AKB 14 Molecular Motors

Time: Tuesday 16:00–18:30 Room: ZEU 255

Invited Talk AKB 14.1 Tue 16:00 ZEU 255

Single-molecules at work - Deciphering the mechanism of a molecular motor — •Jens Michaelis[1,2], Yann Chemla[3], K. Aathavan[3], Thorsten Hugel[2,4], and Carlos Bustamante[3] — [1]LMU München, Department Chemie und Biochemie, 81377 München — [2]Center for Nanoscience, CeNS, LMU München, 80539 München — [3]UC Berkeley, Physics Department, Berkeley, USA — [4]TU München, Zentralinstitut für Medizintechnik, 85748 Garching

Molecular motors are extremely complex and highly evolved nanometer-sized machineries that couple the free energy liberated by a chemical reaction to provide mechanical work. Details about this so called mechano-chemical coupling will not only further our understanding of biological phenomena, but also provide clues for constructing highly efficient nano-machines.

Here, we present our recent data from a viral protein-complex that drives the translocation of DNA into a protein shell, as part of the viral life-cycle.1 Single-molecule force spectroscopy allowed us to elucidate details of the mechano-chemical cycle and identify movement steps and coupling mechanism for this motor-complex. On the other hand, structure-function relationships can be tested using single-molecule fluorescence techniques.

1 Y. Chemla, K. Aathavan, J. Michaelis, S. Grimes, P. Jardine, D. Anderson und C. Bustamante, Mechanism of force generation of a viral DNA packaging motor, Cell 122, (2005), 683-692.

AKB 14.2 Tue 16:30 ZEU 255

Measurement of the distance that walking kinesin holds its cargo away from the microtubule surface — •Jacob Kerssemakers[1], Jonathon Howard[1], Henry Hess[2], and Stefan Diez[1] — [1]MPI of Molecular Cell Biology and Genetics, Dresden, Germany — [2]University of Florida, Gainesville, USA

While much has been learnt about how the heads of kinesin step along a microtubule, little is known about the role that the rod and tail domains play in motility. The tail is thought to be involved in cargo-binding and in generating a compact, autoinhibited conformation, but the role of the rod is not known. Here, we have investigated the extension of the rod during active transport by measuring the height at which microtubules glide over a kinesin-coated surface in the presence of ATP. To perform height measurements with nanometer-precision we utilized fluorescence-interference-contrast (FLIC) microscopy, the principle of which is based on high-resolution interference effects if fluorescent objects are imaged in the vicinity of a reflecting surface. Utilizing a self-calibrating method, we determined the distance by which kinesin molecules lift gliding microtubules above the surface to be 19.3+/-0.8 nm (95% confidence). While significantly shorter than the contour length of the motor molecule, this value is consistent with the segmented structure of the molecule. We propose that the function of the rod is to hold cargo sufficiently far away from the surface of the microtubule so that transport is not interfered with by proteins bound to the microtubules or the organelles.

AKB 14.3 Tue 16:45 ZEU 255

The mitotic kinesin Eg5 is processive and chemical inhibitors can modulate its speed and run length — •Lukas C. Kapitein[1], Benjamin H. Kwok[2], Jeffrey H. Kim[2], Erwin J.G. Peterman[1], Tarun M. Kapoor[2], and Christoph F. Schmidt[1] — [1]Dept. Physics, Vrije Universiteit, Amsterdam, NL — [2]Laboratory of Chemistry and Cell Biology, The Rockefeller University, New York, NY 10021, USA

Small molecule inhibitors of kinesin-5, a protein essential for eukaryotic mitosis, represent important alternatives to anti-mitotic agents that target tubulin, a protein needed in dividing and non-dividing cells. Kinesin-5 inhibitors, like monastrol, are the only known specific inhibitors for microtubule-based motor proteins, and act through poorly understood allosteric mechanisms distinct from those utilized by ATP-derivatives. Moreover, the microscopic mechanism of kinesin-5 motility is not known. Here we characterize the motile properties of a vertebrate kinesin-5 (Eg5) in the absence and presence of monastrol, using a GFP-fusion protein in single-molecule fluorescence assays. We find that Eg5, against common belief, is a processive motor like conventional kinesin. Unlike conventional kinesin, its motility is discontinuous, switching between pause and run states. Monastrol inhibition prolongs the pause states and decreases Eg5*s speed and run length. Our data on the modulation of Eg5's mechano-chemical cycle by a cell-permeable inhibitor provide essential input for the inhibitor's use as a mechanistic probe and for its development as a chemotherapeutic agent.

AKB 14.4 Tue 17:00 ZEU 255

Application of semiconductor nanocrystals to explore molecular motors — •Bert Nitzsche, Cecile Leduc, Jacob Kerssemakers, Felix Ruhnow, Yannis Kalaidzidis, and Stefan Diez — Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden

Employing single molecule fluorescence microscopy and nanometer tracking, recent years have seen great progress in understanding cytoskeletal motors like kinesin or myosin. The used fluorescent labels were either fluorescent proteins or chemical dyes, which both exhibit only moderate brightness and suffer from photobleaching. As a result, temporal resolution and/or observation time can be very limited. Here we attached semiconductor nanocrystals - a new class of sophisticated fluorescent labels that is also called quantum dots (QDs) - to molecular motor systems. Due to their superior photophysical properties (spectacular brightness and high photostability) they have proven ideally suited to study biological (sub)systems such as motor proteins walking along cytoskeletal filaments. We demonstrate that we can combine high temporal and spatial resolution with long observation durations. Beyond this, we show new findings on the trajectories of cytoskeletal motors using QDs, which gives further insights into working mechanisms and functions of molecular motors.

AKB 14.5 Tue 17:15 ZEU 255

Force dependence of motor protein mediated filament depolymerisation — •Gernot Klein, Karsten Kruse, and Frank Jülicher — Max Planck Institute for the Physics of Complex Systems Nöthnitzerstr. 38 01187 Dresden

Many active processes in cells are driven by highly specialised motor proteins, which interact with filaments of the cytoskeleton. Members of the KIN-13 kinesin subfamily are able to induce depolymerisation of the filaments ends[1]. In the mitotic spindle, certain members of the KIN-13 subfamily, which are linked to chromosomes, facilitate pole ward-movement of chromosomes by depolymerising spindle microtubules. In this situation, motors are mechanically coupled and remove subunits under the influence of external forces. Recently we have developed a general description of motor protein induced filament depolymerisation[2]. Based on this description, we study the collective behaviour of depolymerising motor proteins, which are mechanically linked to a common anchoring point, e.g. a bead, and examine the influence of an externally-applied force on motor-induced filament depolymerisation. We find that the depolymerisation velocity can increase as well as decrease for an increasing external force before motors detach from the filament. This behaviour depends on the processivity of the motors during depolymerisation. We compare results obtained in mean-field description with discrete stochastic simulations. The situation studied in our work could be realised by in vitro experiments in which KIN-13 family members are attached to a bead and interact with a filament end. [1] A.W. Hunter, et al., Mol. Cell 11, 445 (2003) [2] G.A. Klein, et al. Phys.Rev.Lett. 94, 108102 (2005)

AKB 14.6 Tue 17:30 ZEU 255

Motor-induced filament interactions in active gels — •Karsten Kruse and Frank Jülicher — Max-Planck-Institut für Physik komplexer Systeme, Nöthnitzer Straße 38, 01187 Dresden

Motivated by active dynamic properties of the cytoskeleton, we develop a general framework for describing the dynamics of systems consisting of polar filaments and active cross-links. In the cytoskeleton, active cross-links are formed by motor proteins that are able to induce relative motion between filaments. The framework is based on an analysis of the momentum flux in the system and allows to calculate the stresses in the filament network generated by the action of motor proteins. We relate the dynamics to continuum theories of active polar gels and show that instabilities occur for sufficiently strong motor activity. We show that the instability can be either in the filament orientation or the filament density, depending on the relative values of the filaments' longitudinal and transversal friction coefficient. Finally, we relate our results to in vitro experiments.

AKB 14.7 Tue 17:45 ZEU 255

Enhanced ordering of interacting filaments by molecular motors — •Jan Kierfeld, Pavel Kraikivski, and Reinhard Lipowsky — Max Planck Institute of Colloids and Interfaces, Science Park Golm, 14424 Potsdam

We theoretically study the cooperative behavior of cytoskeletal filaments in motility assays in which immobilized motor proteins bind the filaments to substrate surfaces and actively pull them along these surfaces. Because of the mutual exclusion of the filaments, the coupled dynamics of filaments, motor heads, and motor tails leads to a nonequilibrium phase transition which generalizes the nematic-isotropic phase transition of the corresponding equilibrium system, the hard-rod fluid. Langevin dynamics simulations show that the motor activity enhances the tendency for nematic ordering. We develop a quantitative theory for the location of the phase boundary as a function of motor density. At high detachment forces of motors, we also observe filament clusters arising from blocking effects.

AKB 14.8 Tue 18:00 ZEU 255

The interplay between crosslinkers and dynamic molecular motor-induced instabilities in the moderation of biopolymer organization — •David Smith[1,2], David Humphrey[2], Falko Ziebert[3], Walter Zimmermann[3], and Josef Käs[1,2] — [1]Institute for Soft Matter Physics, Universität Leipzig, Linné Str. 5, D-04103 Leipzig Deutschland — [2]Center for Nonlinear Dynamics, University of Texas at Austin, Texas 78712, USA — [3]Physikalisches Institut, Universität Bayreuth, D-95440 Bayreuth Deutschland

Structure and function of biological cells rely on the highly-dynamic self-organization of protein filaments to an intracellular cytoskeleton responsive to mechanical and chemical stimuli. While dissolving these complex cellular structures through Brownian motion is inherently slow (tens of minutes), changes in the activity of the molecular motor myosin II cause rapid order-disorder transitions within 1-2 minutes in reconstituted cytoskeletal actin networks. When motor-induced filament sliding decreases, actin network structure rapidly and reversibly self-organizes into various assemblies triggered by a nonlinear instability. Modulation of static crosslinker concentrations allow for a wide phase space of order ranging from nematics to compact asters & dense packing of motor-filament clusters. The observed isothermal transitions between disorder and self-organization illustrate that molecular motors can substantially contribute to dynamic cellular organization.

AKB 14.9 Tue 18:15 ZEU 255

Biotemplated generation of motor protein nanotracks for the directed motion of microtubule transporters — •Cordula Reuther[1], Robert Tucker[2], and Stefan Diez[1] — [1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany — [2]University of Florida, Gainsville, FL, USA

Biological machines have recently found an increasing number of applications in hybrid bionanodevices, where they fulfill tasks of biomolecular transport and manipulation in engineered environments. For example, microtubule-based gliding motility assays have been used to transport micro- and nanometer-sized objects. Spatial control of motility is a crucial criterion for the successful implementation of these nanoscale transport systems. So far, topographic channels with selective surface chemistry have proven to yield the most efficient guiding. However, the fabrication of such structures is labour-intensive and costly, and the channel width might limit the possible size of transported cargo.

Here, we present a method to deposit submicrometer-wide tracks of motor proteins (nanotracks) on unstructured surfaces. We use microtubules themselves as biological templates for the stamping and alignment of motor proteins. Compared to other soft lithography techniques like microcontact printing our approach circumvents protein denaturation due to drying and conformational changes caused by mechanical stress. The generated structures prove very efficient for the guiding of microtubules without topographical barriers. Furthermore, our assay comprises a novel means to study biologically relevant mechanical functions (such as microtubule-microtubule sliding) in vitro.