BP 42: Cytoskeletal filaments (joint BP/CPP)

Time: Thursday 9:30–13:00 Location: H 1028

Invited Talk BP 42.1 Thu 9:30 H 1028

Microtubules adapt to mechanical stress through spontaneous intra-lattice repair — Laura Schaedel[1], Karin John[1], Jeremie Gaillard[1], Maxence Nachury[2], Laurent Blanchoin[1], and •Manuel Thery[1,3] — [1]UMR5168, CEA/CNRS/INRA/Université Grenoble-Alpes, Grenoble, France — [2]Stanford University School of Medicine, CA 94305, USA. — [3]Hôpital Saint Louis, UMRS1160, INSERM/AP-HP/Université Paris Diderot, Paris, France

Microtubule arrays define the shape of axons, cilia and flagella, and provide tracks for intracellular transport. Although microtubules assembled in vitro are stiffer than other cytoskeletal polymers by several orders of magnitude, intracellular forces lead to the formation of highly bent microtubules. It is currently not known how microtubules tolerate the vast forces exerted on them. It is likely that physical constraints affect microtubule structure and stiffness. Using a newly developed microfluidic device, we find that microtubule stiffness decreases incrementally with each cycle of bending and release. Similar to other cases of material fatigue, rather than a homogenous distribution of stress, the concentration of mechanical stresses turns pre-existing defects in the microtubule lattice into larger damages. Strikingly, damaged microtubules are able to recover their initial stiffness by spontaneously incorporating tubulin into their lattice. These findings demonstrate that microtubules are ductile materials with self-healing properties. Microtubule dynamics is thus not exclusive to the ends and intra-lattice incorporation of tubulin enables spontaneous adaptation to mechanical stresses.

BP 42.2 Thu 10:00 H 1028

Molecular wear of microtubules propelled by surface-adhered kinesins — Emmanuel LP Dumont[1], Catherine Do[2], and •Henry Hess[1] — [1]Department of Biomedical Engineering, Columbia University, New York, New York 10027, USA — [2]Institute for Cancer Genetics, Columbia University Medical Center, New York, New York 10032, USA

Wear, the progressive loss of material from a body caused by contact and relative movement, is a major concern not only in engineering but also in biology. Advances in nanotechnology both enable the study of the origins of wear processes at the atomic and molecular scale and demand the prediction and control of wear in nanoscale systems. Here we discuss wear that occurs in an in vitro system consisting of microtubules gliding across a surface coated with kinesin-1 motor proteins, and that energetic considerations suggest a molecule-by-molecule removal of tubulin proteins. The rates of removal show a complex dependence on sliding velocity and kinesin density, which - in contrast to the friction behavior between microtubules and kinesin - cannot be explained by simple chemical reaction kinetics.

BP 42.3 Thu 10:15 H 1028

Diffusible crosslinkers generate directed forces in microtubule networks — Zdenek Lansky[1,2,5], •Marcus Braun[1,2,5], Annemarie Lüdecke[1,2], Michael Schlierf[1], Pieter Rein ten Wode[3], Marcel Janson[4], and Stefan Diez[1,2] — [1]B CUBE, TU Dresden, Germany — [2]MPI-DBG, Dresden, Germany — [3]AMOLF, Amsterdam, The Netherlands — [4]Laboratory of Cell Biology, Wageningen University, The Netherlands — [5]equal contribution

Remodeling of cytoskeletal filament networks is essential to cell division and morphogenesis. The mechanical forces driving the restructuring are attributed to the action of molecular motors and filament dynamics, which both consume chemical energy. By contrast, non-enzymatic filament crosslinkers are regarded as mere friction-generating entities. Here, we experimentally demonstrate that non-enzymatic, diffusible microtubule crosslinkers of the Ase1/PRC1/Map65 family generate directed microtubule sliding when confined between partially-overlapping microtubules. The Ase1-generated forces, directly measured by optical tweezers to be in the piconewton-range, were sufficient to antagonize motor-protein driven microtubule sliding. Force generation can be quantitatively explained by the entropic expansion of confined Ase1 molecules diffusing within the microtubule overlaps. The thermal motion of confined crosslinkers is thus harnessed to generate mechanical work analogous to compressed gas propelling a piston in a cylinder. As confinement of diffusible crosslinkers is ubiquitous in cells, the associated entropic forces are likely to be of importance for cellular mechanics beyond cytoskeletal networks.

BP 42.4 Thu 10:30 H 1028

The Dynamics of cross-linked Microtubules in Neurons — •Maximilian Jakobs — University of Cambridge — Universität zu Köln

Microtubule bundles play a central role in the initiation and growth of cellular processes such as neuronal axons and dendrites. However, a quantitative understanding of the involved mechanisms is still lacking. Here, we developed computer simulations that mimic the 1D dynamics of microtubule bundles, cross-linked by ensembles of molecular motors, to investigate the mechanics of growth. We demonstrated that unipolar motors (such as cytoplasmic dynein and most kinesins) are much more effective in initiating axon growth than bipolar motors (such as kinesin 5). The latter, however, are in turn more efficient in filament sorting. We furthermore investigated axon growth dynamics as a function of the restoring forces acting on MT bundles. Our calculations demonstrated that the maximum force such bundles may exert increases monotonically with the elastic rigidity of the opposing membrane, and that it is insensitive to the polarity of filaments in the bundle. Finally, we found that the motor density must exceed a percolation threshold, which depends on the number of filaments in the bundle, before any force can be exerted. Future experiments and considerations might reveal an important contribution of microtubule-generated forces to neuronal symmetry breaking.

BP 42.5 Thu 10:45 H 1028

Cross-linking proteins facilitate formation of microtubule bundles — •Marcel Prelogovic[1], Lora Winters[2], Iva Tolić[2], and Nenad Pavin[1] — [1]Faculty of science , University of Zagreb, Croatia — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

During mitosis, microtubules (MTs) form a spindle which is responsible for proper segregation of chromosomes. In the fission yeast Schizosaccharomyces Pombe, the spindle is a bundle of MTs emanating from two spindle pole bodies and held together by cross-linking proteins. Our goal is to understand the dynamic properties of MTs interacting with cross-linking proteins and the role of cross-linking proteins in the formation of MT bundles. We introduce a theoretical model of MT bundling which describes angular movement of MTs around the spindle pole body driven by thermal forces and forces exerted by cross-linking proteins, described as elastic springs. If the number of cross-linking proteins connecting the MTs is above a critical number, attractive forces exerted by cross-linking proteins dominate over thermal forces at very small angles between MTs, causing MT-s to bundle. We identify stable bundles as the cases where MTs are more likely to be bundled than not. Theory yields bundling probability as a function of length and cross-linking protein concentration and predicts parameters for which stable bundles form. In conclusion, these results provide an explanation for how the angular brownian motion and cross-linking proteins affect the formation of stable MT bundles.

BP 42.6 Thu 11:00 H 1028

Quantifying protein diffusion and capture on filaments — •Emanuel Reithmann, Louis Reese, and Erwin Frey — Arnold Sommerfeld Center for Theoretical Physics and Center for NanoScience, Ludwig-Maximilians-Universität, München

The functional relevance of regulating proteins is often restricted to specific binding sites such as the ends of microtubules or actin-filaments. A localization of proteins on these functional sites is of great importance. In this respect, recent experimental studies suggested that several key players involved in regulation of microtubules and actin-filaments utilize a one-dimensional diffusive motion on the respective filament to target the functional end. We present a quantitative theory for a diffusion and capture process, where proteins diffuse on a filament and stop diffusion when reaching the filament's end. It is found that end-association after one-dimensional diffusion is highly efficient as compared to direct binding from solution/cytoplasm. As a consequence, diffusion and capture substantially enhances the reaction velocity of enzymatic reactions, where proteins and filament ends are to each other as enzyme and substrate. We show that the reaction ve-

locity ensuing from diffusion and capture can effectively be computed within a Michaelis-Menten framework. We predict that diffusion and capture would significantly beat the (three-dimensional) Smoluchowski diffusion limit for the rate of direct protein association to filament ends for practically all proteins that are known to diffuse on microtubules and actin-filaments.

15 min break

Invited Talk BP 42.7 Thu 11:30 H 1028

Cellular chirality arising from the self-organization of the actin cytoskeleton — •Alexander Bershadsky — Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel — Mechanobiology Institute, National University of Singapore, Singapore 117411, Singapore

Cellular mechanisms underlying the development of left-right asymmetry in tissues and embryos remain obscure. Here, the development of a chiral pattern of actomyosin was revealed by studying actin cytoskeleton self-organization in cells with isotropic circular shape. A radially symmetrical system of actin bundles consisting of α-actinin-enriched radial fibers (RFs) and myosin-IIA-enriched transverse fibers (TFs) evolved spontaneously into the chiral system as a result of the unidirectional tilting of all RFs, which was accompanied by a tangential shift in the retrograde movement of TFs. We showed that myosin IIA-dependent contractile stresses within TFs drive their movement along RFs, which grow centripetally in a formin-dependent fashion. The handedness of the chiral pattern was shown to be regulated by α-actinin-1. Computational modeling demonstrated that the dynamics of radial-transverse fiber system can explain the pattern transition from radial to chiral. Thus, actin cytoskeleton self-organization provides built-in machinery that potentially allows cells to develop left-right asymmetry.

BP 42.8 Thu 12:00 H 1028

Spontaneous polarization in an interfacial growth model for actin filament networks with a rigorous mechanochemical coupling — •Karin John[1], Denis Caillerie[2], Thomas Stoeter[1,3], and Chaouqi Misbah[1] — [1]Université Grenoble Alpes/ CNRS, LIPHY, F-38000 Grenoble, France — [2]Université Grenoble Alpes/CNRS, 3SR, F-38000 Grenoble, France — [3]Otto-von-Guericke Universität Magdeburg

Many processes in eukaryotic cells, including cell motility, rely on the growth of branched actin networks from surfaces. Despite its central role the mechanochemical coupling mechanisms that guide the growth process are poorly understood, and a general continuum description combining growth and mechanics is lacking. We develop a theory that bridges the gap between mesoscale and continuum limit and propose a general framework providing the evolution law of actin networks growing under stress. This formulation opens an area for the systematic study of actin dynamics in arbitrary geometries. Our framework predicts a morphological instability of actin growth on a rigid sphere, leading to a spontaneous polarization of the network with a mode selection corresponding to a comet, as reported experimentally. We show that the mechanics of the contact between the network and the surface plays a crucial role, in that it determines directly the existence of the instability. We extract scaling laws relating growth dynamics and network properties offering basic perspectives for new experiments on growing actin networks.

BP 42.9 Thu 12:15 H 1028

Contractile actin bundles without molecular motors — •Jörg Schnauss[1], Tom Golde[1], Carsten Schuldt[1], Sebastian Schmidt[1], Martin Glaser[1], Dan Strehle[1], Josef Käs[1], and Claus Heussinger[2] — [1]Institute for Experimental Physics I, University of Leipzig , Linnéstraße 5, 04103 Leipzig , Germany — [2]Institute for Theoretical Physics, Georg-August University of Göttingen, Friedrich-Hund Platz 1, 37077 Göttingen, Germany

Since the 1940, interactions of actin and its molecular motor myosin are known as the fundamental process for biological force generation. These interactions convert chemical energy into mechanical work by ATP hydrolysis. The dogma of molecular motors being the basis of all contractile forces has never been disproven. In this study we show an alternative force generation mechanism in the absence of molecular motors. The system is not driven by ATP hydrolysis and solely relies on minimization of free energy based on filament-filament interactions induced by a crowded environment. Dynamics of these contractions behave differently to a single filament pair shown in theoretical and experimental studies. We are able to show that the behavior of contractile actin bundles can be well described as an emergent phenomenon of multiple filament pairs. This crowding regime is well below the macromolecular content of cells and crowding effects have to be considered in cellular systems. We measured contraction velocities ranging from 0.10 to 0.65 μm/s and evaluated a force regime of 0.5 to 3.0 pN. Dynamics and forces of this non-dissipative process correspond to an active behavior of single myosin motors.

BP 42.10 Thu 12:30 H 1028

Organisation 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) differentiate into various cell types. Here substrate stiffness is sufficient to guide hMSCs towards different lineages without additional biochemical stimuli [1]. Stress fibres (SFs)composed of actin filaments, cross-linkers and myosin motor-proteins generate and transmit tension throughout the cell. Myosin inhibition stops the differentiation [1], implying importance of SF tension for this process. Characteristic SF patterns can be detected within 24 hours and used as an early morphological marker [2].

We use 24h long-term live-cell imaging of RFP-Lifeact transfected hMSCs on substrates of different stiffness, recording many cells in parallel for better statistics in comparable conditions. SFs are traced with a sophisticated filament tracking program [3] and a tool to extract filament modes [4], to gain a deeper understanding of SF formation dynamics in early stem cell differentiation. This leads to a non-monotonic dependence of SF polarization on the Young's modulus of the underlying substrate[2].

[1] A. Engler et al., Cell (2006); [2] A. Zemel et al., Nature Physics (2010); [3] B. Eltzner et al., arXiv:1408.4002, 2014; [4] S. Huckemann et al., arXiv:1404.3300, 2014;

BP 42.11 Thu 12:45 H 1028

Elasticity of 3D networks with rigid filaments and compliant crosslinks — •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 — [2]Drittes Physikalisches Institut – Biophysik, Georg-August-Universität, Göttingen

Disordered filamentous networks with compliant crosslinks exhibit a low linear elastic shear modulus at small strains, but stiffen dramatically at high strains. Experiments have shown that the elastic modulus can increase by up to three orders of magnitude while the networks withstand relatively large stresses without rupturing. Here, we perform an analytical and numerical study on model networks in three dimensions. Our model consists of a collection of randomly oriented rigid filaments connected by flexible crosslinks that are modeled as wormlike chains. Under the assumption of affine deformations in the limit of *infinite* crosslink density, we show analytically that the nonlinear elastic regime in 1- and 2-dimensional networks is characterized by power-law scaling of the elastic modulus with the stress. In contrast, 3-dimensional networks show an exponential dependence of the modulus on stress. Independent of dimensionality, if the crosslink density is *finite*, we show that the only persistent scaling exponent is that of the single wormlike chain. Consequently, unlike suggested in prior work, the model system studied here cannot provide an explanation for the experimentally observed linear scaling of the modulus with the stress in filamentous networks.