BP 25: Posters: Molecular Motors

Time: Thursday 17:30–19:30 Location: Poster A

BP 25.1 Thu 17:30 Poster A

Cargo-regulated directionality switching of *S. cerevisiae* Kinesin-5 Cin8 — •Christina Thiede[1], Alice Wiesbaum[1], Adina Gerson-Gurwitz[2], Natalia Movshovich[2], Vladimir Fridman[2], Maria Podolskaya[2], Tsafi Danieli[2], Stefan Lakämper[1], Larisa Gheber[2], Dieter R. Klopfenstein[1], and Christoph F. Schmidt[1] — [1]Drittes Physikalisches Institut, Georg-August-Universität Göttingen, Germany — [2]Department of Chemistry, Ben-Gurion University of the Negev, Israel

In mitotic spindle morphogenesis and dynamics kinesin-5 motors fulfil essential roles as slow, processive microtubule (MT) plus-end directed sliding motors. Eg5, the kinesin-5 from *X. leavis*, switches from a diffusive to a directional mode upon cross-linking a pair of microtubules. The mechanism may be related to kinesin-1 cargo regulation effected by a back-folding of the tail. Recently Cin8, a kinesin-5 from S. *cerevisiae*, was surprisingly found to be able to switch from processive plus-end to processive minus-end motility. Here we have studied the *in vivo* and *in vitro* properties and regulation of Cin8 using single-molecule fluorescence assays. In high salt, Cin8 moved rapidly and processively towards the MT minus-end. In low salt, Cin8 was 10x slower and moved towards the MT plus-end. Phosphorylation sites located in the unique 99 amino acids insert in Cin8's loop 8 influence the switching. The most striking effect was, however, that Cin8 switched from minus- to plus-end directed motility as soon as it was located between two microtubules. This finding suggests that in Cin8 not only the mode of motility is regulated by cargo binding, but also its directionality.

BP 25.2 Thu 17:30 Poster A

The highly-processive kinesin-8, Kip3p, derails from microtubule protofilaments — •Aniruddha Mitra[1,2], Bert Nitzsche[1,2], Volker Bormuth[1,3], Felix Ruhnow[1,2], Marko Stroch[1], Burkhard Rammner[4], Jonathon Howard[1], and Stefan Diez[1,2] — [1]MPI-CBG, Dresden, Germany — [2]B CUBE, Dresden, Germany — [3]Institut Curie, Paris, France — [4]Scimotion, Hamburg, Germany

Kinesin-8 controls microtubule length based on its depolymerization activity at microtubule plus-ends preceded by highly processive motility. However, the mechanism confering high motor processivity even on crowded microtubules in the cytoplasm is not known. We therefore asked if kinesin-8 is capable of switching protofilaments during its plus-end directed motility along the microtubule lattice. We performed in vitro gliding motility assays on surfaces coated with the budding yeast kinesin-8, Kip3p, and measured the rotations of the microtubules around their longitudinal axis using quantum dots in combination with fluorescence-interference contrast microscopy and 2D nanometer tracking. We observed counterclockwise rotations with periodictities unrelated to the microtubule supertwist. Such rotations indicate that the motors do not follow the axes of individual protofilaments but rather switch between them perpetually. We hypothesize that this behaviour, which distinguishes kinesin-8 from the processive protofilament tracker kinesin-1, (i) results from a comparetively long neck linker, non-centrally attached to the motor domain and (ii) is essential for high processivity.

BP 25.3 Thu 17:30 Poster A

Studying collective motor effects by fast optical tracking of gold nanoparticles — •Wiebke Jahr, Rene Schneider, and Stefan Diez — B CUBE - Center for Molecular Bioengineering, Technische Universität Dresden

This study focuses on the usage of gold nanoparticles (GNPs) for the fast optical tracking of microtubules gliding on motor-coated surfaces. GNPs are strong light scatterers and can be functionalized to bind to microtubules via biotin-streptavidin or antibody interactions. In contrast to conventional fluorophores, GNPs show neither photobleaching, photon blinking nor emission saturation. Thus, GNPs are ideal probes to study the motion of filaments on short time scales with high precision. Their signal only depends on their radii and the incoming light intensity so that data acquisition rates are not limited by saturation effects.

In combination with the software package FIESTA [1] (Fluorescence Image Evaluation Software for Tracking and Analysis) stepping events of single and multiple kinesin-1 molecules are investigated at full ATP concentration. Moreover, the effects of roadblocks on the movement of motor proteins is studied. The insights gained from these experiments are applicable to the design of early diagnosis mechanisms for diseases, such as Alzheimer's, which is initiated by overexpression of microtubule-associated proteins, leading to blockages on the motor paths.

[1] F. Ruhnow, D. Zwicker, S. Diez, "Filament localization with nanometer accuracy", Biophys J 98, 363a-363a, (2011).

BP 25.4 Thu 17:30 Poster A

Nanometer precision in filament localization allows for precise off-axis tracking of molecular motors — •Felix Ruhnow[1,2], David Zwicker[3], and Stefan Diez[1,2] — [1]B CUBE - Center for Molecular Bioengineering, Technische Universität Dresden, Dresden, Germany — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany — [3]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany

Measuring the exact path of molecular motors, such as cytoskeletal motor proteins, on their tracks has proven to be difficult without knowing the precise location of the filaments. Up to now, off-axis stepping has therefore mostly been inferred from the tracked positions of the motors with respect to the fitted path of the motors instead of determining the filament centerline. Obviously, this limits the precision of the measurements and may lead to errors due to the sometimes complex three-dimensional structure of the filaments. We developed a filament tracking algorithm to determine the centerline position of fluorescently labeled filaments with nanometer precision. This allowed us to observe the non-parallel movement of kinesin-1 motors with respect to the microtubule centerline, which is consistent with kinesin-1 following a protofilament of a supertwisted microtubule. Combined with methods to measure nanometer heights above substrate surfaces, such as fluorescence interference contrast or parallax, our algorithm presents a promising tool for optical 3D-nanometry.

BP 25.5 Thu 17:30 Poster A

Efficiency of a tightly coupled molecular motor — •Eva Zimmermann and Udo Seifert — II. Institut für Theoretische Physik, Universität Stuttgart

Most molecular motors gain energy for their mechanical motion from the hydrolysis of ATP. We use a simple one-state-model for such a motor protein that performs a one-dimensional (discrete) stepwise motion within an aqueous solution containing non-equilibrium nucleotide concentrations. In our model, the motor protein hydrolyzes one ATP molecule per step which represents tight mechanochemical coupling.

Based on this model we numerically and within an approximation also analytically investigate dynamics and efficiency of the motor protein in the presence of an attached cargo. We are especially interested in the dependence of the motor protein's behavior on different nucleotide concentrations as well as on several internal parameters of the model. Compared with recent experiments, the model seems to capture the basic properties of such a motor protein quite well.

BP 25.6 Thu 17:30 Poster A

Coarse-grained simulations of active protein machines in biological membranes with a solvent — •mu-jie huang[1], raymond kapral[2], alexander s. mikhailov[3], and hsuan-yi chen[1] — [1]Department of Physics and Institute of Biophysics, National Central University, Jhongli 32001, Taiwan — [2]Chemical Physics Theory Group, Department of Chemistry, University of Toronto, Ontario, Canada — [3]Abteilung Physikalische Chemie, Fritz-Haber-Institut der Max-Planck-Gesellschaft, Faradayweg 4-6, 14195 Berlin, Germany.

Protein machines, cyclically changing their conformations, play a fundamental role in the living cells; many of them are found in cellular membranes. Since the cycles of the machines are in the millisecond range, approximate coarse-grained descriptions are needed. Recently, entire operation cycles of some protein machines could already be reproduced by using coarse-grained models, with solvent included. Here, we proceed further and demonstrate that dynamical cycle simulations of machines immersed into a membrane are possible. Our approach combines the elastic-network description for a machine with the multiparticle-collision modeling for the solvent and a reduced description for the lipids. Membrane-mediated synchronization of machine

cycles has been found in our simulations.

BP 25.7 Thu 17:30 Poster A

Spatio-temporal guiding of gliding microtubules by local heating on micro-structured surfaces — •Viktor Schroeder[1,2], Ivan Maximov[3], Till Korten[1,2], Heiner Linke[3], and Stefan Diez[1,2] — [1]MPI-CBG, Dresden, Germany — [2]B CUBE, Dresden, Germany — [3]nmC@LU, Lund University, Sweden

To use gliding microtubules as carriers in molecular sorting devices, methods for spatio-temporal control of transport need to be developed. Our approach is based on composite surfaces where functional kinesin motor proteins are adsorbed onto planar substrates between surface-grafted polymer chains of thermoresponsive poly(N-isopropylacrylamide) (PNIPAM). By external temperature control, we recently demonstrated [1] the reversible landing, gliding, and releasing of motor-driven microtubules in response to conformational changes of the polymer chains.

Based on recent findings that guided microtubule motility along non-topographical motor patterns is possible [2], we now aim to form switchable tracks. Specifically, we report how we apply electrical currents through micro-structured gold layers in order to locally collapse PNIPAM via Joule heating. Consequently, the kinesin motors become accessible on these tracks only and we show that motility can be guided along the routes where electrical currents are applied. We foresee future applications of this novel guiding technique for lab-on-chip devices in the fields of molecular diagnostics and bio-computation.

[1] L. Ionov et al., Nano Lett., 6, no. 9, pp. 1982-1987, (2006).
[2] C. Reuther, et al., Nano Lett., 6, no. 10, pp. 2177-2183, (2006).