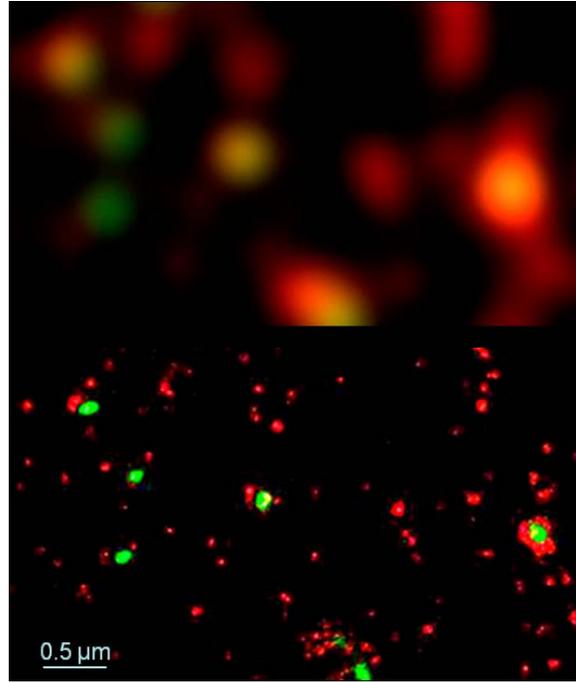
Cytokine receptors are comprised by two or more receptor subunits, which are cross-linked by their ligand, leading to the activation of Janus family kinases (JAKs) associated with the cytoplasmic tails of the receptor subunits. We aim to unravel cytokine receptor assembly, dynamics and effector activation as well as its regulation by plasma membrane organization and specific feedback mechanisms in a quantitative manner. To this end we have established single molecule dimerization assays, which allow visualizing assembly and dynamics of receptor complexes at physiological receptor concentrations in living cells. Thus, ligand-induced dimerization was clearly demonstrated for several cytokine receptors, which was studied in more detail for the type I interferon (IFN) receptor. Strikingly, rapid and very efficient dimerization of the subunits IFNAR1 and IFNAR2 and the formation of a dynamic signalling complex were observed (Figure), in contrast to dimerization experiments with transmembrane receptors reconstituted into polymer-supported membrane *in vitro*. More detailed studies with deletions constructs of IFNAR revealed that the cytosolic receptor domains contribute to stabilizing the signalling complex, probably by interactions between the JAKs. Interestingly, dimerization of IFNAR is modulated by the negative feedback regulator USP18, which binds to the cytosolic domain of IFNAR2. This mechanism can explain differential activities of IFNs, which bind the receptor subunits with different affinities.



Assembly and dissociation of an individual type I interferon receptor complex in the plasma membrane of living cells as monitored by co-tracking (a) and distance analysis (b).

Marina Dietz, Franziska Fricke, Carmen Krüger, Sebastian Malkusch, and Mike Heilemann

## Quantitative single-molecule super-resolution microscopy of membrane proteins



Structural organization of HIV proteins gag (green) and env (red) in the plasma membrane of T cells visualized by widefield (top) and super-resolution microscopy (bottom).

In the fluorescence microscopy field, much interest has focused on new super-resolution techniques (collectively known as PALM/STORM, STED, SIM and others) [1] that have demonstrated to bypass the diffraction limit and provide a spatial resolution reaching a near-molecular level. With these techniques, it has become possible to image cellular structures in far greater detail than ever before. Single-molecule based methods such as photoactivation-localization microscopy (PALM) [1], stochastic optical reconstruction microscopy (STORM) [2] and directSTORM (dSTORM) [3] employ photoswitchable fluorophores and single-molecule localization to generate a super-resolution image. These methods are uniquely suited not only to resolve small cellular structures, but also to provide quantitative information on the number of molecules or stoichiometries.

The important next step is to obtain reliable quantitative information from super-resolution images of cellular structures. In this talk, we will introduce our recent efforts towards quantitative single-molecule super-resolution imaging of membrane proteins including receptors such as TNF-R1 [4] and MetR [5] as well as the HIV-1 envelope protein env [6].

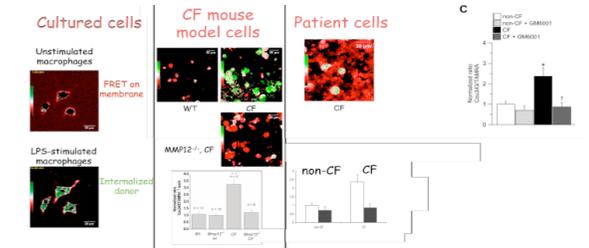
### References

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- [2] Rust, M.J. et al. (2006) *Nature Methods*, **10**: 793-795.
- [3] Heilemann, M. et al. (2008) *Angewandte Chemie*, **47**: 6172-6176.
- [4] Dietz, M. et al. (2013) *BMC Biophysics*, **6**: 6.
- [5] Heidbreder, M. et al. (2012) *BBA Mol Cell Res*, **1823**: 1984-1989.
- [6] Muranyi, W. et al. (2013) *Plos Pathogens*, **9**(2): e1003198.

Carsten Schultz

## Searching for the perfect fluorescent probe

Many biological questions can be best answered by monitoring the crucial factors and events by realtime imaging, most commonly through fluorescence microscopy. Obviously, the success relies largely on the availability and performance of specific fluorescent probes. Hence, when designing realtime imaging experiments the choice of the sensor or reporter molecule has a major impact on the outcome and the quality of the data set. Since the discovery and establishment of green fluorescent protein many sensor molecules are genetically encoded and are fairly comfortably applied to intact cells and via transgenic animals even to entire organisms. The alternatives are fluorescent reporters that are based on small molecules. Especially when it comes to FRET-based probes, these molecules often show superior optical properties, dynamics, and sensitivities compared to their genetically encoded counterparts. However, the development of these probes requires significant amounts of chemistry. In addition, the admission of the probes to cell interior or special subcellular locations is often a challenge. It would therefore be desirable to combine the advantages of both probe types and develop a genetically encoded sensor type that is equipped with small molecule dyes. While this steep goal has not been achieved yet, I will show new developments for introducing artificial amino acids for in vivo protein labeling via click chemistry that will bring us closer to FRET sensors based on small molecule dyes. In addition, I will report on new developments of improving standard genetically encoded FRET probes and show that small molecule based FRET probes can be useful for detecting chronic lung inflammation in patients (Figure).

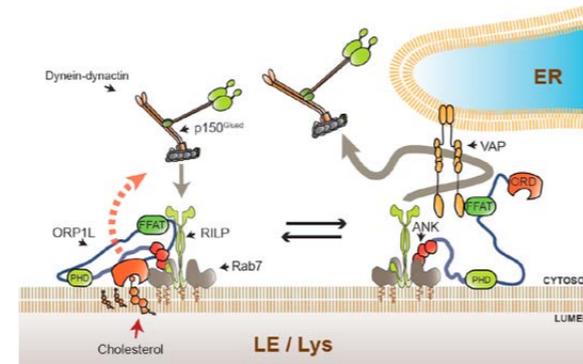


The lipidated peptidic FRET reporter LaRee1 monitors macrophage elastase activity relevant in lung emphysema formation on cells in culture, from a cystic fibrosis (CF) mouse model and from CF patients.

Ilana Berlin, Rik van der Kant, Marlieke Jongma, Petra Paul, Jeroen Bakker, Lennert Janssen, and Jacques Neefjes

## The strange dance of MHC class II molecules: motors, motors and more motors

MHC class II molecules present peptides acquired in endosomal compartments to the immune system that then respond by activating various steps in immune processes such as B cell activation resulting in antibody production. MHC class II is also the most strongly associated factor to almost all auto-immune diseases. MHC class II molecules are targeted to late endosomes by the invariant chain, which is degraded at that location and replaced by a proper peptide. The late endosomes then move to the plasma membrane in a rather regulated fashion. Movement of these structures requires motor protein activities. Since life-imaging shows late endosomes with MHC class II moving in a bidirectional manner, MHC class II transport in these compartments requires motor proteins of the kinesin, dynein and myosin family. How these motors recognize their cognate vesicle in a time and spatial manner, is unclear. I will discuss how the dynein motor on late endosomes is controlled by cholesterol and the ER, how kinesin motors find these vesicles and how one dynein motor determines regulated secretion of MHC class II in activated dendritic cells. Uncoupling yields immature dendritic cells with a mature phenotype. The many motors ultimately control the timing of immune responses and other biological processes.

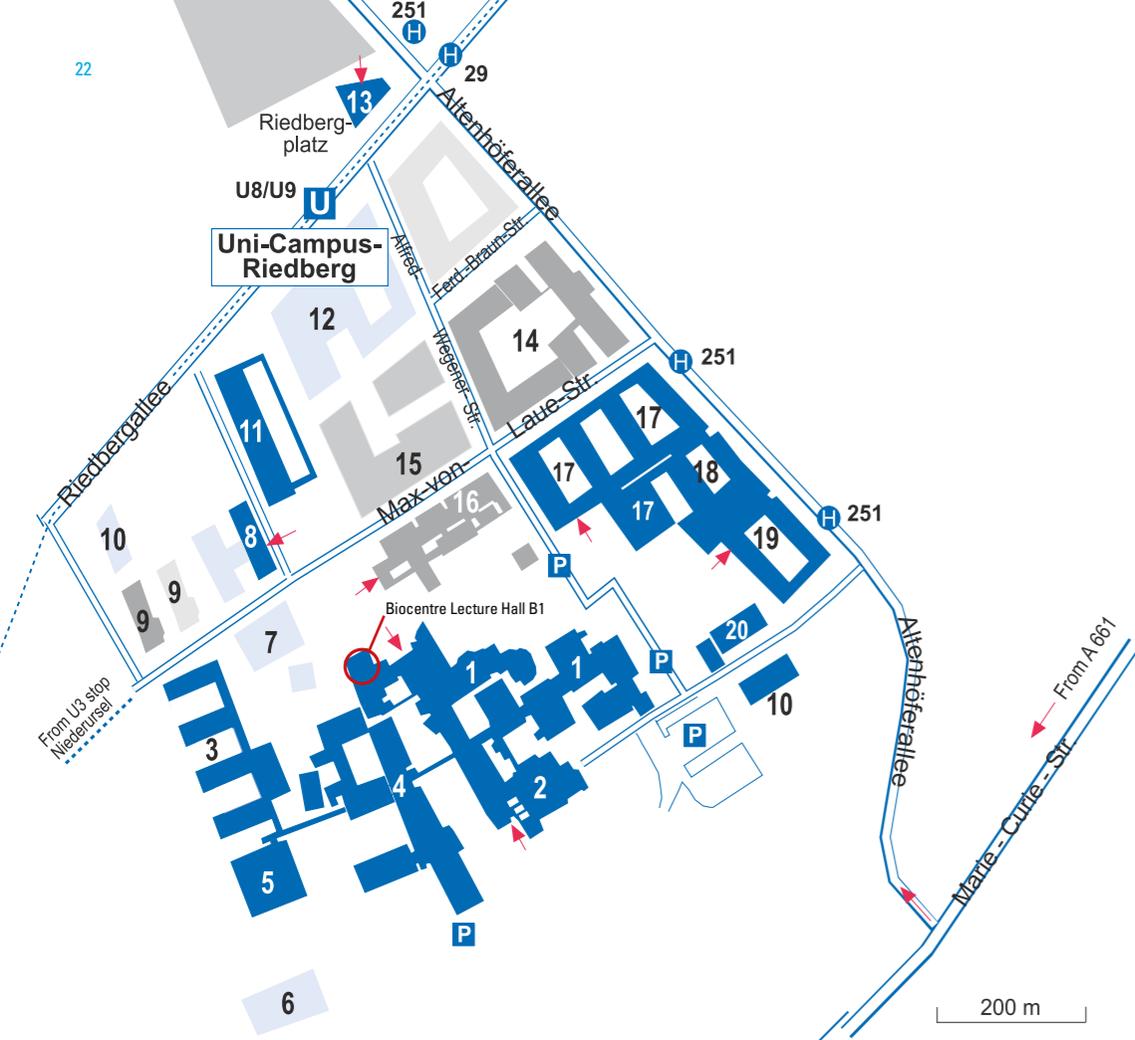


Peter Friedl

## Mechanics of cancer cell invasion in vitro and in vivo

Different modes of cancer cell invasion contribute to local tissue invasion and initiation of metastasis, however the underlying mechanisms of each migration program, their limits and their relevance to metastasis remain unclear. In models for melanoma, sarcoma and breast cancer, within the same cancer lesion in vivo both single-cell and collective invasion mediate cell dissemination. Using intravital multiphoton microscopy, we here show the how tissue microniches impose diverse cancer invasion modes, either as barrier precluding migration, or as invasion-promoting tracks that enable either collective, single-cell or combined invasion modes. As main routes, non-destructive contact-guidance along preformed multi-interface perimuscular, vascular and -neural tracks of 1D, 2D and 3D topography were identified. Using in vitro analysis of engineered low- and high-density environments, the underlying physical and molecular limits of cancer cell invasion, showing nuclear deformability and ECM space as rate-limiting determinants and modulation by MMPs and mechanotransduction. Using in vivo targeting of beta1 / beta3 integrins, unexpected plasticity of invasion, including de novo development of amoeboid dissemination, was associated with enhanced micrometastasis, implicating integrin-independent dissemination as major route to metastasis. In conclusion, cancer invasion and metastasis result from adaptive physicochemical programs that balance cell-intrinsic adhesion and mechanocoupling with encountered physical and molecular cues.

	Fibrillar collagen	Micro- / Macrotracks
Random	<b>A</b>  1D 3D Tumor cell Collagen fiber	<b>C</b> <i>Counter-scaffold:</i> adipocyte, collagen fiber, other tumor cell  Adipocyte Basement membrane <i>Scaffold:</i> adipocyte surface
Aligned	<b>B</b>  1D 3D	<b>D</b> <i>Counter-scaffold:</i> myofiber, collagen bundle, blood / lymph vessel  <i>Scaffold:</i> myofiber, nerve track
Scaffold components	Collagen fibrils with interfibril gaps	Basement membrane, adjacent cell surfaces or collagen bundles bordering gap-like track
Type of topography	1D / 3D	2D / 3D
Mode of invasion	Individual, multicellular streaming (collective)	Collective
Frequency of occurrence	low	high



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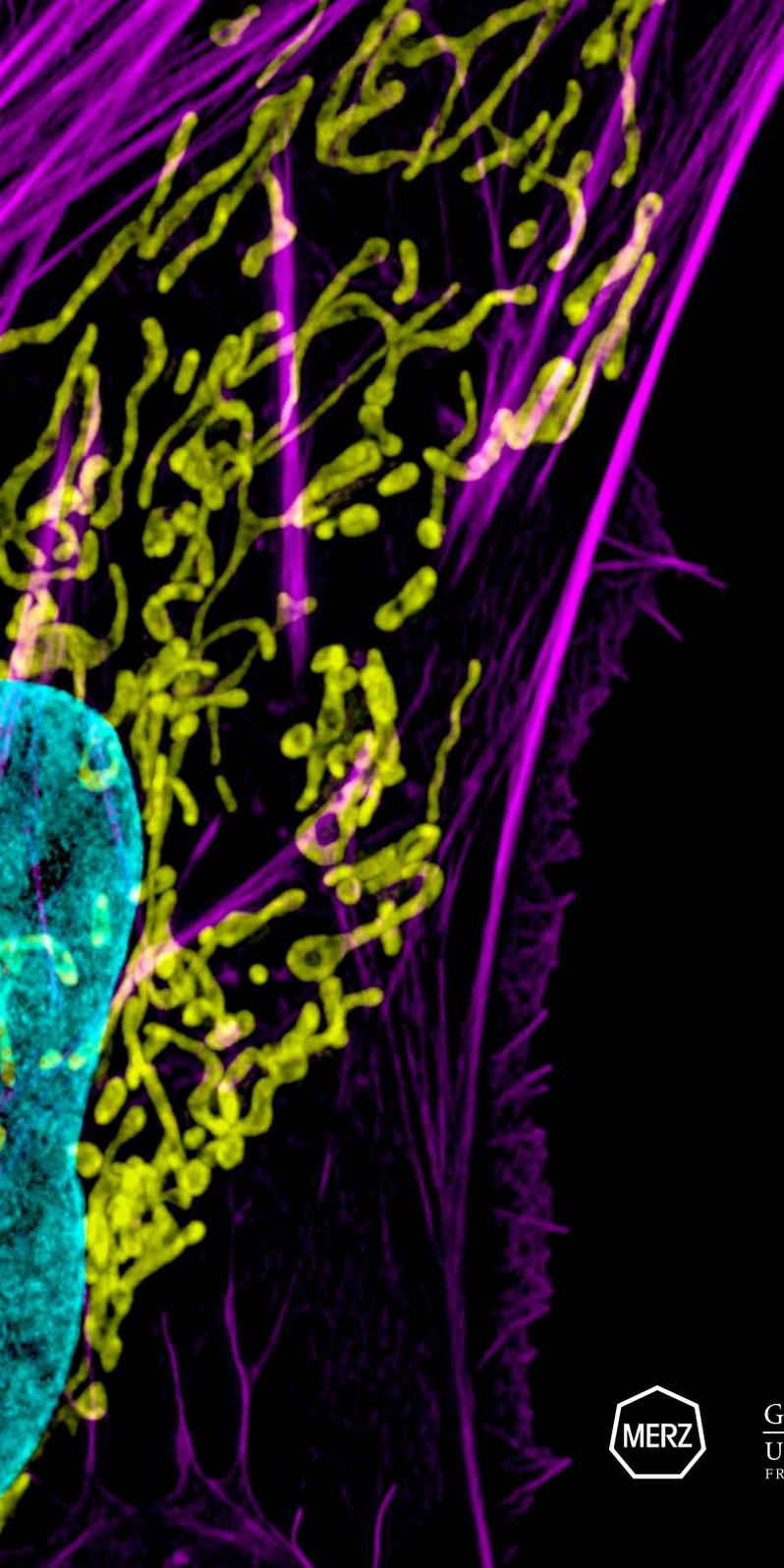
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