gradients, starting from unexpected experimental observations of stochastic response time of individual neutrophils after sudden exposure to spatial chemoattractant gradients. We propose that neutrophils orientation is achieved by the synergy between localized temporal sensing through expanding pseudopods and whole-cell integration of the temporal information by microtubules. In our model, microtubules play functional roles in the local positive feedback via stabilization near membranes experiencing localized temporal concentration increases, and provide global signal integration via scarcity and redistribution inside cells. Experiments using chemical inhibitors of microtubules support the hypothesis that microtubules could play a key role in cell orientation in the presence of spatial chemoattractant gradients. Modeled cells can not only detect the direction of a spatial gradient, but at the same time remain responsive to further changes in the direction of the gradient. Better understanding of neutrophil activity could have practical implications in clinical conditions of inflammation and during immune responses against bacteria and injuries.

3248-Pos Board B295
How Does The Bacterial Flagellar Motor Of Rhodobacter Sphaeroides Stop - Using A Clutch Or A Brake?
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The bacterial flagellar motor is a rotary molecular machine ~50 nm in diameter enabling some bacterial species to swim. It is embedded in the cell envelope and connected to an extracellular helical propeller. The motor is powered by the flow of ions down an electrochemical gradient across the cytoplasmic membrane into the cell. Most of our knowledge on motor function comes from work on the *E. coli* motor, which can switch between clockwise and counterclockwise rotation, allowing the bacterial cell to change direction in response to different stimuli.
A proton-driven flagellar motor of *Rhodobacter sphaeroides* achieves the same goal as the bi-directional *E. coli* motor, that of changing cell direction in response to the external environment, but does so by stopping and rotating in only one direction.
We employed several techniques to monitor and manipulate the motor to find out how the stop is achieved. The rotation of a 0.83μm polystyrene bead attached to a truncated flagellum was monitored using back-focal-plane laser interferometry. This allowed us to observe stops in motor rotation with a high temporal (up to 0.1 ms) and angular (~1 degree) resolution. In separate experiments we tethered cells down to glass coverslips by their flagella and applied external torque with an optical trap using the cell body as a handle.
Here we characterize mechanical properties of the motor and show how the motor stops rotating - by putting the brakes on.

3249-Pos Board B296
Experimental Evidence for Conformational Spread in the Bacterial Switch Complex
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The allosteric regulation of proteins has classically been understood in terms of the Monod-Wyman-Changeux (MWC) or Koshland-Nemethy-Filmer (KNF) models. These are recognized as limiting cases of a general allosteric scheme that has recently been described in a model of conformational spread. A candidate proposed for testing the model is the bacterial switch complex, an ultrasensitive multimeric protein ring responsible for controlling the direction of rotation of the bacterial flagellar motor. The complex is too large for MWC-type interactions to be applicable and cooperative binding studies have ruled out the KNF model. Here we use high-resolution back-focal-plane interferometry to resolve intermediate states of the complex predicted by conformational spread, and demonstrate detailed quantitative agreement between our measurements and simulations. Individual switch events are not instantaneous, but follow a broad distribution of switch times with mean ~ 20 ms, incomplete switches occur at a bias-dependent frequency and intervals between switches are exponentially distributed at all values of bias.

3250-Pos Board B297
Direct Observation Of [Ca2+]i Changes In Motile Sperms With 50 msec Time Resolution
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Ejaculated motile sperms swim against flow in oviduct toward egg. For fertilization, acrosome reaction and regulation of sperm motility including hyperac-

tivation and control of flagellar beat are important events. $[Ca^{2+}]_i$ plays a major role in all the important sperm functions that occur after ejaculation. Much work on sperm Ca^{2+} signaling has used agonists and activators rather than flow, because the small size of sperm presents inherent difficulties in direct observation of motile sperms. Indeed, the $[Ca^{2+}]_i$ in motile sperm has not been directly recorded in flow in microfluidic environment. We will report the system to record $[Ca^{2+}]_i$ in motile sperm with and without flow, and investigated the correlation between velocity of motile sperm and $[Ca^{2+}]_i$ distribution in sperm. Sperm motions in microfluidic environment and $[Ca^{2+}]_i$ changes in the motile sperms were investigated by high-time resolution confocal fluorescent microscopy with high magnification. To record $[Ca^{2+}]_i$, human sperm suspensions stained with FLUO-3AM were injected into a microfluidic channel fabricated by soft-lithography, and confocal fluorescent 4D images were reconstructed with time resolution of 50 msec/frame. $[Ca^{2+}]_i$ changes in the head, midpiece, and tail of the sperm were observed. We found a positive correlation between motile sperm velocity and maximum fluorescent intensity, corresponding to $[Ca^{2+}]_i$, in the midpiece of a sperm. Based on the studies on sperm chemotaxis, $[Ca^{2+}]_i$ is accumulated in the midpiece of sperm, and the Ca^{2+} ions in the midpieces are used for the regulation of flagellar beat mode. We can suggest that $[Ca^{2+}]_i$ elevation in the midpiece would be necessary for high-speed movement of the flagellar.

3251-Pos Board B298
Temperature-dependence Of Torque Generation Of The Na ± driven Chimeric Flagellar Motor And Visualization Of The Stator Proteins In E.coli
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Bacterial flagellum is a supramolecular complex and consists of a basal body, a helical filament, and a hook. A basal body embedded in cell membrane functions as a rotary motor driven by electrochemical potential of specific ion, and rotates flagellar filament like as screw. The rotor consists of MS-ring (FliF) and C-ring (FliG, FliM, and FliN).
To learn roles of the electrostatic interaction between stator and rotor in the mechanism of torque generation, we examined the motor response over the temperature range 5-50 degree. At low temperature (23-5 degree), rotational speeds linearly decreased with decreasing temperature. With increasing temperature, however, sudden drops of speeds were observed over ~30, ~40 and ~50 degree. When the temperature returned back to 23 degreee, the speed was restored mostly in several minutes. The drop and recovery of the speed were coincided with stepwise change in the generated torque.
And, we constructed fusion proteins of rotor components and Green Fluorescent Proteins, and investigated whether rotor components are exchanged in a functional motor by FRAP analysis for a single motor labeled with GFP.
In the tethered cell that was produced each GFP fusion, a fluorescent spot was localized at the rotational center. Each GFP fusion was probably incorporated into flagellar motor as a rotor component. In order to investigate the exchange of rotor components, we carried out FRAP analysis using evanescent light. GFP-FliN or FliM-GFP recovery of fluorescence at the rotational center was observed as time passed. On the other hand, the recovery of fluorescence was not observed in the cell producing GFP-FliG. These results suggest that some rotor components assemble to motor even after functional motor is constructed.

3252-Pos Board B299
Distribution Of Traction Forces Associated With Shape Changes In Migrating Amoeboid Cells
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Amoeboid motility results from the cyclic repetition of a repertoire of shape changes leading to periodic oscillations of cell area (motility cycle). This study aimed to identify the dominant shape changes and their association to the regulated activity and localization of molecular motors. For this purpose, we applied Principal Component Analysis (PCA) to time-lapse measurements of cell shape, traction forces and fluorescence from the F-actin-binding protein limEΔcoil-GFP in migrating *Dictyostelium* cells. This method provides the most significant cell shape changes of the motility cycle, together with maps of the traction forces and F-actin distribution associated with each shape change mode. It also sorts these modes according to their contribution to the variance of the cell area oscillations observed during the motility cycle. Using wild-type cells (*wt*) as reference, we investigated myosin II activity by studying myosin II null cells (*mhcA-*) and essential light chain null cells (*mlcE-*). The results revealed that *wt, mlcE-* and *mhcA-* cells implement similar shape changes during their motility cycle, although they are implemented at a slower pace in myosin mutants. The repertoire of shape changes is surprisingly reduced as only three modes are

enough to represent 67% of the variance in cell area in *wt, mlcE*- and *mhcA*- cells. The three principal shape modes are dilation/elongation, a half-moon shape and bulging of the front/back. The second of these modes represents sideways protrusion/retraction, is associated to lateral asymmetries in the cell traction forces / F-actin distribution, and is significantly less important in *mhcA*- cells. These results indicate that the mechanical cycle of traction stresses and cell shape remains similar but is slowed down when myosin function is lost, probably due to a reduced control on the spatial organization of the traction stresses.

3253-Pos   Board B300
Bihelical waves: A novel form of eukaryotic cell motility exhibited by African trypanosomes
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Flagella and cilia play a critical role in eukaryotic cell motility. Among the most notable waveforms exhibited by eukaryotic flagella are planar and helical waves observed in mammalian sperm and protozoa. Here we report on a high-speed study of the flagellar motility of the protozoan parasite Trypanosoma brucei responsible for the African sleeping sickness whose vector is the tsetse fly. In this organism, the flagellum is physically attached along the length of the tapering cell body, unlike the case of mammalian sperm where the flagellum is attached to the body only at one attachment site. Earlier studies had reported that propulsion was driven by helical waves propagating from the flagellar tip to the base with left-handed helicity. Using a millisecond-timescale microscope, we discovered a novel form of eukaryotic cell motility, in which alternating left-handed and right-handed helical waves (termed "bihelical waves") propagate along the flagellum and are separated by a moving kink. These bihelical waves produce torsion in the cell body that is resolved by a rocking motion but - unlike the case of mammalian sperm or the existing model for T. brucei - without net rotation. We also observed the rapid motion of the flagellum tip, for which we recorded velocities up to 673 nm/ms, about 96 times greater than the velocity of dynein motors in vivo. The forward translational movement of the body is coupled to both the rocking of the posterior cell body about its own axis and the axis of locomotion as well as the propagation of the bihelical waves and kinks. Our results demonstrate that millisecond-timescale microscopy is essential for studies of cell locomotion in microorganisms.

3254-Pos   Board B301
Timing the Start of Division in E. coli: a Single-Cell Study
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Precise determination of morphology dynamics during growth and division of bacterial cells is restricted by optical resolution and micron size of the object. We have developed a method for high precision cell edge detection in a phase-contrast image allowing continuous follow up of the cell contour with about 30 nm accuracy. This approach is used to analyze the entire life cycle of single E. coli cells and provides a detailed morphological characterization of the cell division process. We show that initiation of the envelope constriction occurs much earlier than the appearance of a visible constriction, and is also manifested in a break in the length dynamics corresponding to the addition of new poles formation. We use simple rescaling of variables to provide a global view of the entire cell population. In particular, the data for the dynamics of the constriction width for all the cells in the population collapses to the vicinity of the function predicted by our theoretical model. Some of the parameters that describe cell division obey certain quantitative relations. In addition, we have developed an algorithm for analysis of the spatial distribution of the division initiator protein, tubulin-like FtsZ, in fluorescent images of single cells. With this algorithm, profile and positional dynamics of the FtsZ constriction ring were analyzed, revealing a time gap between the ring maturation and the start of constriction. This gap is presumably required for assembly of the other division proteins forming the divisome. This information provides new constraints on the possible molecular mechanisms involved in the formation of both the divisome and the cell septum.

3255-Pos   Board B302
High-pressure Microscopy For Modulating The Torque Generation Of Bacterial Flagellar Motors
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The bacterial flagellar motor converts the specific ion flux across the cell membrane to the rotational motion. The torque generation is achieved by the intermolecular interaction between rotor and stator complexes. The motor can spin both directions; binding activated CheY molecules induces switching from counter-clockwise (CCW) to clockwise (CW). Here, we show a novel assay that changes the rotational speed and direction of the flagellar motor by specially designed high-pressure microscopy. E. coli cells lacking *cheY* that rotate exclusively in the CCW direction, were tethered to the observation window of high-pressure chamber. At less than 800 atm, all cells rotated in the CCW direction and their speeds were not affected seriously. At more than 1000 atm, some cells started to rotate in the CW direction, and the rotational speed in both directions decreased steeply with pressure. Application of pressure generally works to modify the intermolecular interaction between protein and water molecules, resulting in changing the structure and function of molecular machines. Thus, applied pressure seems to modify directly the intermolecular interaction between rotor and stator units. The pressure-induced effects could inhibit the torque generation of the flagellar motor, and change the rotational direction, as if the activated CheY molecules bind to the rotor.

3256-Pos   Board B303
Enhancement of Bacterial Motility due to Speed-Dependent Absorption
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Marine bacteria often reach high swimming speeds, either to take advantage of evanescent nutrient patches or to beat Brownian forces. Since this implies that a sizable part of their energetic budget must be allocated to motion, it is reasonable to assume that some bacteria are able to increase their nutrient intake by increasing their speed *v*. We formulate a model that uses the concept of internal energy depot originally developed by Schweitzer, Ebeling, and Tilch to investigate this hypothesis. We postulate that the nutrient absorption rate is of the form $q(v) = q_0 + Av$, with q_0 and A being constants. If the fraction c of energy spent non-mechanically is low, we find that there is a single stable velocity v_1^*, but if c is large, there is a critical value of A, A_c, below which only the $v = 0$ solution is stable. Above the bifurcation point A_c a second stable solution appears, whose value v_2^* increases monotonically with A. The mechanical efficiency of the molecular motors is also shown to increase with A. The description of the motion is further clarified by the use of the Fokker-Planck formalism. Solutions obtained using realistic parameter values indicate that the speed increase due to the enhanced nutrient absorption may be substantial.

3257-Pos   Board B304
Quantification of Leaf Vein Patterning
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Vein networks are essential in transporting nutrition effectively into all cells of an organism. In plant leaves these vein networks are formed by the opposite transport mechanism, the retraction of the plant hormone auxin. The so formed auxin flow pattern is consistent with the vascular network of the mature leaf. Key factor in the non-uniform transport are auxin carriers from the PIN protein family.
We investigate a microscopic model for the directed auxin transport by carrier proteins performing both computer simulations and analytic calculations. These enable us to identify the relevant biological processes which should be considered for leaf vein patterning. Quantitative results help us to suggest observables and experimental scenarios to measure the kinetic rates governing the active transport.

3258-Pos   Board B305
Stochastic Effects On Biodiversity In Cyclic Coevolutionary Dynamics
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The formation of out-of-equilibrium patterns is a characteristic feature of spatially-extended, biodiverse, ecological systems. Intriguing examples are provided by cyclic competition of species, as metaphorically described by the 'rock-paper-scissors' game. Both experimentally and theoretically, such non-transitive interactions have been found to induce self-organization of static individuals into noisy, irregular clusters. However, a profound understanding and characterization of such patterns is still lacking. Here, we theoretically investigate the influence of individuals' mobility on the spatial structures emerging in rock-paper-scissors games. We have devised a quantitative approach to analyze the spatial patterns self-forming in the course of the stochastic time evolution. For a paradigmatic model originally introduced by May and Leonard, within an interacting particle approach, we demonstrate that the system's behavior - in