In recent years, a considerable number of DNA-based molecular devices have been developed whose motion can be controlled by nucleic acid ''effector'' strands. For instance, the paradigmatic DNA ''tweezers'' system consists of two double-stranded arms connected by a flexible single-stranded ''hinge'' that can be closed or opened by the addition of so-called ''fuel'' strands. We have recently shown that this motion can also be driven with RNA rather than with DNA effectors. In order to realize an autonomously running biochemical system, we now utilized an artificial gene regulatory circuit in vitro to control the temporal behavior of the DNA tweezers. The gene circuit is a minimalistic feedback system that contains two genes from which regulatory RNA molecules are transcribed. The regulators mutually influence their production in an activatory and inhibitory manner, respectively,resulting in oscillatory network dynamics. We experimentally demonstrate how this transcriptional oscillator can be used to ''clock'' the motion of the DNA nanodevice in a variety of different ways. Furthermore, we investigate the robustness of the oscillator system with respect to increasing ''load'', i.e., tweezers concentrations.

Minisymposium 3: Tug of War: Molecular Motor Interactions

2226-MiniSymp

Opposite-Polarity Motors Activate One Another to Trigger Cargo Transport in Live Cells

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Intracellular transport, unlike in vitro, is typically bi-directional – consisting of a series of back and forth movements. Kinesin-1 and cytoplasmic dynein require each other for bi-directional transport of intracellular cargo along microtubules i.e. inhibition or depletion of kinesin-1 abolishes dynein-driven cargo transport, and vice versa. Using Drosophila S2 cells, we demonstrate that replacement of endogenous kinesin-1 or dynein with an unrelated motor of the same directionality, and targeted to peroxisomes, activates peroxisome transport in the opposite direction. However motility-deficient versions of motors, that retain the ability to bind microtubules and hydrolyze ATP, do not activate peroxisome motility. Thus any pair of opposite-polarity motors, provided they move along microtubules, can activate one another. These results demonstrate that mechanical interactions between opposite-polarity motors are necessary and sufficient for bi-directional organelle transport in live cells.

2227-MiniSymp

Motor Number Controls Cargo Switching at Actin-Microtubule Intersections *in vitro*

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Cellular activities such as endocytosis and secretion require that cargos switch between the microtubule (MT) and actin filament (AF) networks. Previous evidence suggests that switching may be regulated through a tug-of-war between MT and AF motors. To test the hypothesis that motor number can be used to direct the outcome of this tug-of-war, we reconstituted cargo switching at MT-AF intersections in a minimal system. We attached varying numbers of myosin-V and dynein-dynactin molecules to polystyrene beads and used an optical trap to position these beads near MT-AF intersections. Beads displayed a median pause time of 9 s at the intersection before exiting on a track. At least 23% of beads underwent rotation at intersections suggesting that competing motors apply a torque on their cargo. Force measurements to quantify the number of actively engaged motors show that stall force scales with the number of myosin-V motors as has previously been shown for kinesin-1 and dynein. Largely independent of whether it enters the intersection on the MT or AF, a bead with a myosin-V:dynein-dynactin force ratio of 0.5 (1 myosin-V to 4 dynein-dynactins) has a $>$85% probability of exiting on the MT. A bead with a myosin-V:dynein-dynactin force ratio of 1 (1 myosin-V to 2 dynein-dynactins) has an approximately equal probability of exiting on the MT, exiting on the AF, or remaining at the intersection. A bead with a myosin-V:dynein-dynactin force ratio of 4 (2 myosin-Vs to 1 dynein-dynactin) has a $>$95% probability of exiting on the AF. We have developed a statistical model that delineates the relationship between switch probability and motor number. Thus, cargo switching can be tuned via combinations of 1-4 myosin-V and dynein-dynactin motors through a simple force-mediated mechanism. Supported by P01 GM087253.

2228-MiniSymp

Myosin Va and Myosin VI Engage in a "tug of war" on Actin Tracks While Transporting Cargoes *in vitro*

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Myosin Va (myoVa) and myosin VI (myoVI) are processive molecular motors that transport cargo in opposite directions on actin tracks. Since myoVa and myoVI may colocalize to the same cargo *in vivo*, these motors may undergo a tug of war. Therefore, we sought to characterize the stepping dynamics of single myoVa and myoVI motors *in vitro* as they mechanically interact when linked together by a Qdot cargo. Expressed myoVa-HMM with an N-terminal biotin tag were labeled with streptavidin-Qdots (565nm) while expressed dimerized myoVI-HMM were Qdot(655nm)-labeled on an exchanged calmodulin. The effective tug of war on actin filament tracks (25mM KCl, 2mM ATP, 22°C) was observed in TIRF with 6nm resolution, allowing individual steps to be detected. MyoVa won ~80% of the time and regardless of which motor won, its stepping rate was reduced ~50% below its unloaded value due to the resistive load of the opposing motor. Interestingly, as the winning motor stepped forwards (myoVa, 73nm; myoVI, 56nm) the opposing motor stepped backwards (myoVa, 68nm; myoVI, 65nm) at the same rate, although myoVI appeared to be dragged at times. Why does myoVa dominate when its stall force is similar to myoVI? Given the probability that both myoVa and myoVI take occasional backsteps and experience a 2-3-fold reduction in stepping rate when winning, we estimate based on optical trapping data (Altman et al., 2004; Kad et al., 2008) that myoVa exerts a 50% greater resistive load compared to myoVI, providing a potential advantage to myoVa. Differences in the length of the myoVa and myoVI constructs could lead to each motor experiencing different vectorial force components, the potential that this may influence the outcome of the tug of war is being investigated.

2229-MiniSymp

Collective Behavior of Antagonistically Acting Kinesin-1 Motors

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Active cellular transport along microtubules is driven by the concerted operation of molecular motors. This often leads to complex dynamic behaviors such as stop-and-go or bidirectional movements. An important situation arises when motors act antagonistically in a tug-of-war scenario. In order to mimic the action of antagonistic motors, we performed gliding motility assays of antiparallel microtubule doublets driven by kinesin-1. In this configuration the lengths of the individual microtubules of the doublet determined the numbers of motors available to act against each other. At high motor density, we found two possible modes of movement: slow movements, where the doublets were almost stalled, and fast movements, where the doublet velocity was close to the velocity of single microtubules. Moreover, we observed a range of microtubule length differences where both modes coexisted. We developed a theoretical description that quantitatively describes the experimental data. In order to account for the two modes of movement, as well as for the possibility of their coexistence, it was necessary to take into account (i) the finite stiffness of the linkers by which the motors are connected to the substrate, (ii) the load-dependence of the detachment rate of single motors, and (iii) a non-linear force-velocity relationship of single motors. Our results show that mechanical interactions between motors can generate coexisting transport regimes with distinct velocities.

2230-MiniSymp

Iinteractions between Motor Proteins can Explain Collective Transport of Kinesins

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The collective function of multiple motor proteins is central to a variety of transport processes in cells. Yet, how key transport parameters depend on motor number and inter-motor interactions remains unclear. Recent experiments[1] have allowed the dynamic properties of two coupled kinesin-1 molecules to be examined using 'single-molecule' biophysical techniques. These studies have revealed that negative motor cooperativity plays a significant role in collective kinesin dynamics. Current theoretical models that neglect intermolecular interactions cannot capture this behavior. We propose a new theoretical approach, based on discrete-state stochastic models, which allows us to describe complex aspects of coupled kinesin dynamics. By treating intermotor interactions explicitly, these models can be used to reconcile important differences between predictions based on non-cooperative (additive) behaviors, and observations of negative kinesin cooperativity.

[1] A.R. Rogers, J.W. Driver, P.E. Constantinou, D.K. Jamison and M.R. Diehl, *Physical Chemistry Chemical Physics*, **11**, 4882 (2009).

2231-MiniSymp
Cargo Transport by Two Teams of Molecular Motors
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Intracellular transport is accomplished by molecular motors which pull cargos along cytoskeletal filaments. Many intracellular cargos are transported by two teams of molecular motors, for example by a team of kinesins and a team of dyneins. Such a cargo is observed to move bidirectionally along a microtubule, switching direction every few seconds.
We have developed a theoretical model for cargo transport by two motor teams, which describes the interaction of the motor teams as a stochastic tug-of-war, and which incorporates the results of single-molecule experiments [1]. This model can explain experimental in vivo data previously thought incompatible with a tug-of-war, in particular fast bidirectional motion and the response to perturbations. In addition, the model shows that the finite processivity, the low detachment force, and the low backward velocity of biological motors are favorable for bidirectional cargo transport, leading to fast motion, enhanced diffusion, and enhanced processivity of the cargo.
A similar model for cargo transport by a team of actin- and a team of microtubule based motors also results in enhanced processivity of the cargo on each filament type, in agreement with recent experimental results [2].
[1] M.J.I. Muller, S. Klumpp, R. Lipowsky, Proc. Natl. Acad. Sci. USA 105: 4609 (2008)
[2] F. Berger, M.J.I. Muller, R. Lipowsky, EPL 87: 28002 (2009)

Platform AO: Protein Dynamics II

2232-Plat
Functional Dynamics in the Enzyme Adenylate Kinase
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An important question in biology is how the energy landscape of enzymes can enable efficient catalysis of chemical reactions. We have undertaken an in-depth analysis of conformational exchange dynamics in the enzyme adenylate kinase (adk). Adk is an essential enzyme in higher organisms that catalyzes the reversible interconversion of AMP and ATP into two ADP molecules. The enzyme is modular and is composed of distinct ATP and AMP binding subdomains and in addition a core subdomain (where core is responsible for global thermodynamic and thermal stability). We have previously shown that substrate binding is accompanied by rate-limiting spatial displacements of both the ATP and AMP binding motifs. Here, we present a solution state chemical shift based NMR approach to probe the native energy landscape of adenylate kinase with and without its natural substrates present. Binding of ATP induces a dynamic equilibrium in which the ATP binding subdomain populates both open and closed conformations with equal weights. A similar scenario is observed upon AMP binding in the AMP binding subdomain. These structural ensembles represent complexes that are populated transiently during the enzymatic reaction cycle. Our proposed dynamic mode of protein-ligand interaction in adk stands in contrast to the traditional view of substrate/enzyme complexes as rigid, low entropy states. Finally, by using a combination of protein engineering and hydrogen to deuterium exchange experiments we have shown that the individual subdomains in adk can fold independently of each other. Independent folding of subdomains can, in principle, be utilized to accommodate the structural change during the functional open to closed transition.

2233-Plat
Molecular Details of the Apolipoprotein E and the Amyloid Beta Peptide Interaction: Analysis of a Potential Binding Site Responsible for ApoE4 Misfolding
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The relationship between Apolipoprotein E (ApoE) and the aggregation processes of the amyloid β (Aβ) peptide has been shown to be crucial for Alzheimer's disease (AD). ApoE4 is considered as a contributing risk factor for AD. Although various mechanisms have been proposed to explain the physiological and pathological role of this relationship, the detailed molecular properties of ApoE4 interacting with Aβ peptide are unknown. In our studies, a peptide-protein docking approach has been used to investigate the process of Aβ interaction with the N-terminal domain of the human ApoE4 isoform. The use of molecular dynamics simulations (10 ns in water) has allowed studying the interaction mechanism between the protein and the peptide. Our results show that ApoE4 forms a partially unfolded intermediate (molten globule) stabilized by the interaction with Aβ. The initial SDS-induced α-helix used as Aβ peptide model, becomes unstructured due to the interaction with ApoE4. Peptide interaction with the different ApoE isoforms changed the pattern of the salt bridges network in ApoE4 compared to ApoE4 alone. By analysis and statistics of these electrostatic interaction patterns, we present a model for the salt bridge network in the ApoE4- Aβ complex, crucial for the understanding of the interaction mechanism and relevant for potential drug design and therapeutics.

2234-Plat
Structural Dynamics by Time-Reolved EPR and Transient Time-Resolved FRET: Application to Myosin
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We have used three complementary spectroscopic techniques, pulsed EPR (DEER), time-resolved fluorescence resonance energy transfer (TR-FRET) and transient time-resolved FRET [$(TR)^2$FRET], to elucidate the mechanisms of energy transduction in myosin - an important motility protein involved in a variety of processes including muscle contraction. We engineered two double-Cys myosin mutants that were labeled with optical probes or spin labels, and determined structural changes in a single structural element of the myosin motor domain, the relay helix, as affected by nucleotide binding and hydrolysis. In all cases, time-domain detection permitted the reliable determination of the interprobe distance distribution, quantitating both order and disorder, and resolving coexisting structural states. While DEER offered superior distance resolution, $(TR)^2$FRET permitted detection of transient structural changes after rapid mixing (stopped-flow) with ATP. All methods demonstrated two structural states of myosin during the recovery stroke, corresponding to straight and bent conformations of the relay helix, in parallel with straight and bent conformations of the entire myosin head. A narrower interprobe distance distribution in the post-recovery state shows ordering of the relay helix structure during the recovery stroke. These experiments reveal changes in both structure and dynamics of the relay helix and identify it as a key player in the interdomain coupling mechanism. This methodology is applicable to any enzyme in which double Cys mutants can be engineered in a key region of energy transduction, permitting resolution of structural states and dynamics in real time during the transient phase of a biochemical reaction.

2235-Plat
Protein-Exchange Dynamics at GPCR Micro-Domains: a Case Study with the Parathyroid Hormone Receptor
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Na/H exchanger regulatory factor-1 (NHERF1) is a cytoplasmic protein that contains two PDZ domains and assembles macromolecular complexes and regulates localization, trafficking and mobility of a number of membrane transporters and receptors. Interactions among NHERF1 and its target proteins are stabilized by the bimolecular interaction of PDZ and PDZ-binding domains. The PTH receptor (PTHR) contains a PDZ-binding domain that enables direct association with NHERF1 and tethers the PTHR to the actin cytoskeleton. After activation, the PTHR is trafficked to clathrin. The fate of NHERF1 is unknown. We used PTHR as a model to identify the fate of NHERF and to determine the dynamic interactions with the PTHR. Fluorescence Recovery after Photobleaching (FRAP) and confocal fluorescence microscopy were used to measure mobility of PTHR and NHERF1 and the behavior of these proteins at the cell membrane. Fluorescent mCherryNHERF and Green Fluorescent Protein (GFP)-tagged PTHR were coexpressed in rat osteosarcoma cells. We observed a significant reduction in the mobility of PTHR in the presence of NHERF1. Upon stimulation of the receptor with PTH, PTHR internalization was triggered, while NHERF1 remained near the cell membrane. Furthermore, when the PTHR was immunologically immobilized at the cell membrane, mCherryNHERF mobility was high and equivalent to that of NHERF with non-immobilized PTHR. However, PTH interaction with PTHR caused a rapid and greater increase of NHERF1 mobility, even when the receptor was completely immobilized at the membrane. These results support the view that NHERF1 exhibits dynamic interactions with the PTHR, with receptor occupancy followed by rapid dissociation of NHERF1.