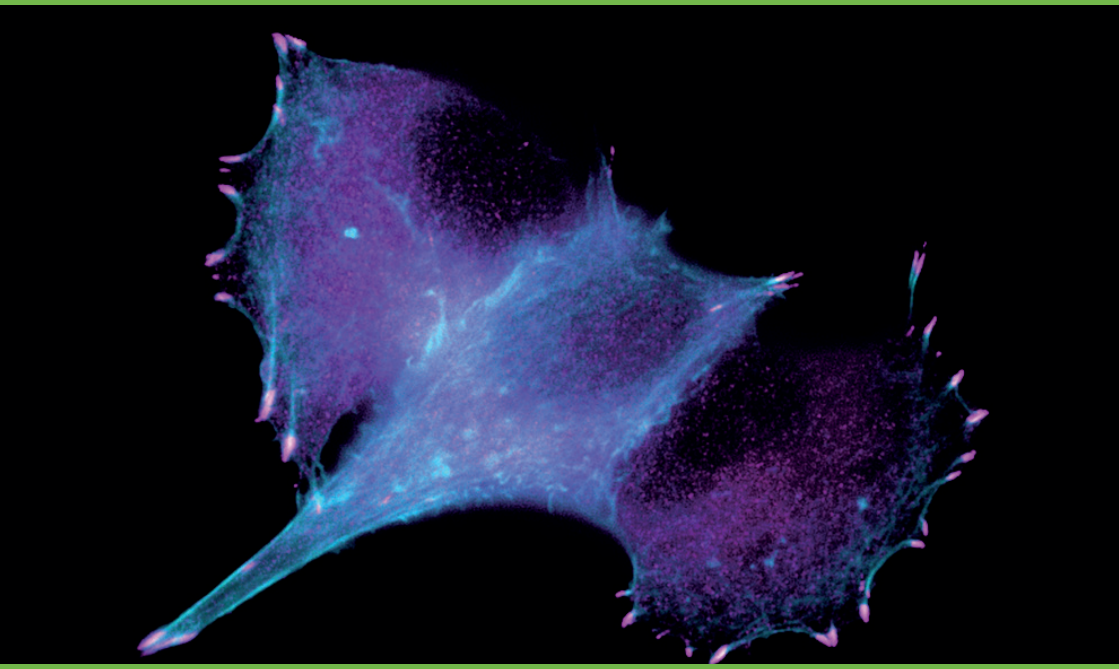
BOOK OF ABSTRACTS



Cell Physics 2021

29 September – 1 October 2021
Saarbrücken

SFB 1027

UNIVERSITÄT DES SAARLANDES

DFG

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29 September – 1 October 2021

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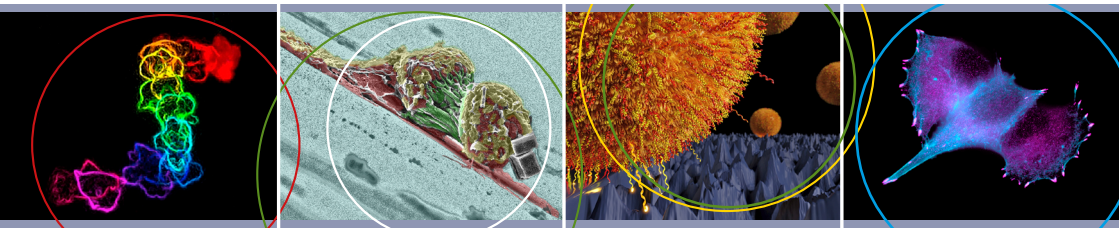
29. September – 1. Oktober | Saarbrücken

TOPICS

- Membranes proteins
- Membrane organization
- Cellular interfaces and compartments
- Cytoskeleton
- Mechanobiology

INVITED SPEAKERS

Rosalind Allen (University of Edinburgh, UK)
Ed Chapman (University of Wisconsin–Madison, USA)
Oliver Daumke (Max Delbrück Center for Molecular Medicine, Germany)
John Eriksson (Turku Bioscience Centre, Finland)
Luis Escudero (University of Sevilla, Spain)
Sandrine Etienne-Manneville (Institut Pasteur, France)
Bernd Fakler (University of Freiburg, Germany)
Daniel Fletcher (University of California, Berkeley, USA)
Nir Gov (Weizmann Institute of Science, Rehovot, Israel)
Bert de Groot (Max Planck Institute for Biophysical Chemistry, Germany)
Olivier Hamant (École normale supérieure de Lyon, France)
Stefan Jakobs (Max Planck Institute for Biophysical Chemistry, Germany)
Paul Janmey (University of Pennsylvania, Philadelphia, USA)
Lukas Kapitein (Utrecht University, Netherlands)
Sarah Keller (University of Washington, USA)
Kinneret Keren (Technion – Israel Institute of Technology, Israel)
Jan Lammerding (Cornell University, USA)
Ilya Leventhal (University of Virginia, USA)
Jan Lipfert (LMU Munich, Germany)
Matthieu Piel (Institut Curie, France)
Sahand Jamal Rahi (EPFL, Lausanne, Switzerland)
Félix Rey (Institut Pasteur, France)
Jelger Risselada (Leiden University, Netherlands)
Georgia Schäfer (University of Cape Town, RSA)
Manuel Théry (The Cell & Plant Physiology Laboratory, France)
Viola Vogel (ETH, Zürich, Switzerland)



ORGANIZERS (UdS)

Ludger Santen
Heiko Rieger

http://www.cell-physics.uni-saarland.de

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Welcome...

...to the conference "Cell Physics 2021" at the Saarland University in Saarbrücken, Germany, 29.09.-01.10.2021. The conference is organized and financed by the Collaborative Research Center SFB 1027 "Physical modeling of non-equilibrium processes in biological systems".

It is intended to be an interdisciplinary platform for scientific exchange between participants from cell biology and biophysics, both represented in roughly equal numbers, and focusses centrally on theoretical concepts in conjunction with cell biological experiments. Topics include

- Membranes proteins
- Membrane organization
- Cellular interfaces and compartments
- Cytoskeleton
- Mechanobiology

The conference will start Wednesday 29.09.2021 at 8:20 a.m. and finish Friday 01.10.2021 at 2:00 pm. It consists of invited talks, contributed oral presentations and poster sessions.

Heiko Rieger, Ludger Santen
(Saarland University, Germany)

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9:00–9:30	Marco Fritzsche	Mechanobiological control of T-cell activation **I.12**
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P.16	Marc Finkler	A methylation-directed, synthetic pap switch based on self-complementary regulatory DNA reconstituted in an all E. coli cell-free expression system

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P.18	Juan Carlos Gil-Redondo	Viscoelastical properties of MCF-7 cells modulated by substrate stiffness
P.19	Daniel Granz	Characterization of the membrane-regulated dynamics of Mga2 from baker's yeast
P.20	Rahul Grover	Investigating vesicular cargo transport driven by multiple motors
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P.65	Renping Zhao	Targeting the microtubule-network rescues CTL killing efficiency in dense 3D matrices

Abstracts of Invited Talks

I.1 Phase separation and biomolecular condensates in biology and disease

Simon Alberti[1]

[1]*Biotechnology Center, Tatzberg 47/49, 01307 Dresden, Germany*

Condensates formed by liquid-liquid phase separation can create functionally distinct membraneless compartments consisting of proteins and RNAs, which have major roles in cellular organization and physiology. RNP granules are a specific type of condensates that assemble by phase separation of RNA-binding proteins (RBPs) and RNA. Recent data also suggest that aberrant phase transitions of RBPs into RNA/protein aggregates may be closely tied or even causative to the pathogenesis associated with diseases such as amyotrophic lateral sclerosis.

In this talk, I will discuss how the concept of biomolecular condensates has expanded our view of biology and disease. I will introduce in vitro reconstitution systems based on condensation that now allow us to rebuild complex structures such as RNP granules in the test tube. Using these reconstitution systems, we have gained important insights into the molecular rules of RNP condensates, such as the molecular grammar and conformational changes underlying condensate assembly, the driving forces and amino acids that govern aberrant phase transitions, and molecular mechanisms of condensate regulation and control. I will further discuss how the concept of phase separation has allowed us to identify novel functions of RNP condensates, and I will demonstrate how phase separation can be used by cells to sense and respond to changes in the environment and regulate fundamental cellular processes such as protein synthesis.

I.2 A biomechanical switch regulates the transition towards homeostasis in mouse esophageal epithelium

Maria P. Alcolea

Wellcome – MRC Cambridge Stem Cell Institute, University of Cambridge, Puddicombe Way, Cambridge, CB2 0AW, UK

Epithelial cells are highly dynamic and can rapidly adapt their behavior in response to tissue perturbations and increasing tissue demands. However, the processes that finely control these responses and, particularly, the mechanisms that ensure the correct switch to and from normal tissue homeostasis are largely unknown. Here we explore changes in cell behavior happening at the interface between postnatal development and homeostasis in the epithelium of the mouse esophagus, as a physiological model exemplifying a rapid but controlled tissue growth transition. Single cell RNA sequencing and histological analysis of the mouse esophagus reveal significant mechanical changes in the epithelium upon tissue maturation. Organ stretching experiments further indicate that tissue strain caused by the differential growth of the mouse esophagus relative to the entire body promotes the emergence of a defined committed population in the progenitor compartment as homeostasis is established. Our results point to a simple mechanism whereby the mechanical changes experienced at the whole tissue level are integrated with those "sensed" at the cellular level to control epithelial cell behavior and tissue maintenance.

Nat Cell Biol. 2021 May;23(5):511-525. doi: 10.1038/s41556-021-00679-w. Epub 2021 May 10.

I.3 Physics models for how antibiotics kill bacteria

Rebecca Brouwers[1], Sharareh Tavaddod[1], Leonardo Mancini[2], Elizabeth Tatham[1], Pietro Cicuta[2], Waldemar Vollmer[3] and **Rosalind J. Allen**[1]

[1]*School of Physics and Astronomy, University of Edinburgh, Edinburgh, UK*
[2]*Cavendish Laboratory, University of Cambridge, Cambridge, UK*
[3]*Biosciences Institute, Newcastle University, Newcastle, UK*

Antibiotics cure infections by killing bacteria or preventing them from growing – yet much remains to be understood about how this works. Many of the most widely used antibiotics target bacterial cell wall synthesis. These antibiotics often kill bacteria by causing them to explode, or lyse. We have investigated experimentally the efficacy of the cell wall targeting antibiotic mecillinam, which is used to treat urinary tract infections, for bacteria growing under different conditions. We show that a simple physics model that takes into account the growth of bacterial cell volume and surface area, can explain non-trivial experimental observations.

I.4 Kinetic transitions in individual recombinant exocytotic fusion pores

Edwin R. Chapman[1]

[1]*Howard Hughes Medical Institute and Department of Neuroscience, University of Wisconsin, Madison WI, USA*

Understanding the mechanism(s) by which proteins catalyze the merger of lipid bilayers hinges on elucidating the structure and dynamics of the first crucial intermediate in the membrane fusion pathway, the fusion pore (Jackson and Chapman, 2006; Lindau and Almers, 1995). We recently developed new tools that allow us to interrogate, electrophysiologically, recombinant fusion pores held in their nascent open state, thus yielding the first µsec time-resolved measurements of these channel-like structures (Bao et al., 2018; Das et al., 2020). This was accomplished by reconstituting v-SNAREs into nanodiscs (ND) and t-SNAREs into planar lipid bilayers (called black lipid membranes [BLM]). As v- and t-SNAREs assemble into trans-SNARE complexes to form a fusion pore between the ND and BLM, the rigid membrane scaffold that forms a belt around the ND limits expansion, trapping the pore in an open state. We combined this approach with single molecule fluorescence experiments to address how regulatory proteins control the assembly and disassembly of trans-SNARE complexes to drive the formation and dissolution of fusion pores, respectively. These studies have begun to relate structural changes in the fusion machinery with kinetic transitions in fusion pores in a fully defined, experimentally accessible, model system.

[1] Bao, H., Das, D., Courtney, N.A., Jiang, Y., Briguglio, J.S., Lou, X., Roston, D., Cui, Q., Chanda, B., and Chapman, E.R. (2018). Dynamics and number of trans-SNARE complexes determine nascent fusion pore properties. Nature 554, 260-263. 10.1038/nature25481.

[2] Das, D., Bao, H., Courtney, K.C., Wu, L., and Chapman, E.R. (2020). Resolving kinetic intermediates during the regulated assembly and disassembly of fusion pores. *Nat Commun 11*, 231. 10.1038/s41467-019-14072-7.

[3] Jackson, M.B., and Chapman, E.R. (2006). Fusion pores and fusion machines in Ca2+-triggered exocytosis. Annu Rev Biophys Biomol Struct 35, 135-160.

[4] Lindau, M., and Almers, W. (1995). Structure and function of fusion pores in exocytosis and ectoplasmic membrane fusion. Curr Opin Cell Biol 7, 509-517.

I.5 The mechanism of dynamin in membrane constriction - From static snapshots to a dynamic model

O. Ganichkin[1], R. Vancraenenbroeck[2,3], G. Rosenblum[3], H. Hofmann[3], AS Mikhailov[4,5], Jeffrey K. Noel[1,4], **Oliver Daumke**[1,6]

[1]Crystallography, Max Delbrück Center for Molecular Medicine, Berlin, Germany
[2]Department of Structural and Molecular Biology, University College London, London, United Kingdom
[3]Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel
[4]Department of Physical Chemistry, Fritz Haber Institute of the Max Planck Society, Berlin, Germany
[5]Computational Molecular Biophysics, WPI Nano Life Science Institute, Kanazawa, University, Kanazawa, Japan
[6]Institute for Chemistry and Biochemistry, Free University of Berlin, Berlin, Germany

Dynamin is a mechano-chemical GTPase that assembles at the neck of clathrin-coated vesicles and catalyzes membrane scission in a GTPase-dependent reaction. Here, I will describe previous structural data on dynamin from our and other groups. I will then explain our efforts toward translating the structural snapshots into a dynamic model of membrane constriction. We incorporated our experimental rate measurements and single molecule FRET-based force measurements into a model that resolves individual powerstrokes within a protein filament wound around a deformable membrane tube.

I.6 The molecular dynamics of potassium channel permeation, selectivity and gating

Bert L. de Groot[1]

[1]*Computational biomolecular dynamics, Max Planck Institute for biophysical Chemistry, Göttingen, Germany*

Ion channels facilitate the passive, selective permeation of ions such as sodium, potassium and chloride across biological membranes and as such are essential for cellular electrical signalling. Molecular dynamics simulations are used to study ion permeation across potassium channels at the atomic level. Together with crystallographic analyses and electrophysiological experiments these provide insight into the mechanisms of selective and efficient permeation of potassium, as well as the complex and subtle conformational changes involved in the gating of these channels.

I.7 Intermediate filaments – Dynamic structures between biomechanics and signaling

John E. Eriksson[1]

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Intermediate filaments (IFs) have received significant attention due to the broad range of diseases they have been shown to be associated with. There is accumulating evidence to show that many of the numerous IF-related diseases are associated with compromised tissue homeostasis as well as failing tissue regeneration and healing. Our research has established that IFs act as signaling scaffolds, organizers, and gate-keepers that are able to integrate and direct signaling machineries, with direct consequences for cell fate in tissues. Our accumulated results imply that IFs have a key role in homeostatic and regenerative signal integration. IFs have a key role in fibroblastic migration, which is of key physiological importance in wound healing. We have already demonstrated that vimentin affects migration through functions related to actomyosin complexes and show that vimentin affects directionality by guiding focal adhesions in fibroblasts. The results demonstrate that vimentin intermediate filaments are in dynamic bidirectional interplay with focal adhesion proteins, thereby controlling the maturation, stability, dynamics, arrangement, and overall orientation of focal adhesions, with a net effect on focal adhesion coordination during directional migration. In this way the also affect the organization of extracellular matrix, which in turn will be reflected upon the sensory systems of the cell that in turn will give cues to the signaling machinery that is coupled to IFs.

I.8 Biophysical approaches to explain the 3D cellular packing of epithelia.

Pedro Gómez-Gálvez[1,2,†], Pablo Vicente-Munuera[1,2,†], Samira Anbari[3,4,†], Antonio Tagua[1,2,†], Carmen Gordillo-Vázquez1,2, Ana M. Palacios[1,2], Antonio Velasco[1], Carlos Capitán-Agudo[1], Clara Grima5, Valentina Annese[1,2], Rafael Robles[5], Alberto Márquez[5], Javier Buceta[6,*], **Luis M. Escudero**[1,2,*]

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Epithelial cell organization and the mechanical stability of tissues are closely related. In this context, it has been recently shown that packing optimization in bended/folded epithelia is achieved by a surface tension energy minimization mechanism that leads to a novel cellular shape: the *scutoid* [1]. However, further cellular and tissue level implications of this new developmental paradigm remain unknown. I will present new data on the relation of this complex cellular shape and the connectivity between cells. We address this problem using a combination of computational, experimental, and biophysical approaches in tubular epithelia. We dissect the contribution of the energetic drivers inducing the complex three-dimensional packing of these tissues. We conclude that tubular epithelia satisfy a novel principle, the "Flintstones' law", that links tissue geometry and energetic profiles with the average cellular connectivity in epithelia. Our study unveils a quantitative morphogenetic law with key physiological consequences.

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I.9 Microtubules regulate mechanosensitive cell migration

Shailaja Seetharaman, Benoit Vianay, Vanessa Roca, Aaron Farrugia, Chiara De Pascalis, Batiste Boëda, Florent Dingli, Damarys Loew, Stéphane Vassilopoulos, Alexander Bershadsky, Manuel Théry, **Sandrine Etienne-Manneville**[1]

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Cells sense the mechanical properties of the substrate through integrin-mediated focal adhesions and transduce these mechanical cues into biochemical signals by a process known as mechanotransduction. In turn, cells adapt and perform specific functions that regulate various cellular processes such as cell migration. Although the role of microtubules in cell adhesion and migration has been well established, their involvement in mechanotransduction remains unclear. Using a combination of microfabrication methods, biophysical approaches and imaging techniques, we show that substrate rigidity affects a tubulin post-translational modification, namely acetylation, through β1 integrin-mediated downstream signalling in astrocytes. The enzyme responsible for microtubule acetylation, αTAT1, interacts with focal adhesion protein Talin in a tension-dependent manner. Moreover, we demonstrate that αTAT1 reorganizes the actomyosin network, increases traction force generation and promotes cell migration on stiff substrates. Our results suggest a feedback mechanism involving microtubules and actin in mechanotransduction at focal adhesions whereby, cells sense the rigidity of the substrate through integrin-mediated adhesions, modulate their levels of microtubule acetylation, which then controls the actomyosin cytoskeleton, force transmission on the substrate and promotes cell migration.

I.10 Fast Ca^{2+}-transport by PMCA-Neuroplastin complexes

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Plasma membrane Ca^{2+}-ATPases (PMCA), or Ca^{2+}-pumps, terminate Ca2+-signals in any type of cell by extruding Ca2+ ions from the cytosol to the extracellular space. In neurons and epithelial cells, Ca^{2+}-extrusion occurs within tens of milliseconds, a speed that is not compatible with current knowledge on PMCA activity.

Based on our recent identification of native PMCAs being assembled from ATPase subunits and the auxiliary proteins Neuroplastin or Basigin, we have revisited the transport velocity of these Ca^{2+}-pumps in the plasma membrane of in intact cells. We found that PMCA2-Neuroplastin complexes, the most abundant Ca^{2+}-transporters in the mammalian brain, provide Ca^{2+}-clearing in the low millisecond-range. Freeze-fracture derived immuno-EM data on densities of Ca^{2+}-source(s) and Ca^{2+}-transporters translated these kinetics into transport rates for PMCA2-Neuroplastin complexes of more than 6000 cycles/s. Direct comparison with the Na^{+}/Ca^{2+}-exchanger NCX2, an alternate-access transporter with fast kinetics, indicated similar efficiencies in Ca^{2+}-transport. Thus, our results unveiled PMCA-Neuroplastin complexes as Ca^{2+}-transporters with unanticipated high transport rates and demonstrate that under cellular conditions ATPases may operate in the kHz range.

I.11 Mind the gap: Spatial organization and signaling at cell-cell contacts

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Membrane interfaces formed at junctions between cells are often associated with characteristic patterns of membrane protein organization, such as in epithelial tissues and between cells of the immune system. Together with the influences of receptor clustering, lipid domain formation, and cytoskeletal dynamics, cell surface molecular height can directly contribute to the spatial arrangement of membrane proteins and downstream signaling. This talk will introduce an optical method for characterizing molecular height on cell surfaces and discuss several biological problems where cell surface molecular height plays a key role in regulating cell-cell signaling.

I.12 Mechanobiological control of T-cell activation

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New perspective of mechanobiology is currently emerging across multiple disciplines in thebiomedical sciences. In contrast to conventional believes, recent evidence indicates that cellsregulate their cell mechanics not downstream of signalling events triggered by ligand–receptor binding, but that cells employ a diversity of feedback mechanisms to dynamicallyadjust their mechanics in response to external stimuli. Quantifying cellular forces hastherefore become an contentious challenge across multiple disciplines at the interface ofbiophysics, cell-biology, and immunology. Mechanical forces are especially important for theactivation of immune T cells. Using a suite of advanced quantitative super-resolution imagingand force probing methodologies to analyse resting and activated T cells, we demonstrateactivatingTcellssequentiallyrearrange theirnanoscale mechanobiology,creatingapreviously unreported ramifying actin network above the immunological synapse (IS). Weshow evidence that the kinetics of the antigen engaging the T-cell receptor controls thenanoscale actin organisation and mechanics of the IS. Using an engineered T-cell systemexpressing a specific T-cell receptor and stimulated by a range of antigens, forcemeasurements revealed that the peak force experienced by the T- cell receptor duringactivation was independent of the kinetics of the stimulating antigen. Conversely,quantification of the actin retrograde flow velocity at the IS revealed a striking dependence onthe antigen kinetics. Taken together, these findings suggest that the dynamics of the actincytoskeleton actively adjusted to normalise the force experienced by the T-cell receptor in anantigen specific manner. Consequently, tuning actin dynamics in response to antigen kineticsmay thus be a mechanism that allows T cells to adjust the length- and time- scale of T-cellreceptor signalling.

I.13 Cellular shapes driven by curved membrane proteins that recruit the actin cytoskeleton: lamellipodia, ruffles and motility

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How eukaryote cells control their shapes is an open puzzle, although many of the protein components involved in this process have been characterized. We explore theoretically the spontaneous formation of shape patterns and dynamics that are driven by the coupling of curved membrane proteins and the actin cytoskeleton that they recruit. When the proteins are convex and they recruit the protrusive forces aof actin polymerization this mechanism leads to symmetry-breaking and to the formation of lamellipodia-like structures [1,2]. In the presence of adhesion we find that the same mechanism provides a minimal model for polarized cell motility [3].

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I.14 Are microtubules their own mechanosensors?

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In plant cells, cortical microtubules (CMTs) generally control morphogenesis by guiding the synthesis of stiff cellulose microfibrils in the wall. CMT orientation has been proposed to depend on geometrical cues, with microtubules aligning with the cell long axis in silico and in vitro. Yet, CMTs are usually transverse in vivo, i.e., along predicted maximal cortical tension, which is transverse for cylindrical pressurized vessels. Here, we confined protoplasts laterally to impose a curvature ratio and modulated pressurization through osmotic changes. We find that CMTs can be longitudinal or transverse in wall-less protoplasts and that the switch in CMT orientation depends on pressurization. In particular, longitudinal CMTs become transverse when cortical tension increases, consistent with observations in planta. To search for upstream regulators, we investigated the contribution of Receptor-Like Kinase (RLK) to the CMT response to tensile stress. We found that both CMT and RLK pathways independently control the mechanical integrity of the cell. Conversely, when both RLK signaling and CMTs are impaired, plant cells behave like passive material. Altogether, these results reveal the key role of microtubule response to cortical tension in plant cell morphogenesis, and further support the idea of an autonomous microtubule mechanosensing pathway.

I.15 Focusing on the mitochondrial inner membrane with light and electron microscopy

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Mitochondria, the 'powerhouses of the cell', are double membrane organelles that are essential for eukaryotic life. Because of their inner-cellular mobility, their small size and their complex architecture, they are notoriously challenging objects for high-resolution light microscopy [1]. We employ STED super-resolution microscopy and other microscopies to investigate the folding of the mitochondrial inner membrane. We aim at understanding how mitochondria develop and maintain their complex inner architecture [2-4]. This talk will summarize our recent progress in investigating inner-mitochondrial protein distributions and cristae dynamics.

[1] Jakobs S. et al., Annu Review Biophys 49, 289-308 (2020)
[2] Pape et al., PNAS 117, 20607-20614 (2020)
[3] Weber et al., Nature Photonics 15, 361-366 (2021)
[4] Stephan et al., EMBO J, e104105 (2020)

I.16 Role of intermediate filament networks in compression stiffening of cells and protection of nuclei

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Networks of stiff or semiflexible polymers become stiffer when deformed to increasingly large shear strains, but these networks generally soften in uniaxial compression, as stiff filaments buckle. This feature was first observed for the extracellular matrix networks formed by fibrin or collagen, but it also occurs with crosslinked networks of F-actin or microtubules. Intermediate filament networks, however, stiffen with both increasing shear and compressional strains, consistent with the expiation that IFs are too flexible to undergo bucking transitions in networks of the mesh size present in vitro or in the cytoskeleton. As a result, normal fibroblasts containing vimentin IFs stiffen when compressed, but vim -/- fibroblasts do not. These results emphasize the unique role of IFs in protection of cells and their nuclei from uniaxial compressive stresses.

I.17 Matrix elasticity gradients guide neuronal polarity by controlling microtubule network mobility

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Neuronal polarization and axon specification depend on extracellular cues, intracellular signaling, cytoskeletal rearrangements and polarized transport, but the interplay between these processes has remained unresolved. The polarized transport of kinesin-1 into a specific neurite is an early marker for axon identity, but the mechanisms that govern neurite selection and polarized transport are unknown3,4. Here we show that extracellular elasticity gradients can control polarized transport and axon specification, mediated by Rho-GTPases whose local activation is necessary and sufficient for polarized transport. Selective Kinesin-1 accumulation furthermore depends on differences in microtubule network mobility between neurites and local control over this mobility is necessary and sufficient for proper polarization, as shown using optogenetic anchoring of microtubules. Together, these results explain how mechanical cues can instruct polarized transport and axon specification.

I.18 Yeast tune their vacuole membranes to phase separate at their growth temperatures

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When yeast consume ample glucose, they grow rapidly. When that glucose is depleted, yeast enter a "stationary stage", and striking changes occur. One of the changes is that the membrane of the vacuole (an organelle) phase separates into micron-scale domains [1]. This transition is reversible: at high temperature the vacuole membrane is uniform, and at a low temperature the membrane phase separates again [2]. The transition temperature scales with the growth temperature of the yeast cells, implying that the cells actively tune the composition of their vacuole membranes to maintain proximity to the phase transition. The process of the cell changing its membrane composition in response to temperature is slow. Specifically, when yeast in the stationary stage are held just above their transition temperature for one hour, their vacuole membranes remained uniform. The membranes returned to a phase-separated state only when the temperature was decreased again. The distribution of domains on the vacuole surface is reminiscent of patterns attributed to modulated phases or microemulsions [3].

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[2] S.P. Rayermann et al., Biophys. J. 113, 2425-2432 (2017).
[3] C.E. Cornell et al., Biophys. J. 115, 690-701 (2018).

I.19 Dynamics of contracting actomyosin networks with turnover

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Contracting actomyosin networks have essential roles in many processes including cell division, intracellular transport and cell motility. To fulfill these functions, the networks must undergo continuous reorganization facilitated by rapid actin turnover. Despite extensive research, the contractile network behavior in the presence of turnover is still not well understood. To address this issue, we rely on an in vitro system based on cytoplasmic Xenopus egg extracts encapsulated into cell-sized water-in-oil droplets. Thanks to the presence of physiological turnover rates, our system exhibits contractile flows that persist for hours and self-organize into a wide array of spatiotemporal patterns. Interestingly, we observe a size-dependent transition in the contractile behavior of the system, going from continuous contraction in smaller droplets to periodic contraction in the form of waves and spirals in larger droplets. The periodicity increases with network contraction rates while the characteristic length-scale for the appearance of waves decreases. Computational modeling suggests that the coupling of the contractile gel mechanics with turnover is indeed key to the pulsatile behavior in large droplets.

I.20 Confined Migration Induces Heterochromatin Formation and Alters Chromatin Accessibility

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Cell migration is required for many physiological and pathological functions. *In vivo*, cells frequently migrate through tight spaces, requiring extensive deformation of the cell body and nucleus. We hypothesized that nuclear deformation associated with such confined migration could alter chromatin organization and function. Studying cells migrating through collagen matrices and microfluidic devices that mimic interstitial spaces in vivo, we found that confined migration results in increased H3K9me3 and H3K27me3 heterochromatin marks that persist for several days. This confined migration-induced heterochromatin ("CMiH") was distinct from heterochromatin formation during migration initiation. CMiH predominantly decreased chromatin accessibility at intergenic regions near centromeres or telomeres, suggesting heterochromatin spreading from existing heterochromatin sites. Consistent with the overall decrease in chromatin accessibility, global transcription was decreased during confined migration. Inhibiting CMiH reduced migration speed, suggesting that it promotes confined migration. Intriguingly, we also identified increased accessibility at promoter regions of gene linked to chromatin silencing, tumor invasion, and DNA damage response. Together, our study indicates that confined migration can induce chromatin changes that regulate confined migration and other cellular functions.

I.21 The lipidomic, biophysical, and functional asymmetry of the mammalian plasma membrane

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A fundamental feature of cellular plasma membranes (PMs) is an asymmetric lipid distribution between the bilayer leaflets. However, neither the detailed, comprehensive compositions of individual PM leaflets nor how these contribute to structural membrane asymmetries have been defined. We report the distinct lipidomes and biophysical properties of both monolayers in living mammalian PMs. Phospholipid unsaturation is dramatically asymmetric, with the cytoplasmic leaflet being approximately twofold more unsaturated than the exoplasmic leaflet. Atomistic simulations and spectroscopy of leaflet-selective fluorescent probes reveal that the outer PM leaflet is more packed and less diffusive than the inner leaflet, with this biophysical asymmetry maintained in the endocytic system. The structural asymmetry of the PM is reflected in the asymmetric structures of protein transmembrane domains. These structural asymmetries are conserved throughout Eukaryota, suggesting fundamental cellular design principles.

I.22 A Tethered Ligand Assay to Probe SARS-CoV-2: ACE2 Interactions

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SARS-CoV-2 attaches to the ACE2 receptor on human hosts cells via its receptor-binding domain (RBD) on the Spike protein. This critical first step occurs in dynamic environments, where external forces act on the binding partners, creating an urgent need for assays that can quantitate SARS-CoV-2 interactions with ACE2 under mechanical load. We present a tethered ligand assay that comprises the RBD and the ACE2 ectodomain joined by a flexible peptide linker. Using magnetic tweezers [1,2] and atomic force spectroscopy, we investigate the RBD:ACE2 interaction over the whole physiologically relevant force range [3]. Combined with steered molecular dynamics simulations, we observe and assign fully consistent unbinding and unfolding events across the three techniques and establish ACE2 unfolding as a molecular fingerprint. We quantify the force dependence and kinetics of the RBD:ACE2 bond in equilibrium and find significant differences between SARS-CoV-1 and 2, which helps to rationalize the different infection patterns of the two viruses [3]. Finally, we probe how different RBD mutations affect force stability and speculate how mechanical coupling promotes increased transmissibility in variants of concern.

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I.23 Emerging imaging technologies to study cell architecture, dynamics and function

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Powerful new ways to image the internal structures and complex dynamics of cells are revolutionizing cell biology and bio-medical research. In this talk, I will focus on how emerging fluorescent technologies are increasing spatio-temporal resolution dramatically, permitting simultaneous multispectral imaging of multiple cellular components. In addition, results will be discussed from whole cell milling using Focused Ion Beam Electron Microscopy (FIB-SEM), which reconstructs the entire cell volume at 4 voxel resolution. Using these tools, it is now possible to begin constructing an "organelle interactome", describing the interrelationships of different cellular organelles as they carry out critical functions. The same tools are also revealing new properties of organelles and their trafficking pathways, and how disruptions of their normal functions due to genetic mutations may contribute to important diseases.

I.24 An active rigidity percolation transition in the actomyosin cortex drives a minimal morphogenetic and motile machinery

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Most metazoan cells can display a spontaneous motile behavior. This locomotion capacity of single cells is generally attributed to the fundamental properties of the actomyosin network which can polarize and produce directed forces. Experimental as well as theoretical work have converged to define the general properties of actomyosin systems. Nevertheless, there has been so far no time-resolved description of actomyosin networks dynamics down to the single filament in live motile cells. Here, we combine confinement to induce the formation of stable blebs - motile structures with a simple shape - with high numerical aperture total internal reflection fluorescence microscopy, to investigate the assembly of the flowing actin cortex from single actin filaments. Our study points to the importance of the active advection, powered by rear located myosin motors, of a rigid, passive solid-like state of the actin cortex. We propose a physical model that shows how a self-organized spatial patterning of the actin filaments network emerges due to the active advection by motors, combined with a rigidity percolation transition. This spatial organization gives stability and mechanical properties (soft front and solid back) to the network and constitute a minimal locomotion mechanism that can produce robust and fast migration through complex environments.

I.25 Optimal checkpoint strategies

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Why biological quality-control systems fail has often remained mysterious. Checkpoints in yeast and animals are overridden after prolonged arrests allowing self-replication to proceed despite the continued presence of errors. Although critical for biological systems, checkpoint override is not understood quantitatively or at the system level by experiment or theory, even though the genes and circuits involved in many checkpoints have been researched extensively.

To uncover potential patterns obeyed by error correction systems, we derived the mathematically optimal checkpoint strategy, balancing the trade-off between risk and opportunities for growth. The theory predicts the optimal override time without free parameters based on two inputs, the statistics i) of error correction and ii) of survival.

We applied the theory experimentally to the DNA damage checkpoint in budding yeast, an intensively researched model for other eukaryotes, whose override is nevertheless not understood quantitatively, functionally, or at the system level. Using a novel fluorescent construct which allowed cells with DNA breaks to be isolated by flow cytometry, we quantified i) the probability distribution of repair for a double-strand DNA break (DSB), including for the critically important, rare events deep in the tail of the distribution, as well as ii) the survival probability after override. Based on these two measurements, the optimal checkpoint theory predicted remarkably accurately the DNA damage checkpoint override times as a function of DSB numbers, which we also measured for the first time precisely. Thus, a first-principles calculation uncovered hitherto hidden patterns underlying the highly noisy checkpoint override process. Our multi-DSB results revise well-known bulk culture measurements and show that override is a more general phenomenon than previously thought. Further, we show that override is an advantageous strategy in cells with wild-type DNA repair genes.

The universal nature of the balance between risk and self-replication opportunity is in principle relevant to many other systems, including other checkpoints, developmental decisions, or reprogramming of cancer cells, suggesting potential further applications.

I.26 Class II membrane fusion proteins in hantaviruses and beyond

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Hantavirus are rodent-borne human pathogens [1] also transmitted from human to humans via aerosols [2,3] for which no vaccine nor therapeutic treatment are available. The 'New World' hantaviruses Andes virus and Sin Nombre virus cause the hantavirus cardiopulmonary syndrome in the Americas, reaching 40 % case fatality rates [4]. The 'Old World' hantaviruses Puumala, Hantaan, and other viruses are endemic in Eurasia and cause hemorrhagic fever with renal syndrome [5]. Structural studies have shown that the hantavirus particles are pleomorphic, but display a regular surface lattice, formed by heterodimers of glycoproteins Gn and Gc, that encloses the viral membrane [6]. This surface lattice is sensitive to acid pH, which induces dissociation of the Gn/Gc heterodimers followed by a fusogenic conformational chain of Gc to induce fusion of the viral envelope with the membrane of the endosome of a target cell [7]. This step allows the release of the viral genetic material into the cytoplasm to infect the cell. Gc was shown to have a typical class II fusion protein fold, as found in other unrelated enveloped viruses and also in cellular proteins involved in cell-cell fusion. In my talk, I will describe the organization of the hantavirus surface lattice and its implications for immunogen design. I will also address the evolutionary links among class II fusion proteins in general.

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I.27 Facial recognition of biological lipid membranes

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The present field of targeted drug design is predominantly protein-centric not just because of historical reasons but especially because of the persisting lack of an efficient design platform for drugs that enable selective targeting of biological membranes beyond the level of molecular structure. Design of (peptide) drugs able to selectively target distinct, collective structural features in biological lipid membranes (e.g., leaflet curvature and lipid composition) is severely challenged by the dynamic, disordered fluid nature of lipid membranes, i.e. its fluid interface, rendering existing molecular structure-based peptide drug design strategies ineffective. Our evolutionary molecular dynamics (evoMD) approach [1] overcomes these current limitations by 'learning' peptides how to optimally recognize distinct fluid membrane interfaces via evolutionary optimization (artificial intelligence) directed by highly efficient free energy calculation approaches. What makes this inverse design approach particularly valuable is that the obtained insights can simultaneously gain an unique mechanistic understanding on how native proteins recognize membrane interfacial features such as membrane curvature or lipid composition and especially how protein-membrane interactions have determined the evolution of these proteins. To demonstrate the utility of the evoMD method, we will first resolve the trans-membrane domain sequence which maximally attracts/clusters cholesterol [1]. Surprisingly, the global solution features an unusual short hydrophobic block, consisting of typically only eight short chain hydrophobic amino acids, surrounded by three successive lysines. We will discuss the underlying molecular mechanism of cholesterol attraction and compare our findings with recent experimental results [2] as well as the proposed cholesterol recognition amino acid consensus (CRAC) motif [3]. Last but not least, we will demonstrate the utility of evoMD in the targeted design of broad-spectrum antiviral peptide drugs.

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I.28 Exogenous vimentin supplementation transiently affects early steps during HPV16 pseudovirus infection

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Understanding and modulating early steps in oncogenic Human Papillomavirus (HPV) infection has great cancer-preventative potential. We have previously identified cell-surface expressed vimentin as a novel HPV16 pseudovirus (HPV16-PsVs)-binding molecule modulating its infectious potential [1]. To further explore its mode of inhibiting HPV16-PsVs internalisation, artificial supplementation with exogenous recombinant human vimentin (rhVim) revealed that only the globular form of the molecule (as opposed to the filamentous form) inhibited HPV16-PsVs internalisation in vitro. Further, this inhibitory effect was transient and not sustained over prolonged incubation times as demonstrated in vitro and in vivo, possibly due to full entry molecule engagement by the virions once saturation levels have been reached. The rhVim -mediated delay of HPV16-PsVs internalisation involved multiple steps during the virus' interaction with the host cell and was found to affect both heparan sulfate proteoglycan (HSPG) binding as well as subsequent entry receptor complex engagement. Interestingly, decreased pseudovirus internalisation (but not infection) in the presence of rhVim was also observed for the oncogenic HPV types 18, 31 and 45.

Together, these data demonstrate the potential of rhVim as a modulator of HPV infection but need further refinement with regard to stabilisation and formulation before development as an alternative prophylactic means.

[1] G. Schäfer et al., Journal of Virology 91, 16 (2017)

I.29 Molecular Mechanobiology of Extracellular Matrix: Functional implications in healthy and diseased organs.

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Reciprocal mechanical signaling between cells and their environment is key to the spatio-temporal coordination of tissue growth and regenerative processes, and if miss-balanced can tip the niche towards pathological transformations. Yet, these reciprocal processes and how they tune cell function are difficult to quantify in real organs. Translating what was learned in Mechanobiology mostly on single cells to the tissue level is hampered by at least two challenges, the lack of nanoscale sensors to probe forces or tissue fiber tension in organs, as well as appropriate de novo grown 3D microtissues to mimic essential aspects of healthy versus diseased tissue niches. To address these challenges, we developed a peptide-based nanosensor that can read out the tensional state of ECM fibers, and validated it using either tissue cryosections or upon injection into living animals. We will discuss our recent insights by comparing healthy versus diseased organ tissues as compared to cancer. Discoveries into the molecular mechanobiology of ECM at the tissue level are prone to open new diagnostic and therapeutic avenues.

[1] Viola Vogel (2021).

Abstracts of Contributed Talks

C.1 Adhesion dynamics and organization of neurons and glial cells on nanocolumnar TiN substrates

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Many biomedical applications such as deep brain stimulation with electrodes for neurodegenerative diseases like Parkinson's disease rely on fine-tuned coupling of biomaterials and biological tissue. Cell survival, proliferation, and biochemical function depend on the surface topography and chemistry at the interface between electrode and biological material. In addition to in vivo implementations, lab-on-a-chip devices such as multielectrode arrays offer new perspectives in in vitro assessments of cellular behavior ranging from neural network formation to drug testing by electrical coupling of the cells. In our study, we investigate the interaction of neurons (SH-SY5Y) and glial cells (U-87 MG) with electrode materials such as titanium nitride (TiN) and TiN with a nanocolumnar surface patterning in contrast to gold and indium tin oxide (ITO) substrates. TiN nano exhibits a lowered self-impedance important for miniaturization of multielectrode systems. Employing single-cell force spectroscopy, we analyze the short-term cell adhesion forces for different contact times on the electrode samples. Results are compared with measurements of cell proliferation, spreading dynamics, network building, and cluster formation on longer time scales of several days. To this end, we implement a radial autocorrelation function of cellular positions on the samples in combination with a K-means cluster algorithm to quantify cell-surface interaction and cell organization. Adhesion forces exerted by glial cells are almost independent of the electrode material and spreading dynamics stop after one day of culture with homogeneous cell distributions. In contrast, adhesion behavior of neurons varies with different substrate types and cells tend to spread more and form larger clusters on TiN and TiN nano. We conclude that TiN with a nanocolumnar surface patterning offers great potential as a bioactive material for building miniaturized microelectrode arrays in combination with neuronal cells.

C.2 Do the Loops in the N-SH2 Binding Cleft Truly Serve as Allosteric Switch in SHP2 Activation?

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The SH2 domain containing phosphatase SHP2 is a critical regulator of signal transduction, being implicated in cell growth and differentiation. Activating mutations cause developmental disorders and act as oncogenic drivers in hematologic cancers. SHP2 is activated by phosphopeptide binding to the N-SH2 domain, triggering the release of N-SH2 from the catalytic PTP domain. Based on early crystallographic data, it has been widely accepted that opening of the N-SH2 binding cleft serves as the key allosteric switch driving SHP2 activation [1]. To test the putative coupling between binding cleft opening and SHP2 activation as assumed by the allosteric switch model, we critically reviewed structural data of SHP2 and we used extensive molecular dynamics (MD) simulation and free energy calculations of isolated N-SH2 in solution, SHP2 in solution, and SHP2 in a crystal environment. Our results demonstrate that the binding cleft in N-SH2 is constitutively flexible and open in solution, and that a closed cleft found in certain structures is a consequence of crystal contacts. The degree of opening of the binding cleft has only a negligible effect on the free energy of SHP2 activation. Instead, SHP2 activation is greatly favored by the opening of the central β-sheet of N-SH2. We conclude that opening of the N-SH2 binding cleft is not the key allosteric switch triggering SHP2 activation [2].

[1] P. Hof et al., Cell 92, 441 (1998).
[2] M. Anselmi and J.S. Hub., Proc Natl Acad Sci USA 118, e2025107118 (2021).

C.3 Shape deformation of RBCs in a doublet

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The high deformability of Red Blood Cells (RBCs) influences the blood flow and blood circulation as well as RBC aggregation. The deformability of the RBCs is determined by the elastic properties of the membrane and their high surface area to volume ratio.

Normally the blood flow is sufficient to prevent formation of RBC aggregates. However, in low shear rates or some pathological conditions the aggregation of RBCs occur [1]. The shape of RBCs in an aggregate differs from the equilibrium biconcave morphology. This change in the morphology of the cells is coupled to the contact areas between two cells in a doublet and as a result the strength of interaction energy.

We induce well-defined long range attraction between RBCs by adding mono disperse rod-like bacteriophage viruses with a high length to diameter ratio. The interaction is tuned by varying the concentration of the rod-like particles which results in different shape of the doublets. We investigate the deformation of a single RBC in a doublet from 3-D reconstructed confocal images. Our results show an increase in the bending energy of single RBCs in a doublet which is coupled to a decrease in the excluded volume for the rods with increasing the adhesion energy.

[1] P Ziherl, S Svetina, Flat and sigmoidally curved contact zones in vesicle–vesicle adhesion. Proc. Natl. Acad. Sci.104, 761–765 (2007).

C.4 Generation of Fluid Flows On The Skin of *Xenopus* Embryo

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Multiciliated cells (MCC) are ubiquitous in living systems. They support physiological functions ranging from the locomotion of marine organisms to the transport of fluid in brain and respiratory tracts [1]. Such ciliated epithelia is also found on the skin of the embryo of amphibian frog, *Xenopus laevis*, when its immune system is not yet functional and then disappears during development [2]. This suggests that the ciliated epithelium supports the function of pathogen clearance by generating fluid flows at the surface of the embryo. In *Xenopus* epithelium, MCCs are regularly distributed and the implication of this precise distribution on the flow pattern is unknown. By combining an experimental setup using cultured explant and numerical simulations, we show how the organization of MCC is affecting the fluid flow. We use a 2D numerical model based on the Lattice Boltzmann method to predict the flow fields generated by the beating ciliated cells and then validate the model using experimental data [3]. We also show how the spatial coverage of MCC within an epithelium is optimized for the emergence of fluid flow and its associated biological function.

[1] Mitchell D.R, Advances in Experimental Medicine and Biology, vol 607, Springer (2007)
[2] Boutin, C., Kojabachian, L., CURR Opin Genet Dev; 56. 1-7 (2019)
[3] Gsell, S., Loiseau, E., D'Ortona, U. *et al.* Hydrodynamic model of directional ciliary-beat organization in human airways. *Sci Rep* 10, 8405 (2020)

C.5 A continuous complete RNA translocation cycle by the DEAH-box helicase Prp43 in atomic detail

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Understanding conformational cycles of complex macromolecular machines in atomic detail remains a central goal of molecular biophysics. Here, we focus on helicases that are crucial for every living organism to carry out functions such as DNA/RNA transcription, translation, DNA/RNA repair,recombination and splicing. The largest group among helicases is the Superfamily 2 (SF2), which includes the DEAD- and DEAH-box helicases as key players in the splicing pathway. Despite the wide interest in understanding the detailed mechanism of ssRNA translocation during splicing, the exact movements are still unknown. Using molecular dynamics simulations and enhanced sampling techniques, we observed a complete RNA translocation cycle of the DEAH-box helicase Prp43 in atomic detail. The simulations reveal the collective behaviour of the three main domains RecA1, RecA2and CTD, like the detachment and the formation of the interface of the RecA domains or the rotation of the CTD. Additionally, the simulations give detailed insight in the essential and atomistic processes during the large domain motions,e.g. a movement cascade induced by an arginine finger in the ATP binding side, the conformational change of a serine loop to a helical state, the cleavage and formation of various hydrogenbonds, including the so called Hook-loop and Hookturn, the behaviour of the ssRNA during the process and more.

C.6 Molecular events during selective ER-phagy

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Living cells constantly remodel the shape of their lipid membranes. In the endoplasmic reticulum (ER), the reticulon homology domain (RHD) of the reticulophagy regulator 1 (RETR1/FAM134B) forms dense autophagic puncta that are associated with membrane removal by ER-phagy. Through molecular dynamics (MD) simulations of FAM134B in flat and curved membranes, we relate the dynamic RHD structure with its two wedge-shaped transmembrane helical hairpins and two amphipathic helices to FAM134B functions in membrane-curvature induction and curvature-mediated protein sorting. In MD simulations, FAM134B-RHD spontaneously forms clusters, driven in part by curvature-mediated attractions. At a critical size, as in a nucleation process, the FAM134B-RHD clusters amplifies the membrane shaping effect to induce the formation of membrane buds [2]. The kinetics of budding depends sensitively on protein concentration and bilayer asymmetry. Our MD simulations shed light on the role of FAM134B-RHD in ER-phagy and show that membrane simulations can be used to study various aspects of membrane remodelling, inaccessible to experiments.

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[2] M. Siggel et al., *J Phys Chem Lett 12*(7), 1926-1931 (2021).

C.7 Design of 3D printed hydrogel biofilm mimics

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Bacteria demonstrate an ability to attach surfaces and grow in ubiquitous communities called biofilms.[1] The structural, physical, mechanical and chemical properties of biofilms are influenced by the surrounding environment.[2] The EPS provides protection to the bacteria against chemical and mechanical stresses, as well as facilitates their nutrition. It has features of both, solids and liquids, and thus it is known to be viscoelastic in nature.[3] To elucidate their complex behavior and understand the role of physical interactions, biofilm mimics using hydrogels have been developed to better understand and imitate them.[3] Thus, in this project, we developed a 3D printable hydrogel-bacteria system to mimic biofilms, and to study the effect of the mechanical properties on bacterial behavior and responses. Viscoelastic Pluronic F127 polymer solutions were used as bioinks for the 3D printing. Variations in bacterial growth, metabolic activity, intracellular stress, secreted biomolecules and viability were seen depending on their ability/inability to modify the surrounding matrix. We believe that this work would give us a reliable and consistent biofilm mimic and help us learn further about the bacterial subpopulations and growth behavior in such synthetic matrices.References:

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C.8 Fatty Connective Tissue Interaction changes Cancer Cell Mechanics

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The tumor micro-environment plays an important role in tumorigenesis and during tumor progression. While already significant findings on the influence of the ECM on cell mechanics [1] and the role of fatty connective tissue in tumor invasion [2] were made, the impact of fatty connective tissue onto the biomechanics of cancer cells remains elusive even though tumors often grow in the direct vicinity.

Here we show that adipocytes of fatty tissues and cancer cells significantly interact with each other. As a result the cancerous cells drastically change their phenotypical and biomechanical properties. We quantified these viscoelastic changes with the optical stretcher. Further we show that the observed viscoelastic changes are time-dependent and reversible. The cancer cells regain their initial mechanical state when the co-culturing and the crosstalk are stopped. Our coculture experiments show that cells adapt their mechanical properties not only depending on the ECM and its stiffness but are also influenced by the interaction with adipocytes of the fatty connective tissue.

From a biophysical perspective, our results indicate an essential impact of adipocytes and fatty connective tissue on cancer, potentially affecting cell motility and possibly inducing an unjamming transition.

[1] Northcott et al, Frontiers in Cell and Developmental Biology 6 (2018).
[2] Kubitschke et al., Scientific Reports 9 (2019).

C.9 Red blood cell deformability in erythrocyte sedimentation and in vivo partitioning

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Erythrocytes, also known as red blood cells, are the most abundant type of cells in the human body. Among the mechanical peculiarities of these cells are their specific shape and high flexibility. These properties are known to be critical for the cells to travel in the capillaries of our circulatory system, whose cross section is sometimes smaller than the erythrocytes diameter. In this presentation, we will see what influence this flexibility has on two extreme regimes of erythrocytes transport: their *in vivo* partitioning in small vessel bifurcation and their *in vitro* sedimentation [1,2]. While the former is a phenomenon occurring at high shear rate and is relevant for oxygen delivery in living organisms, the second occurs at virtually no shear and is used as a diagnostic tool to assess potential inflammation. We will present experimental evidences and characterization that the mechanical properties of erythrocytes have a significant influence on their behavior in both cases. For their sedimentation rate, we also introduce an original model considering this influence and the resulting properties of erythrocytes aggregates.

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[2] A. Rabe, A. Kihm, A. Darras *et al*. Biomolecules, vol. 11, no 5, p. 727 (2021).

C.10 Stochastic bond dynamics induce optimal alignment of malaria parasite

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Merozoites, malaria parasites during the blood-stage of infection, invade healthy red blood cells (RBCs) to escape from the immune response and multiply inside the host. The invasion occurs only when the parasite apex is aligned with RBC membrane, making the parasite alignment a crucial step for the invasion. Recent experiments have also demonstrated that there is a considerable membrane deformation during the alignment process. In this work, using mesoscopic simulations we assess the exact roles of RBC deformations and parasite adhesion during the alignment. Using coarse-grained models of a deformable RBC and a rigid parasite, we show that both RBC deformation and parasite adhesion bond dynamics are important for an optimal alignment. By calibrating the parasite's motion properties against experiments, we show that simulated alignment times match quantitatively with the experimental alignment times. We find that the stochastic nature of adhesion bond kinetics is the key for inducing optimal alignment times [1]. We also show that alignment times increase drastically for rigid RBC which signifies that parasite invasion is less probable into already infected RBC and that membrane deformations during the parasite alignment. Finally, we will demonstrate the importance of parasite shape in the alignment process [2].

[1] Hillringhaus, S., Dasanna, A. K., Gompper, G., & Fedosov, D. A., Stochastic bond dynamics facilitates alignment of malaria parasite at erythrocyte membrane upon invasion. eLife, 9, e56500 (2020).
[2] Dasanna, A. K., Hillringhaus, S., Gompper, G., & Fedosov, D. A., Effect of malaria parasite shape on its alignment at erythrocyte membrane. eLife, 10, e68818 (2021).

C.11 Photoswitchable ICAM1 for immunological synapse studie

Priyanka Dhakane[1], Jingnan Zhang[1], Aránzazu del Campo[1,2], Shrikrishnan Sankaran[1]

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The immunological synapse is formed as a result of tight apposition of Antigen Presenting Cells and Lymphocytes such as T-cells. This structure is a complex assembly of spatially organized concentric rings of multiple proteins.[1] The functional role of molecular clustering in the center of immunological synapse is debatable. It is essential for immune response functions such as activation of T-cells and secretion of cytokines and lytic granules leading to death of the APC. Numerous reports have shown that spatio-temporal organization of ligands along with the APC's mechanical properties are vital for the IS to form and function effectively.particularly in provoking discussion on the similarity of intercellular communication controlling disparate biological processes. Recent studies have clarified some of the underlying molecular mechanisms and functions of the IS. For both T cells and natural killer (NK[2]

I will present our work where we established mechanically tunable 2D hydrogels on which dynamically light activatable ligands are anchored. We designed light responsive Intracellular Cell Adhesion Molecule 1, which is an APC transmembrane protein and is essential for early adhesion of a T-cell to an APC, following which the IS forms through multiple steps. For making it light responsive, we fused Light-Oxygen-Voltage domain from Avena Sativa to the N-terminus of extracellular domain 1 of ICAM.[3]

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[3] Y. I. Wu, D. Frey, O. I. Lungu, A. Jaehrig, I. Schlichting, B. Kuhlman, K. M. Hahn, Nature 2009, 461, 104–108.

C.12 Quantitative biophysical characterization of fibroblast activation

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Fibroblasts are one of the most widespread cell type throughout human organism and their activation can be triggered by both biochemical cues1 and mechanotransductive cues, which allows cells to perceive mechanical signal from the environment2. The environmental cues have a dynamic role affecting some of cellular behaviors, one of which is the fibroblast to myofibroblast transition (FMT)3, which cells undergo after an injury to a tissue take place, leading, if not correctly regulated, to loss of functioning and to stiffening of the tissue. In this project, we focus on characterizing the influence of different mechanical and physical cues from the environment, and particularly the simultaneous presence of some of them, on FMT. The latter is especially relevant because, until now, these cues are mostly investigated individually, while in vivo they act simultaneously. We will present our novel experimental setup to subject fibroblasts to controlled combinations of environmental cues and our in vitro module that allows quantifications of how these cues affect cellular parameters throughout the FMT.

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C.13 Mechanotransduction of axonal growth: a journey from microtubules to local phenomena

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The effect of mechanical forces on axonal growth has been sparking great interest in the scientific community. The impact of mechanical tension on axonal outgrowth is now quite well known [1] while little or nothing is known about the molecular mechanisms evoked. To answer this biological question, we have recently developed a method for stretching axons based on magnetic nanoparticle (MNP) labeling and the application of forces via an external magnetic field [2–3]. We found that the MNP-mediated forces promoted the extension of axons, as well as sprouting and maturation. The elongation was a real mass addition as we observed an accumulation of endoplasmic reticulum cisternae and non-reduction of caliber in stretched axons. As further evidence, there was the block of the elongation by the treatment with an inhibitor of protein synthesis. We have also seen that the stretching stimulated microtubule (MT) polymerization and modulation of intracellular calcium levels [3]. We have observed an alteration of axonal transport in stretched axons, as well as an involvement of local translation. Considering that many structures involved in local translation are transported at the axonal level, we hypothesize that tension stimulates the creation of a cross-talk between local phenomena, where MTs, formerly known as tension sensors [4], could play a key role.

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[4] O. Hamant, D. Inoue, D. Bouchez, J. Dumais, and E. Mjolsness. Nat. Commun., 10:2360 (2019).

C.14 Sculpting vesicles with active particles

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Biological cells are able to generate intricate structures and respond to external stimuli, sculpting their membrane from inside. Simplified biomimetic systems can aid in understanding the principles which govern these shape changes and elucidate the response of the cell membrane under strong deformations. We employ a combined simulation and experimental approach to investigate different non-equilibrium shapes and active shape fluctuations of vesicles enclosing self-propelled particles [1]. Interestingly, the most pronounced shape changes are observed at relatively low particle loadings, starting with the formation of tether-like protrusions to highly branched, dendritic structures. At high volume fractions, globally deformed vesicle shapes are observed. The obtained state diagram of vesicles sculpted by active particles predicts the conditions under which local internal forces can generate dramatic cell shape changes, such as branched structures in neurons.

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C15 Hemidesmosome-like Adhesion Mimicked through Nanopatterning of Laminin-332

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Hemidesmosomes (HD) are multiprotein-complexes that firmly anchor cells to the basement membrane through the interconnection of the cytoplasmic intermediate filaments, keratin, with extracellular laminin- 332 (Ln-332) [1]. This type of cell-matrix adhesion is of fundamental importance in the integrity and normal function of multiple tissues and act as signaling centers, their failure can result in various diseases [2]. Considerably less attention has been paid to HDs compared to focal adhesions in single-cell structures due to the lack of suitable in vitro model systems.

In this work, nanopatterns of Ln-332 are created to direct and study the formation of HD assemblies in adherent Hacat cells, mimicking the cell-matrix interface. Protein nanopatterns of size 100, 300, 500, and 1000 nm were fabricated and used to study cell adhesion, showing that Hacat cells adhere and form hemidesmosome-like adhesions after 3 hr. Hemidesmosome-like junctions formed on larger patterns were more well-defined, imaged by colocalization of α6 integrin with Col 17 or Pan cytokeratin. While cells on flat surfaces and 100 nm patterns expressed a higher level of vinculin protein which suggests the formation of more mature focal adhesion structures compared to HDs. The results also show that the size of nanopatterned could altered cell spread area but not attached cell numbers.

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[2] D. Tsuruta, et al., Journal of dermatological science 62.1 (2011): 1-7.

C.16 Biomechanics and architecture of unsupported and biomimetic lipid membranes: insights on geometrical constrains and lipid chemistry

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The creation of model lipid membranes as free-standing platforms to study processes occurring across the cellular membrane has been so far a field of remarkable interest [1]. Such a design enables studies without drastically modifying the physico-chemical properties and the dynamic nature of lipid bilayers compared to cells. Starting from the pore spanning membrane technology to produce unsupported membranes, we developed a platform for atomic force microscopy and spectroscopy investigation and propose this platform as model setup for systematic studies. The mechanical parameters Young's modulus, packing density and bilayer tension are assessed and morphological features are imaged with regard to the influence of cholesterol and sphingomyelin for lipids of natural origin in a composition mimicking the plasma membrane. In addition, preliminary investigations in presence of proteins are also reported.

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C.17 Lrig1 and Wnt signaling instruct partitioning of melanocytes and resident immunocytes into distinct epidermal niches

Susanne C. Baess[1,2,3], Annika Graband[1,2,3], Kristin Seré[4,5], Martin Zenke[4,5], Catherin Niemann[3,6] and **Sandra Iden**[1,2,3]

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The barrier-forming, self-renewing mammalian epidermis comprises keratinocytes (KCs), pigment-producing melanocytes (MCs) and resident immune cells as first-line host defense. In murine tail skin, interfollicular epidermis (IFE) patterns into pigmented 'scale' and non-pigmented 'interscale' IFE. Why and how mature MCs confine to scale IFE is unresolved. Here, we explored interdependencies of epidermal cell types in skin patterning. Intriguingly, during postnatal development MC clusters co-segregated with newly forming scale IFE, whereas both Langerhans cells (LCs) and Dendritic Epidermal T cells (DETCs) partitioned into interscale IFE, suggesting functional segregation of pigmentation and immune surveillance in this tissue. Analysis of non-pigmented mice and of mice lacking MCs or resident immunocytes revealed that immunocyte patterning is independent of MCs and melanin, and, *vice versa*, LCs and DETCs do not control MC localization. Instead, progressive scale IFE fusion upon genetic Lrig1 loss showed that MCs and immunocytes dynamically trail epithelial scale:interscale patterns. Importantly, disrupting TCF/Lef function in KCs caused MC mislocalization to interscale IFE, implicating Wnt signaling in tissue-level orchestration of epidermal pigmentary units. Together, this work revealed cellular and molecular principles underlying compartmentalization of tissue functions in skin.

C.18 Premature senescence by lamin A/C alterations correlates to changes in cell viscoelastic behavior

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Lamin A/C, a protein of the nuclear envelope, contributes to the mechanical properties of the nucleus such as stability, shape, and rigidity. Mutations in lamin A/C gene *LMNA* induce pathologies called laminopathies, with common phenotypes such as nuclear abnormal shape or cell premature senescence. Laminopathies display varying severity and can be tissue-specific, like Type 2 Familial Partial Lipodystrophy (FPLD2) where only adipose tissues are affected, or multi-systemic, like Progeria where the entire body ages prematurely. The relationship between *LMNA* mutations, mechanical alterations in cells and laminopathy severity remains unknown, resulting in a lack of diagnosis and treatment. So far, most studies focused on Progeria and whether the observed alterations are Progeria-specific or common to all laminopathies is not answered yet. Here, we combined high-throughput microfluidic measurements on the second timescale with semi-automated image analysis and a rheological model to extract mechanical properties of human fibroblasts. We showed that fibroblasts prematurely senescent due to lamin A/C alterations (associated to FPLD2 or artificially induced) exhibit a more viscous behavior. The cell mechanical response depends both on the nucleus and on actin and microtubule networks. Our results suggest that lamin A/C alterations impact the nucleus and its link to the cytoskeleton.

C.19 Differential microrheological properties of actin isoforms

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Actin is one of the most abundant proteins in cells and is the backbone behind a manifold of integral cellular structures, such as stressfibers, sarcomeres and the cortex. As such, actin has become a major research interest and a plenitude of dedicated in-vitro experiments have been conducted in the last few decades to elucidate its properties. However, potential differences between the distinct actin isoforms themselves have only been addressed scarcely.

The presented work focuses on the different microrheological properties of actin networks made out of distinct actin isoforms measured via VPT (video particle tracking). While skeletal α-actin has been rigorously studied in the last decades, the properties of such networks constituted of cytoplasmic actins remains scarce. To that end, we conducted experiments with commercially available cytoplasmic actin as well as with purified β- and γ-actins extracted from modified yeast [1].

Surprisingly, we found striking differences regarding the microrheological properties of said bulk networks. For instance, the bulk networks constituted of muscular α-actin displayed a greater stiffness than networks of their cytoplasmic counterparts. Interestingly, the cytoplasmic β- and γ-actins displayed differential microrheological properties. These results may indicate further differences regarding the functional identity of each isoform.

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C.20 Connective Tissue and Cancer Cross-Talk: Treatment Implications and Biomechanical Signature?

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The most crucial step in cancer progression, often deciding about treatment options, life and death of patients, is the formation of metastases. Unfortunately, in the last decades, progress was slim to treat metastases better. Currently, the best survival strategy is to prevent metastases by detecting the primary tumor as early as possible and to surgical resect it as well as possible.

One step towards a better survival rate is to identify tissues at risk. The second step is elucidating the interaction of tumors and their microenvironment, which often is fatty connective tissue. Here we show that cervical cancers spread from the tissue of origin to tissues of ontogenetic proximity in reverse order of embryogenic development [1]. This "inverse morphogenesis" provides a unique roadmap for tissues at risk of cancer infiltration.

Further, the cross-talk between cancer cells and fatty connective tissue is essential for the mechanical phenotype of cancer cells. We show that stiffness changes of cancer cells are drastic when growing them in proximity to fatty tissue and are one of the largest recorded in optical stretcher measurements. Here, we present the framework for elucidating this emerging field of mechanical phenotyping under tissue cross-talk.

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C.21 Curvature: a dynamic regulator of cell migration mode and motility

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The intrinsic architecture of tissues subject cells to geometrical cues in the form of mesoscale curvatures [1]. While the effect of nano- and micro-scale topographical cues have been extensively studied, surprisingly little is known about the cell response to geometries larger than cell size. To study the effect of curvature in a systematic and high-throughput manner, we developed a microfabricated chip containing arrays of concave and convex structures with a wide range of size (μm to mm) [2]. We observed distinct cell migration modes on these structures: on concave surfaces cells showed undirected but fast migration, whereas migration on convex surfaces was persistently directed towards the direction that imposes the least cell bending. Moreover, we found an unexpected negative correlation between migration speed and persistence that emerged universally across all structures and for all tested cell types. The scale-dependent effect of mesoscale curvature was found to be sensitive to cell phenotype and activation state and was accompanied by changes in the F-actin organization and the levels of phosphorylated myosin and nuclear lamin-A. Thus, our findings demonstrate a complex interplay between cell contractility, nuclear mechanics, and adhesion morphology in dynamically guiding cell migration on physiologically-relevant 3D structures.

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C.22 Theoretical modelling of competitive microbial range expansion with heterogeneous mechanical interactions

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Microbial range expansion experiments provide insight into the complex link between dynamic structure, pattern formation and evolutionary dynamics of growing populations. Our work is inspired by experiments of range expanding *N. gonorrhea* strains which can display different levels of piliation. In the case of equal mean division times the non-piliated strain outcompetes the piliated strain [1]. Even more remarkable the outcompetition is still observed when the piliated strain divides twice as fast.

In our work [2], we develop a theoretical model in order to investigate the interplay of growth statistics and mechanical interactions which are implemented as division driven pushing and swapping of cells. For the case of the competitive growth of a strongly and a weakly interacting strain we investigate the influence of different mean division times, as well as different mechanical interactions on the development of the colony. Our results show that the susceptibility to cell division induced pushing has a much stronger influence on the structure of the colony than cell sorting towards the colony's perimeter. In particular, we show that for the initial conditions realized in the experiments a pure swapping mechanism is not able to reproduce the experimental observed patterns. Rather a heterogeneous susceptibility to pushing is sufficient even in the absence of any swapping.

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[2] E. Maikranz and L. Santen, Physical Biology 18, 016008 (2021)

C.23 Probing cell volume in compressed tissues with Brillouin light scattering

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Volume regulation is key in maintaining important tissue functions, such as growth or healing [1]. This is achieved by modulation of active contractility, as well as water efflux that change molecular crowding within individual cells. Local sensors have been developed to monitor stresses or forces in model tissues, but these approaches do not capture the contribution of liquid flows to volume regulation. Here we use a new tool based on Brillouin light scattering (BLS) that uses the interaction of a laser light with inherent picosecond timescale density fluctuations in the sample [2]. To investigate volume variations, we induced osmotic perturbations with a polysaccharide osmolyte, Dextran (Dx), and compress multicellular spheroids (MCS). During osmotic compressions we observe an increase in the BLS frequency shift that reflects local variations in the refractive index and compressibility. Comparison of local cell compressions within the tissue with small Dx to macroscopic compressions with large Dx reveals the dominant contribution of cell volume to BLS response. To elucidate these data, we propose a model based on a mixing law that describes the increase of molecular crowding upon reduction of the intracellular fluids. Comparison with the data suggests a non-linear increase of the compressibility due to the dense crowding that induces hydrodynamic interactions between the cellular polymers.

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C.24 Elucidating Receptor Cluster Formation in CD95 Signaling via DNA Origami and Multiparametric Image Spectroscopy

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Cell fate is decided by the CD95 oligomerization state. Cluster of Differentiation 95 (CD95) is a membrane receptor protein that signals for controlled cell death (apoptosis) after ligand-induced activation. Some types of cancer show a deregulation of this CD95 apoptosis mechanism leading to proliferation rather than cell death[1]. As the molecular conditions leading to either proliferation or apoptosis are fundamentally not understood, we investigate the structural preconditions on the membrane as decisive mechanism to trigger a particular cell fate decision. However, measuring the oligomerization state of proteins on a cell membrane remains challenging. Here, we present two strategies to uncover the receptor activity states: on the one hand, we use DNA origami sheets exhibiting nanoscale tunable CD95 ligand arrangements. By mimicking characteristic receptor geometries, the sheets act as signaling platforms and enable to derive molecular benchmarks in apoptosis signal initiation[2]. On the other hand, we use calibrated time-resolved FRET experiments to quantitatively measure the supramolecular multimeric state of the CD95 receptor on live membranes directly. We further develop novel confocal methodologies in EGFP-bleaching experiments supported by super-resolved STED images to obtain an unambiguous interpretation of the CD95 oligomerization state.

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C.25 The influence of vimentin on actin dynamics and force generation in RPE1 cells

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The cytoskeleton is a network of filaments in cells, it consists of actin filaments, intermediatefilaments and microtubules. Actin stress fibers are involved in force generation, cell retraction andcell protrusion during migration. The polymerization and depolymerization of actin filamentsregulate cell migration and are influenced by the activity of actin binding proteins. Vimentin is alsoinvolved in cell migration, although it is not associated with molecular motors, and thereforecannot create forces directly. To better understand the mechanism of cell migration, it is importantto understand how cytoskeleton filaments interplay. The roles of vimentin filaments and actinfilaments in cell migration have been studied separately to date; however, their interactionsremain to be defined fully. Therefore, understanding the role of vimentin on actin dynamics, and its implicationin actin force generation are thetwo main interests of this project.

We first measured actin dynamics in vimentin depleted cells using fluorescence recovery after photobleaching [1, 2]. We showed that silencing of vimentin expression slows down actin dynamics but does not affect the fraction of actin monomers that participate. In addition, we showed that plectin as a vimentin-actin cross-linker protein does not have the same effect. Finally, we studied actin force generation using traction force microscopy [3-5]. We showed that silencing of vimentin disarranges the distribution of traction forces and adhesion sites but does not impact the magnitude of traction forces.

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C.26 Long-term nuclear regulation of cancer cells under confinement

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The physical properties of the tumor microenvironment are strongly modified during tumor growth and participate in the development and invasion of cancer cells [1], including not only stiffness, but also compression [2].

In particular, the nucleus is critically affected during compression [3] and is appearing as an important mechanosensor of deformations [4,5]. Nevertheless, most studies focus nowadays on short-term cell response (from minutes to few hours). New questions are open on the long-term adaptation to deformations and the mechano-sensing mechanism involved. We have recently developed a new agarose- based microsystem copping with media renewal impediment to investigate cell response to prolonged confinement [6].

We used this device to apply a tunable and controlled 1D confinement on the colorectal cancer cell line HT-29 up to several days. We evidenced a decrease of the nuclear volume after 24 hours under confinement. The overall nuclear shape is also dynamically regulated with the apparition of transient nuclear blebs. We are currently analyzing the mechanisms and consequences of such adaptation on cell division, transcription activity and protein expression. Such long-termed adaptation to mechanical constrains may be of importance for cancer cell plasticity and play a role in their resistance to treatments.

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C.27 Unravelling the Mechanobiology of Living Cells while Interacting with their Environment

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Active forces in biological systems define the interactions between single molecules, growing cells and developing tissues. To this end, atomic force microscopy (AFM) remains the only technique that offers premium resolution of the analyzed biological systems at near physiological conditions, while being able to simultaneously acquire information about the sample's mechanical properties.

Cells adapt their shape and react to the surrounding environment by a dynamic reorganization of the F-actin cytoskeleton. We will demonstrate the application of high-speed AFM (down to 1 frame/s) to study membrane ruffling and actin cytoskeleton rearrangement in living KPG-7 fibroblasts and CHO cells.

External mechanical stress is known to influence cell mechanics in correlation to the differences in actin cytoskeleton dynamics. A crucial aspect of investigating cellular mechanobiology is to go beyond purely elastic models. We have therefore performed rheological measurements to characterize sample response at different time scales and measure viscoelastic properties in mammalian cells over a large frequency range (0-500 Hz).

Cell-cell and cell-substrate interactions through cytoskeletal modulation, determine cell fate, shape and spreading. We will demonstrate the application of single cell force spectroscopy for quantifying the adhesion between individual cells, and cell matrix substrates.

C.28 Nonequilibrium mechanics of cross-linked actomyosin networks probed with microrheological techniques

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Living cells are active matter, they are a non-equilibrium system. To study the applicability of the fluctuation dissipation theorem far from equilibrium, we embed colloidal particles in those materials - primarily cells and reconstituted, ATP driven actomyosin networks – measure the systems' linear response to thermal fluctuations and compare with equilibrium systems or measurements that don't rely on Brownian motion (active deformation). Additionally, we want to compare in vivo measurements with artificial systems to evaluate to which extent cellular mechanics are a phenomenon emerging from the interaction of hundreds of different biomolecules. Simplified model systems are a useful and popular way to reduce the immense complexity and to access crucial information about living systems, but they also bear the risk of oversimplifying properties that arise from a variety of interactions. A major research interest in the field of biophysics is the creation of artificial cells. Our goal is a small contribution in the form of understanding cortical non-equilibrium mechanics.

C.29 Quantitative Study of Heterogeneity in Membrane Protein Interaction in Cancer Cells using Liquid-Phase Electron Microscopy

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We developed a new method for the parallel detection of single molecules of two membrane proteins from the same family of the human epidermal growth factor receptors (EGFR also named HER1), and its protein family member, HER2. In several types of cancer, such as breast cancer, these receptors are overexpressed, and thereby trigger uncontrolled cell growth and cancer cell spreading through homo- and heterodimerization. We here demonstrate how the dimerization of both receptors can be studied at the single-molecule, subcellular, and single-cell level, and up to surface densities of >2.000 receptors/µm2 [1]. Applying labels consisting of small, specific binding proteins, and two types of quantum dots, a total of 41 breast cancer cells were studied, yielding data of >200.000 of receptor positions. Statistical analysis with the pair correlation function *g(r)* disclosed significant differences in the dimerization behavior of both receptors, depending on the dynamics of the local environment of the plasma membrane. In addition, different receptor interaction profiles were found in small cellular subpopulations [2]. These new possibilities of quantitative analysis of receptor interactions offer a deeper understanding of cancer cell heterogeneity, which is a major cause for drug resistance and disease progression. In addition, by using other binding proteins this method can be tailored to study the interaction of other membrane proteins as well.

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C.30 Spatial-Stochastic Model of Cell Fate Decisions in Early Mouse Development

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The delicate balance necessary for sustaining cell plasticity and ensuring reliable specification of cell lineages is an intriguing problem in developmental biology. In the mouse embryo, the Nanog/Gata-6 (N/G) gene regulatory network plays a crucial role in the delicate balance of the early cell fate decision process. Although stochasticity is an inherent feature of any gene expression process, the current approaches to this problem still primarily rely on deterministic modelling techniques, while generally treating noise as an ad hoc property. Therefore, we are developing a multi-scale event-driven spatial-stochastic simulator for emerging-tissue development. This allows us to perform realistic-yet-efficient simulations of intracellular biochemical dynamics, tissue-scale biomechanical interactions, and intercellular communication. We use well-established event-driven simulation schemes and adapt them for incorporating suitable tissue-scale phenomena, such as cell division and growth. Whenever possible, important biophysical and morphological parameters are fixed by values provided by experimental collaborators or found in recent literature. Otherwise, numerical optimization techniques are implemented to infer biologically-feasible regimes for relevant parameters. We begin by studying the biochemical characteristics of the N/G network in a single-cell setting. We simulate and analyze the dynamics of its core network motif components and their interplay. We subsequently extend the study to a multi-cellular setting, in order to understand the importance of cell-to-cell communication, and how positional information is robustly achieved and preserved. These efforts converge toward a versatile framework capable of efficiently simulating emerging- tissue dynamics coupled with intracellular biochemical processes, in a biophysically faithful fashion.

C.31 Brain microvascular endothelial cell cytoskeletal reorganization inresponse to strain in a microvessel-on-chip

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Blood flow-derived forces are key regulators of microvascular structure and function. Although endothelial cells (ECs) from various vascular beds have been shown to exhibit humoral, metabolic, and structural responses to flow, much less is known about the flow responsiveness of brain microvascular ECs. Using a microvessel-on-chip, we are investigating how human brain microvascular ECs (HBMECs) respond to cerebral blood flow.

An HBMEC-lined microvessel is fabricated in a soft collagen hydrogel using the templating technique. Controlling luminal flow and intraluminal pressure allows subjecting the cells to different levels of circumferential stretch. Results to date indicate that in response to increased circumferential stretch, HBMEC actin filaments reorient in the stress direction and form prominent ventral stress fibers. In contrast, nonoticeable changes are observed in the organization of microtubules or intermediate filaments. Contrary to what has been reported in other EC types, HBMECs do not change shape or alignment in response to stretch despite the actin remodeling, and no change in nuclear shape is observed. Tension also induces a switch from linear to zig-zag cell-cell junctions, and sustained stresses provoke junctional rupture. These findings suggest that strain induces structural responses that are likely to profoundly influence HBMEC barrier function.

C.32 Mechanics and dynamics of cytoskeletal components and phase separated droplets studied with optical tweezers correlated to fluorescence microscopy

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Mechanical properties of cellular substructures and compartments are tightly regulated by the cell and allow for fast adaptation to environmental requirements. The cytoskeleton with its filamentous components (actin, intermediate filaments, microtubules) is crucial for maintaining morphology and structural integrity as well as intracellular transport and (mechanical) signaling. More recently, the role of membrane-less organelles in intracellular organization has moved into focus. Also here, mechanical and dynamic properties of these biomolecular condensates play a key role in their physiological functions. Aberrant behavior can lead to loss of function and pathological conditions.

In this talk, we introduce the C-Trap microscopy platform that correlates optical trapping for highly precise force measurements with fluorescence imaging capabilities from the single-molecule to the filament and organelle level. We outline selected experiments and assays that enable the quantification of mechanical properties and the dynamic behavior of the aforementioned intracellular structures.

C.33 Modeling cellular spreading and motility on curved surfaces

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Cells often adhere and migrate on curved surfaces, such as the fibers of the extra-cellular matrix (ECM), cylindrical protrusions of other cells etc. However, most of the cell biological studies examine cell migration mechanisms using cells on flat surfaces. We study the spreading and migration of a cell-like vesicle on curved surfaces, such as cylinders, sinusoidal surface etc. The vesicle is composed of curvature-sensitive proteins, that diffuse on its surface, and also recruit actin polymerization, which applies an active protrusive force [1]. We note that on the outside of a cylinder, the vesicle coils or migrates circumferentially, rather than axially. For a cylinder of smaller radius, however, they prefer not to coil or migrate, but spreads weakly. In contrast, inside a cylinder, the vesicle prefers to elongate or migrate along the axial direction. On a sinusoidal surface with alternating dips (minima) and peaks (maxima), the vesicle behaves in similar way as inside and outside of a cylinder, respectively. The vesicle prefers to stay in the dip and align axially. However, if placed on the peak, it prefers to slide down to the dip. While migrating from one dip to another, it crosses the peak at higher angle and larger speed. Our results are in agreement with experiments, and offer an explanation for some of the observed curvature-sensitivity of cell migration patterns.

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C.34 A minimal lattice model of lipid membranes with liquid-ordered domains

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Binary or ternary mixtures of lipids and cholesterol featuring liquid-ordered (Lo) domains serve as model systems for studying the formation of lipid rafts in complex biological membranes. In the talk, I will present a new lattice model of a binary mixture of saturated DPPC lipids and cholesterol, which is the simplest model system exhibiting Lo domains in the sea of liquid-disordered (Ld) matrix. Simulations of mixtures of thousands of lipids and cholesterol molecules on time scales of hundreds of microseconds reveal a phase diagram that is in complete agreement with the well-established phase diagram of this mixture. The simulations provide important information on the morphologies and lifetime of the Lo domains which are not available by more detailed computational approaches. Strikingly, the lattice simulation also reproduces local structures that have been observed in atomistic simulations. Specifically, we find that the Lo domains are highly heterogeneous and consist of gel-like hexagonally packed clusters of ordered DPPC chains, surrounded by cholesterol-rich regions at the domain boundaries. The simulation results are explained by a new thermodynamic mechanism that considers the disparity between the packing interactions of ordered lipid chains with cholesterol and each other.

C.35 Vimentin intermediate filaments stabilize dynamic microtubules by direct interactions

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The cytoskeleton determines cell mechanics and lies at the heart of important cellular functions. Growing evidence suggests that the manifold tasks of the cytoskeleton rely on the interactions between its filamentous components—actin filaments, intermediate filaments, and microtubules. However, the nature of these interactions and their impact on cytoskeletal dynamics are largely unknown. Here, we show in a reconstituted in vitro system that vimentin intermediate filaments stabilize microtubules against depolymerization and support microtubule rescue. To understand these stabilizing effects, we directly measure the interaction forces between individual microtubules and vimentin filaments. Combined with numerical simulations, our observations provide detailed insight into the physical nature of the inter-actions and how they affect microtubule dynamics. Thus, we describe an additional, direct mechanism by which cells establish the fundamental cross talk of cytoskeletal components alongside linker proteins.

C.36 BK-channel as a fast and precise Ca^{2+}sensor: application to PMCA pump strength measurements

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Ca^{2+} diffusion within cells and penetration of Ca^{2+} through their membrane engages a wide field of theoretical and experimental research. Therefore, the monitoring of rapid changes of the Ca^{2+} concentration beneath the cell membrane is of great interest. Here, we make use of BK-type Ca^{2+}-activated K+ channels to determine the Ca^{2+} activity of PMCA, which transport Ca^{2+} ions out of cells. Due to their large conductance and their particular gating kinetics the BK channels may be used as fast and reliable sensors for intracellular Ca^{2+} - concentration beneath the plasma membrane. Experimentally we monitor the PMCA-mediated Ca^{2+} clearance (or transport) by the decay of BK-currents following their activation by a short (0.8 ms) period of Ca^{2+} -influx through Cav2.2 channels. To relate the experimentally observed temporal evolution of the K+ current to the underlying temporal evolution of the Ca^{2+} concentration we implement a theoretical model for the Ca^{2+}-dependence of the BK-current and of the PMCA pump strength. Next to the transport in and out of a cell and the diffusion of Ca^{2+} ions within the cell, we expand our model by the reaction of the Ca^{2+} concentration with a buffer solution, as well defined EGTA concentration is present in all experimental measurements. We fit the PMCA pump strength by the best match of the predicted time course of the K+ current with the experimental data. It turns out that this pump strength is at least 2 orders of magnitude larger than what has been assumed so far.

C.37 Sensing their plasma membrane curvature allows migrating cells to circumvent obstacles

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Cell migration is a hallmark out-of-equilibrium process in biology. In addition to persistent self-propelled motion[1,2,3], many cells display remarkable adaptive behaviors when they navigate complex environments[4,5]. Combining theory and experiments, we find a curvature-sensing mechanism underlying obstacle avoidance in immune-like cells. The genetic perturbation of this machinery leads to a reduced capacity to evade obstructions combined with faster and more persistent cell migration in obstacle-free environments. We propose that the active polymerization of the actin cytoskeleton at the advancing edge of migrating cells is locally inhibited by the curvature-sensitive BAR-domain protein Snx33 in regions with inward plasma membrane curvature. This coupling between actin and membrane dynamics leads to a mechanochemical instability that generates complex protrusive patterns at the cellular front. Adaptive motility thus arises from two simultaneous curvature-dependent effects, the specific reduction of propulsion in regions where external objects deform the plasma membrane and the intrinsic patterning capacity due to the membrane-actin coupling that promotes spontaneous changes in the cell's protrusions. Our results show how cells utilize actin- and plasma membrane biophysics to sense their environment, allowing them to adaptively decide if they should move ahead or turn away.

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C.38 Rationalizing the optimality of the gap gene system by ab-initio derivation of optimal ensembles of morphogenetic patterns

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Early embryogenesis is driven by spatio-temporal patterns that specify distinct cell identities ac- cording to their locations in the embryo. This process is highly reproducible, although it results from regulatory interactions that are individually noisy. Despite intense study, we still lack comprehensive, biophysically realistic models that simultaneously reproduce quantitative data and rigorously explain high developmental precision. Moreover, traditional approaches fail to explain why particular pattern- ing mechanisms evolved, and why they favor particular parameter values. We address both questions during early fly embryo development. In *Drosophila*, the gap gene expression patterns were shown to optimally encode positional information. We therefore asked whether one can mathematically derive the gap gene network—without any data fitting—by maximizing the encoded positional information. To this end we built a generic, biophysically accurate spatial-stochastic model of gene expression dy- namics, where genes respond to morphogen input signals and mutually interact in an arbitrary way, and optimized its parameters for positional information. Firstly, our results show how the experimentally observed precision can be achieved by basic biochemical processes under known resource and time con- straints. Secondly, we find that a rich ensemble of optimal solutions exists and systematically analyse its characteristics, finding that some optimal solutions closely correspond to the real gap gene pattern. Finally, we explore a broad range of "mutated" optimal ensembles in which relevant components of the wild-type system are altered or fully discarded, and systematically map out how this affects positional information and other pattern properties; this allows us to rationalize the design of the wild-type gap gene system and the possible roles of its specific components. To our knowledge our work provides the first successful ab-initio derivation of a nontrivial biological network in a biophysically realistic setting. Our results suggest that even though real biological networks are hard to intuit, they may represent optimal solutions to optimization problems which evolution can find.

C.39 Understanding cell behavior in complex multi-cue environments

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In vivo, cells are embedded in the extracellular matrix, which provides the cells with a variety of physical, (bio)chemical and mechanical cues. To understand cell behavior in this complex environment, researchers often isolate single environmental cues and subject cells to these cues in vitro. Although this approach gives useful insights, the environment is oversimplified. The response of cells to a combination of cues like what they experience in vivo is still poorly understood. Here we present a novel experimental platform to explore the cell response to a combination of curvature- and contact-guidance cues. Contact guidance cues are applied using photo-patterning of ECM proteins to a 2.5D cell culture chip containing a library of convex and concave curvatures mimicking tissue geometry. When subjected to opposing contact- and concave curvature-guidance cues, the cells showed an alignment response in the direction of the protein pattern. On convex curvatures, however, cells reorient to avoid a bent morphology, consistent with the results using curvature guidance cues only.

C.40 A-to-I RNA editing of Filamin A (FLNA) regulates cellular adhesion, migration and mechanical properties

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A-to-I RNA-editing by ADARs is an abundant epitranscriptomic RNA-modification in metazoa [1]. Flna pre-mRNA in mammals harbors a single conserved A-to-I RNA editing site that introduces a Q-R amino acid change in Ig-repeat 22 of Rod2 domain of the encoded protein [2-4]. Previously, we showed that FLNA editing regulates smooth muscle contraction in the cardiovascular system and cardiac health [5]. The present study investigates how ADAR2-mediated A-to-I RNA editing of Flna affects actin crosslinking, cell mechanics, cellular adhesion and cell migration. Cellular assays and AFM measurements demonstrate that the edited version of FLNA increases cellular stiffness and adhesion but impairs cell migration in both mouse fibroblasts and human tumor cells. In vitro, edited FLNA leads to increased actin crosslinking forming actin gels of higher stress resistance. Our study shows that Flna RNA editing is a novel regulator of cytoskeletal organization, mechanical propertiesy of cells and mechanotransduction.

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C.41 Collective Search Strategies

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How long does it take to find N targets by M searchers? This question arises, for example, if animals search for food or immune cells chase for pathogens. The usual goal is to minimize the time needed to catch all targets. One obvious possibility would be to increase the number of non-interacting searchers another to search collectively by utilizing communication between the searchers. It is known, that cells of the immune system talk to and influence one another by secreting small proteins that bind to and activate each other. For instance, T cells (a type of lymphocyte) are chemotactic, i.e., they move in response to a chemical stimulus, however, it is unknown if chemotaxis is important for the coordination of the search for pathogens. We use a simulation model of chemotactic active particles together with a self-generated chemorepellent in order to test the possibility and the benefit of collective search strategies in microbiological systems.

Poster Abstracts

P.1 The role of actin, myosin II and cadherins in the cortex of living cells.

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Adhesion induces dramatic morphological and mechanical changes to cells, which are reflected by changes to the actin cortex. Among the many different proteins involved in this sub-membranous layer, motor proteins (e.g., nonmuscle myosin II) and actin nucleators (e.g., Arp2/3, formins) are known to have significant influences on its dynamics and structure. In this work, we present the interplay between the dynamics, structure, and mechanics of the actin cortex in adhered cells and in cells in suspension and the different roles of NMII, Arp2/3, and formins. We will outline how we plan to continue this study to investigate the structure of the actin cortex of healthy and cancerous cells in the context of E-cadherin based cell-cell contacts. Our data build towards a comprehensive understanding of the actin cortex. This understanding allows the prediction and control of cortical changes, which is essential for the study of general cellular processes, such as cell migration, metastasis, and differentiation.

P.2 Microfluidic generation of soft microgels as a tool for studying the influence of 3D microenvironments on the cellular responses upon external stimuli

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Microencapsulation of living cells in biopolymer microgels has been attracting interest due to the microgel's capability to mimic 3D microenvironments on a microscale platform [1-3]. Cells embedded in 3D gel matrices can transduce external physicochemical cues into intracellular biochemical signals, therefore exhibit *in vivo*-like physiological responses.

In this work, we demonstrate that loading gelatine microgels with human liver cells (HepG2 cell line) or probiotic bacteria (*Lactobacilllus plantarum*) using droplet microfluidics can result in: i) the ability to reflect 3D-like toxicity responses of HepG2 cells, as well as ii) the increase the survival rate of *L. plantarum* during exposure to adverse conditions in the gastrointestinal tract. The cell-loaded microgels produced in this process are covalently crosslinked with a naturally derived crosslinker, genipin. This allows the mechanical stiffness of the microgel matrix to be controlled by tuning the crosslinking degree.

Based on this *in vitro* platform, we demonstrate the optimisation of microfluidic parameters for the on-chip droplet generation system and finally determine the mechanical properties of the microgel matrix. We then compare the dose sensitivity of ethanol between 3D-encapsulated and 2D-grown HepG2 cells, and the viability of *L. plantarum* under low pH conditions under encapsulated and non-encapsulated milieux.

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P.3 Phospholipids Motility at the Surface of Model Lipid Droplet

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The dynamic partitioning of certain proteins between a bilayer and lipid (triolein) droplets is key to understand the function of these cellular sub-organelles. As a model for the surface of a lipid droplet that is covered with a bilayer leaflet, we use a triolein-water interface covered by a phospholipid monolayer. The motility of the phospholipids is investigated using fluorescence recovery after photobleaching (FRAP) method on such labelled phospholipids. For that we provide a quick bleaching in a certain area of the phospholipid monolayer and record the fluorescent recovery due the diffusive lateral movements of the phospholipid monolayer. The recovery analysis reveals the diffusion constant of the bleached phospholipids as function of the LD molecular composition. Thus, these findings proves the important ability of surface LDs motility as they need to contact and communicate with other organelles in the cell.

P.4 Stabilizing and elongating actin filaments alters the position of nuclei in migrating cells in confinement

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As one of the main components of the cytoskeleton involved in many mechanics, targeting actin dynamics seems to be a desirable goal. Miuraenamide A (MiuA), a compound that can pass through the cell's membrane, stabilizing actin filaments and promote their polymerisation, aroused our interest. Here I'm going to describe the effects of MiuA on stress fibres in geometrically controlled RPE-1 cells in a quantitative way and show its stabilizing and elongating effects. Furthermore, I will present that when those MiuA treated cells were put in confinement (PDMS microchannels for migration) although their migratory behaviour seemed to be unaltered, the position of their nuclei was shifted towards the centre.

P.5 Investigation of the Electron Beam Dose Tolerance of GFP in Liquid

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The investigation of biological specimens with electron microscopy (EM) is often hampered by radiation damage of samples, in particular because typical biological specimens are electron beam radiation sensitive. Traditionally, sample preparation for EM includes drying or freezing of the specimens but the observation of biological samples and processes in their native, liquid environment is of major interest in EM research for which liquid phase electron microscopy is a new option [1]. To accomplish this, it is necessary to know the dose tolerance of the specimens such that specimens can be kept intact during microscopy.

Here, the electron dose tolerance of biological specimens in liquid was investigated. As a model, the green fluorescent protein (GFP) was used. For sample preparation, GFP was bound to SiN microchips via biotin-streptavidin binding. The fluorescence intensity loss of GFP was investigated upon electron beam radiation in transmission electron microscopy (TEM) and scanning electron microscopy (SEM) with varying electron flux and electron dose. The loss of fluorescence intensity was associated with the loss of protein function and the degradation of the protein [2].

The fluorescence intensity was analyzed over the electron dose from 0.001 – 10 $e^-/Å^2$. The electron flux varied from 0.001 – 10 $e^-/Å^2s$ to analyze the impact of electrons passing the sample per unit area per unit time. It was found that in SEM, the fluorescence intensity decreased and was comparable to background level at an accumulated electron dose of 1$e^-/Å^2$. At this dose, GFP lost its fluorescence function and was considered as no longer intact. Varying the electron flux did not have a large impact on the electron dose threshold of GFP.

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P.6 NK cell cytotoxicity and protein microarrays predict efficacy of melanoma immunotherapies

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Notwithstanding the impressive advances in melanoma-directed immunotherapies, resistance is common and many patients still succumb to the metastatic disease. In this context, natural killer (NK)-cells, although sidelined in the recent development of melanoma immunotherapy, could provide therapeutic benefits in the future. To identify molecular determinants of NK-cell-mediated melanoma killing (*NKmK*), we quantified NK-cell cytotoxicity against a panel of genetically-diverse melanoma cell lines and observed a highly heterogeneous susceptibility. Melanoma cell protein microarrays revealed a correlation between protein abundance/activation and *NKmK*. A"protein-killing-signature", identified metabolic factors as essential regulators of *NKmK*. Using 2D and 3D killing assays and melanoma xenografts, we demonstrated that the PI_3K/Akt/mTOR signaling-axis controls NKmK via expressional regulation of NK cell-relevant surface proteins. Moreover, we developed algorithms to predict *NKmK* of additional melanoma cell lines and the response of melanoma patients to anti-PD-1 checkpoint therapy. Our findings identify novel NK-cell-related prognostic biomarkers and might thus contribute to improved and personalized melanoma-directed immunotherapies.

P.7 Active fluctuations of Microtubules

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Microtubules are a key part of the cytoskeleton of eukaryotic cells. They are responsible for a wide range of mechanical properties and serve many different tasks within the cell e.g. they play an important role during mitosis. In order to be able to fulfill these tasks the microtubules have to be able to adapt their shape. However, Gittes et al have shown in vitro [1] that isolated microtubules have a very high thermal persistence length, which in fact means that they are very rigid and therefore do not show a high degree of bending. In this work the bending spectra of microtubules within a cell are investigated and their persistence length is analysed. It can be shown that the persistence lengths of the filaments in vivo are two orders of magnitudes smaller than in vitro and the bending spectrum of the filaments has a fundamentally different shape compared to purely thermally fluctuating ones. Besides the analysis of the experimental data a theoretical model is developed where active cross-linkers deform the microtubule and therefore lower their persistence length. This model for the dynamics of microtubules is able to reproduce experimental results and explain a possible origin of the high deformations of microtubules in living cells.

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P.8 Sensing lipid saturation: biochemically reconstituting a signal amplifying mechanism

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Biological membranes are complex materials consisting of proteins and lipids. The lipid composition of a membrane determines its physical properties and impacts its function. To maintain these properties, the lipid composition must be sensed and tightly controlled. The sensory machineries that monitor membrane properties must sense signals encoded in the motion of lipid molecules on the nanosecond timescale, but their output functions are robust and on the time scale of milliseconds to hours. Little is known how membrane property sensors amplify and transduce the signals that they pick up from the membrane. We study the endoplasmic reticulum (ER) resident lipid saturation sensor Mga2 of Saccharomyces cerevisiae to investigate its mechanism of signal amplification. Increased levels of saturated lipids in the ER lead to the activation of Mga2, which involves the covalent attachment of a chain of ubiquitin molecules onto Mga2. A theoretical model of signal amplification suggests that minimal changes in the rate of ubiquitin attachment upon ubiquitin chain assembly could indeed be harnessed to amplify a signal originating from the membrane. We aim to i) biochemically reconstitute signal amplification by Mga2 with its ubiquitylation machinery, ii) establish a quantitative kinetic model, and iii) study the role of deubiquitylation in vitro and in vivo. We can already show that the reconstituted Mga2 sensor is ubiquitylated in vitro. The absolute amount of Mga2 and its several distinct ubiquitylated species can be sensitively quantified using an in-gel fluorescence detection

P.9 Simulation of Liquid Jet Explosions and Shock Waves Induced by X-Ray Free-Electron Lasers

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X-ray free-electron lasers (XFELs) produce X-ray pulses with very high brilliance and short pulse duration. These properties enable structural investigations of nanocrystals or single biomolecules, and they allow resolving the dynamics of biomolecules down to the femtosecond timescale. To deliver the samples rapidly into the XFEL beam, liquid jets are used. The impact of the X-ray pulse leads to vaporization and explosion of the liquid jet, while the expanding gas launches shock wave trains traveling along the jet that can affect biomolecular crystals before they have been probed. Here, we used atomistic molecular dynamics simulations to reveal the structural dynamics of shock waves after an X-ray impact. Analysis of the density in the jet revealed shock waves that form close to the explosion center and travel along the jet. A trailing shock wave formed after the first shock wave, similar to the shock wave trains in experiments. Although using purely classical models in the simulations, the resulting explosion geometry and shock wave dynamics closely resemble experimental outcomes, and they highlight the importance of the jet surface in shock wave propagation and attenuation.

P.10 Hydra: a possible dependence of Wnt/β -Catenin signaling on the microtubule cytoskeleton during early regeneration and axis formation

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The phylogenetically old genus Hydra (class Hydrozoa), has the ability to regenerate from fragments. This process shows a striking similarity to the development of early embryos of other animal species regarding genes expressed and patterns formed. Hydra is an established model organism that can give us insights into development of embryonic tissue, establishment of body axis, regeneration of the tissue and the evolution of multicellular organisms. Here, we investigate the mechanisms of axis formation. At the axis defining moment, the early hydra embryo exhibits a strong sensitivity to external mechanical perturbations [1]. We develop the idea that these mechanical fluctuations induce the orientation of microtubules, which contribute to β-catenin nuclear translocation increasing the organizer-forming potential of the cells. We will investigate this hypothesis, among others, by applying mechanical forces on hydra spheres with magnetic tweezers.

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P.11 Insight into the topology of the monotopic hairpin protein UBXD8 in endoplasmic reticulum bilayer and lipid droplet monolayer membranes

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Lipid droplets (LDs) are ubiquitous organelles that act as an "energy sink" to sequester excess metabolic energy in the form of neutral lipids, particularly triacylglycerides and sterol esters. They originate from the endoplasmic reticulum where the local accumulation of neutral lipids within the phospholipid bilayer membrane triggers the formation of new LDs that eventually consist of a hydrophobic neutral lipid core that is encapsulated by a phospholipid monolayer. The surface of LDs is decorated with several proteins, including metabolic enzymes that regulate LD functions. Class I LD proteins are initially inserted into the ER bilayer membrane from where they can partition to the LD monolayer surface. They can associate with the membranes using amphipathic helix motifs or by adopting a monotopic hairpin topology. How such proteins are targeted and topologically arranged in ER and LD membranes is still not fully understood. In this study, we combined a cysteine solvent accessibility method (PEGylation assay) with molecular dynamics (MD) simulations to obtain structural insight into the topology of the ER/LD protein UBXD8 in both, ER and LD membranes. We could precisely map the membrane-embedded UBXD8 hairpin domain that is buried inside the ER bilayer and revealed that this domain is more solvent exposed on the LD monolayer. MD simulations corroborate our experimental observations and suggest that UBXD8 adopts a deeply embedded V-shape topology in the phospholipid bilayer with two anti-parallel α-helices facing each other. In contrast, on the LD monolayer, UBXD8 is more solvent exposed by adopting a shallow conformation resulting from the opening of α-helices. These findings allow us to raise new working hypothesis on the molecular mechanisms underlying ER-to-LD protein partitioning.

P.12 Developing a tunable biomaterials platform to mimic the intercellular interface

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Cells form specialized types of adhesions with their surrounding environment, amongst them the integrin-based focal adhesions (cell-ECM) and cadherin-based adhesions (cell-cell) [1]. Both of these cell-adhesion types are able to sense mechanical environmental cues and influence cell behavior [2, 3]. Moreover, there is evidence that they can establish a close mechanical crosstalk, with some studies showing a competitive relationship [4, 5] and others a cooperative [6]. To further study the nature of the interplay between integrin and cadherin-based adhesions, a platform that allows for the fine tuning of the mechanochemical properties of two orthogonal interfaces is needed. Using poly(acrylamide), an inexpensive, cytocompatible material widely used for mechanobiology studies [7], our group is developing a 2.5D artificial microenvironment that allows for independent, orthogonal tuning of both mechanical and chemical properties. The method involves a soft molding step allowing for the separate polymerization of two hydrogel layers with different coupling chemistries. This platform has the potential to become a powerful tool for studying mechanosensing at the cell adhesion level, as well as for unraveling the intricacies of the crosstalk stablished between cell-cell and cell-matrix adhesions.

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P.13 Deregulation of Histone Modification Associates to Alternative Splicing of Developmental Genes

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Alternative exon usage is known to affect a large portion of genes in mammalian genomes. Importantly,different splice forms sometimes lead to distinctly different protein functions. We analyzed data from theHuman Epigenome Atlas (version 9) whereby we connected the differential usage of exons in variousdevelopmental stages of human cells/tissues to differential epigenetic modifications at the exon level. Intotal, we analyzed 19 different human tissues in adult and cultured cells that mimic early developmentalstages. We found that the differential incidence of protein isoforms across developmental stages was oftenassociatedwithchangesinhistonemarksatexonboundaryregions.Manyofthosegenesthataredifferentially regulated at the exon level were found to be functionally associated with development andmetabolism. We concluded that the analysis pipeline was suitable for providing a mechanistic view to cellreprogramming events at molecular level that is meaningful for the study of cell identity, differentiation, and development.

P.14 Effect of oral biofilm on the formation and persistence of fluoride layers on dental enamel

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The use of dental care products containing fluoride for caries prophylaxis in the daily oral hygiene is established for many decades. However, little is known about the impact of oral biofilm on the formation and persistence of fluoride layers on enamel. The continuously growing oral biofilm develops immediately after oral hygiene starting with the adsorption of salivary macromolecules to enamel surfaces. The subsequent adhesion of microorganisms begins within hours. Here we studied the effect of oral biofilm on fluoridation with the most commonly used fluoride compounds NaF and olaflur (10,000 ppm). Fluoride application was performed in vitro either on biofilm-free or on 3 min-biofilm covered bovine enamel test specimens for 5 min. To examine the fluoridation persistence under in vivo conditions, 3 min-biofilm covered specimens were fluoridated and then exposed orally for 24 h before final evaluation. The analyses of all specimens were performed by scanning electron microscopy and energy dispersive X-ray spectroscopy. Both NaF and olaflur formed homogeneous and persistent fluoride layers on biofilm-free enamel and on 3 min-oral biofilms. The simulation of the in vivo conditions showed for both substances that fluoride is detectable on the tooth surface up to 24 h after oral exposure. However, in comparison to NaF fluoridation with olaflur was about twice as persistent.

P.15 Impact of Narrow Constraint on Single Cell Motion

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Carcinoma progression is associated with of a loss of epithelial characteristics in tumor cells, combined with a gain in mesenchymal ones, a process known as the epithelial-to-mesenchymal transition (EMT) [1]. During carcinoma development and invasion, epithelial cells assume an elongated morphology and disarranged polarity in order to migrate and degrade the surrounding extra-cellular environment, intravasate into the lymphatic and circulatory system, and reach distant tissues and organs [2]. At a molecular level, tumor tissues appear to exhibit an abnormal expression of epithelial markers together with a higher expression of proteins associated to cell migration [3].

In this study, we assess the potential invasiveness of five human breast carcinoma cell lines through single cell migration assays in confinement. For this purpose, we use a novel microfluidic device to investigate the ability of both mesenchymal and epithelial breast carcinoma cells to deform and migrate through narrowing microstructures upon chemoattractant stimulation. We find that normal epithelial cells are able to migrate through the narrowest micro-constrictions as the more invasive mesenchymal cells. We also demonstrate that migration of epithelial cells through a highly compressive environment can occur in absence of a chemoattractive stimulus, thus evidencing that they are just as prone to react to mechanical cues as invasive cells.

We then evaluate the expression of vimentin and cytokeratin (CK) intermediate filaments (IFs) in our cell lines through immunostaining and western blots, and observe how the IF cytoskeletal network responds to the compressive stress applied by narrow micro-channels, and whether differences in IF protein expression affect the migratory behavior of our cells. We find no remarkable difference between the mechanical behaviors of the keratin and vimentin networks during migration through our micro-constrictions. Moreover, since vimentin positive mesenchymal cells did not display invasive behavior, while other vimentin negative cells did, we conclude that vimentin protein expression does not strongly correlate to single cells invasive behavior in confinement.

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P.16 A methylation-directed, synthetic pap switch based on self-complementary regulatory DNA reconstituted in an all E. coli cell-free expression system

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Pyelonephritis-associated pili (pap) enable migration of the uropathogenic Escherichia coli strain (UPEC) through the urinary tract. UPEC can switch between a stable 'ON phase' where the corresponding pap genes are expressed and a stable 'OFF phase' where their transcription is repressed. Hereditary DNA methylation of either one of two GATC motives within the regulatory region stabilizes the respective phase over many generations. The underlying molecular mechanism is only partly understood. Previous investigations suggest that in vivo phase-variation stability results from cooperative action of the transcriptional regulators Lrp and PapI. Here, we use an E. coli cell-free expression system to study molecular functions of the pap regulatory region based on a specially designed, synthetic construct flanked by two reporter genes encoding fluorescent proteins for simple readout. Based on our observations we suggest that Lrp and the conformation of the self-complementary regulatory DNA play a strong role in the regulation of phase-variation. Our work not only contributes to better understand the phase variation mechanism, but it represents a successful start for mimicking stable, hereditary and strong expression control based on methylation. Since the regulatory DNA cruciform conformation corresponds to a Holliday Junction, gene expression is expected to respond if opposite arms of the junction are separated.

P.17 Nascent fusion pore opening monitored at single-SNAREpin resolution

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Vesicle fusion with a target membrane is a key event in cellulartrafficking and ensures cargo transport within the cell and between cells. The formation of a protein complex, called SNAREpin, provides the energy necessary for the fusion process. In a three-dimensional microfluidic chip, we monitored the fusion of small vesicles with a suspended asymmetric lipid bilayer. Adding ionchannels into the vesicles, our setup allows the observation of a single fusion event by electrophysiology with 10-μs precision. Intriguingly, we identified that small transient fusion pores of discrete sizes reversibly opened with a characteristic lifetime of∼350 ms. The distribution of their apparent diameters displayed two peaks, at 0.4±0.1 nm and 0.8±0.2 nm. Varying the number of SNAREpins, we demonstrated that the first peak corresponds to fusion pores induced by a single SNAREpin and the second peak is associated with pores involving two SNAREpins acting simultaneously. The pore size fluctu-ations provide a direct estimate of the energy landscape of the pore.By extrapolation, the energy landscape for three SNAREpins does not exhibit any thermally significant energy barrier, showing that pores larger than 1.5 nm are spontaneously produced by three or more SNAREpins acting simultaneously, and expand indefinitely. Our results quantitatively explain why one SNAREpin is sufficient to open a fusion pore and more than three SNAREpins are required for cargo release. Finally, they also explain why a machinery that synchronizes three SNAREpins, or more, is mandatory to ensure fast neurotransmitter release during synaptic transmission.

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P.18 Viscoelastical properties of MCF-7 cells modulated by substrate stiffness

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Cells sense stiffness of surrounding tissues and adapt their activity, proliferation, motility and mechanical properties based on such interactions. Cells probe the stiffness of the substrate by anchoring and pulling to their surroundings, transmitting force to the extracellular matrix and other cells, and response to the resistance they sense, mainly through changes in their cytoskeleton [1]. Cancer and other diseases alter stiffness of tissues, and the response of cancer cells to this stiffness can also be affected [2]. In the present study we show that MCF-7 breast cancer cells seeded on polyacrylamide gels have the ability to detect the stiffness of the substrate and alter their mechanical properties in response. MCF-7 cells plated on soft substrates display lower stiffness and viscosity when compared to those seeded on stiffer gels or glass. These differences can be associated with differences in the morphology and cytoskeleton organisation, since cells seeded on soft substrates have a round morphology while cells seeded on stiffer substrates acquire a flat and spread morphology with formation of actin filaments, similar to that observed when seeded on glass. These findings show that MCF-7 cells can detect the stiffness of the surrounding microenvironment and thus, modify their mechanical properties.

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P.19 Characterization of the membrane-regulated dynamics of Mga2 from baker's yeast

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Biological membranes define the boundaries of living organisms and organelles. Their physicochemical properties are determined by the lipid and protein composition. An important parameter is the degree of lipid saturation which determines lipid packing, water permeability, and membrane fluidity. Key for maintaining such physicochemical properties are sensor proteins that examine and regulate lipid compositions. However, so far little is known about the mechanisms that sense and control the different membranes properties. An excellent model to study the regulation of the lipid acyl chain composition is the OLE pathway that controls the production of unsaturated fatty acids in *Saccharomyces cerevisiae*. Within the OLE pathway the ER-resident transcription factor Mga2 gets ubiquitylated by the E3-ligase Rsp5 when the cell requires the production of unsaturated fatty acids and gets processing by the proteasomes which releases an active form that induces the expression of the Δ9-fatty acid desaturase OLE1.

Our aim was to investigate the sensing mechanism and the signal transmission from Mga2's transmembrane region to its ubiquitin site. The data from our Förster Resonance Energy Transfer (FRET) experiments indicates that the juxtamembrane region with its structural dynamics could serve as an amplifier or lever for the fluctuating signal from the sensory TMH.

P.20 Investigating vesicular cargo transport driven by multiple motors

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Vesicular cargo transport inside a cell is carried out by multiple molecular motors such as kinesins and dyneins walking along a microtubule network. Various cargos have to be delivered to specific locations at specific times during the cell cycle. This is regulated by many factors such as number of motors carrying a cargo, vesicle fluidity etc. How an ensemble of motors co-ordinate to transport vesicular cargo is not very well understood. In this study, we aimed to understand the influence of motor density on cargo transport. Thereby, we developed in vitro liposomal stepping motility assay using kinesin-3 motor, KIF16B, which can bind directly to liposomes via its C-terminal tail and walk along microtubules with its N-terminal motor heads. Using TIRF microscopy and single particle tracking, we found that the velocity of the liposomes decreases with increasing motor concentrations. However, at low motor concentration the liposomes pause more frequently compared to high motor concentration where the transport was slower but more robust. In contrast, previous studies have reported that velocity of a rigid cargo such as a bead doesn't vary with change in motor concentration [1]. Our results demonstrate that mechanical coupling of multiple motors via a diffusive lipid bilayer influences the transport properties of a vesicular cargo.

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P.21 The adhesion strength of Candida albicans yeast cells to tooth enamel quantified by Fluidic Force Microscopy

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Caries is one of the most prevalent diseases worldwide, which is characterized by a degradation of the mineralized tooth enamel. The opportunistic pathogen *Candida albicans* is considered as a major causal agent for caries in children.[1] Although tooth enamel specimens have been well characterized by atomic force microscopy,[2] this technique has not been used yet to study the adhesion strength of *C. albicans* on the same material.

We applied Fluidic Force Microscopy-based single-cell force spectroscopy to determine the key adhesion parameters "maximum adhesion force", "rupture length" and "de-adhesion work" of single yeast cells on tooth enamel with or without adsorbed salivary molecules, in presence and absence of human saliva on the yeast cell surface.

We observed maximum adhesion forces in the lower nanonewton range for all tested conditions. Significant increases in all adhesion parameters, however, were noticed for naïve and saliva-pretreated yeast cells probed on saliva-pretreated, and on *in situ*-formed pellicle of the enamel when compared to the uncovered enamel surface. These data suggest that the initial adhesion of naïve *C. albicans* yeast cells to tooth enamel occurs through strong interactions between yeast cell wall-associated adhesins and the salivary pellicle formed on teeth, through which this fungus might be well adapted to the conditions seen in the oral cavity.

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P.22 Pure protein membranes made from fungal hydrophobins: Assembly and mechanical properties

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Compartmentalization of an aqueous solution is of utmost importance in biology. Typically, the matrix of the membranes forming the compartments is a phospholipid bilayer. Thereby, the amphiphilicity of the lipids is necessary for bilayer formation. Yet, for applications in, e.g., biomedicine or synthetic biology, phospholipids are limited in their variety in mechanical and biochemical properties and thus, alternative building blocks are needed. Proteins are promising candidates due to their biocompatibility and versatility via genetic engineering. A special family of strongly amphiphilic proteins, hydrophobins, appears to be particularly suited. In this study, we utilize fungal hydrophobins which self-assemble at water-interfaces into stable monolayer films. Contacting two interfacial films, stable bilayer membranes resembling lipid bilayers can be produced [1]. We study the assembly process of the monolayers and the mechanical properties of mono- and bilayers. At the interface, the proteins organize in clusters in which the proteins obtain a crystalline order [2]. In AFM membrane stretching experiments, we determine the elastic modulus of these monolayer films. We find a remarkably high value, very likely owing to the high cohesion in the 2D crystal structure. Yet, in the bilayer form, the modulus seems to be reduced, hinting at a protein reordering during bilayer formation.

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P.23 Adhesion profiles and viability of *Staphylococcus aureus* cells on structured surfaces

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Understanding and controlling microbial adhesion is an important biomedical problem. While it is known that adhesion is mediated by thermally fluctuating cell wall macromolecules [1], many properties, such as the distribution of adhesive strength over the cell wall, are still unknown. In addition, it is still unclear how different materials and their natural or artificial structuring affect bacterial adhesion and viability. Here, we use a combination of single-cell force spectroscopy (SCFS) and wet plating to determine the adhesion and viability of *Staphylococcus aureus* on structured surfaces. The resulting 'adhesion profiles' in SCFS, force-distance curves, can be interpreted with the help of Monte Carlo simulations: By changing the parameters in the simulations, different phenomena can be decoupled, for example, the mechanical stretching of macromolecules from the area accessible for bacterial adhesion. We find that simple geometric considerations of accessible bacterial surface area are insufficient to explain the adhesion profiles. Rather, angle dependent molecule-substratum interactions are responsible for 'bathtub'-like adhesion profiles. Furthermore, several distinct spots of high adhesion capability are responsible for peaked adhesion profiles, corroborating the view that a bacterium can be seen as a 'patchy sphere'.

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P.24 Free energies of stalk formation in the lipidomics era

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Many biological membranes are asymmetric and exhibit complex lipid composition, comprising hundreds of distinct chemical species. Identifying the biological function and advantage of this complexity is a central goal of membrane biology. Here, we study how membrane complexity controls the energetics of the first steps of membrane fusions, that is, the formation of a stalk. We first present a computationally efficient method for simulating thermodynamically reversible pathways of stalk formation at coarse-grained resolution. The new method reveals that the inner leaflet of a typical plasma membrane is far more fusogenic than the outer leaflet, which is likely an adaptation to evolutionary pressure. To rationalize these findings by the distinct lipid compositions, we computed ~200 free energies of stalk formation in membranes with different lipid head groups, tail lengths, tail unsaturations, and sterol content. In summary, the simulations reveal a drastic influence of the lipid composition on stalk formation and a comprehensive fusogenicity map of many biologically relevant lipid classes.

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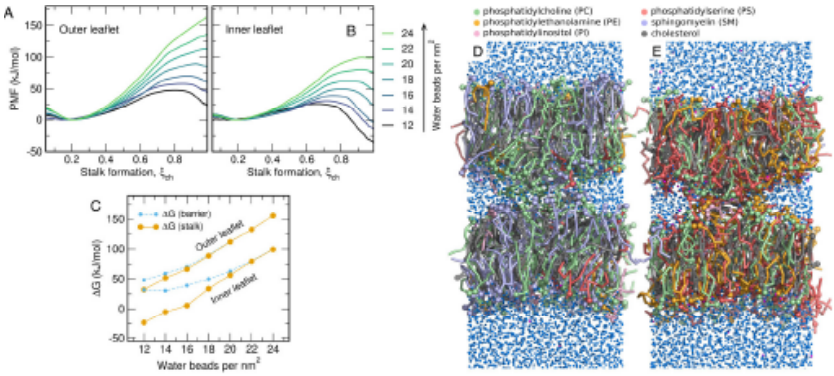

P.25 Time- and Zinc-Related Changes in Biomechanical Properties of Human Colorectal Cancer Cells Examined by Atomic Force Microscopy

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Monitoring biomechanics of cells or tissue biopsies employing atomic force microscopy (AFM) offers great potential to identify diagnostic biomarkers for diseases, such as colorectal cancer (CRC). Data on the mechanical properties of CRC cells, however, are still scarce. There is strong evidence that the individual zinc status is related to CRC risk. Thus, this study investigates the impact of differing zinc supply on the mechanical response of the in vitro CRC cell lines HT-29 and HT-29-MTX during their early proliferation (24–96 h) by measuring elastic modulus, relaxation behavior, and adhesion factors using AFM. The differing zinc supply severely altered the proliferation of these cells and markedly affected their mechanical properties. Accordingly, zinc deficiency led to softer cells, quantitatively described by 20–30% lower Young's modulus, which was also reflected by relevant changes in adhesion and rupture event distribution compared to those measured for the respective zinc-adequate cultured cells. These results demonstrate that the nutritional zinc supply severely affects the nanomechanical response of CRC cell lines and highlights the relevance of monitoring the zinc content of cancerous cells or biopsies when studying their biomechanics with AFM in the future.

P.26 A neuron specific alternative STIM1 splice variant recruits new signaling complexes to affect presynaptic release

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Store-operated Ca^{2+} entry is a ubiquitous mechanism that contributes to the regulation of basal and receptor-triggered Ca^{2+} concentrations thereby governing signaling and cell homeostasis. The two known isoforms STIM1 and STIM2 sense the ER Ca^{2+} content and oligomerize to trigger Ca^{2+} entry by gating Orai channels. We have characterized a novel STIM1 splice variant, STIM1B, where neuronal-specific insertion of an additional short exon (11B) results in a C-terminally truncated STIM1 lacking 145 amino acids including part of the C-terminal inhibitory domain (CTID), microtubule associated EB binding sites, the S/P rich region and the polybasic domain. STIM1B shows slower kinetics of cluster formation and I_{CRAC} activated by STIM1B shows reduced slow calcium-dependent inactivation (SCID). STIM1B is the predominant STIM1 isoform in cerebellar Purkinje neurons but also displays prominent expression in hippocampal as well as other neurons where it preferentially localizes to neurites in contrast to a more somatic STIM1wt localization. Specifically, in autaptic hippocampal neurons, STIM1B, but not STIM1 causes synaptic facilitation upon high frequency stimulation, demonstrating that cell-type specific splicing may adapt neuronal SOCE to support synaptic function [1]. Current work aims at delineating the targeting of SOCE components in neurons using CRISPR/Cas9-mediated epitope tagging of endogenous proteins, understanding frequency dependent regulation of transmitter release by intracellular Ca^{2+} stores, identifying the specific sequence and the mechanisms responsible for exon specific function as well as investigating the role of recruited additional channels and regulators.

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P.27 Development of microtentacles in suspended cells upon weakening of the actin cortex

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Circulating Tumor Cells (CTCs) pose a significant threat due to their role in metastasis: It has been proposed that CTCs are able to escape the blood stream and reattach to the tissue by the formation of so-called microtentacles. Microtentacles are microtubule-based membrane protrusions with a diameter of less than 1 µm and a length of tens of µm. In this work we show that (and how) microtentacles can be generated in noncancer cells in suspension by weakening the actin cortex against the force of growing microtubules. We particularly analyzed the structure of the actin cortex and quantified the number and length of the generated microtentacles. To demonstrate the dynamics of microtubule-based protrusions against soft barriers we developed a stochastic model for growth of microtubules with length-dependent dynamics at their tips. This allows prediction of the influence of barrier stiffness on the microtentacle length distribution.

P.28 Free Energy Simulations of Pore Formation

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Lipid membranes define biological cells by establishing a semi-permeable barrier. Pore formation plays a role in processes such as membrane fusion and fission, the killing of bacterial cells with antimicrobial peptides, and others. Although pores are heavily studied with a variety of methods, the free energy landscape of the initial stages of the pore formation is still not fully understood. We use molecular dynamics simulations to study the mechanisms and energetics of pore formation. We overcome the challenge of exploring the free energy landscape using umbrella sampling along a recently developed reaction coordinate[1, 2]. Here, we here study the effects (i) of electric fields on the free energies of pore formation, as applied during electroporation to allow cellular uptake of drugs or genes, and (ii) of the common small antifungal drug itraconazole and the solvent DMOS[3]. The potentials of mean force (PMFs) show that electric fields greatly stabilize open pores and lower the barrier for pore formation. We also compare two methods for establishing transmembrane potential in an MD simulation – external electric field and charge imbalance. Due to itraconazole low solubility in water there are several liposome-based formulations but the release mechanisms remain unclear. Interestingly, whereas itraconazole has only a small effect on the structure of planar, intact membranes, it strongly stabilized open pores[3].

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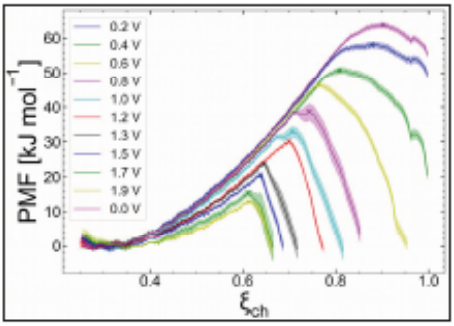

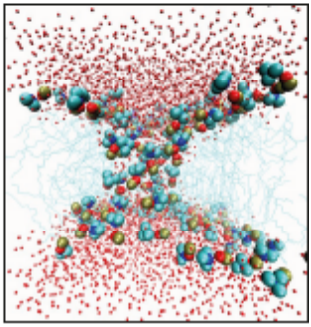

P.29 Light sensitivity of Cell free expressed Archaerhodopsin-3 in microfluidics

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Many studies have highlighted the importance of transmembrane receptor proteins in the plasma membranes of biological cells. Photoreceptors such as Archaerhodopsin (Arch) constitute a class of transmembrane proteins that are highly sensitive to light. Light sensitivity of proteins has been intensely investigated during the past decades. However, there is a lack of information regarding the electrophysiological properties of the pore forming Archaerhodopsin (Arch), as studied in vitro. Here, we use a PDMS microfluidic chip with two channels that form unsupported bilayers between them. The technique enables simultaneous optical and electrical assessments of the bilayer in real time. In this work we recombinantly produced Arch-3 using a cell-free expression system. We synthesized a GFP labelled variant of Arch-3 to track its synthesis, using fluorescence microscopy. The electrophysiological properties of Arch-3 incorporated in a suspended bilayer were studied using a green laser for excitation. From the measurements we estimated the pore size of Arch-3 as 1.2 nm. Due to rapid cell-free prototyping just by changing the DNA, our setup enables us to study also photoelectrical properties of modified transmembrane protein constructs with ease. Moreover, Arch possesses the generic transmembrane structure of G-proteins. Our work represents a first step to study their cascade signaling in conjunction with coupled receptor proteins.

P.30 PEG-Methylsulfone (MS) Based Hydrogels for 3D Culture of Invasive Breast Cancer Organoids: Studying the Effects of Biochemical and Biophysical Cues on Organoid Response

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Breast cancer invasion is accompanied with changes in biochemical and biophysical (mechanics, architecture) properties of complex native extracellular matrix (ECM).[1] Understanding the effects of ECM derived cues on cancer cell response is critical to discover possible interventions. Thus, engineering in vitro 3D models to systematically study cell-matrix interactions has been an important focus in the field. To this hand, synthetic poly(ethylene) glycol (PEG) based 3D hydrogels introduce tunability to individually tailor cell-instructive matrix properties.[1] Here, we use 3D PEG-MS hydrogels, that are crosslinked with cell-degradable peptide crosslinkers, to encapsulate PyMT breast cancer organoids, with non-invasive luminal and invasive basal cells that show mechanosensitivity. We individually tune the biochemical cues by using specific cell-adhesive peptide ligands from fibronectin, collagen I or laminin 5. We control the degree of crosslinking and network mechanics via polymer (PEG) density. We investigate the invasive potential of organoids in different matrices by studying the positioning of basal cells, nuclear localization of mechanosensitive proteins, organoid growth and morphology. We finally aim to translate our knowledge to gradually design more complex biomimetic cancer microenvironments via combinatorial cues and control over matrix architecture next to mechanics.

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P.31 DNA opening during transcription initiation by human RNA polymerase II in atomic details: implications of hydrogen bonds between protein loops and DNA

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RNA polymerase II (RNAPII) is a macro-molecular complex that synthesizes RNA by reading the DNA code, a process called transcription. During the initiation step of the transcription, the RNAPII opens the double-stranded DNA in order to read the DNA code. Since the formation of the DNA transcription bubble remains poorly understood, we used molecular dynamics simulations to provide atomic-level insights into this crucial step of transcription.

Here, by steering the simulations with a combination of (i) guided DNA rotation and (ii) path collective variables [1], we obtained for the first time continuous atomic trajectories of the complete DNA opening process. The simulations provide insights into the role of loop dynamics and protein-DNA interactions during DNA opening.

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P.32 Binding mode characterization of PfFNT' inhibitors through Docking and MD simulations

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Malaria is a key threat to public health worldwide. Recently, Plasmodium-faciparum formate-nitrite-transporter (PfFNT) has been identified as the malaria parasite's lactate transporter and as a novel drug target[1]. A few putative inhibitors for PfFNT have been identified[2]. However, their mechanism of binding and inhibition is not well understood. Here, we used molecular dynamics simulation to study the function and inhibition of PfFNT at an atomic level. The ligands MMV007839 and BH267.meta have been identified as potential inhibitors. For these ligands, we derived new parameters based on GAFF2. To do this, we used the HTMD Parameterize tool[3] complemented with Stochastic Conformational Analysis at the semi-empirical level with ab initio refinement. The new parameters reproduce the dihedral potentials of these ligands at the DF-MP2-aug-cc-pVTZ level of theory. This is a remarkable improvement relative to initial GAFF2 parameters. In silico, we docked the ligands into the putative binding site in the PfFNT structure. Our initial simulations are in agreement with the reported experimental results.

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P.33 Baker's yeast as a model for studying chronic diseases related to ER stress

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An increasing number of studies indicate that saturated lipids can be metabolically harmful and their role in inflammatory diseases such as type II diabetes is actively discussed [1]. Further there is collective evidence linking type II diabetes to chronic ER stress [2]. Using the baker's yeast as a model, we provide evidence for a direct role of aberrant lipid compositions in the ER for the onset and progression of chronic ER stress. We show that increased lipid saturation causes chronic ER stress with direct consequences for the viability of cells. Our studies reveal that the so-called unfolded protein response (UPR) can perpetuate ER stress thereby contributing to dramatic changes in ER morphology. We seek to understand the molecular events and mechanisms that switch the UPR from a beneficial, homeostatic response to a detrimental, cell death inducing program. Our working hypothesis, supported by genetic, functional and lipidomic data is that

1. An overly saturated lipidome causes UPR activation
2. UPR activation upregulates lipid biosynthesis
3. Increased lipid synthesis leads to the production of even more saturated lipids which cause a perpetuation of the UPR forcing the cell to enter and be trapped in a vicious cycle that ultimately gives rise to the formation of non-fluid gel phases in the ER

Our studies reveal that the UPR may contribute to disease development, progression and the chronification there of by perpetuating a stress-inducing condition.

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P.34 Molecular motors from a 3D perspective: motion and torque generation of kinesins

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Kinesin motor proteins organize the mitotic spindle by cross-linking and sliding microtubules, which orchestrates the complex process of cell division. Some kinesins, such as kinesin-5 and kinesin-14, not only move straight along a microtubule, but exhibit a lateral stepping component. This results in a sideward motion, which has not been fully investigated so far due to technical limitations. Here we explore how kinesin-5 and kinesin-14 slide microtubules and estimate the rotational forces (torques), that the motors produce. Using a 3D motility assay, we show that both kinesin-5 and kinesin-14 drive the rotation of short microtubules around long, suspended microtubules along helical trajectories. Further, we develop a microtubule coiling assay in which sliding by kineisn-5 and kinesin-14 twists microtubules, indicative of torque generation. A theoretical simulation allows the quantification of the torque from the observed microtubule bending. These results reveal that kinesin-5 and kinesin-14 both rotate microtubules around each other and generate torque. We hypothesize that this behavior serves to organize spindle fibers, to circumnavigate roadblocks and to break symmetry through chirality of the mitotic spindle.

P.35 Optimal Search Strategies of Auto-chemotactic Walkers

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Stochastic search processes are ubiquitous in nature and are expected to become more efficient when equipped with a memory, where the searcher has been before. A natural realization of a search process with long-lasting memory is a migrating cell that is repelled from the diffusive chemotactic signal that it secrets on its way, denoted as auto-chemotactic searcher. To analyze the efficiency of this class of non- Markovian search processes we present a general formalism that allows to compute the mean first passage time (MFPT) for a given set of conditional transition probabilities for non-Markovian random walks on a lattice. We show that the optimal choice of the n-step transition probabilities decreases the MFPT systematically and substantially with an increasing number of steps. For a single auto-chemotactic searcher, an optimal coupling between the searcher and the chemical reduces the MFPT to 1/3 of the one for a Markovian random walk. This best point consists in a compromise between a blind diffusive search corresponding to the case with no interaction, and an ineffective ballistic search for the strong interaction case. However, when more searchers are present, this optimal searcher-cue coupling is modified to become infinitely strong as the density of walkers is high.

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P.36 Hydroxyapatite pellets as versatile model surfaces for systematic adhesion studies on enamel

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Research into materials for medical application draws inspiration from naturally occurring or synthesized surfaces. Particular attention must be paid to biocompatibility, osseointegration and bacterial adhesion behavior. To understand their properties and behavior, experimental studies with natural materials such as teeth are strongly required. The results, however, may be highly case-dependent because natural surfaces have the disadvantage of being subject to wide variations, for instance in their chemical composition, structure, morphology, roughness, and porosity. Synthetic surfaces which mimic enamel in its performance with respect to bacterial adhesion and biocompatibility, therefore, facilitate systematic studies. We performed single-cell force spectroscopy with single *Staphylococcus aureus* cells to measure adhesion-related parameters such as adhesion force and rupture length of cell wall proteins binding to enamel and synthetic hydroxyapatite (HAp) pellets. We examine the influence of blood plasma and saliva on the adhesion properties of *S. aureus* and match these results to water wettability, elemental composition of the samples and the change in the macromolecules adsorbed over time on the surface. We found that the adhesion properties of *S. aureus* were similar on HAp and enamel samples under all conditions and we therefore conclude that HAp pellets are a good alternative for natural dental material [1].

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P.37 In vitro study of Influenza virus-like particles with a model cell membrane

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A crucial step for viral infections is the penetration of a virus particle in a cell where it might be replicated. In principle, the trimeric viral hemagglutinin protein binds to sialic acid, commonly Neu5Ac, to adhere to host cells (1). We are using virus-like particles (VLP), a non-infectious variant of influenza viral particles, and study their fusion properties with a model cell membrane. As model membrane, a bilayer is formed in a 3D microfluidic device by contacting two lipid monolayer decorated water-oil interfaces. After the formation of the bilayer, VLPs are dispersed near the bilayer and their fusion with the bilayer is studied by fluorescence microscopy and electrophysiological measurements (Patch Clamp). Our model system aims at defining the interactions between the virus particles and cell membranes which is essential to combat viral infections and improve viral vectors as therapeutic agents.

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P.38 DNA oligomer binding in competition reveals interactions beyond stacking

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DNA hybridization, the binding of two complementary DNA single strands forming a double helix, is a highly sequence specific molecular recognition process that plays important roles in biology and nanotechnology. However, DNA binding in complex situations in presence of the competing partner is poorly understood. We find that the ratio of the binding affinities in competition changes compared to pairwise measurements. This is the signature of the interaction among the competitors on the probe. We additionally compare the pairwise binding constants of DNA oligonucleotide strands from fluorescence anisotropy to the values from fluorescence correlation spectroscopy. For a specific case with a mismatched sequence we observe that the binding constants from both techniques differ by two orders of magnitude. We emphasize that the situations beyond a simple helix formation require careful interpretation, considering the employed measurement technique and the type of interaction at the binding site. These situations may involve other molecular conformations than an undisturbed double helix.

P.39 Modeling intracellular transport by multiple kinesin and dynein motors

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Intracellular transport is essential for the functionality of the cell. Microtubule-based transport is carried out by opposite-directed kinesin and dynein motors. How the cell regulates bidirectional transport by multiple dynein and kinesin motors remains an open question. Motor number, ATP or roadblock concentrations [1], as well as the cargo itself might regulate bidirectional cargo transport. By developing mathematical kinesin and dynein models, which are based on known single motor properties [2], we use Monte Carlo simulations to understand the underlying processes of the experiment. We found that motor type and number determine the transport direction in bidirectional gliding assays, while ATP or roadblocks have no effect [1].

Intracellular cargos often have a membrane composed of a lipid-bilayer, on which motors can diffuse [3]. How the membrane diffusion influences cargo transport is not well understood. Experimental data of membrane-bound cargo transport (see abstract by Rahul Grover et al.) shows directed motion with frequent pausing events. The simulation predicts that pausing comes from some passive motors. These passive motors could be a result of a) non-functional motor heads or b) the geometry of the experimental assays, which might interrupt the mobility of motors on MT.

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P.40 Substrate morphometry enhances the understanding of bacterial adhesion on nanostructured surfaces

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Surface topography plays a decisive role for the formation of biofilms and the behaviour of adhered microbials such as cells or bacteria. Newer studies revealed that a description of surface roughness by a root mean square (RMS) value only is a too simple model to capture the topography involved in bacterial adhesion [1]. We show that the RMS could not explain the change in the adhesive behavior of *Staphylococcus aureus* on black Si samples featuring a variable nanoroughness. However, the Minkowski functionals from integral geometry provide a comprehensive and robust characterization of complex random structures [2] that have been successfully applied to a variety of applications, including material science [3] and pattern formation [4]. Minkowski functional analysis of nanorough surfaces can reliably distinguish topographies with similar RMS values of surface roughness. Thus, shape analysis based on Minkowski functions yielded a quantitative agreement between the fraction of the surface accessible to the tethering macromolecules and the adhesion forces determined experimentally [1].

In this poster we will focus on the intuitive interpretation of the scalar and tensorial Minkowski functionals and discuss their advantage compared to the RMS. Furthermore, we will analyze the surface topography of dental ceramics and black Si by atomic force microscopy and show a way to calculate the Minkowski measures in dependence of height, which leads to quantitative comparison of the different types of roughness.

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P.41 Vesicles from natural proteins (HFBI): characteristics and chances

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Hydrophobins are a family of surface-active proteins known for their small size and strong amphiphilicity. HFBI is a protein from this family produced by *Trichoderma reesei*. We use these proteins to form artificial bilayers [1] and vesicles [2]. Compared to ordinary lipid bilayers, hydrophobin bilayers are similar in thickness but can maintain a much higher lateral tension [1], which makes them interesting for artificial vesicles. These artificial vesicles could be of great importance for drug delivery, but also for vaccines, which have recently attracted a lot of interest. A fundamental property of drug carriers is the exchange with their environment. The water permeability of these protein vesicles is therefore a crucial parameter to explore. We measured the permeability of HFBI membranes using the droplet interface bilayer technique. By measuring the volume change of two droplets of different salt concentration connected by a bilayer, the permeability can be derived. Our experiments showed that HFBI bilayers have a very low water permeability, about two orders of magnitude lower than conventional lipid bilayers, which are known for their low permeability. They also withstand much higher osmotic pressure than lipid membranes. This ensures safe packaging of potential compounds in the vesicles. Furthermore, we can manipulate these properties by inserting other proteins or channels, as we have already done with a hydrophobin mutant or the rather simple ion channel gramicidin A [2]. In this context, we are exploring a vesicle formation technique to create a large number of vesicles that can be filled with drugs or specific solutions. We achieve this with a combination of microfluidics and centrifugation.

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P.42 Lateral force transductor on epithelial monolayers based on polymer structures

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Epithelial cells transmit signals through chemical and mechanical signals during different processes like wound healing. [1][2] Several studies have been done to investigate the mechanical forces transmitted through the extracellular matrix with techniques like traction force microscopy, where the passive reaction of the cells has been explored but an active force interaction.[3][4] We propose a method to interact with the epithelial cells conserving the integrity of the monolayer. Based on deformable environments and a stretcher device, the force can be transmitted directly to the lateral junctions of the cells to imitate the signal of the monolayers. The captured deflection of the structures proved that the system is capable of interacting with the cells. This study is the base to explore the behavior of the cells under different stress conditions directly on the monolayer.

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P.43 Lipid specificity of Viral Fusion Proteins

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Viral fusion proteins drive fusion of viral and host cell membranes in a series of complex structural transition events. Although the structure of several fusion proteins has been solved, the characterization of viral protein-membrane interactions at atomistic resolution is still missing. Membrane interactions of fusion proteins are conserved and occur via fusion peptides (FPs) in class I and fusion loops (FLs) in class II/III proteins. Previously, we had characterized the glycerophospholipid binding in class II fusion protein glycoprotein C (gC) of Rift Valley fever virus (RVFV) [2] and the studies revealed specific binding pocket for PC lipid. Here we aim to understand if specific lipid binding site also exists in class I and III viral fusion proteins and dependence of lipid headgroup type, tail length and degree of lipid tail unsaturation for protein binding. Molecular dynamics (MD) simulations is an excellent technique to understand how proteins associates with lipid membrane at atomistic resolution and here we make use of MD simulations to gain structural insights into lipid contact sites and membrane insertion of FP / FL residues.

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P.44 Properties of Reconstitute Model Lipid Droplets in a Phospholipid Bilayer using a 3D Microfluidic Platform

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Lipid droplets (LDs) are the main energy storage organelles in cells. The energy is stored in the form of neutral lipids such as triglycerides and sterol esters. The monolayer of LDs embedded into the cell membrane is decorated with a specific set of proteins where the targeting and removal of these proteins are being studied to understand the biology of diseases, such as obesity, diabetes and atherosclerosis. The partition mechanism of these proteins between LD and bilayer is still under investigation. Here, a 3D microfluidic platform is developed to explore the partition dynamics of these proteins in a free-standing lipid bilayer enriched with LDs. The lipid bilayer is formed by contacting two oil-water interfaces that are decorated with a phospholipid monolayer. The bilayer is characterized by electrophysiological measurements and fluorescence microscopy. Using confocal microscopy, the 3D geometry of the reconstituted bilayer-embedded LD is determined with a remarkable spatial resolution. It appears that the bilayer-embedded LDs have a radial dimension in the micrometer range and present a characteristic lens shape. Based on wetting theory, we demonstrate that this lens shape geometry corresponds to an equilibrium shape.

P.45 T cell stiffness is enhanced upon formation of immunological synapse

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To exert their effector functions, T cells need to form an intimate contact with their cognate target cells, which is termed the immunological synapse (IS). Mechanobiology has been receiving increasing attention, given its indispensable and previously ignored role in regulating cell functions. In terms of T cells, they can sense the stiffness of targets/substrates and generate force upon IS formation, which are important for their effector functions. However, how the stiffness of T cells per se is regulated upon IS formation still remains elusive. In this work, we determined stiffness of different cell parts in detail during the processes of IS formation in T cells. To this end, we established a method to investigate live T cells on functionalized co-verslips by atomic force microscopy (AFM) based Peak Force Quantitative Na-noscale Mechanical Characterization (Peak Force QNM), which enables simulta-neous determination of the surface profile and stiffness of live T cells. Using primary human CD4⁺ T cells, we found that upon IS formation, T cells were substantially stiffened at the cell body as well as at the lamellipodia. In general, the stiffness at the lamellipodia is significantly higher than that at the cell body. Furthermore, we identified that calcium is involved in regulation of this IS formation-induced T cell local stiffening at lamellipodia [1].

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P.46 Entropic force acting on a flat wall by a grafted F-actin

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Actin filaments have a significant impact on the deformation of the cell membrane [1]. In the cell cortex, some of the barbed ends of F-actins polymerize, whereas their pointed ends are attached to the cortex gel. The region beneath the membrane, where these filaments have thermal fluctuation is called the semiflexible region (SR) [2]. In SR, the fluctuating F-actins exert force on the membrane. We study the force acting on a flat wall by a grafted F-actin using an MD simulation. We focus on the distribution and magnitude of the entropic force exerted by the grafted F-actin on the different regions of the wall. Our results demonstrate that the filament's tip has a radial distribution. Moreover, we study effects of the increasing of the compression on the radial distribution of the filament's tip and the amount of the entropic force. The scale of the entropic force is about pN, which is in good agreement with experimental data.

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P.47 Placing transcription factor complexes into gene regulatory networks

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Interactions between transcription factors and DNA are important for gene transcriptional regulation. Cooperativity amongst TFs can lead to improvement in DNA binding specificities. Recent studies show that cooperative binding events are evolutionarily conserved and show more effects on gene transcription than single TFs. A gene regulatory network, built with TF complexes showing significant cooperativity as regulatory drivers, can be helpful for mechanistic understanding of transcriptional regulation and may have predictive value for diseases like cancer. Main aim of this work is to develop an automated pipeline to map putative transcription factor complexes onto gene regulatory networks. We consider the raw RNA-seq data of two groups of monocytes - classical and non-classical. We compare the differential complexes obtained from the stand-alone software CompleXchange[1] and TF-gene interactions predicted by, our in-house web server, TFmiR2[2] to extract common TFs and target genes. We use the tool Fimo[3] from the MEME suite to scan the TF motifs in the promoter regions of their common genes and are subjected to constraints to filter out non-essential complexes. Cytoscape[4] is used to visualize the initial network. Our results indicate that many of the individual TFs targeting common genes in the regulatory network are part of protein complexes.

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P.48 Effect of different plant extracts on the salivary bacteria and oral biofilm – an ex vivo and *in situ* study

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Aim: To evaluate the antimicrobial effects of various plant extracts on salivary flora and oral biofilms.

Methods: The effect of plant extracts (curcuma, liquorice, rosemary, and hops) in various concentrations (0.2 % to 8.3 %) on the salivary flora was tested *ex vivo*. The bacteria from saliva were individually incubated with each extract for 10 min and analyzed using fluorescence microscopy after LIVE/DEAD staining. To investigate the effects of plant extracts on the oral biofilm, dental specimens were mounted on acrylic splints and exposed intraorally for 48 h. The extracts (10 ml of 0.2 % hops or 0.4 % rosemary) were used as mouth rinsing and applied for 30 s each 6 h. Water served as a control. The biofilm samples were analyzed using fluorescence microscopy, scanning and transmission electron microscopy.

Results: All *ex vivo* tested plant extracts showed an antibacterial effect at high concentrations. Hops and rosemary showed strong effects also at low concentrations and were therefore tested in situ. They induced a significant reduction in bacterial colonization and biofilm vitality. Moreover, the biofilm thickness on dental specimens was significantly reduced *in situ*.

Conclusions: The tested plant extracts showed different antimicrobial activity depending on their concentration. Hops or rosemary rinsing induced a significant inhibition of biofilm formation in the oral cavity.

P.49 Molecular fingerprints of a stressed endoplasmic reticulum

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Biological Membranes are complex and dynamic assemblies comprising thousands of different lipid and protein species. Physical and compositional properties of membranes determine their identity and functionality in the cellular context.

The endoplasmic reticulum (ER) is the site of activation of the unfolded protein response (UPR), a large-scale transcriptional program crucial for protein and lipid homeostasis. Conditions triggering the UPR are collectively termed ER stress. These include overpopulation of the ER lumen with un- or misfolded proteins or lipid perturbations. Unmitigated ER stress has been linked to diseases like type II diabetes and cancer[1,2]. We investigate the role of the ER membrane in these processes and elucidate the molecular basis of cytotoxicity during prolonged ER stress.

To address this, we have established a versatile immunoisolation strategy for subcellular membranes. Quantitative lipidomics revealed substantial remodeling of the ER membrane lipidome upon ER stress. Based on these data, combined with molecular dynamics simulation, we suggest *in vitro* model systems that mimic the physical properties of the ER membrane better than prevailing ones. Quantitative proteomics reveals that the stressed ER is accumulating proteins of the late secretory pathway, suggesting a general block of secretion as a basis for adverse effects during prolonged ER stress.

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P.50 Immune cells in an obstacle park

Zeinab Sadjadi[1], Doriane Vesperini[2], Heiko Rieger[1] and Franziska Lautenschläger[2]

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We study how migration and search efficiency of immune cells is influenced by spatial arrangement of obstacles. We focus on the topographical influence of the environment on cell migration in absence of a chemical gradient and visualize the migration of ameoboid cells in pillar parks with different pillar densities. The pillars are periodically arranged on square and triangular lattice sites. In experiments, a microfluidic device is designed to track the neutrophil cells in quasi-2D environments whose thickness vary from 3.5 to 6 micrometers. Our device consists of monosispersed pillars.

We calculate the mean first passage time and dynamical properties of the cells in different densities and configurations of pillars and compare the results with numerical simulations to understand the mechanism underlying cell migration strategies.

P.51 Kinetic and Mechanical Properties of Interfacial Self-organized Film formed by Class II Hydrophobins

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Hydrophobins are a class of amphiphilic proteins with a high potential for making new kind of vesicles useful in e.g. drug delivery. Contrary to lipid vesicles, they can be formed with either hydrophobic or hydrophilic shell [1]. Hydrophobins form self-organizing films at the interface between an aqueous and any hydrophobic phase. The adsorption kinetics of class II hydrophobins HFBI and HFBII were studied by ellipsometry measurements. These reveal unusual linear kinetics. Attaching bulky side groups, via biomolecular engineering, restore usual Langmuir kinetics [2]. Since conformational changes, due to the high stability, can be neglected, only diffusion and their interactions should determine their kinetics. To confirm this, we introduced a stochastic model which includes microscopic diffusion and electrostatic, van der Waals and orienational interactions between proteins. We show that the kinetics of the system are determined by two factors: the diffusive motion close to the interface and the supply rate of this area [2]. Additionally, we studied the expansion of the film by simulation. We repeated the process of increasing the area followed by a relaxation process. Atomic force microscopy measurements demonstrated a honeycomb structure for the self-assembled film [3]. We guarantee that an orientational energy function must be considered to keep this structure.

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P.52 The Effect of Transmembrane Domains on the Free Energy of Stalk Formationduring Membrane Fusion

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The nucleation of the stalk is the first step in membrane fusion. The overall fusion process including the stalk formation is facilitated by fusion proteins anchored in the membrane by transmembrane domains (TMDs). Although TMDs of fusion proteins have been suggested to play an active role during fusion, little quantitative or mechanistic understanding of putative TMD effects has evolved. We used molecular dynamics simulations to analyze the influence of TMDs of the SNARE complex and of viral fusion proteins on the free energy of stalk formation. The stalk free energy was computed highly efficiently via potential of mean force (PMF) calculations along a newly designed reaction coordinate together with the Martini coarse-grained force field[1, 2]. The results reveal a decrease in both, the free energy barrier of stalk nucleation as well as the free energy of the final stalk structure, when TMDs are present in the membrane. However, the observed TMD effect strongly depends on the lipid composition and on the hydrophobic mismatch between the TMD and membrane core.

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P.53 The functional pas de deux of v-SNARE transmembrane domains and lipids in membrane fusion

Madhurima Dhara, Maria Mantero Martinez, Mazen Makke, **Yvonne Schwarz**, Ralf Mohrmann and Dieter Bruns

Membrane fusion is initiated once the assembled trans-SNARE complexes as the core fusion machinery exert the mechanical force necessary on the adjoining phospholipid membranes. Our recent work suggested an active role of SNARE transmembrane domains (TMDs) in promoting membrane merger. Yet the its molecular mechanism remained unclear. Our results demonstrate that naturally occurring v-SNARE TMD isoforms, varying in the number of helix-destabilizing, ß-branched valine or isoleucine residues, differentially regulate fusion pore dynamics. Thus, TMD flexibility represents an unrecognized mechanistic determinant adapted by v-SNARE variants to promote transmitter release. Furthermore, we show that membrane-incorporated lipids like lysophosphatidylcholine or oleic acid affected fusion induction and subsequent pore expansion in a membrane leaflet-specific fashion and in a manner that correlated with their intrinsic curvature preference of highly bent fusion intermediates. Thus, membrane mechanics represent a rate-limiting energy barrier for Ca2+-triggered fusion of chromaffin granules, which proceeds via the formation of a membrane stalk intermediate into a lipidic fusion pore. Collectively, our results suggest that both, support of membrane curvature by v-SNARE TMDs and SNARE force generated membrane bending promote fusion pore formation and progressive expansion.

P.54 Correlated dynamics of migrating immune cells enhances the efficiency of their search for pathogens

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Migration of immune cells is believed to be optimized in the course of evolution to reduce their search time. Nevertheless, so far the optimality of the search for pathogens and other targets by immune cells has not been verified. Mediated by retrograde actin flows, the speed of migrating cells is coupled to their directional persistence (i.e. the straightness of trajectories) in such a way that they decelerate to change the direction of motion. We show that such a correlated dynamic enables immune cells to reduce their search time [1]. We introduce a new class of optimal search strategies based on tuning the strength of coupling between factors influencing the search efficiency and prove that the correlated motion is advantageous for optimizing search efficiency when the persistence length of the searcher is much smaller than the size of the environment in which they search. Understanding the mechanisms of adaptive search and clearance in the immune system opens the way toward more effective cancer immunotherapies and vaccine design. Our findings also may open new possibilities to design artificial intelligent searchers.

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P.55 Molecular dynamic simulations of hydrophobins: pure-protein bilayers and lipid-protein interactions

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Hydrophobins are a family of proteins characterized by a large exposed hydrophobic region, rendering them highly amphiphilic. Class II hydrophobins such as HFBI self-assemble into monolayers at water-air or water-oil interfaces, revealing long-range ordered hexagonal 'honeycomb' structures[1]. This property allows for the preparation of pure-protein bilayers and vesicles[2] with unexpectedly low permeability for water and ions. To provide a molecular explanation for these properties, we carried out atomistic and coarse-grained molecular dynamics (MD) simulations of HFBI bilayers. Our results indicate that the proteins have to rearrange upon monolayer contact in order to form a stable dense bilayer.

In contact with lipid bilayers, hydrophobins were found to modulate the stability of the membrane in electroporation experiments. Coarse-grained simulations suggest that the proteins aggregate inside the pore and bind to the exposed hydrophobic membrane core, therefore leading to the observed stabilization.

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P.56 Towards automated tracking and analysis of individual killer cell cytotoxicity

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Individualized immune therapy of cancer is at the cutting edge of medical advances. To assess the efficacy of new therapeutic treatments, the killing efficiency of human natural killer (NK) cells, specifically targeted at cancer cells needs to be assessed. Although population-based assessment of NK cell killing efficiency is an established analysis method, it inherits several shortcomings. For example, cell counts can vary on a frame-to-frame basis, due to temporary miss-detections or cells migrating out of the field of view. The fate of these cells needs to be heuristically determined, which can lead to biased analysis results. In addition, analyses on a population level cannot reflect alterations in induced cell death by individual killer cells, which is a critical factor for the success of a therapy. There is indeed emerging evidence for heterogeneity among single NK cells, ranging from inefficient killers to "super killers".

Building on a novel time-resolved single-cell cytotoxicity assay, which allows the assessment of quality, quantity, and kinetics of target cell death induced by single primary human NK cells (Backes et al 2018, PMID: 30190323), we are developing measurements and analysis methods to allow automated quantification of single NK cytotoxicity on a large scale. To overcome the above-mentioned shortcomings, we propose an individual tracking and analysis on a per-cell level. This will not only generate more accurate analyses on population level but will most importantly allow the fate determination of each NK and cancer cell, their respective contacts, and the time point of cell death induction. The killing history of individual NK cells will give many insights into single NK cell cytotoxic efficacies.

P.57 Spatial evolution of intermediate filaments organization in astrocytes

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Intermediate filaments (IF) constitute cytoskeletal networks which interacts with other structural networks such as filamentous actin network. While the actin organization has been studied for decades and proven to be very dynamic and varying within the cell, the organization of intermediate filaments remains mainly descriptive. In the present work, we investigate the organization of two IF proteins, vimentin and Gliary Fibrillary Acidic Protein (GFAP) in relationship to the organization and movement of the actin network. To this end, we develop mathematical model of transport for which one can tune different interactions between networks. Based on the actin flow speed, the model will evolve over time to "steady state" organizations of IF networks which we compare to experimental data. In order to gather a large number of data, we base our work on the average IF spatial distribution in rat astrocytes which are forced to a square shape, obtained by micro-patterning of the cells. Moreover, keeping the same overall shape, we are able to tune the adhesion points of cells by changing the shape of the pattern, namely using X shape or H shape.

P.58 Role of Extracellular Vimentin in Cancer-Cell Functionality and Its Influence on Cell Monolayer Permeability Changes Induced by SARS-CoV-2 Receptor Binding Domain

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The cytoskeletal protein vimentin is secreted under various physiological conditions. This extracellular vimentin can be primarily categorized into two types: one which is intact to the cell surface and another which is secreted into the extracellular space. While surface vimentin has been shown to be involved in processes such as viral infections and cancer progression, secreted vimentin controls inflammation by reducing the neutrophil infiltration, helps in bacterial elimination in activated macrophages and promotes axonal growth in astrocytes by activating IGF-1 receptor in the same signaling pathway as IGF-1. IGF-1R is overexpressed in cancer cells and IGF-1/IGF-1R pathway plays significant role in general cellular functions. In this study, we demonstrate the functional role of extracellular vimentin in cancerous and non-cancerous cells by evaluating parameters such as cell migration, proliferation, adhesion and membrane permeability. Our findings show enhanced migration, proliferation and adhesion in cancerous cells (MCF-7) than the non-cancerous cells (MCF-10a) upon extracellular vimentin treatment. Whereas membrane permeability is reduced in monolayers of MCF-7 compared to MCF-10a upon extracellular vimentin treatment. RBD domain of the SARS-CoV-2 spike protein alters blood-brain barrier integrity and surface vimentin has been shown to act as a co-receptor between SARS-CoV-2 spike protein and the cell-surface angiotensin-converting enzyme 2 (ACE2) receptor. Here, we checked for membrane permeability in MCF-7 and MCF-10a monolayers upon SARS-CoV-2 RBD treatment in presence of extracellular vimentin. Our finding suggests that extracellular vimentin bound to the cell surface enhances membrane permeability on both cell lines. But extracellular vimentin directly bound to SARS-CoV-2 RBD inhibits this effect in MCF-7 monolayers.

Further we also aimed at the verification of cell surface and secreted vimentin using macrophage activation as a model. Results showed that activated macrophages do express vimentin on their cell surface and that its structure is distinctly different from intracellular filamentous vimentin. Further investigation revealed cell surface vimentin is expressed in a polarized manner on macrophages. This polarization effect is also strongly promoted on activation. Analysis with macrophages expressing GFP tagged vimentin also confirmed the secretion of vimentin in the extracellular surrounding on activation. A higher amount of vimentin was observed in the environment when activation was performed for longer periods. Furthermore, we would like to check if this secreted vimentin has any influence on phagocytosis and migration of the macrophages.

P.59 Rewiring of protein interactions between stimulated and unstimulated immune cells

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The immune system is a collaboration of diverse cell types performing functions that are in general aimed at defence mechanisms against infection. Each immune cell develops from stem cells in the bone marrow and differentiates into specialised cells with targeted functions. Differentially analysing various immune cell types would capture the hallmark characteristics that distinguish a particular immune cell type from other cell types in certain conditions. Here we retrieved the RNAseq data of 25 stimulated and unstimulated types of immune cells processed by Calderon et al. (2019)[1]. A complete protein interaction network (PPIN) provides a superset of the existing interactome of a cell and but does not contain characteristic information specific to the cell and condition. Hence, the in-house computational tool PPIXpress[2] was used to construct condition-specific PPIN for all 25 immune cells (stimulated and unstimulated) by pruning the global PPIN to those genes/transcripts that are covered by at least a single sequencing read. Subsequently, we used another in-house tool termed. PPICompare[3] to compare and analyze the condition-specific PPINs of immune cells. The results showed the rewired interaction events between various cell types and conditions and the causes of rewiring either due to differential expression (loss or gain of interacting partners) or alternative splicing (isoform-switch of transcript) as large text files. Furthermore, we developed a new tool that can be used downstream of PPICompare. It executes automatic biological interpretation of the PPICompare results based on Gene Ontology and KEGG pathway enrichment analysis. Here, it reported the most enriched annotations of proteins affected in rewirings between unstimulated and stimulated immune cells. The tool has a feature to filter out and perform enrichment analysis on how the PPI rewirings affect certain groups of proteins of interest such as transcription factors, chromatin readers and splicing factors and their binding proteins. When comparing the unstimulated and stimulated versions of an immune cell, the top enriched GO terms showed the characteristics involved in the stimulation of the immune cell. Similarly, by comparing two immune cell types such as CD8 and NK cells, the enriched GO terms suggested the characteristics of CD8 lost or gained in the NK cell.

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P.60 Bayesian Sequential Analysis of T-Cell Migration Data

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We consider CD8+ cytotoxic T lymphocytes, one of the main cytotoxic killer cells present in the human body to eliminate target cells that are pathogen-infected or tumorigenic. During their search, T-cells pass through various complex biological microenvironments, of which the extracellular matrix (ECM) is one of the core elements. Collagen matrices of different densities were used to simulate the ECM of different tissues and to examine the migration behavior of the T-Cells. We use the single cell tracking data obtained in [1] as an input for an analysis method developed by Metzner et al. [2]. In this method, an autoregressive process of order 1 (AR-1) with time varying parameters is imposed as the governing model of motion and the distribution of the parameters is inferred via a Bayesian sequential updating scheme per cell and per time step. It is found that some cells undergo an abrupt parameter switch, which can be attributed to a fast and slow motion of the cell. Especially for the lowest density collagen matrix, this leads to two distinct parameter regimes in the time and ensemble averaged distribution. When the slow moving cells migrate through the collagen matrix, they interact with it thus causing deformation by forming channels in the matrix. Other cells can enter these channels and hence it is presumed that the fast motility mode arises through cells moving inside the channels.

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P.61 Is the proteomic composition of the salivary pellicle dependent on the substrate material below?

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The use of dental restorative materials is a routine task in clinical dentistry. All orally exposed surfaces are covered with a continuously growing biofilm due to the adsorption of salivary proteins and other macromolecules at the fluid/solid-interface. Each substrate material has different physico-chemical properties supposed to influence the composition of the initial biofilm, termed pellicle. This study aimed at characterizing and comparing the individual pellicle proteome of five subjects formed on six dental restorative materials and natural enamel. Substrate material-specific adsorption patterns were analyzed by comparing the proteomic profiles of the 3-min pellicle and the corresponding saliva. The combination of chemical elution and tandem nano-mass spectrometry resulted in the identification of 1348 different pellicle proteins, out of which 187 to 686 proteins were present in individual 3-min pellicles. Unexpectedly, quantitative analyzes based on the molecular weights, isoelectric points, and molecular functions of the identified proteins yielded in similar distribution patterns independent of the substrate material. Overall similar fold changes were obtained for the major part of commonly enriched or depleted proteins in the 3-min pellicles. These results point to a minor important role of the substrate material on the proteomic composition of the 3-min pellicle.

P.62 Persistence length of distinct actin isoforms

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The physiological differences of actin isoforms in living cells are well studied. However, differences in basic mechanical properties of the isoforms in *vitro* have not been investigated yet. Therefore, the persistence length of 3 different actin isoforms was determined in the presented study. Skeletal α-actin was extracted from mammalian cells, cytoplasmic β- and γ-actin were produced by yeast and modified so that they were more similar to vertebrae actin. To determine the persistence length, actin was polymerized into single filaments and fluorescently labelled with phalloidin. The movements of the filaments in a plane were recorded with a confocal fluorescence microscope and the persistence length could be determined from their shape by using a corresponding software [1].

Differences in the persistence length of the isoforms were found, especially between β- and γ-actin. Overall β-actin has a higher persistence length than γ-actin, but the results are also much more variable than those of γ-actin. This suggests a higher sensitivity of β-actin to external influences.

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P.63 Antibacterial effect of structured titanium surfaces using ultrashort pulsed direct laser interference patterning

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Titanium is a ubiquitous surface material for medical implants (e.g., dental implants, stents, and orthopedic devices), but also a common ground for implant associated infections (IAI), demanding surface modifications that reduce microbial adhesion to the implant material[1–8]. Here we report on a heat treatment and laser-based structuring method producing a microstructured titanium surface that displays a reduced binding capacity for bacterial biofilm-formers such as Escherichia coli and Staphylococcus aureus.

Titanium surfaces were first heat-treated to ensure a titanium dioxide (Rutile) layer formation and subsequently microstructured using ultrashort pulsed direct laser interference patterning (DLIP)6-10. Surface topographies of all processing steps were characterized by different methods, including scanning force microscopy (SFM). The surfaces were subsequently tested for their bacterial binding capacities by classical microplate-based adhesion assays and single cell force spectroscopy (SCFS).

We found that a titanium dioxide layer produced by heat treatment already reduced the capacity of the bacteria to stick to the surface, which could be further reduced when the heat-treated titanium surfaces were also microstructured by DLIP.

Our findings may help to create novel titanium-based implants that display a reduced risk for bacterial biofilm formation and thus IAI.

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P.64 The more aggressive the softer – comparing the mechanical properties of breast cancer cells

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During tumor progression, cells change biochemically and morphologically. Adhesive properties, cell-to-cell contacts, and cytoskeletal arrangements occur, which influence the mechanical properties of tissue and cells. In many tissues, mechanical signaling plays a crucial role in maintaining integrity proper function. In cancer, these pathways get disrupted as the disease progresses. Here, we compare the mechanical and viscoelastic properties of the non-malignant breast epithelial cell line MCF10A to MCF7 breast cancer cells and to the triple-negative breast cancer cell line MDA-MB-231. Indentation and microrheology studies were performed via atomic force microscopy (AFM) force spectroscopy. Results show that both cancer cell lines appear much softer than the non-malignant MCF10A cells, with the triple-negative – the most aggressive cell line – to be the softest.

P.65 Targeting the microtubule-network rescues CTL killing efficiency in dense 3D matrices

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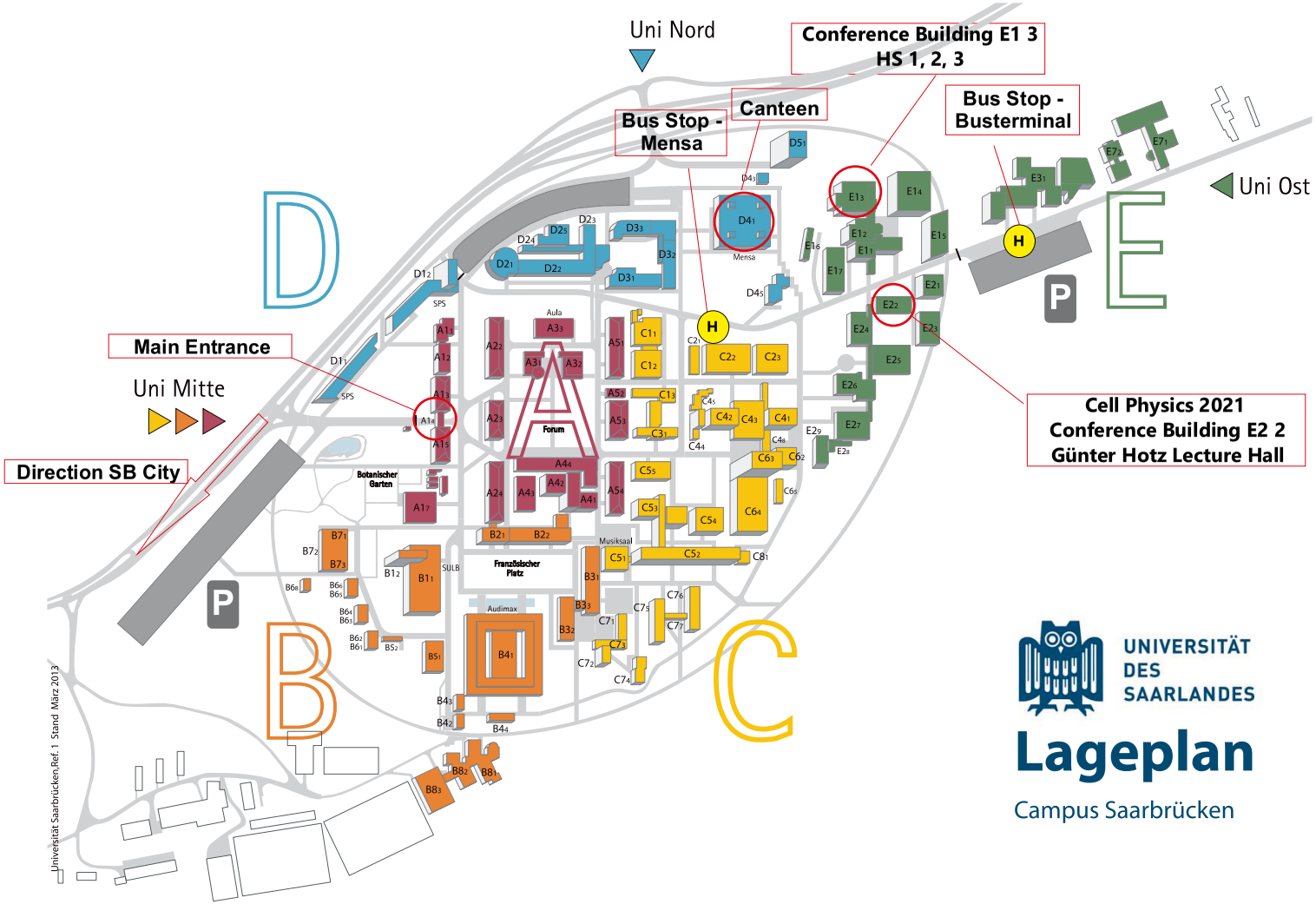
Dense three-dimensional (3D) matrix, a prominent physical feature of the solid tumor environments, is one of the potential reasons to impair immune surveillance against tumor cells. Cytotoxic T lymphocytes (CTLs) are one of the key players to eliminate tumor cells. Here, using the real-time 3D killing assay, we found that the killing efficiency of primary human CTLs was substantially impaired in dense collagen matrices (4 mg/ml and 5 mg/ml) compared to less dense collagen matrix (2 mg/ml). Although dense collagen did not significantly affect the expression of cytotoxic proteins in CTLs, dense collagen impaired CTL motility, leading to decreased searching efficiency of CTLs. Furthermore, we identified that two physical features of dense collagen matrices, high stiffness and small pore size, contributed to impaired CTL motility and reduced killing efficiency. Notably, in the 3D collagen matrix, CTL migration velocity was positively correlated with nucleus deformability. In dense matrices, microtubule disruption with nocodazole led to enhancement in nucleus deformability, CTL migration, searching efficiency, and killing efficiency. Moreover, treating CTLs with vinblastine, which is a chemotherapy drug targeting microtubules, rescued impaired CTL killing efficiency in dense matrices. Our findings suggest targeting the microtubule network as a promising strategy to enhance the efficacy of CTL-based immunotherapy against solid tumors.

Author Index

Registration takes place on Wednesday (29.9.) 7:30-8:20

Wednesday (29.9.)	Thursday (30.9.)	Friday (1.10.)
8:20-8:30 Opening		
8:30-10:30 **Cytoskeleton M/A** Kapitein Fritzsche Keren Gov	8:30-9:30 **Membranes** Levental Risselada 9:00–11:00 **Mechanobiology** Vogel Hamant Etienne-Manneville	8:30-10:30 **Molecular Biophysics** Rey Lipfert Daumke Rahi
10:30-11:00 Coffee	11:00-11:30 Coffee	10:30-11:00 Coffee
11:00-13:00 **Cytoskeleton A/V** Piel Janmey Eriksson Schäfer 13:00-13:15 Lumicks Company/ Roman Renger	11:30-13:00 **Multicellular Aggregates** Allen Alcolea Escudero	11:00-13:15 **Cytoskeleton** Laura Aradilla Zapata (née Schaedel) Mostajeran Sadhu Falconieri Fedosov Kaub Nietmann Weber Jebane **Signalling** Iden Schmidt D'Urso van der Putten **Multicellular Aggregates** Bhusari Maikranz Wysocki Abend Monnier
13:15-14:15 Lunch & Poster	13:00-14:00 Lunch & Poster	13:15-14:00 Lunch & Poster

14:15-15:30		14:00-15:30		Fin
Membranes Sarkar Sitarska Kurniawan Ghorbani Griffo	**Molecular Biophysics** Bhaskara Anselmi Becker Komaragiri **Genes Developmen** Sokolowski Sierra	**Membrane Proteins** Monzel Dhakane Peckys **Cancer** Blauth Kubitschke Mouelhi	**Cell Hydrodynamics** Dasanna Darras Babaki Baby **Mechanobiology** Neumann Ramón-Lozano	
15:30-16:00 Coffee		15:30-16:00 Coffee		
15:20-16:20 **Cell compartments** Lippincott-Schwartz Alberti		16:00-16:30 **Mechanobiology** Lammerding 16:30–17:30 **Membrane Proteins Spengler** Groot Fletcher 17:30–18:30 **Cell Compartments** Jakobs		
17:00-18:00 **Membrane Proteins** Fakler Chapman		16:30-17:30 **Adhesion** Smith Gaub		
18:00-19:30 **Posters**		18:30-20:00 **Posters**		
19:30 Social dinner		20:00 Speaker dinner		



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