flagellum (wavelength and amplitude), effect swimming characteristics. MATLAB tracking and analysis algorithms are used to extract motility parameter quantities.

3279-Pos Board B434

Helical Flow of Surface Protein required for Bacterial Locomotion

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Cells of *Flavobacterium johnsoniae* and of many other members of the phylum *Bacteroidetes* exhibit rapid gliding motility over surfaces by a unique mechanism. These cells do not have flagella or pili, and instead rely on a novel motility apparatus comprised of Gld and Spr proteins. SprB, a 669 kDa cell-surface adhesion, is required for efficient gliding. SprB was visualized by electron microscopy as thin 150 nm long filaments extending from the cell surface. Fluorescence microscopy revealed movement of SprB proteins toward the poles of the cell at approximately 2 μm/s. The fluorescent signals appeared to migrate around the pole and continue at the same speed toward the opposite pole along an apparent right-handed helical closed loop. Movement of SprB, and of cells, was rapidly and reversibly blocked by the addition of CCCP, which dissipates the proton gradient across the cytoplasmic membrane. In a gliding cell, some of the SprB protein appeared to attach to the substratum. The cell body moved forward and rotated with respect to this point of attachment. Upon reaching the rear of the cell, the attached SprB was often released from the substratum, and apparently recirculated to the front of the cell along a helical path. The results suggest a model for *Flavobacterium* gliding, supported by mathematical analysis, in which adhesins such as SprB are propelled along a closed helical loop track, generating rotation and translation of the cell body.

3280-Pos Board B435

Imaging Colonization Dynamics and Rheological Properties of a Host and its Developing Microbiome by Light Sheet Microscopy

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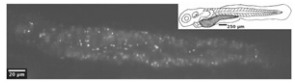
For microbes colonizing an animal host, the mechanical properties of the host environment are of great importance, affecting motility and therefore (presumably) the ability to establish a stable population. Indeed, some species possess the ability to affect the fluidity of their environment, both directly by chemically modifying it, and indirectly by influencing the host's production of secretory cells. By utilizing the unique strengths of light sheet fluorescence microscopy combined with the techniques of microrheology, we can witness early encounters between colonizing bacteria and an initially germ-free host, and directly measure the material properties of the intestinal environment. We performed three-dimensional imaging of the entire larval zebrafish gut for twenty-four hours following bacterial inoculation, yielding highly resolved spatiotemporal information about the interplay between microbes and host. Additionally, by driving magnetically doped micron-scale probes, the rheology of the mucosal layer within the fish can be measured over three decades of frequency, adding physical knowledge of the environment to quantitative observations of a complex biological system's maturation.



3281-Pos Board B436

Exploration of Bdellovibrio Chemotaxis and Predation using Microfluidics

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Bdellovibrio bacteriovorus is a predatory, gram-negative bacterium that preys on other gram-negative bacteria. It has long been hypothesized that B. bacteriovorus can sense prey in the environment and move toward them, and recent genomic sequencing indicates that B. bacteriovorus has approximately 20 methyl-accepting chemotaxis receptor proteins and the full flagellar machinery necessary for chemotaxis. Nonetheless, B. bacteriovorus chemotaxis has never been demonstrated in the laboratory. As a result, the molecules it might use to target and track its prey have not been identified. A road block to prior research has been the limitations on traditional chemotaxis assays; B. bacteriovorus does not form colonies on agar media plates and it has been known to move up to 100 body lengths per second, which makes it difficult to track its growth or movement in response to a specific chemoeffector. To address these issues, we have designed a microfluidic device to measure the reaction of B. bacteriovorus to specific chemoeffectors. The small dimensions and controlled flow in a microfluidic device allow us to introduce B. bacteriovorus to a gradient of chemoeffectors such as sugars, metabolites, and signaling molecules. With multiple outlets containing a range of chemoeffector concentrations, we can observe both attractive and repellent responses, as well as score the degree to which B. bacteriovorus reacts to these chemicals. Thus a microfluidic device provides significant advances over classic ''on/off'' chemotaxis assays, allowing us to explore for the first time the target molecules and affinity of B. bacteriovorus chemotaxis receptors.

3282-Pos Board B437

On Time Reversal Symmetry and Bacterial Chemotaxis

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Motility of polar flagellated bacteria is typically forward and backward in rapid succession. We recently found that one of the marine species, Vibrio alginolyticus, incorporates a flick movement at the end of the backward swimming interval, breaking the time reversal symmetry of the swimming trajectory. A flick in this bacterium is functionally equivalent to a tumble of peritrichously flagellated bacteria, such as Escherichia coli, causing the cell body to deflect in a new direction before the next run starts. Since V. alginolyticus is capable of swimming in both forward and backward directions, it raises an interesting question about how the chemotaxis behavior of this bacterium is regulated. Herein, we provide experimental evidence showing that the marine bacterium differentiates chemical signals detected in the two swimming intervals and responds in the manner that is consistent with the chemotaxis strategy where the forward swimming interval is exploratory and the backward interval is exploitative.

3283-Pos Board B438

Chemotactic Response of Escherichia Coli to Repellents, CoCl2 and NiCl2

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Negative chemotaxis refers to the motion of microorganisms away from harmful chemicals. Soft agarose gel assay has been traditionally used to characterize the response to various repellents. In this study, we use the ''chemical-in-plug'' method to quantify the motion of Escherichia coli in the presence of repellents, NiCl2 and CoCl2, over a broad range of concentrations. These experiments were complimented with drift velocity measurements of individual bacteria in controlled gradients using a capillary assay. The latter also revealed the tumbling frequency and steady state clockwise bias for varying concentrations of repellents thereby providing insight into adaptation to repellents. The experimental technique yielded the motion of the bacteria in space and time and further related the motion to the evolving concentration profile of the repellent. Results show that the bacteria exhibit logarithmic sensing to the repellents, i.e., the drift velocity of E.coli is proportional to the logarithmic concentration gradient suggesting Weber law. The predictions of a standard population based model agreed with the observed linear behavior when the binding of the repellent to the receptor was sub-sensitive. This was borne out by a low value of Hill coefficient ($n<<1$) used to describe the binding characteristics of the receptors. The analysis shows that the binding characteristics for the repellents was sub-sensitive in contrast to an ultra-sensitive response observed for attractants suggesting a negative cooperative behavior of receptors. The above experiments suggest that negative cooperativity allows the cells to respond to harmful chemicals without saturation even at high concentration.

3284-Pos Board B439

Role of the Pseudomonas Aeruginosa Flagellar Motor in Swimming Motility and Chemotaxis

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Flagellar-driven swimming motility is well-established in some bacterial model organisms, and it is best described in the case of Escherichia coli. However, increasing genetic and structural data show that diversity in flagellar motors exists across the bacterial kingdom, where new paradigms of swimming motility may be discovered. In this report, we describe the flagellar motor function of monotrichous P. aeruginosa, and show that unlike E. coli, it is a motor that rotates in both counter-clockwise (CCW) and clockwise (CW) directions giving rise to a 'run-and-reverse' trajectory. Additionally, the flagellar motor exhibits multiple speeds in the CCW but not the CW direction. using a microfluidic-based assay, we show that in the presence of a chemoattractant (serine), the cells alter their run-length, switching frequency and motor speeds in order to move toward favorable environments. Therefore, in chemotaxis, apart from varying the switch frequency, the P. aeruginosa flagellar motor has an

additional mechanism that allows it to favor the higher rotation speed state. These findings are validated in a computational model of P. aeruginosa swimming and chemotaxis.

3285-Pos Board B440

Effect of Run and Tumble Time on Rheological Behavior of a Suspension of Escherichia Coli

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Microorganisms like microalgae and bacteria inject mechanical energy into the suspending fluid for their movement. Pusher-type bacterial cells such as Escherichia coli propel by rotating their flagella in a screw-like fashion. We measure the viscosity of a suspension of E. coli of different wild type and mutant strains at varying cell densities. The strains of E. coli differed in their run speeds, tumble time, and run time. The viscosity profile of the nonflagellated E. coli strain (BL21-DE3) increases linearly with cell density and agrees well with the prediction for a suspension of rod-shaped particles. Experiments were complimented with Small Angle Light Scattering (SALS) studies to observe the cell orientation in shear. The measured viscosity for all the strains were correlated with the chemotactic property of individual cells such as run speed, run time and tumble time. For smooth swimmers, we experimentally demonstrate the presence of instability at a critical cell density beyond which the viscosity decreases with increase in cell density.

3286-Pos Board B441

Structure and Dynamics of a Phase-Separating Active Colloidal Fluid

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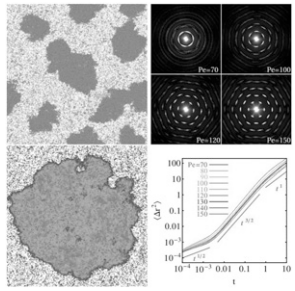
We examine a minimal model for an active colloidal fluid in the form of self-propelled Brownian spheres that interact purely through excluded volume. Despite the absence of an aligning interaction, this system shows the signature behaviors of an active fluid, including anomalous number fluctuations and phase separation behavior. using simulations and analytic modeling, we quantify the phase diagram and separation kinetics. The dense phase is a unique material that we call an active solid, which exhibits the structural signatures of a crystalline solid near the crystal-hexatic transition point, but the rheological and transport properties associated with a viscoelastic fluid.



3287-Pos Board B442

Investigating Stator Dynamics of the Escherichia Coli Flagellar Motor

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The bacterial flagellar motor (BFM) of the Escherichia Coli is an elegant molecular nano-machine that regulates bacterial motility. Powered by the proton-motive force, each motor generates mechanical torque via proton flux through numerous associated stator units that surround the rotor complex. These stator units freely diffuse in the cytoplasmic membrane and temporally engage with the BFM to rotate helical flagellar filaments and propel the bacterium to favorable environments. However, a fundamental understanding of stator dynamics of the BFM is still needed. We are employing a tweezer set-up that is capable of applying external torque to individual tethered E. Coli cells and therefore allows us to investigate mechanisms of the BFM. By adjusting the external load torque on the motor, we can physically control motor rotation, such as inducing forward rotation, backward rotation, and moments of stall to observe the behavior of the BFM with a temporal resolution of a few milliseconds. These results will further elucidate the dynamic role of stators in the BFM and in bacterial motility.

3288-Pos Board B443

Dynamic Conformational Changes of Flagellar Filament Observed by High-Pressure Microscopy

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The bacterial flagellar motor is a molecular machine that rotates a flagellum in both directions. CCW rotation allows the left-handed helical filaments to form a bundle that propels the cell smoothly, whereas CW rotation of a filament leads to change the shape of filament in right-handed helix and break the bundle, and inhibits smooth swimming of the cell, called a tumble. The switching in the helical structure is thought to be caused by directional mechanical actions arising from abrupt change of exerted torque by the motor rotation. Here, we show that application of pressure can also change the helical structure of flagellar filaments. The flagellar filaments in *E. coli* cells were fluorescently labeled, and then the images were acquired by using a high-pressure microscope [1, 2] with some modifications. We measured the diameter and pitch of the individual filaments and then classified them into 11 possible waveforms which are predicted from structural data. At 0.1 MPa (ambient pressure), all flagellar filaments formed left-handed helical structure (normal form). At 40 MPa, we found left-handed forms (normal and coiled forms) and right- (curly I (or II)). At 80 MPa, 80% flagellar filaments took curly I (or II) forms. After the pressure was released, most filaments returned to the initial left-handed structures. The application of pressure is thought to enhance the structural fluctuation and/or association of water molecules with the exposed regions of flagellin molecules, and results in switching the helical from left- to right-handed structure.

[1] Nishiyama M. and Y. Sowa. 2012. Microscopic Analysis of Bacterial Motility at High Pressure. *Biophys. J.* 102:1872-1880.
[2] Nishiyama M. and S. Kojima. 2012. Bacterial motility measured by a miniature chamber for high-pressure microscopy. *Int. J. Mol. Sci.* 13:9225-9239.

3289-Pos Board B444

Young's Modulus of *B. Subtilis* Cell Wall: Measuring and Modeling the Elasticity of Rod-Like Bacteria

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The peptidoglycan layer is the principle structural component of load baring cell wall in rod-like bacteria. As a polymer cross-linked into a rigid scaffold, peptidoglycan is responsible for characteristic shape of bacterial cells, their mechanical strength and durability. Atomic force microscopy (AFM) is an ideal tool to make highly precise measurements, to analyze multiple isolated cells, and to compare the mechanical properties between the individual bacterial cells. We can then investigate how the biology of the cells (deletion of specific proteins) or treatment and stresses to the cells (exposure to antibiotics) can affect the mechanics of their cell walls. Although there have been many studies of the mechanics of bacteria using AFM, many of them treat the force-indentation relationship in the terms of the standard Hertz model (i.e. approximate the cell as a uniform elastic solid). Of the few studies that treat the compression of a cell as the deformation of a thin elastic shell, none treat bacteria as a rod-like structure, which it resembles. We used large radii colloidal probes to obtain force-compression curves on multiple individual cells of wild type *B. Subtilis* and a mutant deficient in the protein mbl (Δmbl) that plays a key role in cell wall synthesis. To interpret the data in a quantitative manner, we developed a variety of analytical models for a rod-like elastic shell filled with incompressible fluid. We applied these models to describe the stretching of the cell wall and to calculate the Young's modulus of peptidoglycan in hydrated rod-like bacteria. Compared to wild type cells, the Young's modulus of the peptidoglycan in mutant bacterial cells is reduced by a third.

3290-Pos Board B445

Exploring the Mechanics of Magnetically Driven Motility in Magnetotactic Bacteria through Genetic Regulation

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Magnetotactic bacteria guide themselves to optimal growth environments by a process termed magneto-aerotaxis, in which chains of intracellular magnetic nanoparticles, known as magnetosomes, orient along the Earth's geomagnetic field lines as a guide to efficiently locate oxygen-poor regions. From an external standpoint, this unique magnetotactic navigation system is regulated by key components: a magnetic nano-compass (magnetosome chain), a propulsion system (flagellar motility), and some magnetically-activated sensor (signal transduction). We hope to gain insight into these external systems by deconstructing the internal regulation of magnetotactic navigation from a genetics perspective. While genomic regions have been identified that encode magnetosome-related genes, little is known about how these genes regulate magnetosome production and how they interact with flagellar and cytoskeletal components to achieve guided motility. Here, we explore the genetic response of *Magnetospirillum magneticum* strain AMB-1 to an applied electromagnetic field as a means to identify genes activated by magnetic stimulation, focusing