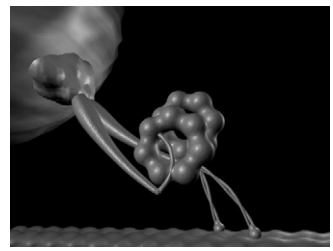
3181-Plat
Direct Measurement of the Force-Velocity Relationship for Multiple Kinesin-1 Motors by Magnetic Tweezers
Veikko F. Geyer, Stefan Diez.
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.
Transport of intracellular cargo is known to be achieved by the concerted operation of multiple motor proteins. However, force generation by multiple motors remains a matter of debate even though the load-bearing properties of single motors have intensively been characterized by various in vitro assays. Here, we report a novel assay to study cooperative transport in the presence of external load. In particular, we designed a magnetic-tweezers setup that is capable of exerting horizontal forces of up to 100 pN on superparamagnetic beads that are attached to microtubules gliding on a surface coated with kinesin-1 motors. Dependent on the magnitude and direction of the applied load, we demonstrate the redirection, stalling and backward slipping of moving microtubules. Moreover, for transport events involving less than 10 motors, we precisely determine the force-dependent gliding velocities from the fluorescent signal of the magnetic bead using an automated tracking algorithm. At constant load, we observe velocity-steps which we hypothesize to result from transitions in the number of engaged motors. At variable load, i.e. by monotonically increasing or decreasing the magnetic force, we directly measure force-velocity curves during multi-motor transport. Our method, which allows the characterization of a dynamic multi-motor system in terms of forces and velocities, is expected to elucidate general properties of intracellular cargo transport by a small number of kinesin-1 or other microtubule motors.

3182-Plat
A Little Motor, a Big Switcher! Bidirectional Membrane Tube Movement Driven by Collections of Nonprocessive Motors
Paige M. Shaklee[1,2], Line Bourel-Bonnet[3], Marileen Dogterom[2], Thomas Schmidt[1].
[1]Leiden University, Leiden, Netherlands, [2]AMOLF, Amsterdam, Netherlands, [3]Equipe de Biovectorologie, Illkirch, France.
Motors proteins are essential players in intracellular transport, often working in groups to move cargo across the cell. Yet how multiple motors coordinate to mediate cargo movement is still unclear. Inspired by the motor-driven network of the endoplasmic reticulum, we examine the organization and transport of membrane material by active motors in a minimal model system using Giant Unilamellar Vesicles (GUVs) as a membrane reservoir. We find that motors attached to the outside of a GUV, in the presence of microtubule (MT) tracks, collectively exert forces large enough to deform the GUV and extract membrane nanotubes. Processive kinesin motors (motors that take many steps before falling off a MT) had previously been shown to extract membrane tubes, but surprisingly, nonprocessive ncd motors (motors that only take a single step before falling from a MT) can also mediate membrane tube formation. Moreover, tubes formed by nonprocessive motors show distinct phases of persistent growth, retraction, and an intermediate phase characterized by dynamic switching between the two. We probe the physical mechanism by which nonprocessive motors collectively mediate membrane tube dynamics with image correlation spectroscopy and fluorescence recovery after photobleaching. Nonprocessive motors at the interface between the underlying MT track and the membrane tube cargo show a diffusive behavior with a diffusion constant 1000 times smaller than that of a freely-diffusing lipid-motor complex. We interpret the small diffusion constant as an indicator that nonprocessive motors dynamically bind and unbind to the MT in order to maintain a continuous interaction between the membrane tube and MT. We consequently develop a model that describes the membrane tube dynamics through a balance between motor density fluctuations and membrane tube tension [Shaklee et al PNAS 2008; Biophys J accepted].

3183-Plat
ATP Hydrolysis in Eg5 Kinesin Involves a Catalytic Two-Water Mechanism
Courtney L. Parke, Edward J. Wojcik, David K. Worthylake, **Sunyoung Kim**.
LSU Health Sciences Center, New Orleans, LA, USA.
Motor proteins couple steps in ATP binding and hydrolysis to conformational switching both in and remote from the active site. In our kinesin• AMPPNP crystal structure, closure of the active site results in structural transformations for appropriate microtubule binding and organizes an orthosteric two-water cluster. We conclude that a proton is shared between the lytic water, positioned for gamma-phosphate attack, and the second water that serves as a general base. To our knowledge, this is the first experimental detection of the catalytic base for any ATPase. Deprotonation of the second water by switch residues likely triggers subsequent large-scale structural rearrangements. Therefore, the catalytic base is responsible for initiating nucleophilic attack of ATP and for relaying the positive charge over long distances to initiate mechanotransduction. Coordination of switch movements via sequential proton transfer along paired water clusters may be universal for NTPases with conserved active sites, such as myosins and G-proteins.

3184-Plat
A Coupling of Structural and Kinetic Models Reveals the Stepping Mechanics of Dynein
Denis Tsygankov, Adrian W.R. Serohijos, Nikolay V. Dokholyan, Timothy C. Elston.
University of North Carolina, Chapel Hill, NC, USA.
Using coarse-grained structural model for two-headed dynein and a hybrid of molecular dynamics and Monte Carlo simulations, we explore the mechanical properties of dynein stepping, including: a structurally biased search for binding sites, step-size distribution, mechanical modulation of transition rates, and the dependence of these features on the orientation and flexibility of the motor's interaction with the microtubule. By simulating the processive motion of dynein, we demonstrate the consistency of our structural model and earlier kinetic studies (JCP 130, 025101) with experimental observations.



3185-Plat
Cytoplasmic Dynein Travel Cut Short by a Neurodegenerative Mutation in its Tail
Jing Xu[1], Kassandra M. Ori-McKenney[2], Steven P. Gross[1], Richard B. Vallee[2].
[1]Department of Developmental and Cell Biology, University of California, Irvine, Irvine, CA, USA, [2]Department of Pathology and Cell Biology, Columbia University, New York, NY, USA.
Cytoplasmic dynein is a minus-end directed microtubule motor responsible for cellular functions including fast retrograde axonal transport. The Loa mouse strain carries a mutation within the tail domain of the dynein heavy chain gene, and develops progressive motor neuron degeneration similar to amyotrophic lateral sclerosis, associated with a decreased rate of retrograde axonal transport. To understand the molecular basis for transport impairment and its role in motor neuron degeneration, we have conducted a detailed biochemical and biophysical study of purified mutant and wild-type mouse dynein. The mutant dynein was identical in subunit composition to the wild type protein but showed mild, but reproducible dissociation during sucrose gradient centrifugation (Ori-McKenney et al., 2009, MBC abstr., in press). ATPase activity for the mutant dynein exhibited a higher Km for microtubules, and the mutant's microtubule binding was reduced in the presence of ATP. Using optical trap and quantum dot assays, we found that Loa mutation drastically reduced the single motor processivity of dynein without affecting its velocity or force production. Surprisingly, small increase in buffer ionic strength further exaggerated the difference between the mutant and wild-type travel. Our analysis of motor motion under load showed no difference in the stepping behavior between mutant and wild-type dynein. We propose that the Loa mutation introduces defects in the interhead communication leading to decreased processivity, possibly via weakened heavy chain-intermediate chain interaction. Our study provides the first indication that a tail mutation can affect dynein function. Furthermore, our results provide the first link between altered processivity and disease. Supported by RO1GM070676, AHA0825278F, GM47434, and the CUMC MNC.
Authors Jing Xu and Kassandra M. Ori-McKenney contributed equally to this work. Steven P. Gross and Richard B. Vallee are co-senior authors.

3186-Plat
3D Arrangement, Conformation and ATP-Induced Structural Change of Inner and Outer Dynein Arms Revealed by Electron Cryo-Tomography
Takashi Ishikawa[1], Khanh Huy Bui[1], Tandis Movassagh[1], Hitoshi Sakakibara[2], Kazuhiro Oiwa[2].
[1]ETH Zurich, Zurich, Switzerland, [2]KARC, NICT, Kobe, Japan.
Axonemal dyneins form inner and outer arms, cause sliding among nine microtubule doublets to generate bending motion of eukaryotic flagella/cilia. However, it is not clear how the linear motion of dynein is integrated into well-coordinated bending. To understand the mechanism of flagellar/ciliary bending motion, we reconstructed 3D structure of inner and outer dynein arms at various nucleotide states using the technique of electron cryo-tomography and single particle averaging. Our averaged tomogram visualized three heavy chains of outer arms