phosphorylation, and found no significant differences between NEB KO and wt muscle. Our mechanical studies revealed that NEB KO fibers had increased tension cost (5.9 vs. 4.4 pmol mN^{-1} mm^{-1} s^{-1}) and reductions in k_{tr} (4.7 vs. 7.3 s^{-1}), calcium sensitivity (pCa_{50} 5.74 vs. 5.90), and cooperativity of activation (n_H 3.64 vs. 4.38). Our findings indicate that in skeletal muscle (1) nebulin increases thin-filament activation, and (2) that through altering crossbridge cycling kinetics, nebulin increases force and efficiency of contraction. In addition to nebulin deficient murine muscle, we also studied nebulin-deficient muscle fibers from patients with Nemaline Myopathy (NM). We found increased tension cost, and reductions in k_{tr} and calcium sensitivity in NM fibers when compared to human control fibers, consistent with the findings from nebulin-deficient murine muscle. This novel role of nebulin in regulating muscle contraction adds a new level of understanding to skeletal muscle function, and might provide a mechanism for the muscle weakness in patients with nebulin-based Nemaline Myopathy.

Platform AF: Bacterial Motility

2146-Plat

High Resolution, Long Term Characterization of Bacterial Motility Using Optical Tweezers

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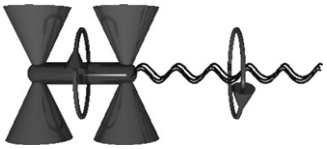

We present a single-cell motility assay[1], which allows the quantification of bacterial swimming in a well-controlled environment, for durations of up to an hour and with a temporal resolution greater than the flagellar rotation rates of ~100 Hz. The assay is based on an instrument combining optical tweezers, light and fluorescence microscopy, and a microfluidic chamber. Using this device we characterized the long-term statistics of the run-tumble time series in individual *Escherichia coli* cells. We also quantified higher-order features of bacterial swimming, such as changes in velocity and reversals of swimming direction.

[1] Min, T.L., Mears, P.J., Chubiz, L.M., Rao, C.V., Golding, I. & Chemla, Y.R. (2009) High-resolution, long-term characterization of bacterial motility using optical tweezers. *Nature Methods* (in press)

2147-Plat

Impact of Microscopic Motility Schemes on the Overall Swimming Behavior of Parasites

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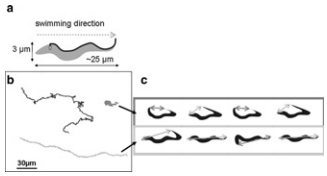

Figure 1. a) schematic of cell body b) typical swimming trajectories c) directionally persistent cells are 'stretched'

In recent work, Engstler et al. showed that the motility of the trypanosomes, causative agents of African sleeping sickness, is essential in their evasion of the host immune response. Our studies reveal that the trypanosome travels in one of three distinct motility modes: random walk, directional persistence, and an intermediate class in which they exhibit a combination of both. To further elucidate the parasite's motility we utilize high-speed videomicroscopy to uncover the microscopic origin of the macroscopic motility modes. Trypanosome swimming is facilitated by a flagellum that runs along the cell body with only a small 'free' segment at the anterior end of the cell. We use a straightforward parameter, namely the distance between the anterior and posterior ends of the cell to characterize trypanosome swimming. Remarkably this parameter is sufficient for extraction of relevant time scales for classification of the motility modes. Further, we find not only that these different motility modes correspond to distinct physical movements but also that a stiffer cell body gives rise to directional persistence.

2148-Plat

A Model for Bacterial Motility Utilizing Helical Cytoskeleton Filaments and Ion-Driven Motors

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The bacterial cytoskeleton determines cell shape and mediates cell division. Recent work indicates that the cytoskeleton mediates cell motility as well. Adventurous (A) motility in Myxococcus xanthus requires actin-like MreB filaments. During the motility, a double helix structure – possibly consisting of MreB – rotates in the cell's cytoplasm. Proteins localized along the helical structure are associated with proton transporting protein complexes homologous to the MotA-MotB stator that drives rotation of the bacterial flagellar motor. These observations suggest an entirely new model for bacterial motility in which motors driven by ion motive force move along the helical cytoskeleton to generate propulsive forces. This mechanism may be widespread in bacteria, since both MreB homologs and MotA-MotB homologs are common across a variety of bacterial species, including species that move in the absence of flagella. We have constructed a biophysical model to test the feasibility of this motility mechanism. Our model explains many intriguing observations in Myxococcus motility, including rotation of the helical cytoskeleton, periodic reversals of cells, and clustering of motility-related proteins at the cell poles and the substrate interface. Previous models assumed that periodic cell reversals are attributed to biochemical oscillators in the cell. This model, in contrast, proposes that reversals result from a mechanical oscillator intrinsic to a system with transmembrane motors traveling on a closed helical track. According to this mechanism, mechanical interactions play an important role in signal transduction.

2149-Plat

Direct Evidences of a Motility Motors in Myxococcus Xanthus

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Myxococcus xanthus, a gram-negative soil bacterium, glides over solid surfaces with two independent motility mechanisms termed social (S) and adventurous (A) motility. S-motility has been shown to be powered by the extrusion, adhesion and retraction of type IV pili. A-motility, however, is much less well understood. Two main models have been proposed to explain A-motility. The first, the ''slime gun'' model, implicates the secretion of polyelectrolyte slime from the cell pole, pushing a cell forward. More recently, we proposed a second model that involves lateral force generation at focal adhesion sites between the cell and substrate. In this model, unknown motors drive cells forward by moving along a filament inside the cell. To date, however, there has been no direct evidence for lateral force generation or the presence of motor proteins in Myxococcus.

We have developed an optical trapping bead assay, which enables us to adhere polystyrene beads to the surface of Myxococcus cells. We find that beads are moved along the cell surface with speeds comparable with those of gliding cells. Beads move in a helical pattern along the cell surface with a pitch size of a few microns. Multiple beads on the same cell can move in the different directions, indicating that beads are carried by individual motors instead of by a global movement of the cell envelope. We also show that in mutant cells lacking key A-motility regulatory genes, beads move in a less coordinated fashion.

2150-Plat

Morphogenesis and Cell Division of E. Coli Under Mechanical Confinement

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Bacteria have characteristic shapes and sizes which are conserved by an elaborate cytoskeletal machinery. Surprisingly, these well-defined shapes are strongly modified in E. coli bacteria in narrow nanofabricated channels. Growth in constrictions where bacteria are squeezed to about twice thinner than their typical diameter leads to flattened cells that laterally are much wider (up to 5 micron) than regular E. coli [1]. We will report on the cell growth, spatial structure and dynamics of cytoskeletal proteins and the nucleoid in this unusual bacterial phenotype. While the physical confinement has a profound effect on the cell shape and the pattern of cell division, it has only a limited effect on the replication rate of the cells. In most cases, broad (multinucleate) cells are still able to segregate chromosomes in roughly equal amounts to two daughter cells. This process typically starts with the formation of a chromosome-free area in the middle of the cell, which propagates asymmetrically to the perimeter of cell.

From this asymmetric location, the cell wall starts to constrict and a septum forms. Our results show that the cellular structure of bacteria has a high degree of plasticity in coping with lateral stress and confinement.

[1] Bacterial growth and motility in sub-micron constrictions, J. Männik, R. Driessen, P. Galajda, J.E. Keymer and C. Dekker, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 14861.

2151-Plat

Morphology, Growth and Size Limit of Bacteria

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Bacterial cell wall is the main structure to maintain a specific cell shape and to resist the osmotic pressure of several atmospheres. Despite many research, some basic questions remain unsolved: Out of many possibilities, why do bacteria only have several defined shapes? What is the relation between growth and morphology of a bacteria? How do rod-like bacteria select and maintain a specific radius, but grow in the axial direction? Is there any size limit for bacteria? What factors determine the size limit if it exists? In this paper, we set up a general growth model for bacterial cell wall and try to answer the previous questions. We found the growth modes and the size limits for coccus, bacillus, vibrio and spirillum, which are consistent with the experiments well.

2152-Plat

Structure and Assembly of CFA/I pili from Enterotoxigenic Escherichia Coli That Cause Traveler's Diarrhea

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Enterotoxigenic Escherichia coli (ETEC) bacteria that cause traveler's diarrhea utilize pili to initiate infection via pilus binding to epithelial cells in the small intestine. According to the World Health Organization, ETEC cause the largest number of recorded community-acquired cases of childhood diarrhea in the developing world, and are the most common cause of Traveler's diarrhea. Through a multi-disciplinary approach that includes x-ray crystallography, electron microscopy, site-directed mutagenesis, and genetic sequence analysis we elucidate the structure and assembly of CFA/I pili expressed on ETEC. We show that the distinction between Class I pili from the chaperone/usher pathway (e.g., P-pili from uropathogenic bacteria) and Class 5 pili from the alternate chaperone pathway (e.g., CFA/I pili), which was based on the lack of genetic sequence homology, does not correlate with any major structural or functional differences between these classes of pili. Pilin subunits transit the outer membrane through an usher that can accommodate single subunits, but not the assembled helical filament. We identify a proline residue in the major pilin, CfaB, that appears to isomerize from the trans to the cis conformation, producing the conformational change required for assembly of the mature pilus filament comprising about 1,000 subunits. Lastly, analysis of genetic variability among clinical strains representative of the eight discrete Class 5 fimbrial subtypes, in combination with structural data, show that each bacterial strain presents a distinct outer surface of CfaB, while the interior and protein-protein interface residues are more highly conserved. These data suggest that protein surface variability facilitates evasion of the immune system by ETEC.

2153-Plat

Force Generation by Type IV pili of *Neisseria Gonorrhoeae*

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Type IV pili are major bacterial virulence factors supporting adhesion, surface motility, and gene transfer. During infection they mediate attachment to mammalian host cells and elicit downstream signals. The polymeric pilus fiber is a highly dynamic molecular machine that switches between elongation and retraction. We used laser tweezers to investigate the dynamics of individual pili of the human pathogen *Neisseria gonorrheae*. We found that the retraction velocity of bacteria adhered to an abiotic surface is bimodal and that the bimodality depends on force and on the concentration of the putative motor protein PilT [1]. When adhered to host cells the bimodality persisted at higher forces compared to an abiotic environment. This increase in average velocity is consistent with an up-regulation of PilT due to interaction with host cells. Bacteria generated considerable force during infection but the maximum force was reduced from (120 ± 40)pN on abiotic surfaces to (70 ± 20)pN on host cells, most likely due to elastic effects. Velocity and maximum force of pilus retraction were independent of the infection period within 1h and 24h post infection [2]. Thus the force generated by type IV pili during infection is high enough to induce cytoskeletal rearrangements in the host cell.

[1] M. Clausen, M. Koomey and B. Maier, Biophys. J. 2009, 96, 1169-1177
[2] D. Opitz, M. Clausen and B. Maier, ChemPhysChem 2009, 10, 1614-1618

Platform AG: Membrane Protein Structure I

2154-Plat

Molecular Modeling and Simulations of the Transmembrane Domain of Human Growth Hormone Receptor

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How transmembrane (TM) domains of membrane proteins transmit the signal across the cell membrane has long been a subject of keen interest in biology. There is a recent paradigm shift in the mechanism of activation for the cytokine receptor superfamily. The role of cytokine hormone binding to the extracellular domain is now recognized as an ''inducer'' of the conformational change of pre-dimerized TM domains that triggers subsequent intracellular responses. This is drastically different from its traditional role as an ''organizer'' whose sole function was to initiate the receptor TM dimer formation. Toward quantitative understanding of the mechanisms and accompanying energetics of TM-induced signaling of various single-pass TM receptors, we have generated TM homodimer models of human growth hormone receptor (hGHR) from primary sequence information using the GBSW implicit membrane model and replica-exchange molecular dynamics (REX-MD) simulations. The conformational clustering shows that hGHR forms right-handed TM dimers with two different interfacial motifs, i.e., LFFQ and GxxG. To test such prediction, we first carried out TOXCAT experiments of two hGHR TM mutants: Gly256Ile and Gly259Ile. Mutation of either position to isoleucine disrupts dimer formation. These results suggest the involvement of the glycine residues in the TM helix interaction through the GxxG motif, although we need more extensive experiments to examine the involvement of other residues in the TM dimer interface, or the existence of an alternate dimerization point. In addition, we have performed MD simulations of various hGHR dimer models extracted from GBSW REX-MD in explicit POPC membranes. The stability and orientational changes of hGHR TM dimers as well as various helix-lipid interactions will be also presented and discussed.

2155-Plat

Molecular Dynamics Simulations of the Dimerization of Transmembrane α-Helices

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The lateral association of transmembrane (TM) α-helices within a lipid bilayer environment is a key stage in the folding of membrane proteins. It may also play a role in signalling across cell membranes. Dimerization of TM helices provides a simple example of such lateral association. Direct atomistic (AT) resolution MD simulation of self-assembly of a TM helix bundle remains challenging. AT-MD may be complemented by coarse-grained (CG) simulations. We demonstrate how CG-MD may be used to simulate formation of dimers of TM helices. We also show how a serial combination of CG and AT simulation provides a *multi-scale* approach for generating and refining models of TM helix dimers. This approach has been applied to a number of examples, including the glycophorin TM helix dimer (a paradigm for helix/helix packing) [1], and the TM domain of the syndecan-2 receptor protein, which contains a GxxxG motif comparable to that of glycophorin. The multi-scale approach has also been applied to a more complex system, the heterodimeric αIIb/β3 integrin TM helix dimer.

[1] Psachoulia, E., P. J. Bond, P. W. Fowler, and M. S. P. Sansom. 2008. Helix-helix interactions in membrane proteins: coarse grained simulations of glycophorin helix dimerization. Biochem. 47:10503-105012.

2156-Plat

Aromatic Interfaces between Transmembrane Helices M1/M4 and M3/M4 Play a Key Role in Cys-loop Receptor Assembly

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Cys-loop receptors, also designated pentameric ligand-gated ion channels (pLGICs) include nicotinic acetylcholine receptors (nAChRs), serotonin type 3 receptors ($5HT_3$Rs), γ-amino butyric acid type-A receptors ($GABA_A$Rs) and glycine receptors (GlyRs). pLGICs function as obligate pentamers linked by non-covalent interactions between the N-terminal extracellular domains of identical or homologous subunits. Here we show that expression of GlyR α1 or $5HT_3$A subunits in two separate fragments (one containing the ectodomain