BP 31: Posters: Biological Machines & Motor Proteins

Time: Thursday 17:15–20:00 Location: P3

BP 31.1 Thu 17:15 P3

Neck-linker-length dependence of processive Kinesin-5 motility — •André Düselder, Christina Thiede, Stefanie Kramer, Christoph F. Schmidt, and Stefan Lakämper — Drittes Physikalisches Institut, Georg-August-Universität Göttingen, Germany

To explore the basic motor activity of the mitotic Kinesin-5, we previously constructed a stable dimeric Kinesin-5 head/Kinesin-1 stalk chimera (Eg5Kin), which contains the motor domain and 14 amino acids of the neck linker of Xenopus leavis Eg5 fused to the neck coiled coil of Drosophila melanogaster Kinesin-1. We have here investigated the effect of varying neck-linker length on the motile properties of Eg5Kin. We generated six Eg5Kin constructs comprising of 13 to up to the 18 amino acids of the native Eg5 neck linker, possibly providing a physiological context.

Using single-molecule fluorescence, we found that all six constructs are active motor molecules capable of processive motility. In a first set of experiments, we found that the neck-linker length influences the run length, but not the velocity of the motor. We thus confirm the findings of Shastry and Hancock (2010, Curr. Biol. 20:939) with a different motor. In addition we used optical-trap assays to investigate the change in the average force the motor constructs generated and found only a small variation. Our data thus suggest that the neck-linker length of Eg5 is at least not the sole determinant for speed and force generation.

BP 31.2 Thu 17:15 P3

Tug-of-war of small ensembles of myosin II motors — •Philipp Albert, Thorsten Erdmann, and Ulrich S. Schwarz — Institute of Theoretical Physics, University of Heidelberg

Myosin II motors are non-processive and therefore have to work together in ensembles in order to generate appreciable levels of force. In the actin cytoskeleton of cells these ensembles are usually small and stochastic effects are therefore expected to be pronounced. The parallel cluster model (PCM) recently developed for small ensembles of myosin II motors takes advantage of the separation of time scales present in the myosin II hydrolysis cycle. The PCM reduces the complex network of stochastic transitions occurring in an ensemble consisting of several myosin II motors to a one-step master equation. We extend the PCM to a bipolar myosin II minifilament, resulting in a model for the stochastic tug-of-war between two non-processive motor ensembles. Stochastic simulations reveal that the movement of the bipolar minifilament can be described by a diffusive process, with a diffusion constant that depends on the size of the minifilament. In order to investigate mechanosensitivity of molecular motors, springs are added to the system as an external elastic element. For sufficiently large ensembles, increasing the stiffness results in a transition from a state with frequent detachment to an attached state.

BP 31.3 Thu 17:15 P3

Kinesin-3 (UNC-104) can act as a dimeric motor during axonal transport *C. elegans* neurons *in vivo* — •Volker Christoph Henschel[1], Alessandro Esposito[2], Christoph Friedrich Schmidt[1], Fred Sylvester Wouters[3], and Dieter Robert Klopfenstein[1] — [1]Drittes Physikalisches Institut, Biophysics, Georg-August-University Göttingen, Göttingen, Germany — [2]MRC Cancer Cell Unit, Hutchison/MRC Research Centre, Cambridge, UK — [3]Laboratory of Cellular and Molecular Systems, Department of Neuro- and Sensory Physiology, Georg-August-University Göttingen, Göttingen Germany

Monomeric Kinesin-3 (UNC-104) is responsible for the transport of presynaptic vesicles to synaptic termini in *C. elegans*. To investigate the role of the endogenous coiled-coils, we introduced point mutations in the motors coiled-coil region in the neck promoting either dimer formation of Kinesin-3 or reducing the likelihood of dimerization. We verify dimerization by cross-linking of purified truncated motors *in vitro*. We show by live *in vivo* imaging, that reducing dimerization of Kinesin-3 leads to decreased vesicle transport velocities and affects the control of muscle contraction. *C.elegans* with reduced dimerization properties exhibit a 45% reduction in anterograde velocity. Additionally, severe motility and a significant egg laying defect are observed. To assess dimer formation *in vivo* we combine Foerster Resonance Energy Transfer (FRET) and anisotropy imaging with spinning-disc laser confocal microscopy. Our data suggest a direct link between dimerization status and transport velocities.

BP 31.4 Thu 17:15 P3

A tetrameric chimera made from a kinesin-1 and a kinesin-5 shows interesting motility properties — •Alok D. Wessel, Christina Thiede, Stefan Lakämper, Stefanie Reiter, and Christoph F. Schmidt — Drittes Physikalisches Institut, Georg-August-Universität Göttingen, Germany

X. laevis Eg5 is a mitotic kinesin-5 that drives relative sliding of antiparallel microtubules (MT) by the processive action of its two opposing sets of dimeric motors. In order to obtain a tetrameric model system with clearly defined properties and motile phases, we have constructed a tetrameric chimera by replacing the Eg5-motor domain and neck-linker by the homologous regions of D. melanogaster Kinesin 1 (DK4mer).

In surface-gliding assays, Dk4mer showed fast motility (553 +- 31 nm/s). Single GFP-tagged DK4mer motors moved processively along the MT at comparable speeds (499 +- 3 nm/s). We observe clearly distinguished directional and diffusional episodes and an overall run length of ~9 microns on average. We further performed relative sliding assays using DK4mer and polarity labeled MTs and show that DK4mer is capable of sliding MT apart simultaneously using both pairs of motor domains. The exact sliding speed was found to depend on ionic conditions. Direction of sliding appeared to alternate. This phenomenon could be due to additional surface-bound motors, to 1D diffusional motion or to an unlikely reversion of motor direction.