KCNE transmembrane peptides are a family of modulatory β-subunits that assemble with many voltage-gated K+ channels, producing physiologically relevant complexes with a diverse array of potassium currents. All five KCNE transcripts have been found in cardiac and other tissues, raising the possibility that two different KCNE peptides can assemble with the same K+ channel to form a heteromeric complex. To determine whether heteromeric KCNE-K + channel complexes form, we developed an electrophysiologically compatible technique that employs a derivatized scorpion toxin that irreversibly inhibits K+ channel complexes that contain a specific KCNE peptide. Using this KCNE sensor and two-electrode voltage-clamp recordings, we measured the electrical output from heteromeric KCNQ1/KCNE K+ channel complexes, which revealed a hierarchy in KCNE modulation of KCNQ1 channels. Moreover, our results demonstrate that KCNQ1/KCNE1/KCNE4 complexes generate a slowly activating current that has been previously attributed to homomeric KCNQ1/KCNE1 complexes, providing a potential functional role for KCNE4 peptides in the heart.

1815-Plat Sodium Coordination Site Reveals A Novel Sodium-sensitive Phenotype In A Kir Channel

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Kir channels are important in setting the resting membrane potential and modulating membrane excitability. A common feature of Kir and many other ion channels that has emerged in recent years is that they all require the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) for activation. Residues in both the C- and N- termini affect PIP_2 binding and/or activation of Kir channels.

Among these, Kir3 channels are activated by PIP_2 only in the presence of gating molecules such as the beta gamma subunits of G proteins or intracellular sodium that enhance channel-PIP_2 interactions. The Na^+ mechanism of Kir3.1/Kir3.4 activation shown to operate during Na^+ accumulation, was suggested to be involved in the direct electrophysiological effects of cardiac glycosides, drugs widely used in heart failure or for improvement of the inotropic state of the heart.

We have identified the coordination site of Na^+ in Kir3 channels to be located in the C-terminus in the vicinity of a conserved arginine (R225 in Kir3.4) that has been shown to affect channel-PIP_2 interactions. Via mutagenesis of residues, which are a part of the coordination site, we have been able to remove sodium sensitivity in Na^+-sensitive Kir channels, and introduce sodium sensitivity in a Na^+-insensitive Kir channel. Based on the residues involved in the coordination site, we have identified a novel sodium-sensitive phenotype in yet another Kir channel. Experimental data obtained by recording macropatch activity of the channel expressed in Xenopus oocytes and mutagenesis experiments confirmed its sodium sensitivity.

1816-Plat A pH-Sensing Residue in the S1-S2 Loop Modulates Ion Selectivity of *Candida albicans* TOK, an 8TM-2P K^+ Channel

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Candida albicans is a polymorphic human fungal pathogen that causes fatal infections in immunocompromised individuals. Changes in pH are an important environmental stimulus for morphological differentiation associated with candidal virulence (Davis, 2003. *Curr. Genet.* 44; 1–7). We previously described CaTOK to be structurally and functionally similar to its *Saccharomyces* forebear: a K^+-selective, outward-rectifier whose threshold for ion flux is sensitive to the transmembrane K^+ gradient. Here, we assessed CaTOK regulation by extracellular pH (pH_o). CaTOK current magnitude was augmented in dose-dependent fashion by pH_o such that acidification from 7.6 to 5.0 induced a two-fold increase in peak current (pK_a ~6.5, Hill = 1.0). Furthermore, acidic pH_o shifted CaTOK current reversal potential (E_{REV}) by ~12 mV towards E_K. This hyperpolarizing shift was also observed in the voltage of half-maximal activation ($V^1/_2$) obtained from normalized conductance-voltage relationships ($\Delta V^1/_2$ ~24 mV). E_{REV} measurements with various external ionic conditions support the notion that changes in current magnitude with lowered pH_o result from increased selectivity for potassium over sodium. Neutralization of a single extracellular histidine following the first transmembrane domain (H144N) virtually abolished all pH_o effects. As predicted, mutation to lysine (H144K) produced changes analagous to those observed when the wild type channel was exposed to pH_o 5.0 (ΔE_{REV} ~23 mV and $\Delta V^1/_2$ ~38 mV towards E_K) whereas mutation to aspartate (H144D) led to positive shifts in E_{REV} and $V^1/_2$ of ~6 mV and ~15 mV respectively. The mechanism whereby protonation of H144 confers alterations in ion selectivity is under investigation. The findings suggest CaTOK may be important to pH-induced responses of *Candida albicans*.

Platform AP: Kinesin & Dynein Family Proteins

1817-Plat Binding and Motility of Kinesin Resolved by Atomic Force Microscopy

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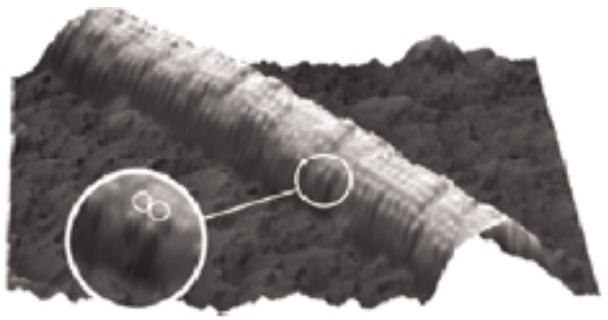
Motor proteins of the kinesin family move actively along microtubules to transport cargo within cells. How exactly a single motor proceeds on the 13 narrow "lanes" or protofilaments of a microtubule remains unknown because the required resolution lies beyond the reach of light microscopy. We have here succeeded to image

kinesin-1 dimers immobilized on microtubules with single-head resolution (see figure) and, in addition, to image single Kinesin-1 dimers in their motion along microtubules with nanometer resolution by Atomic Force Microscopy (AFM).We found that Kinesin-1 dimers:

(i) bind non-cooperatively when décor-ating protofilaments,
(ii) bind to the microtubule with both heads for the major part of the chemical cycle,
(iii) bind to a single protofilament, instead of straddling two, and remain on this track during processive movement.



1818-Plat Experimental Evidence for the Role of Cover-Neck Bundle Formation in the Kinesin Power Stroke

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Despite substantial progress in structural studies and single-molecule motility experiments, current understanding of the molecular details for how kinesin translates ATP binding and ATPase activity to a power stroke has been limited to studying conformational changes in the neck linker and relied mostly on conceptual models. Recent atomistic-level structural analyses coupled with molecular dynamics simulations have revealed a novel mechanism for the force generation in kinesin motility (Hwang, Lang, and Karplus, *submitted*). The simulations implicate the motor head's N-terminal *cover strand*, a previously unstudied short segment, as a crucial element. Specifically, the cover strand is thought to form a β-sheet with the neck linker upon ATP binding (*cover-neck bundle*), creating a conformational bias that drives the neck linker forward, which leads to its subsequent latching to the motor head. We investigate this hypothesis through optical trapping motility measurements of cover strand mutants in comparison to wild-type kinesin. The mutants were designed to have impaired ability to form the cover-neck bundle, and our measurements indeed demonstrate reduced walking speed and stall force. This result supports the proposed role of the cover-neck bundle formation as a force generating mechanism in kinesin motility and provides insight into the longstanding, nresolved structural details underlying kinesin's mechanochemical amplification.

1819-Plat Probing One and Two-Head Bound States Using a Kinesin-2 Homodimeric Construct

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Of the 14 kinesin families, Kinesin-2 motors are unique in having two different motor domains. These motors are involved in intraflagellar transport as well as cytoplasmic transport of melanosomes. Mechanistic differences between Kinesin-1 and Kinesin-2 motors may provide insights into their underlying hydrolysis cycles. We used an optical tweezer to manipulate polystyrene beads functionalized with single Kinesin-1 or Kinesin-2 motors, and measured run lengths and velocities under minimal loads. Mouse KIF3A/B was compared to homodimeric chimeras, KIF3A/A and KIF3B/B and to conventional Kinesin-1 motors. At saturating ATP, KIF3A/B moved at 525 ± 125 nm/s and the homodimers moved at similar speeds, while Kinesin-1 moved at 702 ± 136 nm/s. The run lengths of all three KIF3 motors were approximately 600 nm, while the run length for Kinesin-1 was three-fold higher. Similar performance of GFP-labeled motors were observed in single-molecule TIRF assays. When ATP concentrations were reduced from 1 mM down to 1 μM, Kinesin-1 run lengths were constant, consistent with previous reports. This implies that Kinesin-1 waits in the same mechanochemical state regardless of the time it takes for ATP to bind. In contrast, the run length of KIF3A/A increased nearly three-fold when the ATP was lowered from 1 mM ATP to 1 μM. This implies that during the time the motor waits for ATP to bind at limiting ATP levels, the motor transitions to a different chemomechanical state, resulting in a lower probability of detachment following ATP binding. One interpretation of this ATP dependence is that at saturating concentrations, ATP binds to the motor while both heads are bound to the microtubule. This result puts constraints on the kinetic cycles for Kinesin-1 and Kinesin-2 motors.

Supported by NIH.

1820-Plat Illuminating The Way Kinesin-1 Walks Using FRET Between The Motor Domains

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Kinesin-1 is a motor protein that walks processively along microtubules in a hand-over-hand manner driving intracellular transport of vesicles and organelles. Each step of 8 nm requires the hydrolysis of one ATP and takes about 10 ms at cellular ATP concentrations. Key aspects of kinesin's walking mechanism are not fully understood. One important question concerns the configuration of the two motor domains during processive motion. In particular it is unclear what the relative position and orientation of the domains are when one is waiting for ATP to bind. Are both bound to successive binding sites, or is only one bound and the second in another fixed orientation or mobile?

Here, we use a novel assay based on single-molecule confocal fluorescence microscopy to characterize Kinesin-1's stepping mechanism *in vitro*. A key advantage of our approach over conven-

tional wide-field methods is that our time resolution is far better, less than 100 μs. We apply this approach to isolated kinesin constructs that are labeled with a donor fluorophore on the one motor domain and an acceptor on the other and we follow the conformational changes and distances of the motor domains during stepping with Förster Resonance Energy Transfer. We use kinesin construct with dye molecules attached to different sites of the motor domain. Our results provide a detailed insight in the exact choreography of both motor domains during kinesin's processive motion.

1821-Plat Mechanisms of Kinesin-1 Regulation and Coordination

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The kinesin-1 motor protein has a well-established mechanism of movement, but relatively little is known about how it starts or stops. In the absence of cargo, kinesin-1 adopts a regulated, folded conformation having very low ATPase and microtubule-binding activity. In this conformation the conserved IAK sequence in the tail inhibits kinesin's initial microtubule-stimulated ADP release, preventing full-length kinesin from engaging in ATPase activity or movement on microtubules in the absence of cargo. The tail also has regulatory activity on kinesin heads that are bound to microtubules. In motility assays, the presence of kinesin tails slows and frequently stalls movement.Our experiments have produced evidence for a direct interaction between the kinesin head and tail. We site-specifically cross-linked kinesin heads and tails in solution and visualized the conformation of a kinesin head-tail cross-link on microtubules by cryo-EM. The tail interacts with the α3/Switch I region of the head, positioning the IAK sequence where it may stabilize bound ADP in the head. This regulatory interaction could potentially enable both a fully regulated state for kinesin in solution and an idle state for kinesin on microtubules. To determine the mechanism of kinesin regulation, we are performing experiments to visualize the movements of mutant kinesins within an intact bidirectional transport system in live S2 cells. We currently have a structural picture of kinesin motility. Combined, these experiments will enable a structural understanding of kinesin regulation and coordination.

1822-Plat The Effect of Randomness on the Collective Behavior of Kinesin-1

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The motor protein kinesin-1 converts the energy from ATP hydrolysis and Brownian motion into directed movement. There is increasing evidence suggesting that several kinesin-1 motors cooperate to transport a cargo. Recent experiments also suggest that the collective behavior of kinesin differs significantly from single-molecule behavior. Previously, we developed a mechanistic model that describes the mean transient and steady state behavior of kinesin-1. A transient description is essential when studying the behavior of multiple coupled kinesin molecules, as interactions between motors result in time-varying loads imposed on each individual motor. Recently, we extended our mechanistic model to include stochastic chemical kinetics, allowing investigation of the effect of randomness on collective transport. The stochastic chemical kinetics are modeled through the single-molecule Michaelis-Menten equation, derived by Kou et al. (J. Phys. Chem. *109*:19068). We tailored metrics to characterize the dynamics of nonlinear, non-smooth, stochastic systems such as motor proteins. These metrics include the complex order parameter, correlation dimension, and proper orthogonal decomposition. Our simulations suggest that stochasticity in the chemical kinetics has a significant impact on the collective dynamics. The coupling between motors through the cargo acts to drive the motors towards a synchronized state. However, the randomness of the ATPase cycle of each motor, resulting in a stochastic dwell time between steps, perturbs the motors away from this synchronized motion. Therefore, the degree of synchronization increases with load and cargo linker stiffness, but motors never reach a completely synchronized motion. Instead, stochasticity drives fluctuations around a synchronized state.

1823-Plat Force Measurements and Submillisecond Tracking of Dynein- and Kinesin-Driven Cargoes in Living Cells

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Although recent particle tracking studies in living cells have revealed individual steps of molecular motors, force measurements were not conducted despite their necessity for making mechanistic claims about kinesin- and dynein-driven active transport. Here, we demonstrate a label-free, high time resolution optical manipulation assay for simultaneous tracking and trapping of actively transported organelles in living cells. Using a quadrant detector and piezo stage-based positional and force feedback, we can detect individual steps at constant force (0–5 pN) and measure the stall forces for single and multiple motor-driven cargoes with $<$150 us time resolution and nanometer spatial precision. This assay allows us to address the controversial relationship between the number of motors and velocity, the force-dependent step size of dynein, and the behavior of multiple motor-driven cargoes in the cellular environment.

1824-Plat The Heterodimeric Axonemal Dynein-f : Its Mechanical and Enzymatic Properties and Possible Roles in Beating of Flagellar Axonemes

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The relation between microtubule landing rate and surface density of dynein-f were well fitted by the first-power dependence, as expected for a processive motor. To study this processivity further, we observed single dynein-f molecules labeled with quantum dots at

their tail domains under a total internal reflection fluorescence microscope. Although dynein-f occasionally showed one-dimensional diffusion, imaging of the quantum dot with nanometer precision indicated processive stepping behavior of individual dynein-f molecules mainly with 8 nm step and without large steps (>24nm). In an axoneme, this slow and processive dynein-f could impede microtubule sliding driven by other faster dyneins. To obtain further insights into the *in vivo* roles of dynein-f, we measured the sliding velocity of microtubules driven by a mixture of dyneins -c and -f at various mixing ratios. The velocity was modulated as a function of the ratio of dynein-c in the mixture. This modulation suggests that dynein-f acts as a load in the axoneme, but forces pushing dynein-f molecules forward seem to accelerate their dissociation from microtubules.

Platform AQ: Molecular Mechanics & Force Spectroscopy

1825-Plat Probing Equilibrium Binding Energies With Chemical Force Microscopy

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Force spectroscopy experiments reveal a wealth of kinetic behaviors exhibited by individual intermolecular bonds under external loading. While the conventional description of force spectroscopy has focused on probing kinetics the bond rupture under non-equilibrium fast loading conditions, using bond rupture measurements as a way to probe equilibrium potential energy surfaces remains virtually unexplored. We use an analytical approach and numerical simulations to show that measuring rupture forces as a function of the probe stiffness in the near-equilibrium regime could provide an estimate of the free energy of bond dissociation. We have tested this prediction in the experiment using chemical force microscopy measurements of the interactions between a small number of well-defined functional groups, and show that these measurements can estimate the free energy of an individual hydrogen bond. In another application we have used chemical force microscopy to measure the strength of the interactions of single chemical functional groups with the sidewalls of vapor-grown individual single-wall carbon nanotubes. We show that it is possible to combine the results of ab initio calculations and the rigorous kinetic description of the force spectroscopy experiment to predict binding force distributions for a single molecular contact that match the experimental results. Interestingly, our analysis reveals the important role of molecular linkage dynamics in determining interaction strength at the single functional group level.

1826-Plat Effects of Multiple Bonds Rupture in Force Spectroscopy Measurements of Interactions between Biotin and Streptavidin

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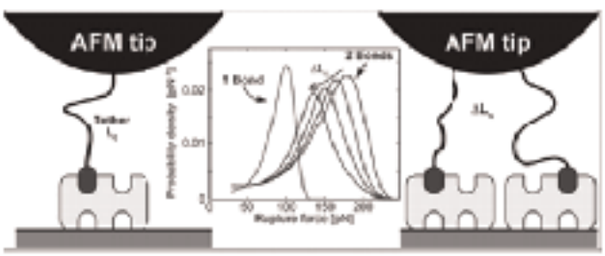
Force spectroscopy is becoming a widespread experimental tool in biophysical research. Often it has been used to uncover molecular details of protein folding and receptor-ligand interactions. Kinetic parameters that include dissociation rate and distance to the activation barrier are commonly extracted from experiments. Accuracy of these parameters depends on the single-molecular nature of the measured rupture forces. In the atomic force microscopy (AFM) studies of receptor-ligand pairs it is often difficult to ensure that the measured rupture forces correspond to interactions between single molecules. Moreover, the distribution of rupture forces in AFM measurements frequently does not follow the distribution predicted based on the single-molecule models and complicated kinetic theories have been proposed to explain this discrepancy. Here we propose that the deviation from the single-molecule distribution of rupture forces comes from occasional ruptures of double-bonds that occur during one rupture event. An analytical theory that takes into account difference between lengths of the tethers that attach ligands to AFM probes is developed and applied to successfully explain the distribution of the measured rupture forces between biotin and streptavidin molecules.



1827-Plat Direct Detection of Double Stranded poly(A) Conformations by Single Molecule Atomic Force Spectroscopy

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Eukaryotic mRNA is modified at its 3′ end by a stretch of ~200 adenines. Because of the biological significance of this adenine tail, the structure of polyadenylic acid, (polyA) has been extensively studied. In bulk measurements poly(A) was found to form single-stranded helices under neutral or alkaline pH, but double-stranded (ds) helices under acidic pH. To the best of our knowledge such ds-poly(A) structures have never been examined at a single-molecule