3524-Pos Board B385
Heat Shock Proteins Regulate Structure of Intermediate Filament Networks
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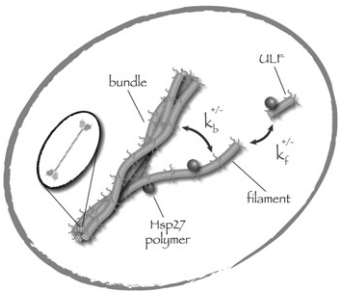
Living cells exhibit an enormous bandwidth of mechanical and morphological properties. These are mainly determined by the cytoskeleton, a composite network constituted of three classes of biopolymers: actin filaments, microtubules and intermediate filaments. While the interaction of actin filaments is mediated by a variety of cross-linking proteins, keratin intermediate filaments exhibit an inherent tendency to interact with each other under physiological salt conditions. Here we show, how small heat shock proteins can modulate such interactions in reconstituted keratin networks resulting in a drastic alteration of network structure and morphology. This mechanism provides an essential tool for cells to regulate the organization of their intermediate filament cytoskeleton.



3525-Pos Board B386
Sperm Cell Crawling in the Nematode Caenorhabditis Elegans
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Cell motility is important in biological processes, such as the immune response and cancer cell metastasis. Actin is implicated in most amoeboid cell movement, but *Caenorhabditis elegans* sperm cells lack actin, and their motility is driven by the Major Sperm Protein (MSP) cytoskeleton. MSP and actin form filament systems that are functionally similar, and the sperm cell therefore represents a simplified cellular model for studying the general properties of biopolymer-based movement.
We use the sperm cell to test how membrane tension affects movement and cytokeleton dynamics. We relax or tense the cell membrane with biochemical treatments or osmotic shock and we observe that membrane tension reduction is correlated with a decrease in cell displacement speed, whereas an increase in membrane tension enhances motility. We show evidence for the idea that membrane tension optimizes motility by streamlining polymerization in the direction of movement, thus adding a layer of complexity to our current understanding of how membrane tension enters into the motility equation.
We also use the sperm cell to study the effect of adhesion on retrograde flow and movement. We prepare substrates of varying compositions, and observe the interplay between cytoskeletal flows and the efficiency of displacement.
In the long term, study of the simplified MSP system and comparison to actin based movement should lead to a better understanding of the fundamental principles cell motility.

3526-Pos Board B387
Electron Tomography of MSP Filaments Derived from the Amoeboid Sperm of Ascaris Suum
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Locomotion of nematode sperm is remarkably similar to that of most other crawling cells but is powered by a system of filaments composed of major sperm protein (MSP) instead of the actin-myosin machinery typically associated with amoeboid motility. The MSP motility apparatus has been reconstituted *in vitro*, and individual MSP filaments can be generated by the addition of ATP to detergent-treated *Ascaris* sperm cytosol. Filaments formed in this way have been examined using both electron cryo-tomography and also conventional tomography of negative-stained samples. This has allowed for the structural analysis of filaments formed in the presence of MSP accessory proteins. Subvolume averaging has been applied to individual filaments sampled along their lengths within complex filament meshworks, and the resulting physiological models were compared to earlier models derived using purified MSP, including a helical reconstruction of filaments polymerized in ethanol (King et al. 1992. JCS 101:847) and an x-ray crystal model of MSP subfilaments (Bullock et al. 1998. NSB 5:184). Comparisons suggest important differences between filaments formed under physiological and nonphysiological conditions. There are currently six *Ascaris* sperm proteins known to modulate MSP filament dynamics in sperm; these same proteins are absent from filaments prepared using purified MSP. Comparing MSP filament models with and without these accessory proteins allows us to map the binding sites of these accessory proteins and provides a greater understanding of how they effect MSP filament dynamics and influence motility. Supported by NIH Grant R37 GM29994 and by the American Heart Assoc.

3527-Pos Board B388
Control of Directionality of Individual Kinesin-5 Motors
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Kinesin-5 motors fulfill essential roles in mitotic spindle morphogenesis and dynamics as slow, processive microtubule (MT)-plus-end directed motors. The Saccharomyces cerevisiae kinesin-5 Cin8 was found, surprisingly, to switch directionality. Here we have examined Cin8 directionality control using single-molecule fluorescence motility assays and live-cell microscopy. On spindles, Cin8 motors mostly moved slowly towards the midzone, in the plus-end direction of the interpolar MTs. Occasionally, Cin8 also moved faster towards the spindle poles, in the minus-end direction of the MTs. In vitro, individual Cin8 motors could be switched by ionic conditions from rapid and processive minus-end to slow plus-end motion on single MTs. At high ionic strength, Cin8 motors rapidly alternated directionalities between antiparallel microtubules, while driving steady plus-end relative sliding. Deletion of the uniquely large insert in loop 8 of Cin8 induced bias towards minus-end motility and affected the ionic-strength dependent directional switching of Cin8 in vitro. In vivo, the deletion mutant exhibited reduced midzone-directed motility and efficiency to support spindle elongation, indicating the importance of directionality control for the anaphase function of Cin8.

3528-Pos Board B389
Myosin-6 Mobility at the Plasma Membrane of Cultured Mammalian Cells
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Myosin-6 is a reverse-directed, actin-based, molecular motor responsible for moving cellular cargo towards the minus-end of actin filaments. It is involved in clathrin-mediated endocytosis and intracellular transport. We have used TIRF microscopy to study the movement and localisation of the ''short insert'' isoform of eGFP-myosin-6 within live 3T3 fibroblasts and human endothelial cells. Individual GFP tagged myosin-6 particles were automatically tracked by computer. Diffusion of membrane associated proteins is usually controlled by lipid mobility and individual trajectories follow a simple ''random walk'' in which mean squared displacement (MSD) is proportional to time interval (dT) and the lateral diffusion coefficient. We found the movement of myosin-6 shows anomalous diffusive behaviour and D_{lat} values are better described by a single exponential function, with a significant fraction of molecules (~30%) having $D_{lat} < 0.02\ \mu m^2\ s^{-1}$. Close examination of individual trajectories revealed that myosin-6 molecules exhibit periods of free movement interspersed with intervals in which motion is arrested. This phenomenon has been described previously as ''transient confinement''. The length of time during which movement of myosin-6 molecules were arrested had an exponential lifetime distribution ($t_{1/2} \approx 250$ ms). The number of observed particles decreased exponentially with time due to photobleaching. However, the shape of the intensity distribution of the individual particles remained constant and was well described by a fit to the sum of two Gaussian terms.
We conclude that myosin-6 molecules associate with the plasma membrane and bind intermittently with immobile sub-cellular structures, which might be either the actin cytoskeleton or trans-membrane anchoring proteins. The ''short insert'' splice variant of myosin VI is predominantly monomeric.

3529-Pos Board B390
Using a Non-Averaged Displacement Analysis to Characterize Multiple Populations of Single Molecule Motions
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Microscopic motions of fluorescently labeled single molecules often switch between bound, freely diffusing, and uni-directionally moving states, and in many systems (see below) determining the kinetics of transitions between

these states is important for establishing basic molecular mechanisms. Techniques such as a mean squared displacement (MSD) analysis average populations and thus cannot resolve multiple states of motion. By plotting non-averaged rather than mean displacements of simulated trajectories, we show that states of motion can be resolved and analyzed to determine the kinetics of transitions between dynamic states. We have applied this technique to several different dynamic systems. First, we show that submaximal velocities of partially activated thin filament motility result from switching between an ''on'' and ''off'' state, and that the level of activation increases with a decrease in the lifetime of the ''off'' state. Second, we show that submaximal myosin-based actin sliding velocities observed at low myosin densities result from a similar switching between ''on'' and ''off'' states. Next, we use this technique to analyze the dynamics of myosin light chain kinase (MLCK) moving on actin-myosin filaments in smooth muscle cells, where the lifetime of the paused states could give potential insight into the kinetics of smooth muscle phosphorylation by MLCK. Finally, we apply this technique to vesicle motility in 3T3 L1 to determine the kinetics of vesicle pausing. In general, a non-averaged displacement analysis provides a simple approach for determining the kinetics of transitions between dynamic states of single molecules.

3530-Pos Board B391
Universal Optimality of Molecular Motors
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Abstract
Molecular motors capable of directional track-walking or rotation are abundant in living cells, and inspired the emerging field of artificial nanomotors. Some biomotors can convert 90% of free energy from chemical fuels into usable mechanical work, and the same motors still maintain a speed sufficient for cellular functions. We have explored how this extreme efficiency-speed trade-off can be achieved in a single-molecule system that is intrinsically prone to ubiquitous thermal fluctuations. Specifically, we found that a regime of universal optimization unfamiliar in macroscopic engines exists for molecular motors. Like Carnot cycle for heat engines, the universally optimized working cycle for molecular motors is infinitely slow. But when a deviation from the ideal conditions reduces energy efficiency linearly from 100%, the speed is recovered exponentially due to Boltzmann's law. Experimental data on a biomotor indicate that this universal optimization has been largely approached in living cells, underpinning the extreme efficiency-speed trade-off in biomotors. I shall also discuss an experimental study by us towards achieving a similar artificial nanomotor in engineered systems.
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3531-Pos Board B392
Watching the Motions of the Cell Wall Synthesis Machinery and Underlying Cytoskeleton in *B. Subtilis*
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Rod-shaped bacteria elongate by the action of cell-wall synthesis complexes linked to underlying dynamic MreB filaments, but how these proteins function to allow continued elongation as a rod remains unknown. To understand how the movement of these filaments relates to cell wall synthesis, we characterized the dynamics of MreB and the cell wall elongation machinery using high-resolution particle tracking in Bacillus subtilis. We found that both MreB and the elongation machinery move in linear paths across the cell, moving at similar rates (~20 nm / second) and angles to the cell body, suggesting they function as single complexes. These proteins move circumferentially around the cell, principally perpendicular to its length. We find that the motions of these complexes are independent, as they can pause and reverse,and also as nearby complexes move independently in both directions across one surface of the cell. Inhibition of cell wall synthesis with antibiotics or depletions in the cell wall synthesis machinery blocked MreB movement, suggesting that the cell wall synthetic machinery is the motor in this system. We propose that bacteria elongate by the uncoordinated, circumferential movements of synthetic complexes that span the plasma membrane and insert radial hoops of new peptidoglycan during their transit.

3532-Pos Board B393
Quantitative Study of Tubulin Distrubution and Mitochondrial Dynamics in Skeletal Muscle Cells
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The comparative studies of regulation of oxidative phosphorylation as a function of mitochondrial organization in healthy and cancerous cardiac muscle cells have revealed that the mechanisms of regulation of the energy metabolism depend on the cellular organization (Biochim Biophys Acta, 2010, 1797: 678-97). The aim of this study is to evaluate mitochondrial dynamics and organization of mitochondria into intracellular energy units in adult skeletal muscle cells as a function of the energy metabolic pattern and mitochondrial interactions with cytoskeletal proteins. Among the latter, intracellular distribution of tubulin isoform was evaluated in rat oxidative and glycolytic skeletal muscle cells by Western blot analysis, immunofluorescence and functional methods. Investigation of mitochondrial dynamics in living skeletal muscle fibers was conducted using fluorescent probe labelling analyzed by fluorescent confocal microscopy. Fast scanning of cells was carried out using a line scanning LSM7 LIVE and LSM710 confocal microscope. The fast and simultaneous line scanning in two fluorescent channels synchronized with the piezo stage axial displacements allow rapid 3D imaging of the mitochondrial dynamics referenced to the structural elements of sarcomers. The analysis of position of mitochondrial fluorescence centres, or virtual mass centres, is studied by applying the gradient clustering algorithm.
Among the results obtained, we report specific tubulin isoforms distribution profiles in oxidative and glycolytic muscles that could rely to their energy meatbolic pattern.
This study was performed in the framework of the ANR project SYBECAR, France.

3533-Pos Board B394
Cytoplasmic Streaming in Drosophila Melanogaster
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The persistent circulation of the cytoplasm, called cytoplasmic streaming, occurs in a variety of eukaryotic cells. One context in which streaming occurs is during the establishment of Drosophila body axes, when Kinesin-1 transports the axes determinants and drives cytoplasmic streaming. Although Kinesin is essential for flows, neither the mechanism by which Kinesin induces streaming nor the impact of these flows on transport are known. We have succeeded in a precise quantitative measurement of the statistical properties of streaming by Particle Image Velocimetry. We have combined these measurements with an in vivo study of the cytoplasm rheology, to calculate the energy dissipation due to streaming. Since Kinesin is required for flows we can relate the energy dissipated to the work done on the fluid by Kinesin and determine the minimum number of motors necessary to drive streaming. Furthermore we have performed these measurements on mutants that effect Kinesin-1 motor activity and found remarkable agreement between our in vivo measurements and in vitro studies.

3534-Pos Board B395
Structural Basis for Activation of the Cytoskeleton Regulatory Protein IRSp53
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The scaffolding protein insulin receptor tyrosine kinase substrate p53 (IRSp53) is a ubiquitous regulator of the actin cytoskeleton that mediates filopodia and lamellipodia formation under the control of Rho-family GTPases. IRSp53 is comprised of an N-terminal IRSp53/MIM homology domain (IMD) that senses negative membrane curvature and a central semi-CRIB motif followed by an SRC homology 3 (SH3) domain that binds to proline-rich regions of a wide range of actin regulators. Here, we present the crystal structure of a complex between activated CDC42 and the CRIB domain of IRSp53. Isothermal titration calorimetry (ITC) experiments confirm the binding of this domain to CDC42, and exclude the possibility of binding by inactive forms of CDC42 or activated forms of RAC1. Moreover, we demonstrate that the SH3 domain also binds to the CRIB domain, suggesting a mechanism of regulation where binding of CDC42 is required to release autoinhibition of the SH3 domain.