BP 1: Molecular Motors

Time: Monday 9:30–13:00 Location: HÜL 386

Topical Talk BP 1.1 Mon 9:30 HÜL 386

What determines the path of kinesin motors along the microtubules? — •Zeynep Ökten — TU München, Germany

In long-range transport of cargo, prototypical kinesin-1 steps along a single protofilament on the microtubule, an astonishing behavior given the number of theoretically available binding sites on adjacent protofilaments. Using a laser trap assay, we analyzed the trajectories of several representatives from the kinesin-2 class on freely suspended microtubules. In stark contrast to kinesin-1, these motors display a wide range of left-handed spiraling around microtubules and thus generate torque during cargo transport. We provide direct evidence that kinesin*s neck region determines the torque generating properties. Disrupting the stability of the neck by inserting flexible peptide stretches resulted in pronounced left-handed spiraling. Mimicking neck stability by crosslinking significantly reduced the spiraling of the motor up to the point of protofilament tracking.

BP 1.2 Mon 10:00 HÜL 386

Microtubule rotations reveal the 3D stepping trajectories of motor proteins on microtubule lattices — •Aniruddha Mitra[1], Felix Ruhnow[1], and Stefan Diez[1,2] — [1]B CUBE, Dresden, Germany — [2]MPI-CBG, Dresden, Germany

Microtubules consist of about 13 protofilaments which act as tracks for motor proteins. It is interesting to know, whether processive motors step strictly along one of these tracks or whether they are able to switch between the tracks. We recently performed in vitro gliding motility assays on surfaces coated with kinesin-1 and kinesin-8 motor proteins and measured the rotations of microtubules around their longitudinal axis using quantum dots in combination with fluorescence-interference contrast (FLIC) microscopy and 2D nanometer tracking. We found that the cargo carrying motor kinesin-1 follows a single protofilament, while the highly processive microtubule-depolymerizing motor kinesin-8, does switch tracks with a bias towards the left. Because the attached quantum dots might interfere with the microtubule rotations, we recently devised a novel quantum-dot free method to obtain microtubule rotation data.

BP 1.3 Mon 10:15 HÜL 386

The kinesin-8, Kip3, frequently switches microtubule protofilaments in a biased random walk — •Michael Bugiel[1], Elisa Böhl[1], and Erik Schäffer[2] — [1]Nanomechanics Group, Biotechnology Center, TU Dresden, Tatzberg 47/49, 01307 Dresden, Germany — [2]Zentrum für Molekularbiologie der Pflanzen (ZMBP), University of Tübingen, Auf der Morgenstelle 32, 72076 Tübingen, Germany

The budding yeast Kinesin-8 Kip3 is a highly processive motor protein that walks to the end of cytoskeletal microtubules and shortens them in a collective manner. Microtubules usually consist of 12 to 15 circularly-arranged tubulin polymer chains, called protofilaments. Left-handed rotations of microtubules in Kip3 gliding assays indicate sideward motion of Kip3 perpendicular to the microtubule axis, i.e. a switching between single protofilaments. Here, we used high-resolution optical tweezers in a force feedback mode to track the path of single Kip3 motors by applying alternating sideward loads. Our studies show that Kip3 steps sideward in both directions, but follows the load on average with a preference towards the left. Based on statistical data analysis and comparison with simulations, we propose a diffusive sideward motion of Kip3 on the microtubule lattice. This diffusive switching mechanism may enable Kip3 to bypass obstacles and reach the microtubule end for length regulation.

BP 1.4 Mon 10:30 HÜL 386

Teams of molecular motors in optical traps from a theoretical perspective — •Florian Berger, Corina Keller, Stefan Klumpp, and Reinhard Lipowsky1 — Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany

Intracellular transport of cargos is achieved by the cooperative action of molecular motors, which pull the cargo along cytoskeletal filaments. A lot of single molecule studies together with theoretical considerations have contributed to develop a common understanding how single kinesin motor respond to an external force. However, it is far from obvious, how an external load force influence the dynamics of a small team of molecular motors. Starting from the single molecule properties, we introduce a theoretical framework for cooperative transport and study the response of a motor team to a constant force (force-feedback trap) and a time dependent force (stationary trap). Within in our simple description, we discuss the basic differences of these two measurement techniques and point out the implications for transport in a soft crowded environment.

BP 1.5 Mon 10:45 HÜL 386

Length regulation of microtubules: the effect of limited resources — •Matthias Rank, Louis Reese, and Erwin Frey — Arnold Sommerfeld Center for Theoretical Physics (ASC) and Center for NanoScience (CeNS), Department of Physics, Ludwig-Maximilians-Universität München

Length regulation of microtubules is strongly associated with the motion and action of molecular motors as kinesin-8 Kip3p. This processive motor has been shown to depolymerise microtubules; its activity thus competes with spontaneous tubulin polymerisation. We describe the kinesin motion in terms of the totally asymetric simple exclusion process (TASEP) with modified dynamic rules at the terminal site. In particular—mimicking situations in real cells—we investigate the effect of limited resources on the dynamics. We find rich phase behaviour which differs fundamentally from the case of an infinite reservoir. We supplement our mean-field theory with Monte Carlo simulations to obtain a complete theoretical picture of the process.

BP 1.6 Mon 11:00 HÜL 386

Direct measurement of the pressure generated by a 1D protein gas confined within microtubule overlaps — •Annemarie Lüdecke[1,2], Marcus Braun[1,2], Zdenek Lansky[1,2], Michael Schlierf[1], Pieter Rein ten Wolde[3], Marcel E. Janson[4], and Stefan Diez[1,2] — [1]B CUBE, TU Dresden, Dresden, Germany — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany — [3]AMOLF, Amsterdam, Netherlands — [4]Laboratory of Cell Biology, Wageningen University, Wageningen, Netherlands

The integrity of the mitotic spindle during anaphase is facilitated by antiparallel microtubule-microtubule overlaps in the spindle midzone. Within these overlaps, motor proteins (e.g. kinesin-5, kinesin-14) as well as passive, non-enzymatic microtubule crosslinkers (e.g. from the MAP65 family) localize and influence the mechanical stability of the overlaps. Here, we show in vitro that the diffusible microtubule crosslinker Ase1, a member of the MAP65 family, can slow down and halt the shortening of microtubule-microtubule overlaps driven by the kinesin-14 Ncd. Using mathematical modeling we show that Ase1 confined in a microtubule overlap behaves like a 1D gas upon compression, i.e. producing an entropic force opposing the compression. Direct measurement of the entropic force by optical tweezers yielded values in the pN-range, comparable to the forces produced by motor proteins present in the spindle midzone. We hypothesize that entropic pressure may be a general mechanism of force production in biological systems.

15 min. break

BP 1.7 Mon 11:30 HÜL 386

Stochastic dynamics of small ensembles of non-processive molecular motors: The parallel cluster model — •Thorsten Erdmann[1,2], Philipp J. Albert[1,2], and Ulrich S. Schwarz[1,2] — [1]Institute for Theoretical Physics, Heidelberg University, Heidelberg, Germany — [2]BioQuant, Heidelberg University, Heidelberg, Germany

Forces in the actin cytoskeleton are generated by small groups of non-processive myosin II motors. For such small groups comprising few tens of myosin II molecules, stochastic effects are highly relevant. Using a three-state crossbridge model with the assumptions of fast power-stroke kinetics and equal load sharing between motors in equivalent states, the stochastic reaction network is reduced to a one-step master equation for binding and unbinding dynamics (parallel cluster model) together with rules for ensemble movement. For constant external load, ensemble dynamics is determined by the catch bond character of myosin II, leading to an increase of the fraction of bound motors under load and firm attachment of small ensembles. This adaptation to load results in a concave force-velocity relation described by a Hill relation. When working against an external linear spring, myosin II ensembles

adjust themselves to an isometric state with constant average position and load. Ensemble dynamics is now determined by the distribution of motors over different bound states. Above a critical spring stiffness, myosin II can no longer perform the power stroke. This stalls ensemble movement but slow unbinding from the pre-power-stroke state protects the ensembles against detachment. [Reference: T. Erdmann, P.J. Albert & U.S. Schwarz, J. Chem. Phys. 139, 175104 (2013).]

BP 1.8 Mon 11:45 HÜL 386

Dynamical Phenomena in Coupled Muscle Myosins — •Lennart Hilbert, Zsombor Balassy, Shivaram Cumarasamy, Anne-Marie Lauzon, and Michael C. Mackey — McGill University, Montréal, Québec, Canada

The length (l) of muscle myosin filaments in smooth muscle cells is distributed following $exp(-l/L_0)$, $L_0 = 0.116 - 0.182\,\mu m$ [Liu et al., J Physiol, 2013]. The kinetics of skeletal muscle myosin groups working on a commonly propelled actin filament qualitatively change at $L_1^c \approx 0.3\,\mu m$ and $L_2^c \approx 1.0\,\mu m$ lengths of interaction between a myosin-coated surface and myosin-propelled actin filaments (L, motility assay) [Hilbert et al., Biophys J, 2013]. In this study, motility assays of smooth muscle myosin showed the same kinetic regimes for increasing L as seen for skeletal muscle myosin: (1) actin arrest to surface, (2) alternating arrest and forward sliding (autocorrelation time $0.3\,s$), (3) continuous forward sliding; transition lengths $L_1^c \approx 0.27\,\mu m$ and $L_2^c \approx 0.75\,\mu m$. The regimes were reproduced by a mathematical model of $N = 5$ to 100 myosin binding sites ($N \propto L$) on individual actin filaments interacting with surface-fixed myosins. Binding site kinetics were mechanically coupled via actin, and dependent on L, a stable focus (arrest) and a limit cycle (sliding) existed that entrained global behavior. The dynamic phenomena and resulting L-dependent statistics from our actin sliding experiments were captured by a reduced mathematical model with two fast variables (exhibiting limit cycle or stable focus) and one slow variable (switching between cycle and focus).

BP 1.9 Mon 12:00 HÜL 386

Single-molecule studies of RNA polymerase I elongation — •Ana Lisica[1], Marcus Jahnel[1], Christoph Engel[2], Patrick Cramer[2], and Stephan W. Grill[1] — [1]MPI-CBG and MPI-PKS, Dresden, Germany — [2]Gene Centre Munich, LMU, Munich, Germany

Eukaryotic RNA polymerases (Pol I, Pol II and Pol III) have a highly conserved core and active center region. This implies that the mechanism of RNA polymerization is similar in all three polymerases. Additionally, Pol I and Pol III have specific surface subunits. Furthermore, the strength of the intrinsic activity of transcript cleavage differs highly: Pol I and Pol III possess a strong RNA cleavage mechanism, while Pol II has a weak one and needs a transcription factor (TFIIS) to cleave the RNA. To observe fine differences in transcription dynamics that stem from structural differences between these machines, we are using high-resolution dual-trap optical tweezers to analyze the mechanochemical details of the Pol I elongation in comparison to Pol II. Here we present the first single-molecule optical tweezers traces of Pol I transcribing a bare DNA template. A comparison with the Pol II dynamics revealed that Pol I is faster, with significantly higher overall and pause-free velocities, it pauses less often than Pol II, exhibits shorter pauses and can transcribe against higher opposing forces. Surprisingly, we find that the intrinsic transcript cleavage ability of Pol I is functional only with backtracked RNAs shorter than about 18 nt. Together, our results contribute to the understanding of unique micromechanical function and cellular role of this essential enzyme.

BP 1.10 Mon 12:15 HÜL 386

Myosin II Activity Softens Cells in Suspension — •Chii Jou Chan[1,2], Andrew Ekpenyong[1,2], Jochen Guck[1,2], and Franziska Lautenschläger[1,3] — [1]Cavendish Laboratory, Department of Physics, University of Cambridge, Cambridge, United Kingdom — [2]Biotechnology Center, Technische Universität Dresden, Dresden, Germany — [3]Department of Physics, Saarland University, Saarbrücken, Germany

The cytoskeleton of cells is crucial for many cellular functions that require shape change or force generation, which is enacted by actin in concert with the motor protein myosin. While studies investigating the contribution of myosin-activity on the mechanical properties of cells have been performed on cells attached to a substrate, we investigated mechanical properties of cells in suspension using an optical stretcher. Both naturally suspended cells and naturally attached cells were treated with myosin inhibitors (Blebbistatin, Y-27632). We find that all cells, once in suspension, stiffen when myosin activity is inhibited and vice versa This is exactly opposite to what has been reported for cells attached to a substrate, which stiffen via pre-stress by myosin activity. Possible reasons for this difference and likely molecular mechanisms will be discussed. Our findings shed new light on the role of myosin II in the control of cell mechanical properties when cells are not attached to flat, rigid surfaces.

BP 1.11 Mon 12:30 HÜL 386

Linking single-motor dynamics to ciliary ultrastructure using single-molecule super-resolution microscopy in Caenorhabditis elegans. — •Felix Oswald[1], Bram Prevo[1], Pierre Mangeol[1], Jonathan Scholey[2], and Erwin Peterman[1] — [1]VU University Amsterdam, Amsterdam, Netherlands — [2]University of California, Davis, Davis, United States of America

Cilia are finger-like protrusions that are present in most eukaryotic cells fulfilling crucial motility and sensory functions. Their shape and structural integrity is determined by microtubules and a variety of other structural components such as transition fibers and Y-shaped links. In Caenorhabditis elegans two kinesin-2-family motors, kinesin-II and OSM-3, are responsible for building and maintenance of the chemosensory cilia. It is known that both kinesins are active on the microtubule doublets and that OSM-3 alone maintains the microtubule singlets. It is not known, however, how other structural features influence their dynamics. In order to address this question we use single transgenes encoding for fluorescently labeled kinesins in combination with single-particle tracking PALM (sptPALM). By localizing single-motor proteins deep inside the living organism we are able to build super-resolution roadmaps that reveal ultrastructural details. This allows us to relate the rich single-motor dynamics to ciliary subdomains and their specific structural features. Our findings are the outset to understand the influence of ciliary ultrastructure on motor dynamics.

BP 1.12 Mon 12:45 HÜL 386

Dynein, microtubule and cargo: a ménage à trois — •Nenad Pavin[1], Vaishnavi Ananthanarayanan[2], and Iva Tolic-Norrelykke[2] — [1]Department of Physics, Faculty of Science, University of Zagreb, Zagreb, Croatia — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

To exert forces, motor proteins bind with one end to cytoskeletal filaments, such as microtubules and actin, and with the other end to the cell cortex, a vesicle or another motor. A general question is how motors search for sites in the cell where both motor ends can bind to their respective binding partners. In the case of nuclear movements in meiotic prophase, we identify the steps of the dynein binding process: from the cytoplasm to the microtubule and from the microtubule to cortical anchors. We found that dyneins on the microtubule move either in a diffusive or directed manner, with the switch from diffusion to directed movement occurring upon binding of dynein to cortical anchors. We explain theoretically how this dual behavior of dynein, together with the two steps of binding, enables dyneins to self-organize into a spatial pattern needed for them to generate large collective forces.

Ananthanarayanan, Schattat, Vogel, Krull, Pavin, Tolic-Norrelykke, Cell 2013.