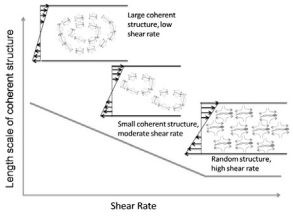
Bacteria Mechanics and Motility

2914-Pos Board B606
Curvature-Dependent Localization of the Bacterial Cytoskeleton Drives De Novo Morphogenesis in Escherichia Coli
Gabriel H. Billings[1], Nikolay Ouzounov[2], Tristan Ursell[1], Joshua W. Shaevitz[2], Zemer Gitai[2], Kerwyn Casey Huang[1].
[1]Stanford University, Stanford, CA, USA, [2]Princeton University, Princeton, NJ, USA.
A peptidoglycan cell wall determines the shape of nearly all bacteria. The cell wall, along with the shape that it adopts, is crucial to cellular physiology. The mechanical integrity and morphology of the cell wall is determined by the spatiotemporal patterning of cell wall synthesis; therefore a rod-shaped cell such as *Escherichia coli* faces the challenge of coordinating the nanoscale proteins responsible for peptidoglycan synthesis to construct a micron-scale sacculus. What are the principles that allow cell wall synthesis proteins to establish order over a range of length scales spanning nearly three orders of magnitude? We approached this question by examining the process of reversion in cell-wall-deficient 'L-forms' of *E. coli,* in which cell-wall synthesis has disrupted by beta-lactam antibiotics. An L-form undergoing reversion begins in a spherical shape without an intact cell wall. When cell wall synthesis inhibiting antibiotics are removed, the cell generates newrod-shaped protrusions, which eventually undergo septation and adopt the normal rod morphology of *E. coli*. The reversion of L-forms thus provides on opportunity to study morphogenesis in bacteria lacking an intact cell wall. We therefore investigated the morphological dynamics of the reversion process in L-forms of *E.* coli, and simultaneously imaged the localization of MreB, a bacterial actin required for the rod-like morphology of many bacteria. We found that MreB localizes to negatively curved regions of the cell (e.g. invaginations), and furthermore targets cell wall synthesis to those regions. Our results therefore suggest a model in which the localization of MreB in response to geometric cues is crucial to morphogenesis in *E. coli*.

2915-Pos Board B607
The Bacterial Brain: Structure and Dynamics of a Bacterial Chemoreceptor Array
Christopher K. Cassidy.
University of Illinois, Champaign, IL, USA.
The ability of all living things, from single cells to large multicellular organisms, to sense and interpret environmental signals is central to life. Bacteria, though relatively simple unicellular organisms, have evolved exquisite protein networks, which they use to detect gradients in certain chemicals in their surroundings and alter their swimming behavior. The head of this protein circuit, a chemoreceptor array, is a remarkably ordered supramolecular complex composed of the histidine kinase, CheA; adaptor protein, CheW; and various methyl-accepting chemotaxis proteins (MCPs). These proteins cluster together by the thousands at the cell pole. The clustering of receptors within the chemoreceptor array gives rise to systems-level network properties of bacterial chemotaxis such as signal amplification, ultrasensitivity, and precise adaptation. We present an all-atom structure, roughly twelve million atoms in size, of a patch of the chemoreceptor array from the thermophile Thermotoga maritima, refined by electron cryotomography data. Molecular Dynamics simulations reveal inter-protein interactions essential to receptor communication. Based on insight into the natural structural mobility of the array from normal modes of the trajectories, steered molecular dynamics was employed to perturb the lattice at multiple sites mimicking chemoreceptor activation. The results suggest a molecular mechanism for signal transduction through the array.

2916-Pos Board B608
Elasticity Mediated Interactions of Motile Bacteria with Anisotropic Viscoelastic Medium
Rishi R. Trivedi.
University of Wisconsin-Madison, Madison, WI, USA.
Bacteria display complex, dynamical and cooperative behaviors that are guided by the mechanical and biochemical environments in which they live. Many such habitats are isotropic in nature: that is, they possess direction-independent physical properties. However, bacteria also colonize microenvironments that have anisotropic properties (e.g., optical, mechanical, and diffusional anisotropy), including those enriched in collagen, cellulose, chitin, and the extracellular matrix in bacterial biofilms. Here we report an investigation of the bacterium Proteus mirabilis dispersed in a model anisotropic viscoelastic active materials comprised of a lyotropic liquid crystal (LC). We observed that LC elasticity-mediated forces drive the non-motile as well as motile P. mirabilis cells to assume orientations in which their long axes are parallel with the director of LC. We have studied complex behaviors of the motile bacteria emerging from the interplay of forces generated by the flagella and the elasticity of the LC and found that in contrast to passive microparticles (including non-motile bacteria) that associate irreversibly in LCs via elasticity-mediated forces, motile bacteria formed reversible end-on-end assemblies in a direction of LC alignment. We have also investigated spatial variations in the LC orientation to guide the trajectory of bacteria in the LC. Overall, these observations provide insights into the fundamental dynamical behaviors of bacteria in complex anisotropic environments and suggest that motile bacteria in LCs are an exciting model system for exploration of principles for the design of active materials.

2917-Pos Board B609
Rheological Behavior of a Suspension of Escherichia Coli with Varying Motor Characteristics
Richa Karmakar, Mahesh S. Tirumkudulu, K.V. Venkatesh.
IIT Bombay, Maharashtra, India.
We determine the rheological response of a suspension of E. coli for varying cell densities and shear rates. Experiments were performed at moderate to high shear rates with five different strains of E. coli varying in motor characteristics like duration of run and tumble. Irrespective of the strains, at low densities and at a fixed shear rate, the viscosity increased linearly with cell density akin to a dilute suspension of passive rods. The strains with low run speeds, short durations of run, and high rotary diffusivities exhibit lower viscosities compared to strains with high run speeds and large run duration. Interestingly, the latter exhibit a sharp decrease in viscosity at a critical volume fraction signaling the presence of strongly coordinated motion. The presence of such coordinated motion and the influence of shear rate and cell density on their lifetimes and length scales were confirmed by visualizing the motion of tracer particles in sheared suspensions. Reduction in correlated motion is observed for both increase in shear rate and decrease in cell density.The critical density depends not only on the magnitude of shear but also the motor characteristics of cells.



2918-Pos Board B610
Stabilizing and Controlling Swimming Bacteria: Shaping a Turbulent Suspension into a Ferromagnetic State
Hugo Wioland[1], Francis G. Woodhouse[1], Jörn Dunkel[1,2], Enkeleida Lushi[3], Raymond E. Goldstein[1].
[1]DAMTP, University of Cambridge, Cambridge, United Kingdom, [2]MIT, Cambridge, MA, USA, [3]School of Engineering, Brown University, Providence, RI, USA.
Dense suspensions of swimming bacteria are famous for self-organizing into large and turbulent jets and swirls. Confining a dense Bacillus subtilis suspension into a flattened drop allows the stabilisation of a vortex ('spin'), the structure of which results from a complex interplay between steric and hydrodynamic interactions [Wioland et al., PRL 110, 268102 (2013)]. We now couple up to 100 of such vortices inside microfluidic devices to create bacterial spin lattices. Depending on the vortex and coupling geometry, we were able to reproduce 'ferromagnetic' and 'antiferromagnetic' bacterial vortex states, that appear to be controlled by the subtle competition between bacterial boundary layer flows and bulk dynamics.

2919-Pos Board B611
Stepping Dynamics of the Bacterial Flagellar Motor
Ashley L. Nord[1], Bradley C. Steel[2], Richard M. Berry[1].
[1]Physics, University of Oxford, Oxford, United Kingdom, [2]Pöyry, UK, Oxford, United Kingdom.
The bacterial flagellar motor (BFM) is a rotary molecular motor which is self-assembled and embedded within the cellular envelope of many species of swimming bacteria. Powered by a flux of ions across the cytoplasmic membrane, the BFM rotates the helical flagellar filaments that propel the cell. It is one of the best characterized large biomolecular complexes. In 2005, a sodium-driven chimeric flagellar motor in *E. coli* at low energization and low expression of torque-generating units was observed to take 26 steps per revolution[1]. However, achieving sufficient spatial and temporal resolution to resolve kinetics and discrete stepping behavior under normal energization conditions and with multiple torque-generating units remains a challenge. We have used a novel objective-type laser darkfield microscope to image small gold nanoparticles attached to the hooks of rotating BFMs with sub-nanometer and microsecond resolution. We present preliminary results for the stepping behaviour of the BFM rotating at up to hundreds of revolutions per second and with a varying number of torque-generating units.

1. Y. Sowa, A. D. Rowe, M. C. Leake, T. Yakushi, M. Homma, A. Ishijima, and R. M. Berry. Direct observation of steps in rotation of the bacterial flagellar motor. Nature, 437(7060): 916-9, 2005.

2920-Pos Board B612

Quantitation of Cell Wall Growth Suggests Feedback Mechanisms that Robustly Build Rod-Like Bacteria

Tristan Ursell, Kerwyn Casey Huang.
Bioengineering, Stanford University, Stanford, CA, USA.

In bacteria, a host of enzymes regulates the reproducible and robust construction of the cell wall, whose mechanical integrity is crucial for viability under osmotic stress. Antibiotics that target these enzymes disrupt cell wall construction, ultimately leading to mechanical failure of the cell. Our work explores the physical mechanisms of cell growth and death, as a guide to understanding antibiotic mechanisms that disrupt mechanical properties of the cell. We use a combination of cell wall fluorescent labeling, high resolution time-lapse microscopy, and computational image processing to characterize where, and with what dynamics, cell wall and outer membrane growth occurs. When cell-shape analysis is combined biophysical simulations of growth, our data strongly suggest that dynamic localization of the bacterial MreB cytoskeleton is part of a curvature sensing and growth feedback mechanism that orchestrates heterogeneous growth to maintain rod-like shape and regulate mechanical stress. Analysis of MreB and cell-surface marker fluorescence indicates that the cytoskeleton is present at sites of active growth and that chemical depolymerization of the cytoskeleton causes homogenous, unstructured growth and eventual cell death by rupture. Quantitative tracking of growth is an effective method for characterizing cell wall mechanical failure, and these techniques pave the way for studying the detailed dynamics of growth-associated proteins and their disturbance by antibiotics.

2921-Pos Board B613

Surviving a Bumpy Ride in the Oropharynx: Bacterial Pili as Nano-Seatbelts that Dissipate Mechanical Energy

Daniel Echelman[1], Jorge Alegre-Cebollada[1], Georgia Squyres[1], Carmelu Fernandez[1], Chungyu Chang[2], Hung Ton-That[2], Julio Fernandez[1].
[1]Biology, Columbia University, New York, NY, USA, [2]Microbiology and Molecular Genetics, University of Texas-Houston Medical School, Houston, TX, USA.

Bacterial pili function in cellular adhesion, and must withstand large mechanical stresses in host environments, such as coughing and chewing. In gram positive bacteria, pili are covalently-linked polymers of single protein subunits, termed pilins. Gram positive pilins uniquely possess intramolecular isopeptide bonds that bridge the peptide backbone to form bypass force transduction pathways. In the crystal structure of Spy0128, a pilin from *Streptococcus pyogenes*, isopeptide bonds link the N- and C-terminal β-strands. Consequently, Spy0128 is mechanically inextensible. Here we report on the mechanical properties of two related pilins, SpaA from *Corynebacterium diphtheriae* and FimA from *Actinomyces oris*, using atomic force microscopy (AFM)-based single molecule force spectroscopy. In the crystal structures of SpaA and FimA, the isopeptide bonds do not directly link the N- and C-terminal β-strands in a single pilin domain. Instead, the isopeptide arrangement creates a ~40 residue polypeptide loop that resembles a slackened seatbelt, which we predict is sensitive to mechanical unfolding. We find that both SpaA and FimA extend to 14 nm under mechanical force, consistent with our structure-based prediction of unfolding of the "nano-seatbelt" from a slackened to a taut conformation. At a loading rate of 400 nm/s, these loops unfold at forces of ~503pN in SpaA and ~665pN in FimA; as such, SpaA and FimA are among the most mechanically stable proteins yet reported. When the force perturbation is removed, the loops refold at a rapid rate of 29 s^{-1} or higher**.** Remarkably, the mechanical stabilities are ~75pN weaker upon refolding, suggesting that gaining full mechanical stability requires maturation. The high mechanical stability and rapid refolding of the nano-seatbelts suggest a mechanism whereby pilin subunits, polymerized as tens-to-hundreds of repeats in pili, readily absorb and recover from mechanical shocks.

2922-Pos Board B614

Pressure-Speed Relationship of the Sodium-Driven Flagellar Motor of Vibrio Alginolyticus

Masayoshi Nishiyama[1], Yoshiki Shimoda[1], Yoshifumi Kimura[2], Masahide Terazima[1], Michio Homma[3], Seiji Kojima[3].
[1]Kyoto University, Kyoto, Japan, [2]Doshisha University, Kyoto, Japan, [3]Nagoya University, Nagoya, Japan.

The bacterial flagellar motor is a molecular machine that converts an ion flux to the rotation of a helical flagellar filament. Motor rotation rate and directions can be changed by environmental factors such as temperature, pH, and solvation. Hydrostatic pressure is also an inhibitor of the rotation of flagellar motors [1, 2]. Our previous results indicated that the application of pressure inhibits the rate of ion tranlocation in the mechanochemical energy translation, but the detailed mechanism is still unknown. Here, we characterized the pressure dependence of the rotational speed of sodium-driven flagellar motor in swimming *Vibrio alginolyticus* cells. The motor in strain NMB136 exclusively rotates in counter-clockwise direction and propells the cell body forward. We monitored the pressure-induced effects on the behavior of the cells that swim freely in solution. The swimming speed exponentially decreased with the increment of pressure. The sodium concentration dependence of the swimming speed at each pressure was well described by a Michaelis-Menten kinetics. The applied pressures decreased the maximum velocity, but increased the Michalis constant. Our results showed that the motor has at least two pressure-sensitive reactions, one of which is the binding process of external sodium ions to the motor. Another is the post-sodium-binding process, suggesting sodium transit and/or its release to inside the cell.

[1] Nishiyama M. and Y. Sowa. 2012. Microscopic Analysis of Bacterial Motility at High Pressure. *Biophys. J.***102**:1872-1880.
[2] Nishiyama M. *et al.* 2013. High Hydrostatic Pressure Induces Counterclockwise to Clockwise Reversals of the *Escherichia coli* Flagellar Motor. *J. Bactetiol.***195**: 1809-1814.

2923-Pos Board B615

Motility Enhancement through Surface Modification is Sufficient for Emergent Behaviors During Phototaxis

Rosanna Man Wah Chau[1], Devaki Bhaya[2], Kerwyn Huang[1].
[1]Bioengineering, Stanford University, Stanford, CA, USA, [2]Plant Biology, Carnegie Institution for Science, Stanford, CA, USA.

The emergent behaviors of communities of genotypically identical cells cannot be easily predicted from the behaviors of individual cells. In many instances, direct cell-cell communication or cell differentiation play important roles in the transition from individual to community behavior. In the cyanobacterium Synechocystis, cells exhibit light-directed motility (phototaxis). This process occurs at both single-cell and community scales. While single cells undergo a biased random walk, an inoculation of cells on an agarose surface can be observed to form dynamic finger-like projections toward a directed light source. These subcommunities consist of a high concentration of cells concentrated at the progressing front, followed by a lower concentration of cells distributed along the finger. Results from time-lapse microscopy suggest that cells secrete an extracellular polymeric substance (EPS) that modifies the physical properties of the substrate, leading to enhanced motility and the ability to detect tracks left by other cell groups. Our quantitative, single-cell tracking results show that the EPS confers no information of directionality or memory of light directionality, suggesting its major role in motility enhancement. Furthermore, the distribution profiles of the movement bias of single cells vary spatially across the inoculation, with cells in finger-like projections having a more pronounced movement bias toward light. We have developed a cellular automata model that demonstrates that indirect, surface-based communication conferred by EPS is sufficient to create distinct motile groups whose shape and bias distributions match our experimental observations, even in the absence of direct cellular interactions or changes in single-cell behavior. Therefore, our modeling and experiments provide a framework to show that the emergent behaviors of phototactic communities involve modification of the substrate, and this form of surface-based communication could provide insight into the behavior of a wide array of biological communities.

2924-Pos Board B616

High throughput 3D Palm Imaging Elucidates Mechanisms of Bacterial Cell Division

Seamus Holden[1], Thomas Pengo[1], Karin Miebom[1], Justine Collier[2], **Suliana Manley**[1].
[1]physics, EPFL, Lausanne, Switzerland, [2]microbiology, University of Lausanne, Lausanne, Switzerland.

We created a high throughput modality of photoactivated localization microscopy, HTPALM, which enables automated 3D PALM imaging of hundreds of synchronized bacteria during all stages of the cell cycle. We used HTPALM to investigate the nanoscale organization of the bacterial cell division protein FtsZ in live C. crescentus. We observed that FtsZ predominantly localizes as a patchy mid-cell band, and only rarely as a continuous ring, supporting a model of "Z-ring" organization where FtsZ protofilaments are randomly distributed within the band and interact only weakly. We found evidence for a previously unidentified period of rapid ring contraction in the final stages of the cell cycle. We also found that induction of the SOS response produced high-density continuous Z-rings which may obstruct