BP 10: Posters: Molecular Motors

Time: Tuesday 9:30–12:30 Location: P1

BP 10.1 Tue 9:30 P1

The role of kinesin-8 in chromosome movements on the mitotic spindle — •Anna H. Klemm[1], Nenad Pavin[2], and Iva M. Tolic-Norrelykke[1] — [1]Max Planck Institute CBG, Germany — [2]Department of Physics, University of Zagreb, Croatia

During cell division, replicated chromosomes move back and forth around the spindle midzone before they are segregated evenly on the two daughter cells. In a screen in the fission yeast Schizosaccharomyces pombe we studied the role of all known fission yeast kinesin-motors, dynein heavy chain and the microtubule (MT) crosslinking protein ase1 on these movements. We found that the movement was only changed by deletion of the kinesin-8 motor klp5/6, but not by deletion of the other motors. It is known that the motor protein kinesin-8 influences chromosome movements by regulating MT catastrophe. However, the mechanics of how the cell coordinates the movement is unknown. Here we saw that in cells lacking klp5/6 replicated centromeres move over the entire spindle length and switch less often the direction of motion, whereas in wild-type cells, the replicated centromere covers only the central third of the spindle. The centromeres spend a significantly longer time in proximity of the spindle pole body in klp5/6-deleted cells in comparison with wild-type cells. Also, comparable to interphase MT and in vitro studies, klp5-GFP accumulates at the plus-end of polar spindle MTs as they grow and then disappears when the polar spindle MT undergoes catastrophe. We conclude that klp5/6 causes centromere movements away from the spindle pole bodies, most likely by increasing microtubule catastrophe in a length-dependent manner.

BP 10.2 Tue 9:30 P1

Synchronization of elastically coupled processive molecular motors and regulation of cargo transport — •Felix Kohler and Alexander Rohrbach — Universität Freiburg, Germany

In biological systems, many energetic processes are quantized by the hydrolysis of ATP-molecules enabling elementary reactions and conformation changes of proteins. In this way, molecular motors step discontinuously along cytoskeletal filaments to transport cargos such as vesicles or to translate filaments for cytoskeletal reorganization [1]. Most motors operate in groups and thereby enable a more efficient cargo transport [2]. A few studies have shown indications for a coordination between the motors and thus a coherent stepping of motors in vitro [3] and in vivo [4]. In order to analyze collective work of motor proteins by a theoretical approach, we introduce the synchronization q as a new observable for elastic coupling, which is identified with the probability to find a stochastic system in its ground state. The synchronization q can be read out from the ratio of the mean times of motor resting and stepping. For the motor proteins myosin V and kinesin I we show that the transport velocity and power can increase significantly for certain motor synchronizations or couplings.

[1] J. Howard, Mechanics of Motor Proteins and the Cytoskeleton
[2] F. Jülicher and J. Prost, prl 75 (1995)
[3] C. Leduc, F. Ruhnow, J. Howard, and S. Diez, pnas 104, (2007)
[4] X. Nan, P. A. Sims, P. Chen, and X. S. Xie, jpc B 109, (2005)

BP 10.3 Tue 9:30 P1

Single and multi motor measurements of the kinesin-like protein KIF20A — •Alice Wiesbaum[1], Nikta Fakhri[1], I-Mei Yu[2], Anne Houdusse-Juille[2], and Christoph F. Schmidt[1] — [1]Georg-August Universität Göttingen, De — [2]Institut Curie, Paris, Fr

The Kinesin-like protein KIF20A is necessary for different processes in human cells. It was found to have an essential role during cell division. There KIF20A is required for chromosome passenger complex mediated cytokinesis. Following phosphorylation by PLK1, KIF20A creates a docking site for PLK1 and recruits it at the central spindle. Knocking out KIF20A leads to defects in the cleavage furrow formation. A down-regulation of KIF20A showed an attenuate growth of pancreatic cancer cells. All this makes KIF20A an interesting object to study.

To understand the genetic structure and the function of single parts of KIF20A, we did in vitro experiments with different KIF20A mutants. KIF20A shows a flexible extension at the N-terminal, of which we partly removed different amounts of amino acids. We investigated KIF20A and the obtained constructs in surface gliding assays, as well as in single molecule tracking, using fluorescent nanotubes bound to the motor protein. In addition we had a look at the shape of KIF20A using small angle x-ray scattering (SAXS).

BP 10.4 Tue 9:30 P1

Bimodal transport in a system of active and inactive kinesin-1 motors — •Lara Scharrel[1,2], Rui Ma[3], Frank Jülicher[3], and Stefan Diez[1,2] — [1]B CUBE - Center for Molecular Bioengineering, Technische Universität Dresden, Germany — [2]Max Planck Institute of Cell Biology and Genetics, Dresden, Germany — [3]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany

Long-range directional transport in cells is facilitated by microtubule-based motor proteins. One example is transport in a neuron where groups of motor proteins, such as kinesin-1 and cytoplasmic dynein, ensure the supply and clearance of cellular material along the axon. Defects in axonal transport have been linked to Alzheimer and other neurodegenerative diseases. However, it is not known how in detail multi-motor transport is impaired if a fraction of motors is defective. To mimic impaired multi-motor transport in vitro, we performed gliding motility assays with varying fractions of active kinesin-1 and inactive kinesin-1 mutants. We found that impaired transport manifests in multiple motility modes: (i) a fast mode with gliding at single-molecule velocity, (ii) a slow mode with gliding at close-to zero velocity or stopping, and (iii) a mode with switches between fast and slow mode. Notably, the transition from fast to slow mode occurred at a threshold fraction of active motors. Furthermore, we developed a theoretical description which explains the bimodal motility as well as the sharp transitions between fast and slow motility. Our results demonstrate that, depending on the fraction of active motors, impaired multi-motor transport is either performed close to full speed or is out of action.

BP 10.5 Tue 9:30 P1

Synthetic molecular motors: a model study — •Amartya Sarkar and Alexander S. Mikhailov — Fritz Haber Institute, Faradayweg 4-6, Berlin 14195

A model of a synthetic molecular machine, whose operation closely resembles that of real molecular motors, is constructed and numerically investigated. The machine, described in terms of an elastic network, is able to perform cyclic conformational motions. These mechanochemical motions result from repetitive and sequential events - binding of a ligand, its conversion to a product, and the product release. The machine has two domains (arms) connected by a flexible hinge; while one of the arms is pinned, the other is able to perform cyclic swinging motions. Due to ratchet interactions between the swinging arm and a stiff filament, internal cyclic motions in the machine become converted into directed translational motion of the filament. Stochastic simulations of this model motor system under the conditions of both weak and strong coupling are performed. Further simulations with a stall force applied to the rigid filament have also been performed. Various statistical and mechanical properties of this model have been studied in detail.

BP 10.6 Tue 9:30 P1

Measurements of single fluorescent motor proteins: The Right Way — •Felix Ruhnow, Linda Kloss, and Stefan Diez — B CUBE, Technische Universität Dresden

Cytoskeletal motor proteins are required in many cellular processes, such as intracellular transport and mitosis. Therefore, the biophysical characterization of motor protein movement along their filamentous tracks is essential. Commonly, stepping motility assays are used to determine the stepping and detachment rates of various molecular motor proteins by measuring their speed, run length and interaction time. However, comparison of these results proved to be difficult because the experimental setup (e.g. bead assay vs. single-molecule fluorescence assay), the experimental conditions (e.g. temperature, buffer or filament preparation) and data analysis (e.g. normal vs. exponential distribution) can influence the results. Here, we describe a method to evaluate traces of fluorescent motor proteins and propose an algorithm to correct the measurements for photobleaching and the limited length of the filaments. Additionally, bootstrapping is used to estimate statistical errors of the evaluation method. The method was tested with numerical simulations as well as with experimental data from kinesin-1

stepping experiments to show that the run length of kinesin-1 is independent of the microtubule length distribution. Our work will not only improve the evaluation of experimental data, but will also allow for better statistical comparison of two or more populations of motor proteins (e.g. motors with distinct mutations or motors linked to different cargos).

BP 10.7 Tue 9:30 P1

Microtubule Guiding on Nano-Patterns of Molecular Motors Generated by Laser-Ablation — •Matthäus Mittasch[1,2], Cordula Reuther[1,2], Stephan Grill[1,3], and Stefan Diez[1,2] — [1]Max Planck Institute of Molecular Biology and Genetics, 01307 Dresden, Germany — [2]B CUBE - Center for Molecular Bioengineering, Technische Universität Dresden, 01307 Dresden, Germany — [3]Biotechnology Center, Technische Universität Dresden, 01307 Dresden, Germany

Molecular motors such as kinesin-1 are highly optimized biological nano-machines that can be immobilized within engineered environments for the transport of cytoskeletal filaments such as microtubules in order to miniaturize and revolutionize hybrid nano-devices. However, for many applications it is of great importance to control the direction of the filament motion on planar surfaces to fulfill unique tasks such as the specific sorting of molecular entities. Here, we demonstrate the generation of highly-localized binding sites for kinesin-1 on poly(L-lysine)-g-poly(ethylene glycol)-coated surfaces using laser-ablation. Thereby, specific patterns of molecular motors with freely programmable shapes and feature-sizes down to a few hundred nanometers were generated. Straight lines, having a width of about 500 nanometers were shown to guide microtubules reliably. Moreover, we tested the guiding behavior on curved lines to investigate experimentally, if complex patterns can be used to sort microtubules, as was recently predicted theoretically (Rupp and Nédélec, Lab Chip (12), 2012).

BP 10.8 Tue 9:30 P1

Dynamics of diffusing particles interacting with directionally moving particles on a polar filament — •Denis Johann, Debajit Goswami, and Karsten Kruse — Saarland University, Saarbrücken, Germany

During cell division, pairing of sister chromosomes and their segregation is driven by the mitotic spindle, which is a stable bipolar structure consisting of overlapping microtubules, motor and other associated proteins. However, how the interplay between these proteins and microtubules render stability to the spindle structure is still unknown. Passive mobile cross-linkers called Ase1 have been found to dynamically adapt the dynamics of microtubule sliding induced by molecular motors to the length of the microtubules' overlap region [1].

Here, we study the effect of Ase1 on molecular motors in presence of steric interactions in terms of a stochastic lattice model. We find that the Ase1 accumulate towards the end of the lattice in the direction of motor hopping and characterise their distribution through a mean-field theory.

[1] Braun et al., Nature Cell Biology 13, 1259-1264 (2011)

BP 10.9 Tue 9:30 P1

Directionality of Single Kinesin-5 Cin8 Molecules is Mediated by the Tail Domains — •André Düselder[1], Christina Thiede[1], Alice Wiesbaum[1], Vladimir Fridman[2], Dieter Klopfenstein[1], Larisa Gheber[2], and Christoph F. Schmidt[1] — [1]Georg-August-Universität, Göttingen, Deutschland — [2]Ben-Gurion University of the Negev, Beer-Sheva, Israel

Cin8, the tetrameric Kinesin-5 from budding yeast, shows the striking ability to move toward both the plus as well as the minus end of a microtubule. The molecular mechanism for this switch of directionality remains unknown. We have investigated this mechanism by examining the role of the C-terminal tail domains of Cin8 in the regulation of its directionality and motor function. To remove only the head-tail-interactions in the otherwise native tetramer, we built a Cin8 variant lacking the tail domains. In contrast to the wild type motor, this construct moves with a low velocity toward a randomly-chosen, but persistent direction. To look solely at the motility-generating subunits of Cin8, we fused the head domains and neck linker of Cin8 to the stalk of Kinesin-1 to construct a dimeric chimera. As a single molecule, this chimera is bidirectional with frequent changes in direction, indicating that the Cin8 head domains are inherently bidirectional. In optical trapping experiments, the probability of a switch of directionality increases with an increase of the force acting on the motor. We performed extensive power spectral analysis of the motor under various loads and different nucleotide conditions. Our findings suggest a force sensitive mechanism for a switch of directionality in Cin8.

BP 10.10 Tue 9:30 P1

Rotation eines Proteinkomplexes in einer Lipidmembran: Molekulardynamik-Simulationen — •Michael Becker — Universität Duisburg-Essen 47057 Duisburg

Wir befassen uns mit der Simulation des F_{0c}-Unterkomplexes der ATP Synthase und dessen Interaktion mit den Membranlipiden bei der Rotation. Zu diesem Zweck wird eine MD Simulation, bestehend aus einer Membran und des in ihr eingebetteten c-Ringes durchgeführt. Die Rotation des c-Ringes wird mittels einer gesteuerten Molecular Dynamik Simulation realisiert, wobei die Geschwindigkeit und somit der Impuls der Atome des c-Ringes alle 0.1 $[ps]$ angepasst wird. Das hieraus resultierende Drehmoment ist durch $\vec{M} = \vec{r} \times \frac{\partial \vec{P}}{\partial t}$ gegeben. Unsere Idee ist es also, dem c-Ring alle 0.1 $[ps]$ einen festen Drehimpuls $\vec{L}$ zu geben und anhand der Änderung dieses Drehimpulses, in der Zeit von eben diesen 0.1 $[ps]$, dass durch die Reibung wirkende Drehmoment mittels $\vec{M} = \frac{\vec{L}(t) - \vec{L}(t0)}{\Delta t}$ zu bestimmen, da im Gleichgewicht dieses Drehmoment dem angelegten entspricht.