BP 33: Cytoskeletal Filaments II

Time: Thursday 15:00–17:15 Location: H 1028

BP 33.1 Thu 15:00 H 1028

High resolution three-dimensional tracking with optical tweezers reveals protofilament switching of the kinesin-8 Kip3 — •Michael Bugiel and Erik Schäffer — Zentrum für Molekularbiologie der Pflanzen (ZMBP), Universität Tübingen, Auf der Morgenstelle 32, 72076 Tübingen, Germany

The budding yeast kinesin-8 Kip3 is a highly processive motor protein that walks to the ends of cytoskeletal microtubules and shortens them in a collective manner. Microtubules consist of circularly arranged tubulin polymer chains, called protofilaments. How exactly Kip3 reaches the end is unclear. Left-handed rotations of microtubules in Kip3 gliding assays indicated sideward motion of Kip3 perpendicular to the microtubule axis, i.e. switching between single protofilaments. We used high resolution optical tweezers in a force-feedback mode to track the trajectories of single Kip3 motors. Previous 2D assays with alternating sideward loads showed that Kip3 performs sideward steps in both directions, consistent with a diffusive sideward motion on the microtubule lattice. Here, we topographically suspended microtubules such that Kip3-coated microspheres can freely rotate around the microtubules in three dimensions. Tracking these motor driven microspheres with a 3D, zero-load force-clamp showed that Kip3 switched protofilaments in discrete steps equally frequent in both directions. A statistical analysis confirmed a diffusive sideward motion of Kip3, consistent with the 2D results. The diffusive protofilament switching may enable Kip3 to bypass obstacles and reach the microtubule end for length regulation.

BP 33.2 Thu 15:15 H 1028

Visualizing acto-myosin dynamics and vortices at a membrane surface using interferometric scattering microscopy — •Darius V Köster[1,2], Nikolas Hundy[3], Gavin Young[3], Adam Fineberg[3], Philipp Kukura[3], and Satyajit Mayor[1,4] — [1]NCBS, Bangalore, India — [2]Warwick Medical School, Warwick, UK — [3]University of Oxford, Oxford, UK — [4]InStem, Bangalore, India

The plasma membrane and the underlying cytoskeletal cortex constitutes an active platform for many cellular processes. Recent work has shown that acto-myosin dynamics modify the local membrane organization, but the molecular details are not well understood due to difficulties with experimentally accessing the associated time and length scales. Here, we use interferometric scattering (iSCAT) microscopy to investigate a minimal acto-myosin network linked to a supported lipid bilayer membrane. Using the magnitude of the interferometric contrast, which is proportional to molecular mass, we detect, image and distinguish actin and myosin filaments. As a result, we can follow the diffusion of single actin filaments attached to the bilayer revealing different types of diffusion depending on filament length and quantify binding kinetics and processivity as a function of ATP concentrations, providing new evidence for the theoretically predicted behavior of ensembles of myosin head domains. Simultaneous observation of long-term network flow and organization enables us to link changes in myosin II filament dynamics with decreasing ATP concentrations to a switch in the acto-myosin network from a remodeling, fluid state to a contractile, and observe the formation of vortices as predicted by theory.

BP 33.3 Thu 15:30 H 1028

Size-dependent phagosomal transport depends on microtubules, actin filaments and associated motors — •Steve Keller, Konrad Berghoff, and Holger Kress — University of Bayreuth, Bayreuth, Germany

The internalization and intracellular degradation of pathogens by macrophages is an essential part of the mammalian immune response. The associated intracellular transport of the phagosome from the cell periphery to the perinuclear region is crucial for the phagosome maturation. To date biochemical factors are known to influence the fate of phagosomes. Here we show that the phagosomal transport is also strongly influenced by the size of the phagosomes and that this size-dependent transport depends on microtubules, actin filaments and associated motors. We found that large phagosomes are transported very persistently to the nucleus with almost no centrifugal motion, whereas small phagosomes show strong bidirectional transport. Our investigation of the molecular basis of this size-dependent transport suggests that dynein motors and the intracellular distribution of microtubules strongly influence the centripetal transport of large phagosomes. Additionally, our findings indicate that actin filament-associated motors and the distribution of actin filaments strongly influence the bidirectional transport of small phagosomes. Our findings suggest that a simple size-dependent cellular sorting mechanism might exist that supports inward transport of large phagocytosed bacteria for facilitating their digestion and that simultaneously supports outward transport of small bacterial fragments for example for antigen presentation.

Invited Talk BP 33.4 Thu 15:45 H 1028

Dynamics and instabilities of contractile actin networks in artificial cells — •Kinneret Keren — Physics Department, Technion-Israel Institute of Technology, Haifa 32000, Israel

Contractile actin network have an essential role in many cellular processes including cell division, intracellular transport and cell motility. While the molecular components involved are largely known, we still do not understand what controls the large scale properties of these networks. We generate bulk actin networks by introducing cytoplasmic Xenopus egg extracts, which contain all the components of the actin machinery, into cell-sized water-in-oil droplets. Importantly, the presence of turnover in our system allows these networks to attain a dynamic steady state characterized by contractile actin flows which persist for hours. We find that under a broad range of conditions, the network undergoes homogenous contraction despite large spatial variations in network density, and that this contraction rate is inversely proportional to the actin disassembly rate. We observe either a symmetric state in which the network contracts towards the center of the droplets and exhibits a spherically symmetric density and flow pattern, or a polar state in which the contraction center is localized near the droplet's boundary. In the symmetric state, the contraction center is actively maintained near the middle of the droplet, reminiscent of actin-based centering mechanisms found in living cells. During symmetry breaking, the system transitions from this symmetric state to a polar state, mimicking cellular symmetry breaking as seen for example during motility initiation or spindle migration in mammalian oocytes.

BP 33.5 Thu 16:15 H 1028

Rotational movement of microtubules driven by thermal forces and by kinesin-5 motors leads to mitotic spindle formation — •Ivana Ban[1], Marcel Prelogović[1], Lora Winters[2], 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 spindle divides chromosomes between two daughter cells. In the fission yeast Schizosaccharomyces pombe, the rod shaped mitotic spindle is composed of antiparallel microtubules (MTs) emanating from two opposite spindle poles, whose formation is mediated by motor proteins. A key question is what are the physical principles underlying the formation of a mitotic spindle. Here we show, experimentally and theoretically, that MTs at one pole search for a MT from the other pole by performing random rotational movement around the spindle pole. When MTs from opposite poles get into close proximity, motor proteins start to accumulate in the region where MTs are close to each other. In our model, minus end directed motors generate forces that drive the formation of an antiparallel MT bundle, thereby forming the mitotic spindle. We identified experimentally the kinesin-5 motor Cut7 as the main force generator in this process. In conclusion, random rotational motion helps MTs from opposite poles to find each other and subsequent accumulation of kinesin-5 motors allows them to generate forces that drive spindle formation.

BP 33.6 Thu 16:30 H 1028

Bistability and oscillations in cooperative microtubule and kinetochore dynamics in the mitotic spindle — •Felix Schwietert and Jan Kierfeld — TU Dortmund University, 44221 Dortmund, Germany

In the mitotic spindle microtubules attach to kinetochores via catch bonds during metaphase. We investigate the cooperative dynamics of a one-sided spindle model consisting of a microtubule ensemble which is attached to a kinetochore via elastic linkers. The model includes

the dynamic instability of microtubules, forces on microtubules and kinetochores from elastic linkers, and an external force on the kinetochore. We use a mean-field approach based on Fokker-Planck equations to analytically solve the one-sided spindle model, which establishes a bistable force-velocity relation of kinetochore motion. All results are in agreement with stochastic simulations. We derive constraints on linker stiffness and microtubule number for the occurrence of bistability. In the full two-sided spindle model, two such bistable systems are coupled in a tug-of-war. This leads to stochastic chromosome oscillations in metaphase (directional instability), which have been observed in several experiments. We also derive constraints on linker stiffness and microtubule number for metaphase chromosome oscillations. With certain modifications the model can be used to explain the effects of additional processes, e.g. microtubule poleward flux or polar ejection forces.

BP 33.7 Thu 16:45 H 1028

Bending dynamics of single-walled carbon nanotubes in viscoelastic media — •Kengo Nishi[1], Fred MacKintosh[2,3,4], and Christoph Schmidt[1,5] — [1]Third Institute of Physics - Biophysics, University of Göttingen, 37077 Göttingen, Germany — [2]Department of Chemical & Biomolecular Engineering, Rice University, Houston, TX 77005, USA — [3]Center for Theoretical Biological Physics, Rice University, Houston, TX 77030, USA — [4]Department of Physics and Astronomy, Vrije Universiteit, 1081HV Amsterdam, The Netherlands — [5]Department of Physics, Duke University, Durham, NC 27707, USA

The mechanics and dynamics of cells and tissues are dominated by semi-flexible polymer networks, whose bending stiffness leads to non-trivial dynamics. Micron-sized beads are commonly used in microrheology approaches to measure the viscoelasticity of such systems. Insertion of such probes can lead to artefacts and is often not possibly in confined geometries in living cells. Here we introduce the use of single-walled carbon nanotubes (SWNTs), themselves semi-flexible polymers with non-photobleaching near-infrared fluorescence, as multiscale stealth probes for microrheology. We investigate the bending dynamics of SWNTs embedded in viscoelastic media and analyze their thermally driven shape fluctuations. We find that we can describe the bending dynamics of SWNTs by a Langevin equation with a bending term and a time-dependent memory function.

BP 33.8 Thu 17:00 H 1028

Tensile elasticity of a hinged wormlike chain — •Panayotis Benetatos — Kyungpook National University, Daegu, South Korea

It is known that local defects in the bending rigidity of double-stranded DNA, such as denaturation bubbles or singe-stranded nicks, significantly affect its configurational properties and elastic response. In this talk, we present an analytic calculation (within the weak bending approximation) of the force-extension relation of a wormlike chain with a fixed hinge defect. We show that the gain in configurational entropy allowed by the defect has a significant effect on the stretching compliance of the polymer. Our results apply to any pair of semiflexible segments connected by a hinge. As such, they may also be relevant to cytoskeletal filaments (F-actin, microtubules), where one may treat the cross-link connecting two filaments as a hinge defect.

P. Benetatos, Phys. Rev. E 96, 042502 (2017)