genistein increased accumulation of the actin-nucleating protein formin-2 (FMN-2) and profilin in the peri-nuclear area. Silencing of FMN-2 by siRNA raised intracellular Ca^{2+} and rendered genistein resistance in decreasing intracellular Ca^{2+} in the cells. To define how actin filament assembly is regulated in the adipogenic differentiation, we determined functional changes in gene expression of actin binding proteins associated with morphological transformation in adipogenesis-induced WJCs. Adipogenic differentiation, as indicated by elevating expression of PPAR-γ mRNA, caused changes in β-actin mRNA expression and protein level. Gelsolin, an actin filament severing protein, also displayed a biphasic change of mRNA expression and protein level in the differentiation. During adiopogenesis mRNA expression levels for FMN-2 and Tm-1 were declined significantly, but no changes for Tm-2 and Tm-4. Taken together, our study resulted in the novel finding that actin-binding proteins act by modulating actin filament assembly for the proliferation and differentiation in human WJCs.

819-Pos

Analysis of De Novo Cell Cortex Assembly in Blebs as a Novel Assay for Probing Cortical Dynamics and Regulation

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The contractile actin cortex drives many cellular processes, such as cell migration and cytokinesis, but little is known about the proteins that regulate its assembly.

To address this question, we developed an assay for assessing the involvement of candidate proteins in the recruitment of the main cortical components, actin and myosin, during cortex build-up. One situation where cortex assembly can easily be studied is during the growth of blebs. Blebs are membrane protrusions that are initially devoid of cortical proteins and that subsequently reassemble a cortical layer prior to bleb retraction. They therefore constitute an ideal system for the study of de novo cortex assembly under physiological conditions. In the developed assay, we use laser ablation of the cell cortex to induce bleb growth in a controlled manner and subsequently quantitatively monitor the recruitment of fluorescently labelled actin and myosin during the bleb life cycle. This allows for the extraction of a range of dynamic parameters of cortical assembly that can be compared between control cells and cells with varying levels of candidate proteins. Preliminary data, obtained with this assay, show that proteins typically involved in actin polymerization, such as profilin and cofilin, influence the rates of cortex assembly in HeLa cells. Additionally, the assay allowed us to precisely characterize the dynamics of cortex assembly in control cells, providing new insights into the mechanisms of bleb growth and retraction. From these first tests, we conclude that the developed assay provides a highly sensitive tool for the study of cortex assembly.

820-Pos

The Actin Cytoskeleton Dynamically Associates with T-Cell Receptor Clusters

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The actin cytoskeleton is a key determinant of cell surface protein organization in many eukaryotic cells. In mammalian T-cells undergoing antigen-mediated activation, the cytoskeleton drives the development of a set of macroscale protein domains, collectively termed the supramolecular activation cluster. These protein domains, composed of T-cell receptors surrounded by adhesion molecules and their ligands, are highly characteristic of T-cell activation and are thought to play an important role in modulating receptor signaling intensity.

Though significant research has been undertaken to elucidate the interactions among various receptors involved in T-cell activation, the nature of the interactions between these receptors and actin remains poorly established. We have used live-cell fluorescence microscopy to image the actin cytoskeleton as it interacts with T-cell receptors in real time. Our results support recent work from our lab that had shown that T-cell receptors are likely to be friction-coupled to the cytoskeleton. Actin density tracking has also extended that work by demonstrating that cytoskeletal velocity may be affected by mobility-limited T-cell receptor clusters, and thus that T-cell receptors may have the capacity to regulate actin flow.

In addition, we have evaluated the time dynamics of the T-cell receptor-actin interaction and found that actin periodically accumulates and dissipates at T-cell receptor clusters. By applying an autocorrelation function to our image stacks, we found that the half-decay time ($Tau_{1/2}$) of the actin fluorescence at regions corresponding to T-cell receptor clusters was significantly increased compared to background, indicating a greater persistence of actin in those regions. Thus we have developed a novel method of analyzing actin kinetics and shown that the actin cytoskeleton dynamically associates with T-cell receptor clusters.

Cell & Bacterial Mechanics & Motility I

821-Pos

Response of the Bacterial Flagellar Motor to Controlled Temperature Change

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The bacterial flagellar motor is a rotary molecular motor capable of rotating at up to 700Hz. To resolve the individual steps that represent the discrete torque-generating step of the motor requires a low load marker and either high time resolution or a slowing of the motor. Previously the motor's speed has been reduced by decreasing the ion-motive force available to the motor[1]. Here we demonstrate two novel generic methods of microscope temperature control capable of slowing the motor while retaining nanometer resolution on the microscope. The first method involves a Peltier-cooled collar acting directly on the objective, and the second uses a chamber of fluid directly on top of the objective. These devices were used to probe the speed and function of the Bacterial Flagellar Motor across 0 °C - 40 °C. We confirmed that at slowing due to cooling was much greater at low loads than at high load, and extended previous chimera torque-speed curves to single-stator, low induction measurements. At high temperature we observed the motor stopping and subsequent resurrection-like behaviour as the motor was cooled. We investigated the membrane voltage response with temperature using the voltage sensitive dye TMR on cells treated with EDTA to allow the dye to penetrate the cell membrane[2]. From this we were able to investigate the cause of these stops at high temperature as a function of ion-motive-force.

[1] Y. Sowa, et. al, *Nature*, vol. 437, Oct. 2005, pp. 916-919.
[2] C. Lo, et. al, *Biophysical Journal*, vol. 93, Jul. 2007, pp. 294-302.

822-Pos

Conceptual Model for a Synthetic Bipedal Stepping Motor

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Biomolecular nanomotors have provided the inspiration for the design and construction of artificial nanoscale motors and machines based on several types of molecule including DNA. However, no synthetic nano-motors have yet been constructed from building blocks of protein-based material even though biomotors themselves are proteins. The HFSP smotor group (1) are in the process of developing a bottom-up approach to the understanding of biomotors by designing and constructing synthetic protein motors and numerically simulating their kinetic properties. One such concept is the ''tripedal tumbleweed'' motor, which is described in (1). In this context we present the results of numerical simulations for a bipedal motor with two connected peptide legs and with some of the properties of the tumbleweed motor. This motor walks on a one-dimensional track of periodically arranged binding sites. The two ''feet'' at the end of the legs represent different ligand-gated binding proteins which can only bind to their specific binding sites on the track when the related ligands are themselves bound to the binding proteins. The sequence of binding sites on the track is AB-AB-AB…. and the motor is powered by a temporally periodic sequence of composite washes which modulate the ligand concentrations and the leg angles. The washes cause the motor to undergo directed motion by a hand-over-hand mechanism on a track with asymmetric spacing between the AB and the BA binding sites. We will show simulation results for both two-dimensional and three-dimensional motor action of our bipedal motor which will include stepping diagrams, stall forces and first passage times for a range of parameters. This motor has the following properties observed for biomolecular motors: binding, power stroke and diffusional search. Extensions of the model will also be discussed. 1. E. Bromley et al. HFSP Journal (2009)

823-Pos

Tug of War: Dynamics of Bacterial Flagellar Motor with Multiple Stators

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In a single flagellar motor, there are multiple stator units that drive the rotation of the flagellar filaments. Here, we introduce a ''tug-of-war'' model for the flagellar motor where each individual stator can generate either positive or negative torque depending on its relative mechano-chemical state with respect to the rotor. The key ingredient of our model is that the instantaneous chemical switching (stepping) rate of a stator depends on the torque it generates: stators that generate negative torque switch faster. We find that the dynamics can be characterized by the waiting and moving time scales of the motor. We show that the experimentally observed torque-speed relationship can be explained

by the different dependences of these two time scales on the load. The model further reveals the dependence of the motor dynamics on the number of stators. In particular, we show that the maximum speed of the motor is independent of the number of stators, which agrees with recent resurrection experiments at near zero loads (Yuan & Berg, PNAS 105, 1182-1185, 2008). We have also used the model to study stepping statistics in single flagellar motor and different noise sources for rotational speed fluctuation. In general, we believe the model maybe useful to study other molecular motor systems with multiple asynchronous power generating units. [Part of the work was published in (Meacci & Tu, PNAS 106, 3746-3751, 2009)].

824-Pos

Understanding Kink Propagation in Spiroplasma

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Spiroplasma are helical bacteria of the class Mollicutes, that lack cell walls and swim by propagating a kink of handedness-change along their helical shaped body. Recent microscopy studies indicate that the major structural component of the cell is a multistranded protein ribbon, bound to the inner cell membrane. The ribbon runs along the whole cell body, following the shortest helical path on the membrane inner surface. Kink propagation is believed to be driven by conformational changes in the ribbon subunits (itself possibly driven by unidentified motor proteins), but the microscopic mechanism is largely unknown. We use simple mechanical models to understand kink propagation in Spiroplasma. Our conclusions differ from earlier work based on purely geometrical considerations in several important ways. This leads us to propose new microscopic mechanisms for the handedness change, and a new interpretation of the observed bend angle in kinked cells.

We further model the mechanochemistry of kink propagation, and find that the kink speed might be limited either by protein friction or a chemical event in the mechanochemical cycle of a ribbon subunit. The two mechanisms might be distinguished based on the randomness of the kink propagation.

Our results offer a qualitatively new understanding of existing observations, and several useful suggestions for future experiments.

825-Pos

Swimming Hydrodynamics of a Run-And-Tumble Bacterium with Helical Flagella

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To study the swimming of a peritrichous bacterium such as Escherichia coli, which is able to change its swimming direction actively, we simulate the ''run-and-tumble'' motion using a bead-spring model to account for the hydrodynamic and the mechanical interactions between the cell body and multiple flagella, the reversal of the rotation of a flagellum in a tumble and the associated polymorphic transformations of the flagellum. The cell body and each flagellum are connected by a flexible hook, so that the flagella can take independent orientations with respect to the cell body. This simulation reproduces the experimentally observed behaviors of E. coli, namely, a three-dimensional random-walk trajectory in run-and-tumble motion and steady clockwise swimming near a wall. We show that the polymorphic transformation of a flagellum in a tumble facilitates the reorientation of the cell, and that the time-averaged flow field near a cell in a run has double-layered helical streamlines, with a time-dependent flow magnitude large enough to affect the transport of surrounding chemoattractants. This new model, which can be refined by using more beads if more quantitative predictions are desired, strikes a balance between accuracy and simplicity that will permit it to be used to determine the migration behavior of particles near a swimming cell, cell-cell hydrodynamic interactions, the effect of the number and geometric distribution of flagella on migration, the mechanism of circular swimming near a wall, details of the tumbling motion, and the effect of the Brownian motion on swimming. We also develop minimal models, inspired by the simple model of Najafi and Golestanian, that contain only 3-5 beads, and can simulate simple ''pusher'' and ''puller'' micro-swimmers, and are also able to include helical flow typically produced by rotary flagellar motion.

826-Pos

Swimming Microorganisms in Gels

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Many swimming microorganisms must move through viscoelastic fluids and gels. I present work on swimming in gels. First, unlike incompressible fluids, a gel can have compressional modes with relative motion between polymer and solvent fractions. In a continuum model for a gel, we show that compressibility can increase the swimming speed of Taylor's swimming sheet. The zero-frequency shear modulus of a gel requires altered boundary conditions on the swimmer. Second, many biological gels are heterogeneous on the lengthscale of swimming microorganisms, necessitating non-continuum models that treat the gel network and swimmer on equal footing. We show that a random network modeled as dilute, immobile spherical obstacles increases the average swimming speed of a Golestanian three-sphere swimmer.

827-Pos

A Tug-of-War Mechanism for Bacterial Surface Movement

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In various bacterial species surface motility is mediated by cycles of type IV pilus motor adhesion and force generation, but it is unclear whether multiple motors cooperate mediate movement. Here we show that 7 ± 1 pili/cell are required for persistent movement of *Neisseria gonorrhoeae* with MSD $\sim t^{1.5 \pm 0.1}$. The unbinding force of individual pili from the surface $F < 30$pN was considerably lower than the stalling force $F > 100$pN, suggesting that density, force, and adhesive properties of the pilus motor have evolved to enable a tug-of-war mechanism for bacterial movement. Consistently, we found that bacteria were unable to move on fluid lipid membranes, most likely because force generation was not translated into bacterial movement due to slippage. Using microcontact printing, we confined the surface motility and microcolony formation to non-fluid islands within a fluid lipid membrane. Our patterning technique used physico-chemical surface properties that did not interfere with bacterial tug-of-war mediated motility and we anticipate that it will be useful for studying differentiation and gene expression within dynamical bacterial clusters and biofilms.

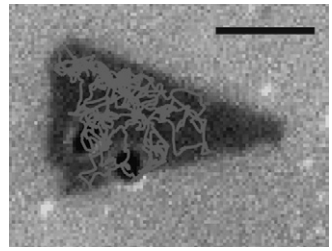

Figure: BSA-triangles on coverslides surrounded by with a DOPC membrane. The trace shows a path over 2min. Scale bar: 5μm.

828-Pos

Examples of X-Ray Scattering Studies of Biological Systems under Extreme Conditions

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Biological systems under extreme conditions often resort to unusual structures to achieve special functions for survival. The ''bioengineering'' principles of such ''extreme'' structures may inspire biomimic designs of functional materials. Here we give several examples of such naturally occurring unusual structures probed with x-ray scattering. i) In bacterial spores, the spore coat appears to comprise of laminar layers of quasi-2D crystals with periodicity of ~1 nm. Such ordered assembly may be responsible for the spore resistance to heat, toxic chemicals, and mechanical disruption. ii) In starved bacterial cells, the DNA packaging protein (DPS) is over-produced to compact the chromosomal DNA close to crystalline density to protect the genomic integrity and facilitate homologous recombination. iii) In bacterial cells with over-produced DNA, mild treatment with antibiotics can lead to liquid-crystalline DNA that responds to external osmotic stress. Future work includes 1) uncovering the molecular basis of characterized structures and 2) extending into systems such as cells under radiation or heat and cancer cells.

829-Pos

Redundant Mechanisms for Stable Cell Locomotion Revealed by Minimal Models

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Crawling of eukaryotic cells on flat surfaces is underlain by the dynamics of the actin network and graded adhesion to the substrate and is regulated via a complex biochemical network. Some crawling cells maintain roughly constant shape and velocity. The paradigm of this stable crawling is the fish keratocyte, a rapidly moving cell that maintains a half-moon shape while translocating. Here we use moving boundary simulations to explore 4 different, minimal mechanisms for cell locomotion and show that all of these are sufficient to produce steady shapes and movements with resulting features that resemble the keratocyte morphology. We begin by considering a diffusion-limited actin model where G-actin transport to the leading edge controls the rate of protrusion of the leading edge.