BP 58: Cytoskeletal Filaments

Time: Thursday 9:30–13:00 Location: H44

Invited Talk BP 58.1 Thu 9:30 H44

Cytoskeletal coordination — •Gijsje Koenderink — FOM Institute AMOLF, Amsterdam, Netherlands

Cell shape and mechanics are determined by the interplay of the plasma membrane with three distinct cytoskeletal networks, made of actin filaments, microtubules, and intermediate filaments. These cytoskeletal polymers markedly differ in their structure and physical properties, and have traditionally been thought to have distinct cellular functions. However, there is growing evidence that they also exhibit strongly coupled functions necessary for cell migration, cell division, and mechanoresponse [1]. Our goal is to resolve physical mechanisms that contribute to cytoskeletal coordination. For this purpose, we study cell-free model systems reconstituted from purified cellular components. I will illustrate this approach by discussing two examples. First, I will demonstrate a model system where we introduced interactions between the actin and microtubule (MT) cytoskeletons via MT end-tracking proteins (+TIPs) that also bind F-actin [2]. We showed that the interaction between growing MT ends and actin is sufficient to capture and re-direct MT growth along actin bundles. Second, I will show how a fourth cytoskeletal filament, septins, interact with actin as well as the plasma membrane.

BP 58.2 Thu 10:00 H44

Overlap microtubules link sister k-fibers and balance the forces on bioriented kinetochores — •Maja Novak[1], Janko Kajtez[2], Anastasia Solomatina[2], Iva M. Tolic[3,2], and Pavin Nenad[1] — [1]Department of Physics, Faculty of Science, University of Zagreb, Bijenicka cesta 32, 10000 Zagreb, Croatia — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany — [3]Division of Molecular Biology, Rudjer Boskovic Institute, Bijenicka cesta 54, 10000 Zagreb, Croatia

During metaphase, forces on kinetochores are exerted by k-fibers, bundles of microtubules that end at the kinetochore. Interestingly, non-kinetochore microtubules have been observed between sister kinetochores, but their function is unknown. Here we show by laser-cutting of a k-fiber in HeLa and PtK1 cells that a bundle of non-kinetochore microtubules, which we term 'bridging fiber', bridges sister k-fibers and balances the inter-kinetochore tension [1]. We found PRC1 and EB3 in the bridging fiber, suggesting that it consists of anti-parallel dynamic microtubules. By using a theoretical model that includes a bridging fiber, we show that the forces at the pole and at the kinetochore depend on the bridging fiber thickness. Moreover, our theory and experiments show larger relaxation of the inter-kinetochore distance for cuts closer to kinetochores. We conclude that the bridging fiber, by linking sister k-fibers, withstands the tension between sister kinetochores and enables the spindle to obtain a curved shape.

[1] Kajtez, Solomatina, Novak et al., Nature Communnications (Accepted)

BP 58.3 Thu 10:15 H44

Why microtubules run in circles — •Falko Ziebert[1], Herve Mohrbach[2], and Igor Kulic[3] — [1]Albert-Ludwigs-Universität, 79104 Freiburg, Germany — [2]Groupe BioPhysStat, LCP-A2MC, Université de Lorraine, 57078 Metz, France — [3]Institut Charles Sadron UPR22-CNRS, 67034 Strasbourg, France

The fate of every eukaryotic cell subtly relies on the exceptional mechanical properties of microtubules. Despite significant efforts, understanding their unusual mechanics remains elusive. One persistent, unresolved mystery is the formation of long-lived arcs and rings, e.g., in kinesin-driven gliding assays. To elucidate their physical origin we develop a model of the inner workings of the microtubule lattice, based on recent experimental evidence for a conformational switch of the tubulin dimer. We show that the microtubule lattice itself coexists in discrete polymorphic states. Metastable curved states can be induced via a mechanical hysteresis involving torques and forces typical of few molecular motors acting in unison, in agreement with the observations.

BP 58.4 Thu 10:30 H44

Microtubule bundle formation is driven by angular diffusion of microtubules and forces exerted by cross-linkers — •Marcel Prelogović[1], Lora Winters[2], Ana Milas[3], Iva Tolić[3], and Nenad Pavin[1] — [1]Department of Physics, Faculty of Science, University of Zagreb, Bijenička cesta 32, 10000 Zagreb, Croatia — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany — [3]Division of Molecular Biology, Ruder Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

During mitosis, microtubules (MTs) form a spindle, which is responsible for proper segregation of the genetic material. Most of the spindle MTs are organized into bundles by cross-linking proteins. A key question is what are the physical principles underlying the formation and stability of MT bundles. Here we show (Prelogović et.al., submitted), by introducing a model and experimentally testing its predictions, that random angular movement of MTs around the spindle pole and forces exerted by passive cross-linking proteins are sufficient for the formation of stable MT bundles. Our model predicts that the time needed for bundle formation depends mainly on the concentration of cross-linking proteins and the angular diffusion of the MT, but weakly on MT length. We confirmed these predictions by experiments in wild-type and ase1Δ fission yeast cells. In conclusion, the angular motion drives the alignment of MTs, which in turn allows the cross-linking proteins to connect the MTs into a stable bundle.

BP 58.5 Thu 10:45 H44

Axonal microtubule bundle polarity is maintained by mechanically mediated depolymerization of ill-oriented microtubules — •Maximilian Jakobs and Kristian Franze — University of Cambridge, UK

The microtubule (MT) bundles found in neuronal axons are highly polar with around 90% of all MTs pointing with their +end away from the cell body. Disruption of this polarity is thought to be involved in a variety of neurodegenerative diseases. Although the MT array polarity has been discovered more than 30 years ago, its origin is still poorly understood. We here tracked growing MT +ends in dissociated primary neurons to look for correlations between bundle polarity, MT growth and transport velocities, and MT growth lifetimes. Even though the +ends moved in the anterograde (away from the cell body) and retrograde (towards the cell body) direction with similar velocities, the fraction of velocities above 15μm/min was larger in the retrograde direction, implying that active transport drives -end out MTs back into the cell body. Additionally, MTs stopped growing more frequently when pointing with their +end towards the cell body than vice versa. This behaviour might be explained by an increased drag force acting on retrogradely growing MTs, which originates from an anterogradely directed viscous flow within the axon. This flow acts as a mechanical barrier for retrogradely growing MTs, facilitating their depolymerization and thus keeping the MT array polar. Understanding the mechanism that polarises the axonal MT array might yield new approaches towards preventing neuronal degeneration during disease.

BP 58.6 Thu 11:00 H44

Small-Angle X-ray Scattering Investigation of Structural and Organizational Changes Induced by Ions on Keratin Filaments — •Clément Hémonnot[1], Daniel Schmitz[1], Manuela Denz[1], Monika Mauermann[2], Harald Herrmann[2], and Sarah Köster[1] — [1]Institute for X-Ray Physics, Uni. Göttingen, Germany — [2]DKFZ, Heidelberg, Germany

Keratin intermediate filaments (IF) proteins play an important role for cell mechanics as they form extended filaments (5 nm radius) and complex, highly ordered intracellular networks, which provide integrity and stability to epithelial cells. We present a study of the assembly of keratin in presence of monovalent and divalent ions by small-angle X-ray scattering (SAXS). As SAXS can reveal structures on the nanometer length scale, we investigate the impact of K+ and Mg2+ ions on the internal structure and organization of keratin filaments and assemblies. We show that the radius of the filaments follows a linear trend with increasing ion concentration. Moreover, we are able to determine where in the filaments the ions accumulate by using a model consisting of a core filament and Gaussian chains representing the N- and C- terminals. Because of Coulomb screening, at low concentrations both ion species accumulate in the side chains; at intermediate concentrations, the ions start to bind to the core of the filament; at high concentration, ions eventually lead to bundling events. Such bundling was not observed in the case of monovalent ions for other IFs such as vimentin.

These results help to understand the differences in structure formation in different IFs, leading to different mechanical roles in the cell.

BP 58.7 Thu 11:15 H44

Mechanical Properties of Single Vimentin Intermediate Filaments — •Johanna Block[1], Andrea Candelli[2], Jordi Cabanas Danes[3], and Sarah Köster[1] — [1]Institute for X-Ray Physics, University of Göttingen, Germany — [2]LUMICKS, Amsterdam, The Netherlands — [3]Physics of Living Systems, VU Amsterdam, The Netherlands

The cytoskeleton plays a fundamental role for the mechanical integrity of biological cells. The cytoskeleton of eukaryotic cells is composed of three types of filaments: microfilaments (MFs) built up from actin monomers, microtubules (MTs) and intermediate filaments (IFs). While MFs and MTs have been of interest for biophysicists since decades, IFs moved to the center of attention only some years ago. The aim of our work is to characterize the mechanical properties of fluorescently labeled single vimentin filaments in solution. Using combined optical tweezers and fluorescent microscopy we test the mechanical properties of the filaments in a very controlled way and image them simultaneously. By analyzing the filament behavior under different stretching conditions and comparing glutaraldehyde-fixed and unfixed filaments, we gain knowledge about the stretchability, the elastic behavior and the involved molecular mechanisms such as subunit gliding or α-helix to β-sheet transition. From our data we hypothesize that many of the specific mechanical properties of IFs are encoded in their molecular architecture, which differs considerably from that of MTs and MFs. By probing single vimentin IFs we further the understanding of these important determinants for cell mechanics.

15 min break

Invited Talk BP 58.8 Thu 11:45 H44

Single molecule studies on myosin motors — •Claudia Veigel — Lehrstuhl Cellular Physiology and Center for Nanosciences (CENS), LMU München

Many types of cellular motility are based on the myosin family of motor proteins. There are now known to be at least 35 different classes of myosins, involved in intracellular transport processes, cytokinesis, muscle contraction, exo- and endocytosis or even signal transduction in vision or hearing. The ability to coordinate the timing of motor protein activation lies at the very centre of this wide range of cellular motile processes. Using a combined approach of recombinant protein expression and single molecule techniques including optical tweezers we study the basic mechanisms of activation, force production and movement of these molecular machines at the single molecule level. In this talk we will report on our recent studies on myosins class XXI and VI, which interact with lipids and transport cargo, such as cytoplasmic vesicles, over micrometer distances along the actin cytoskeleton in the cell.

BP 58.9 Thu 12:15 H44

Stabilization of small myosin II ensembles by mechanical load and ATP concentration — •Thorsten Erdmann, Kathrin Bartelheimer, and Ulrich S. Schwarz — Institute for Theoretical Physics and BioQuant, Heidelberg University, Heidelberg, Germany

Biological systems use ensembles of non-processive myosin II motor molecules to generate contractile forces. In muscle, large myosin II ensembles remain continuously attached to actin filaments to ensure effective force generation. In the cytoskeleton or in reconstituted actomyosin gels and motility assays, by contrast, small myosin II ensembles detach from actin with probabilities depending on both internal and external parameters. We study the influence of mechanical load and ATP concentration on small myosin II ensembles using a five-state crossbridge model. Increasing mechanical load or decreasing ATP concentration both increase the number of bound motors and stabilize ensemble attachment. While ensemble velocity is always reduced by increased mechanical load, lowering ATP concentration can increase ensemble velocity and stall force due to load-sharing between increased numbers of bound motors. To facilitate the use of our model in higher level modelling, we first reduce it to a three-state cross bridge model with conserved mean-first passage times in the motor cycle. Next, we exploit a separation of time-scales in the motor cycle to project the stochastic reaction network to a one-step master equation. We test the validity of each reduction step by comparison to the full model.

BP 58.10 Thu 12:30 H44

Structure and formation 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

During differentiation, pluripotent adult human mesenchymal stem cells (hMSCs) become various cell types like nerve, bone or muscle precursor cells. Here, substrate stiffness is sufficient to guide hMSCs towards different lineages in the absence of additional biochemical stimuli [1]. Key players are stress fibres that generate and transmit contractile forces throughout the cell [3] and mediate cell-matrix mechanics. Characteristic reorganisation of stress fibres is detected within 24 hours and can be used as early morphological marker [2]. Using massive parallel life-cell imaging of RFP-Lifeact transfected hMSCs on substrates of different stiffness during early stem cell differentiation, we detect distinct pattern formation strategies of stress fibres, traced with novel 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 (2015) - to appear;

BP 58.11 Thu 12:45 H44

Force distributions in disordered fiber networks — •Knut M. Heidemann[1], Abhinav Sharma[2], Florian Rehfeldt[2], Christoph F. Schmidt[2], and Max Wardetzky[1] — [1]Institut für Numerische und Angewandte Mathematik, Georg-August-Universität, Göttingen, Germany — [2]Drittes Physikalisches Institut, Georg-August-Universität, Göttingen, Germany

Disordered fiber networks determine the mechanical response of many materials in nature. Due to the filamentous character of these networks, the strain field, and hence the force distributions, can be highly inhomogeneous. Large local stresses can result in an increased susceptibility for local rearrangements due to rupture or unbinding events.

In our study, we introduce a quantitative measure to characterize the emergence of highly stressed one-dimensional paths, so-called force chains, in three-dimensional nonlinear fiber networks. Furthermore, we provide an analytical approach, based on graph theory, that quantitatively describes the force distributions in one-dimensional periodic spring networks. Our analytical results are in excellent agreement with our numerical simulations.