Multiphase Recovery of Escherichia Coli to Hyperosmotic Shock

using the Goldman formula to relate shear stress with force acting on a bacterium. The changes in bacterial position caused by changes in fluid flow are smaller than the changes in length obtained in the AFM experiments, suggesting bacteria bind to the flow chamber surface through more than one pilus.
We measure bacterial displacements with sub-micrometric precision by tracking their centers, and conclude that these displacements are consistent with the coiling and uncoiling of pili observed by AFM measurements but not with bacterial or pili deformation.

750-Pos Board B536
Oxygen Depletion and Speed Switching of a Bacterial Motor
Rainer Kurre, **Berenike Maier**.
University of Cologne, Köln, Germany.
Regulation of motility can enhance bacterial fitness through different strategies. The type IV pilus motor, that mediates bacterial surface motility, works in distinct velocity modes. Here, we addressed the question how environmental inputs control the occupation of the different modes in the human pathogen Neisseria gonorrhoeae. We found that fluctuating oxygen concentrations trigger transcription-independent switching between the high velocity mode (1.5 μm/s) and the low velocity mode (0.5 μm/s) within seconds. In the transition regime, single pili switched between both modes, indicating bistability. Oxygen depletion and switching into the low velocity mode correlated with a significant decrease in proton motive force. The response to oxygen depletion was not conserved between bacterial species, suggesting that their regulatory circuits have evolved differently. We hypothesize that phenotypic switching between fast ''explorers'' and slow ''power savers'' in regions of rapid oxygen consumption may be important for initializing biofilms.

751-Pos Board B537
Multiphase Recovery of Escherichia Coli to Hyperosmotic Shock
Teuta Pilizota, Joshua W. Shaevitz.
Princeton University, Princeton, NJ, USA.
All living cells employ an array of different mechanisms to help them survive changes in extracellular osmotic pressure. The difference in the concentration of chemicals in a bacterium's cytoplasm and the external environment generates an osmotic pressure that inflates the cell. It is thought that the bacterium Escherichia coli uses a number of interconnected systems to adapt to changes in external pressure. This allows these cells to maintain turgor and live in surroundings that range more than two-hundred-fold in external osmolality. Here, we use fluorescence imaging to make the first measurements of cell volume changes over time during hyperosmotick shock and subsequent adaptation on a single cell level in vivo with a time resolution on the order of seconds. We directly observe two previously unseen phases of the cytoplasmic water efflux upon hyperosmotic shock and identify the mechanisms behind them. Furthermore, we monitor cell volume changes during the post-shock recovery and observe two different types of response that depend on the shock level, as well as two different recovery time scales. The initial phase of recovery is fast, on the order of 20 min, and proceeds even in the absence of the two potassium transporters TrkA(G/H) and KdpFABC. A protein-synthesis controlled mechanism then causes the cell to switch to a second, slower recovery phase that lasts on the order of several hours. Interestingly, the occurrence of this secondary phase requires the presence of both TrkA(G/H) and KdpFABC, suggesting that it is triggered by coordinated import of potassium using these two transporters.

752-Pos Board B538
A Microfluidic Device for High Throughput Measurements of Thermotaxis
Michael J. Erickstad, Loic Tadrist, Alex Groisman.
University of California, San Diego, La Jolla, CA, USA.
E. coli bacteria have the ability to sense thermal gradients and congregate in locations with a preferred temperature. Studies of this phenomenon, called thermotaxis, have been complicated by the lack of tools to enable the generation of well-defined thermal gradients and the collection of data on motile *E. coli* in these gradients. Here we report a microfluidic experimental system which allows the collection of data on the migration of bacteria in thermal gradients at a high throughput. In our microfluidic device, a bacterial suspension slowly flows through a 10 mm long, 0.5 mm wide microchannel that has a linear temperature gradient across it. It takes ~100 seconds for cells to reach the end of the microchannel, thus they have sufficient time to sample the temperature gradient and find their preferred position in the gradient. By analyzing the distribution of cells across the microchannel near its end, we collect data on the thermotaxis of >10,000 individual *E. coli* cells per experiment. The temperature gradient is generated by the continuous circulation of temperature-controlled water through two large-diameter channels flanking the microchannel. The temperature gradient is evaluated using a temperature-sensitive fluorescent dye. The microfluidic chip is made from a single cast of PDMS, making it easy to fabricate and replicate with high fidelity. We have used the experimental system to perform preliminary experiments on wild type *E. coli* in a variety of linear thermal gradients and have observed the congregation of cells centered about a temperature of 35–37° C.

753-Pos Board B539
Microfluidic Analysis of Thermotaxis in *Escherichia Coli*
Lynn MacDonald[1], Michael Erickstad[2], Alex Groisman[2], William S. Ryu[1].
[1]University of Toronto, Toronto, ON, Canada, [2]University of California San Diego, San Diego, CA, USA.
Maeda et al. (1976) observed that *Escherichia coli* sense the direction of a temperature gradient, performing thermotaxis (directional motion in a temperature gradient) and aggregating at a preferred temperature. The molecular details of bacterial thermotaxis are still poorly understood. We have developed a microfluidic setup to measure thermotaxis of swimming bacteria in a dilute suspension at high-throughput. Extensive data on thermotactic migration of a given bacterial strain in a stable thermal gradient can be collected in less than 30 minutes. The microfluidic device is made of polydimethylsiloxane (PDMS) and has a 16 mm long, 500 μm wide and 40 μm deep test channel across which a linear, programmable temperature gradient is formed. A bacterial suspension is perfused through the channel at a low speed, such that individual cells have sufficient time to explore the gradient before they are imaged near the channel exit. We have developed imaging software to identify healthy swimming cells and determine their trajectories for taxis analysis. The spatial distribution of the bacterial trajectories is used as a measure of their taxis performance, and we show that the taxis performance is correlated with the steepness of the temperature gradient. Inversion of the thermal response can be induced by chemical adaptation to aspartate and serine, but adaptation to aspartate or serine only give a very weak inversion or null response, respectively.
Maeda, K., Imae, Y., Shioi, J.I., Oosawa, F. (1976). J. Bacteriol. 127:1039–1046.

754-Pos Board B540
Microfluidic Devices for Experiments on Bacterial Aerotaxis and for High-Resolution Imaging of Motile Bacteria
Micha Adler, **Alex Groisman**.
University of California, San Diego, La Jolla, CA, USA.
Bacterial aerotaxis, directional motion of bacteria in gradients of oxygen concentration (often called oxygen taxis), was discovered 130 years ago and has since then been reported in a variety of different bacteria. In spite of numerous studies, quantitative results an bacterial aerotaxis have always been difficult to obtain because of the lack of appropriate instrumentation. Here, we built and characterized an experimental setup consisting of a computer-controlled 3-channel gas mixer and a two-layer microfluidic device, generating stable linear gradients of oxygen concentration, $[O_2]$, across a long test channel, with $[O_2]$ as small as 0.25% (corresponding to microaerobic conditions) in the middle. The test channel was continuously perfused with a suspension of *E. coli* cells and distributions of cells across the channel near its outlet were measured, with data on aerotaxis of ~10^5 different cells collected within <1 hour. Extensive series of experiments on aerotaxis of *E. coli* cells were performed in linear gradients of $[O_2]$ with different slopes and mean concentrations. The experiments indicated that, in contrast to what was believed before, at $[O_2]$ up to ~13%, *E. coli* always prefer highest accessible $[O_2]$.
We also built and characterized microfluidic bacterial culture devices (chemostats) with the culture chamber depths adjustable between ~2 and <0.5 μm with a resolution of ~20 nm. By changing the chamber depth, motile bacterial cells of different species (*B. subtilis*, *E. coli*, *C. crescentus*) were gently immobilized for high-resolution fluorescence imaging and released after the imaging was completed. The technique also enabled culturing motile *C. crescentus* cells in semi-permeable microchambers, thus making it possible to generate *C. crescentus* colonies of exceptionally high density and observe the behavior of cells in these colonies.

755-Pos Board B541
The Heterogeneous Motility of the Lyme Disease Spirochete in Gelatin Mimics Dissemination through Tissue
Michael W. Harman.
University of Connecticut, Farmington, CT, USA.
The Lyme disease spirochete *Borrelia burgdorferi* exists in nature in an enzootic cycle that involves the arthropod vector *Ixodes scapularis* and mammalian