3181-Plat
Direct Measurement of the Force-Velocity Relationship for Multiple Kinesin-1 Motors by Magnetic Tweezers
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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
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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
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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
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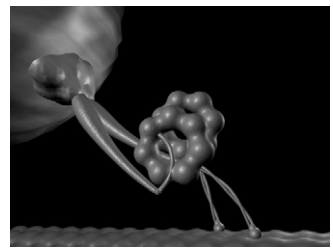
Using coarse-grained structural model for two-headed dynein and a hybrid of molecular dynamics and Monte Carlo simulations, we explore the mechanical properties of dynein stepping, including: a structurally biased search for binding sites, step-size distribution, mechanical modulation of transition rates, 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].
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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
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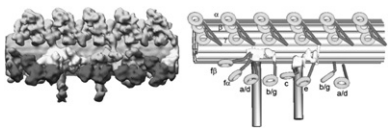
Axonemal dyneins form inner and outer arms, cause sliding among nine microtubule doublets to generate bending motion of eukaryotic flagella/cilia. However, it is not clear how the linear motion of dynein is integrated into well-coordinated bending. To understand the mechanism of flagellar/ciliary bending 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

dyneins from *Chlamydomonas* stack vertically, while eight inner arm dyneins make a horizontal array (Ishikawa et al. (2007) JMB; Bui et al. (2008) JCB) (figure). We also found that the arrangement of inner dyneins and other linkers is not symmetrical among nine microtubule doublets (Bui et al. (2009) JCB). By further image analysis we revealed the shift of the ATPase head of dynein toward the tip of flagella during Pi release. The orientation of the coild-coil stalk is constant. This shift can winch adjacent microtubule. Interestingly apo and nucleotide-bound forms of dynein coexist and they make clusters in flagella, which could explain torsion for bending.



Platform AW: Protein Folding & Stability

3187-Plat
Computer Simulation Models of Protein Stabilization by Osmolytes
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Osmolytes are intracelular organic compounds that protect protein against unfolding in the presence of environmental stresses such as high temperatures, desiccations, or the presence of denaturants. In this work we examine the physics of protein stabilization by osmolytes with modified Go models. The reference Go model is a high-resolution Go model of the 20-residue Trp-cage protein (Linhananta et al., J. Chem. Phys. 122: 114901, 2005). Previously we showed that the Go model Trp-cage in vacuum exhibits cooperative behavior with a scaled folding temperature of $T^* = 4.0$. The model is generalized by immersing the protein in solutions of spherical solvent molecules whose interactions with the protein, controlled by the protein-solvent contact energy parameter, are adjusted to mimic the effects of osmolytes and urea solutions. For osmolyte solutions we setto mimic the repulsive interaction between osmolytes and proteins. Simulations of the models found the scaled folding temperature increases from the reference model value of $T^* = 4.0$ to $T^* > 5.5$. This demonstrates thermal stabilization by osmolytes, since the Trp-cage remains folded up to higher temperatures. We performed analysis to show that this stabilization arises from the osmolyte ability to reduce the entropy and free energy of unfolded states of proteins. Finally we calculate the cooperativity measure of the models to show that this stabilization occurs without any loss in cooperativity of the Go model protein.

3188-Plat
Citrate Binding Stabilizes Human Gamma-Crystallin to Slow Unfolding and Inhibit Aggregation
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Recent studies have demonstrated that small molecules can bind destabilized or aggregation prone proteins to prevent unfolding and aggregation. Cataract, the leading cause of blindness worldwide, is caused by aggregation of proteins in the eye lens. The most abundant proteins in the eye lens belongs to the βγ-Crystallin superfamily, which accounts for 90% of lens protein composition. These proteins are synthesized in utero and must remain stable and soluble throughout life. Damage, or deleterious post-translational modification can destabilize these proteins and induce aggregation-prone conformations. Sodium citrate has been shown to prevent unfolding and aggregation of alpha-antitrypsin by stabilizing secondary structure. In this study, we demonstrate the effects of citrate binding on both wild-type and disease models of Human γD Crystallin. Equilibrium unfolding-refolding experiments show an increase in the ΔG of unfolding with increasing concentrations of sodium citrate, while kinetic experiments show that sodium citrate slows the rate of unfolding in denaturant. UV resonance Raman spectroscopy has been used to examine Trp residues in the protein and monitor vibrational modes as a function of temperature. Preliminary results indicate a resistance to unfolding in the presence of citrate. The effect does not appear to be due to metal ion chelation, and may reflect direct binding to the crystallins, as with anti-trypsin.

3189-Plat
Peptide Folding on Peptide Amphiphile Micelles Determines Micelle Structure and Assembly
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Covalent attachment of a hydrophobic tail to a peptide produces chimeric molecules termed peptide amphiphiles (PAs). Difference in aqueous solubilities between the alkyl tails and peptide headgroup drives PA self-assembly in aqueous environment. We here show that self-assembly further induces peptide folding into secondary structure motifs because of peptide crowding in the micelle corona. Furthermore we present results to the effect that the type (alpha helix Vs beta sheets) and extent of folding is controlled by the chemistry of the linker between tail and the peptide headgroup.
We have prepared a series of peptide amphiphiles consisting of 1) a palmitic tail, 2) a bioactive, 16-amino acid peptide and 3) linkers differing in H-bonding potential, length and hydrophilicity. Circular dichroism and fluorescence spectroscopy were used to monitor shifts in secondary structure while dynamic light scattering, AFM and cryo-TEM provided information on supramolecular structure.
Our results demonstrate that H-bonding availability and linker length are determining factors for peptide folding upon PA self-assembly into worm-like micelles. Alpha helical content present in the control PA (direct amide bond linkage between peptide and tail) decreased with increasing ethylene oxide linker length. Instead, inclusion of 4 alanines as a linker promoted beta-sheet formation.
These changes in PA structure had an effect on micelle length and flexibility. Additionally, in presence of divalent Mg2+ ions elongation or stacking of worm-like structures was observed, when alpha helices and beta sheets were formed respectively.
Our results provide insight on the mechanisms through which headgroup structuring occurs on peptide-based micelles, with implications on the bioactivity, stability and morphology of the self-assembled entities.

3190-Plat
Millisecond Timescale, Atomistic Protein Folding Simulations Yield a Network Theory for Protein Folding
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Understanding protein folding is a classic grand challenge in molecular biophysics; a solution for which could have immediate medical benefits, particularly for protein misfolding diseases like Alzheimer's. Molecular Dynamics (MD) simulations have the potential to provide quantitative models of protein folding but, unfortunately, this potential has yet to be fully realized due to the need to capture long-timescale transitions at atomic resolution. Taking advantage of a new theory for molecular kinetics and the computational power of Graphics Processing Units (GPUs), however, we are now able to reach millisecond timescales at atomic resolution (one million times longer than conventional simulations). But, how can one use these simulations to gain insight? We present a novel network theory which is capable of quantitative prediction of the native states and folding timescales for the villin headpiece and NTL9, which fold on microsecond and millisecond timescales respectively. Furthermore, it leads to experimentally testable hypotheses about the nature of protein free energy landscapes and how proteins fold so quickly. We also reduce these concepts to simpler and more fundamental, humanly comprehensible networks that capture the essence of molecular kinetics and reproduce qualitative phenomena like apparent two-state folding. Models at both the quantitative and qualitative levels are crucial for gaining an intuition for molecular kinetics and for ultimately answering the general question of ''how do proteins fold?''

3191-Plat
Universal Convergence of the Specific Volume Changes of Globular Proteins Upon Unfolding
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Both pressure and temperature are important environmental variables, and in order to obtain a complete understanding of the mechanisms of protein folding, it is necessary to determine how protein stability is dependent on these fundamental thermodynamic parameters. Although the temperature dependence of protein stability has been widely explored, the dependence of protein stability on pressure is not as well studied. In this paper, we report the results of the direct thermodynamic determination of the change in specific volume ($\Delta V/V$) upon protein unfolding, which defines the pressure dependence of protein stability, for five model proteins (ubiquitin, eglin c, ribonuclease A, lysozyme, and cytochrome C). We have shown that the specific volumetric changes upon unfolding for four of the proteins (ubiquitin, eglin c, ribonuclease A, lysozyme) appear to converge to a common value at high temperature. Analysis of various contributions to the change in volume upon protein unfolding allowed us to put forth the hypothesis that the change in volume due to hydration is very close to zero at this temperature, such that $\Delta V/V$ is defined largely by the total volume of cavities and voids within a protein, and that this is a universal property of all small globular proteins without prosthetic groups. To test this hypothesis, additional experiments were performed with variants of eglin c that had site-directed substitutions at two buried positions, in order to create an additional cavity in the protein core. The results of these experiments, coupled with the structural analysis of cytochrome c showing a lower packing density compared to the other four proteins, provided further support for the hypothesis. The deviation of the high-temperature ΔV value from the convergence value can be used to experimentally determine the size of the excess cavities in proteins.