BP 43: Cytoskeletal Filaments

Time: Wednesday 15:00–17:15 Location: HÜL 386

Invited Talk BP 43.1 Wed 15:00 HÜL 386

Diffusive anchorage of molecular motors allows for adaptive force generation — •Stefan Diez — B CUBE, TU Dresden, Germany

Cytoskeletal motor proteins have been well characterized in vitro as single molecules or as ensembles rigidly attached to non-biological substrates. However, in cells, motors are often only loosely coupled to their cargo. Towards understanding the resulting collective transport properties we reconstituted membrane-anchored kinesin-1 gliding motility assays. We found that motor slippage in the membrane rendered the gliding velocity of the microtubules strongly dependent on the number of motors and their diffusivity in the lipid bilayer. Moreover, we investigated the force generation of kinesin-14 motors diffusively anchored with their tail domains on one microtubule while sliding another microtubule. Again, slippage significantly reduced the transport efficiency leading to a strongly reduced force production as compared to rigidly bound motors. Notably, under these conditions the motor forces were low enough to be fully balanced by the entropic forces arising from the diffusive anchorage of non-motor crosslinkers, hence allowing for the adaptive formation of persistent partial microtubule overlaps. Taken together, our results illustrate the importance of motor-cargo coupling, which provides cells with an additional means of regulating transport efficiency and force generation.

BP 43.2 Wed 15:30 HÜL 386

Structure and Dynamics of Stress fibers in adult Stem Cells — •Carina Wollnik[1], Benjamin Eltzner[2], Stephan Huckemann[2], and Florian Rehfeldt[1] — [1]Third-Institute of Physics - Biophysics, Georg-August University Göttingen, Germany — [2]Institute for Mathematical Stochastics, Georg-August University Göttingen, Germany

Adult human mesenchymal stem cells (hMSCs) are capable of differentiation towards various cell types such as nerve, bone, and muscle precursor cells. Strikingly, substrate stiffness as physical stimulus is enough to guide hMSCs towards different lineages in the absence of additional biochemical stimuli [1]. Connecting focal adhesions throughout the cell, stress fibres generate and transmit tension [3], lack of which stops the differentiation process [1]. Characteristic stress fibre reorganisation patterns are detected after 24 hours and used as early morphological marker [2], backed up with genetic evidence [6]. Here, we present data from massive parallel live cell imaging of mechanoguided early stem cell differentiation of RFP-Lifeact transfected hMSCs [7]. Stress fibres are traced with sophisticated tracking algorithms [4,5].

[1] A. Engler et al., Cell (2006) [2] A. Zemel et al., Nature Physics (2010) [3] E. K. Paluch et al, BMC Biology (2015) [4] B. Eltzner et al., PLoS One (2015) [5] S. Huckemann et al., Bernoulli (2016) [6] C. Wollnik et al., in preparation (2016+) [7] C. Wollnik et al., in preparation (2016+)

BP 43.3 Wed 15:45 HÜL 386

Electron Microscopy (EM) and Single Particle Analysis on Myosin — •Dario Saczko-Brack, Christopher Batters, Benoit Rogez, Markus Kröss, and Claudia Veigel — LMU, Department of Cellular Physiology, Schillerstrasse 44, 80336 Munich, Germany

Myosin-IX is critically involved in structural reorganizations of the acto-myosin cytoskeleton in the lamellipodium of migrating cells and in cell polarization in morphogenesis. Using a combination of negative stain EM, single particle image processing, fluorescence spectroscopy and motility assays we discovered that myosin-IXa assembles actin filaments into highly ordered lattices with parallel actin polarity and a repeat distance of precisely 36 nm, matching the helical repeat of actin. We resolved three distinct conformations of myosin-IXa crosslinks in the absence of nucleotide. Furthermore we found that calmodulin binds to a large insert in the motor domain exclusively found in class IX myosins. This creates two coordinated actin binding sites that constrain the acto-myosin interactions which generates the lattices. These might introduce a myosin-related, force-sensing mechanism into the cytoskeleton in cell polarization and collective cell migration.

The cytoskeletal motor myosin VI is involved in many motile processes including cancer cell migration and is the only myosin shown to move towards the minus end of actin. We demonstrate that calcium is the cellular switch that induces a structural rearrangement of this motor which regulates the transition from an inactive to a cargo-binding state and controls the mechanical properties.

Batters, Brack et al. PNAS 2016

BP 43.4 Wed 16:00 HÜL 386

Lateral association and elongation of vimentin intermediate filament proteins: A time-resolved light-scattering study — •Carlos Lopez[1], Oliva Saldanha[2], Klaus Huber[1], and Sarah Köster[2] — [1]Department Chemie, Universität Paderborn, 33098 Paderborn, Germany — [2]Institut für Röntgenphysik, Georg-August-Universitat Göttingen, 37077 Göttingen, Germany

Intermediate filaments constitute one of the three protein filament systems in the cytoskeleton of metazoa. Together with actin filaments and microtubules they form a sophisticated composite network, which has been identified as a main player in cell mechanics. The assembly pathway of the cytoskeletal protein vimentin may be responsible for the mechanical properties of the emerging filaments, such as high flexibility and extensibility, and thus play a key role in cellular mechanics.

Assembly of Vimentin from its tetrameric form can be triggered by addition of a monovalent salt. A two-step assembly mechanism: lateral association and a subsequent elongational step, has been established; however, the elongational step has not be followed in solution.

We present direct in situ observation and modeling of the elongation reaction of the filaments on the relevant length (60-600nm) and time scales, using time-resolved, multiangle static and dynamic light scattering. We thus achieve sufficient spatio-temporal resolution without the need of labeling, staining, or adsorption to substrates. The mass per unit length, hydrodynamic diameter and the end-to-end elongation rate constant of the assembling filaments are evaluated as a function of added salt.

15 min break

BP 43.5 Wed 16:30 HÜL 386

Mechanisms of microtubule nucleation and size in spindles — •Franziska Decker[1,2], Elisa Rieckhoff[1,2], Benjamin Dalton[1,2], David Oriola[1,2], and Jan Brugues[1,2] — [1]Max Planck Institute for the Physics of Complex Systems — [2]Max Planck Institute of Molecular Cell Biology and Genetics

The spindle is the protein machinery responsible for segregating the genetic material into the daughter cells. Microtubules,the main building blocks of the spindle,have a lifetime of 20 sec while the entire spindle remains for minutes or even up to hours. Thus, maintenance of the spindle requires constant creation of new microtubules through microtubule nucleation. Here, we used laser ablation to measure the minus ends of monopolar spindles in Xenopus leaevis egg extract as a proxy to microtubule nucleation. We found that microtubule dependent microtubule nucleation explains the nucleation profile and microtubule density in these structures, with the amount of nucleators activated in chromosomes setting the number of microtubules in spindles. This nucleation mechanism could account for the scaling of spindles with cell volume as observed in early embryogenesis or spindles encapsulated in extract, and provides an alternative prediction to previous models based on microtubule dynamics. To test whether microtubule dynamics or microtubule nucleation are responsible for the scaling of spindles, we performed measurements of microtubule dynamics and nucleation during the early rounds of cell division in Zebrafish embryos.

BP 43.6 Wed 16:45 HÜL 386

Overlapping microtubules establish a microenvironment enabling the autoregulation of molecular motors — •Marcus Braun[1,2], Zdenek Lansky[1,2,3], Agata Szuba[1,2], Friedrich W Schwarz[2], Anniruddha Mitra[1,2], Mengfei Gao[1,2], Annemarie Lüdecke[1,2], Pieter Rein ten Wolde[4], and Stefan Diez[1,2] — [1]B CUBE, TU Dresden, Arnoldstraße 18, 01307 Dresden, Germany — [2]MPI-CBG, Pfotenhauerstraße 108, 01307 Dresden, Germany — [3]CAS, BIOCEV, Prumyslova 595, Vestec 25250, Czech Republic — [4]AMOLF, Science Park 104, 1098 XG Amsterdam, the Netherlands

Collective action of molecular motors is required for the remodeling of microtubule networks underpinning essential cellular processes, such as cell division. Among these motors are microtubule-crosslinking mo-

tors, which slide microtubules along each other. However, additional regulatory proteins are thought to be necessary to establish stable overlaps between the sliding microtubules and to prevent the breakdown of the networks. Here, we show in vitro that human kinesin-14 HSET motors - as they slide overlapping microtubules apart - collectively detect the decrease in overlap length and slow down the sliding in an autoregulatory manner, leading to the formation of stable overlaps. Slowdown is quantitatively explained by the dependence of HSET sliding on the local HSET density in the overlap and the generation of an entropic force antagonizing the sliding. We argue that overlapping filaments, when crosslinked by proteins sensitive to their spatial arrangement, establish envelope-free compartments constituting distinct microenvironments that can locally catalyze biochemical processes.

BP 43.7 Wed 17:00 HÜL 386

Microtubule pivoting and minus end directed motors drive the formation of the mitotic spindle — •Ivana Ban[1], Marcel Prelogović[1], Lora Winters[2], Ana Milas[3], Iva Tolić[2,3], and Nenad Pavin[1] — [1]Faculty of science , University of Zagreb, Croatia — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany — [3]Division of Molecular Biology, Ruder Bošković Institute, Zagreb, Croatia

During mitosis, the genetic material is divided into two equal parts by the spindle. This complex micro-machine is made of chromosomes, microtubules (MTs) and a variety of accessory proteins. In the fission yeast Schizosaccharomyces pombe, the mitotic spindle is a bundle of MTs emanating from two spindle pole bodies, whose formation is mediated by motor proteins. A key question is what are the physical principles underlying the formation of a mitotic spindle. In this work, we combine theory and experiment to describe how angular motion of MTs and forces exerted by motor proteins lead to spindle formation. In our model, MTs explore their environment by performing angular movement around the spindle poles, until two MTs come into close proximity, allowing motor proteins accumulate in the overlap region. In the case of minus end directed motors, this leads to formation of antiparallel bundles. We experimentally observed random angular motion of MTs as well as accumulation of Cut7 motor proteins in the overlap region, followed by antiparallel bundle formation. In conclusion, these results provide an explanation for how the angular Brownian motion and motor proteins drive the formation of a stable mitotic spindle.