BP 8: Cytoskeletal filaments

Time: Tuesday 9:30–12:45 Location: H10

BP 8.1 Tue 9:30 H10

The influence of vimentin on actin dynamics — •Zahra Mostajeran[1], Emmanuel Terriac[1], and Franziska Lautenschläger[1,2] — [1]INM-Leibniz Institute for New Materials, Saarbrücken, Germany — [2]Saarland University, Saarbrücken, Germany

The cytoskeleton is a network of polymers which extends inside the cytoplasm to form and maintain the cell shape. It is composed of three main types of filaments: microtubules (MTs), actin filaments, and intermediate filaments (IFs). Vimentin belongs to the family of IFs and is involved in fixing organelles in the cytoplasm and regulating the direction of cell migration. It forms non-polar filaments and has therefore no known molecular motor directly interacting on it. Vimentin is linked via plectin protein cross-linker to MTs and actin filaments as well as to itself. Vimentin has been shown to colocalize to actin stress fibers (SFs). These are bundles of actin filaments assembled by the molecular motors and crosslinker proteins. Actin SFs play a key role in cell contractility and cell migration. We investigate the effect of vimentin in processes like cell migration and traction forces which are known to be initiated by forces generated by actin filaments and the molecular motor myosin. To understand how vimentin IFs are involved in these processes, we study the dynamics of the actin SFs on cells with different amounts of vimentin. We also consider the role of plectin on our results. We demonstrate that actin SFs are less dynamic in vimentin depleted cells compared to vimentin wild-type cells. We could further show that the dynamics of actin SFs is not influenced by plectin, suggesting a role of vimentin itself on actin SF dynamics.

BP 8.2 Tue 9:45 H10

Cytoskeletal organization within polar cells and interactions between different components — •Carsten Baltes[1,2], Emmanuel Terriac[1], and Franziska Lautenschläger[1,2] — [1]Leibniz-Institut für neue Materialen — [2]Universität des Saarlandes

The cytoskeleton is composed of three main parts: actin filaments, microtubules and intermediate, like for in this study, vimentin. The organization of the cytoskeleton and shape of cells are linked. Here we investigate the effect of imposing shape on the cytoskeletal organization. We further disturb particular cytoskeletal parts in this setup to test their interactions between different filaments. To acquire those defined shapes we used micropatterns of fibronectin achieved with the Primo device (Alvéole Paris). We focused on two circular shapes, full circles as example for a symmetric shapes and 3/4 circle (which resemble the shape of PacMan) to enforce polarity to the cell. Using withaferin A, blebbistatin and rho-associated-protein kinase (ROCK) inhibitor Y27632, we selectively disturbed vimentin organization and myosin II activity. We observed effects on the distribution of the cytoskeletal elements. We further found different behavior between cells treated with blebbistatin and Y27632, leading to the hypothesis that ROCK may be essential in the structure and dynamic of the vimentin network. Our results help to understand the cytoskeletal architecture in cells and the link between the different networks. Our method might be useful for further investigations in the cytoskeletal interplay.

BP 8.3 Tue 10:00 H10

Studying Cytoskeletal Processes, including T-cell activation, at the single-molecule level with optical tweezers and correlated fluorescence microscopy (OT-CFM) — •Ann Mukhortava, Aida Llauró Portell, Roleand van Wijk, Andrea Candelli, and Gerrit Sitters — LUMICKS, Amsterdam, Netherlands

Microtubules, actin and intermediate filaments are highly dynamic cytoskeletal structures that interact with various motor proteins and regulatory factors and thus play fundamental roles in many essential biological processes. A lot of these, including cell division, signaling, and migration, involve mechano-chemical and -biological pathways. Force spectroscopy on a single-molecule and single-cell level permits exploring and manipulating these complex interactions to help understand their nature better. OT-CFM which we commercialized as the C-Trap™ integrates optical tweezers, confocal/STED microscopy, and an advanced microfluidics system in a truly correlated manner. It enables live, simultaneous and correlative visualization and manipulation of molecular interactions with sub-picoNewton (pN) force resolution and microsecond temporal resolution. Here, we present our experiments on visualizing and quantifying the elastic properties of filaments, the motility of cytoskeletal molecular motors and the force-triggered activation of T-cells using the C-Trap™ system. These experiments show that technological advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that opens up new venues in many research areas.

BP 8.4 Tue 10:15 H10

Effect of the molecular architecture on vimentin intermediate filament mechanics — •Anna Schepers, Charlotta Lorenz, Johanna Block, Julia Kraxner, and Sarah Köster — Institute for X-Ray Physics, Georg-August-University Göttingen, Germany

Intermediate filaments (IFs), together with microfilaments (MFs) and microtubulues (MTs), give cells specific and unique mechanical properties in shape of the cytoskeleton. While MFs and MTs are conserved between cell types, IFs are expressed in a cell type specific manner. To understand the mechanisms within IFs that determine the mechanical response to stresses, single IFs are investigated in vitro using a setup that combines optical tweezers, fluorescence microscopy and microfluidics. By changing the environment (pH, buffer, ion valency and ion concentration) different force-strain behaviours of single vimentin IFs are observed in stretching experiments. The IFs show a remarkable dependency on the buffer pH and presence of cations while the cation valency and buffer seem to be neglectable. With these results, we can link the molecular architecture and, in parts, the primary protein structure of vimentin IFs to their mechanical response.

BP 8.5 Tue 10:30 H10

Stress-Strain Behavior of Keratin and Vimentin IFs — •Charlotta Lorenz, Johanna Block, Anna Schepers, Julia Kraxner, and Sarah Köster — Institute for X-Ray Physics, Georg-August-Universität, Göttingen, Germany

The cytoskeleton is vital for cell motility, cell division and mechanical stability of the cell. These tasks are distributed among three different protein classes, microfilaments (MFs), microtubules (MTs) and intermediate filaments (IFs). Unlike MFs and MTs, IFs are expressed in a cell-type specific manner giving the cell a tool to adapt to different mechanical requirements. So far, the mechanical properties of different IFs on a single filament level have not been probed. Therefore, we study the stress-strain behavior of two IFs, vimentin and keratin filaments, by optical trapping in combination with fluorescence microscopy and microfluidics. In comparison to keratin IFs, vimentin IFs are stiffer and exhibit a strong loading-rate depending behavior, which predestines vimentin to act as a cellular "safety belt". Monte-Carlo simulations based on theoretical modelling allow the decoupling of different IF-type depending parameters like the monomer interaction and the number of monomers per cross-section of the IF. The obtained parameter distributions show that more energy is required to extend vimentin IFs than keratin IFs. This behavior can possibly be explained by a compaction step of vimentin during IF assembly which is not observed for keratin IFs.

BP 8.6 Tue 10:45 H10

Germanium nanospheres as high precision optical tweezers probes — •Swathi Sudhakar and Erik Schaeffer — Cellular Nanoscience, Center for Plant Molecular Biology, Eberhard Karls University of Tuebingen, Tuebingen, Germany.

Force spectroscopy on single biological molecular machines is often performed using optical tweezers. Commonly microspheres composed of silica or polystyrene are trapped in a highly focused laser beam and are used as handles to measure the mechanics of motor proteins such as kinesin. The ultimate precision of such experiments is limited by thermal fluctuations and, among others, the size of the microsphere. Thus, ideally, microspheres should be as small as possible. However, since trapping forces scale with the particle volume, maximum trapping forces quickly approach motor-generated forces creating a lower practical size limit of about 200 nm for polystyrene microspheres when studying kinesin motors. Here, we have developed germanium nanospheres with diameters ranging from 30-200 nm. With a high refractive index of 4.4, their trapping efficiency and maximum force per power is more than 10-fold improved compared to equal-sized silica spheres. Using 70-nm-diameter germanium nanospheres, we measured the stepping

behavior of kinesin-1. With an improved precision, we could measure intermediate steps of kinesin. In the long-term, the development and application of novel high-precision probes will provide new insight into the working mechanism of molecular machines.

30 minutes break.

Invited Talk BP 8.7 Tue 11:30 H10

Force generation by actin, microtubules and motors — •Rhoda Hawkins — University of Sheffield, UK

When driven out of equilibrium by the consumption of biochemical energy, cytoskeletal protein filaments alone and in combination with molecular motors are able to generate sufficient forces to deform and move cells as well as to transport cargo within a cell. I will present some of my group's work theoretical work on force generation mechanisms using both analytical calculations and simulations in combination with experimental data.

First I will discuss our work on polymerising branched actin, comparing in vitro data with simulations and analytical calculations. Then I will present stochastic simulations of polymerising branched actin exerting force to deform a model membrane in the context of phagocytosis, which is a process by which immune cells engulf pathogens.

In the second part of the talk I will present analytical calculations and simulations of molecular motors moving along cytoskeleton filaments transporting a cargo. I will compare our results with experimental data of molecular motors on microtubules in vitro and in vivo in axons. In particular I will discuss the differences between processive and non-processive motors and the effects of multiple motors and multiple filaments. Finally, I will discuss the effects of competing molecular motors pulling a cargo in different directions for a deformable cargo and relate this to experimental data on cell nucleus deformation.

BP 8.8 Tue 12:00 H10

Contractile actin flow in Xenopus egg extract droplets — •Jianguo Zhao, Kengo Nishi, and Christoph F. Schmidt — Drittes Physikalisches Institut - Bio-physik, Fakultät für Physik, Georg-August-Universität Göttingen

The actin cytoskeleton of eukaryotic cells is a highly dynamic viscoelastic active material. A typical cell maintains a cortex lining that supports the cell membrane, a polymer network consisting of actin, myosin motors and a plethora of regulatory proteins. Actin turns over between polymeric and monomeric forms on a time scale of minutes. Myosin motors generate active contractile stresses that can induce large-scale actin flow, which is essential for the transport of cytoplasmic components, locomotion as well as shape changes of cells. How exactly so many interacting biochemical processes result in static or dynamic steady states is unclear. Using water-in-oil droplet containing cytoplasmic extract of Xenopus laevis eggs as a model system for an active cytoskeleton, we could produce radially convergent continuous flows of polymerized actin that persist over time scales much longer than the turn-over time of a single actin filament. We mapped the spatiotemporal distribution of this contractile persistent actin flow. Interestingly, we found that macromolecular cargo present in the extract gets transported into the center of the droplet and compacted into a jammed state. We demonstrated this by tracking embedded IR fluorescent single-walled carbon nanotubes as mechanical probes.

BP 8.9 Tue 12:15 H10

The structure and mechanics of the cellular cortex before, during and after adhesion — •Daniel Flormann[1], Emmanuel Terriac[1], and Franziska Lautenschläger[1,2] — [1]Leibniz Institut für neue Materialien — [2]Universität des Saarlandes

The cellular cortex plays an important role in biological processes such as cell migration and division. This thin (roughly 200nm) network beneath the cell membrane is mainly composed of actin, associated motors and linkers. It is highly dynamic and the main contributor to the so-called cortical tension. This tension drives the mechanical properties of cells as well as its shapes. During cell adhesion this cortex is altered. In order to test if such alterations of the actin cortex during adhesion influence cellular mechanics, we compared the mechanical properties of RPE1 cells in adhered and suspended state by atomic force microscopy. This results were then correlated to the local structure of the actomyosin network using electron microscopy. We found indeed differences in the mechanical responses and structures depending on the state of adhesion. Altering the activity of the motor protein myosinII allowed us to change the mechanical properties of the cells in both states and changes of the structure could also be observed. Hence, we describe here a quantitative correlation between the structure of the actin cortex and the mechanical properties of cells both in the frame of adhesion state or by chemical alteration. These results may be promising in understanding the mechanical plasticity of cells in processes like embryogenesis or metastasis.

BP 8.10 Tue 12:30 H10

Experimental Characterization and Theoretic Modeling of Circular Dorsal Ruffles — Julia Lange[1], Malte Ohmstede[1], •Merthe Schwachenwald[1,2], Christof Taxis[2], and Hans-Günther Döbereiner[1] — [1]Institut für Biophysik, Universität Bremen — [2]Fachbereich Biologie, Phillips-Universität Marburg

Circular Dorsal Ruffles (CDRs) are dynamic actin structures propagating on the dorsal cell side. Three factors influence CDR dynamics: Stimulation with growth factors, protein composition, and boundary conditions of cells. In our set-up the latter is ensured by using microcontact printed substrates to receive an even cell shape. The influence of different proteins and growth factor stimulation on CDR dynamics is controlled by microfluidics and examined with light microscopy. We will use optogenetics to control protein expression linking a light sensitive protein to a peptide degron and connect both to an actin regulator. This leads to a degradation of the construct, when exposing it to blue light. Optogenetic manipulation allows a more refined control of protein concentration than with traditional biochemical means. CDR dynamics under changing biochemical conditions is compared with theory through two-dimensional simulations of propagating wave fronts. We aim to verify a bistable model and augment it to include the effects of fluctuations in an active cytosol. We found CDR characteristics to cluster with their number. Moreover, we observe clear long-distance interaction of CDRs. We examine the effects of Jasplakinolide (Actin inhibitor), Wiskostatin (N-WASP inhibitor), and Wortmannin (PI3K inhibitor) on wave characteristics.