be revealed. In order to better understand the mechanosensitive properties of the stators, we combine high-resolution motor torque measurements in *E. coli* with a step detection algorithm with which we can estimate stator stoichiometry in single-motor traces. We detect stator dynamics at both steady-state and after a rapid and controlled change in external load, via a magnetic field.
In this way, we directly probe the mechanosensitivity of the stators, we observe and characterize single stator association and dissociation events triggered by the change in external load, and we quantify for the first time the dependence between stator number and external load. We incorporate these results into an adsorption model of stator kinetics, providing the first step into understanding the mechanism of mechanosensitivity of the BFM.

2794-Pos Board B401
Micro Manipulating a Single Bacterial Cell: Studying the Strength of Bacterial Cell Adhesion
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Bacteria switch their lifestyle from motile individuals to immobile colonies of clustered cells called biofilm on the surface when the environmental conditions such as pH, temperature and nutrients etc. are not favorable. In biofilm, bacteria express extra cellular polymeric substance (EPS) such as proteins, DNA and polysaccharides to protect themselves. Once formed, biofilm is stable and it it difficult to eliminate bacteria in biofilm. However at the initial stage of biofilm development, bacterial cells have to attach the surface before expressing EPS to protect themselves. We have developed a micro manipulation technique to manipulate single cells to study the initial surface attachment. In this presentation I will introduce a single-cell manipulation technique and the quantification of cell-surface and cell-cell adhesion strength.

2795-Pos Board B402
A Cardiolipin-Deficient Mutant of Rhodobacter Sphaeroides has an Altered Cell Shape and is Impaired in Biofilm Formation
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Cell shape has been suggested to play an important role in regulating bacterial attachment to surfaces and the formation of communities associated with surfaces. We found that a cardiolipin synthase deletion mutant of the rod-shaped bacterium *Rhodobacter sphaeroides*—in which synthesis of the anionic, highly curved phospholipid cardiolipin (CL) is reduced by 90%—produces ellipsoidal-shaped cells that are impaired in forming biofilms. Reducing the concentration of CL did not cause significant defects in *R. sphaeroides* cell growth, swimming motility, lipopolysaccharide and exopolysaccharide production, surface adhesion protein expression, and membrane permeability. Complementation of the CL-deficient mutant by ectopically expressing CL synthase restored cells to their rod shape and increased biofilm formation. Treating *R. sphaeroides*cells with a low concentration (10 μg/mL) of the small molecule MreB inhibitor, S-(3,4-dichlorobenzyl) isothiourea (A22), produced ellipsoid-shaped cells that had no obvious growth defect, yet reduced *R. sphaeroides* biofilm formation. This study demonstrates that CL plays a role in *R. sphaeroides* cell shape determination, biofilm formation, and the ability of this bacterium to adapt to its environment.

2796-Pos Board B403
Cross-Kymography Reveals the Structural and Kinetic Parameters of Archaellum
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Motility is critically important for all unicellular microorganisms. The flagellum is the representative motility machinery of bacteria and archaea, but its fluctuation and morphological dynamics have hindered the precise determination of flagella structure and the motor properties. To overcome problems, here we developed an advanced analysis method that we named 'cross-kymography' under total internal reflection fluorescence microscope. The cell body was attached to the glass, and labelled flagella were illuminated by evanescent wave. From the same image sequence, two kymographs, one parallel to the flagella and the other perpendicular to it, were extracted. Notably, the combination of directions of the propagation of spots in two kymographs uniquely indicates both structural helicity and rotation direction at any one time (1). The application to *Salmonella enterica* serovar Typhimurium demonstrated the left-handed helix structure as a normal mode. We observed a rotary filament of archaea, the archaellum, as surface appendage that resembles bacterial flagellum but is homologous to bacterial type IV pilus; little is known about the mechanism by which archaella produce motility. Notably, archaella bundle were always right-handed regardless of the rotation direction of the motor, and rotation rate was estimated to be 22.5 and 22.2 Hz in CW and CCW rotation direction, respectively. Using these structural and kinetic parameters, we computationally reproduced the swimming and precession motion with a hydrodynamic model. The torque was estimated to be 50 pN nm, and the efficiency of the motor is calculated to be 6-10% assuming unitary steps we observed in the motor is caused by single turnovers of chemical reaction of ATP hydrolysis.
(1) Kinosita, Y., Uchida, N., Nakane, D. & Nishizaka, T. Direct observation of rotation and steps of the archaellum in the swimming halophilic archaeon *Halobacterium salinarum*. *Nature Microbiology* 1, 16148.

2797-Pos Board B404
Quantifying Biophysical Differences between Planktonic and Biofilm Bacteria in Response to Antibiotic Application
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While many think of a bacterium as a solitary organism, most bacteria likely spend at least some time living in complex communities called biofilms. In fact, bacteria can exist in two separate forms: a motile planktonic bacterium or a stationary biofilm member. These biofilms might be made of a single species or multiple species, and provide a distinct organization that makes the biofilm resistant to removal by chemical or physical means. This becomes a problem when biofilms are causing infections by growing on indwelling medical devices or fouling the bottoms of boats. However, much of what we know about the antibacterial activity of a chemical compound comes from studying their effect on planktonic cells, and evidence suggest that chemical antibiotics will have a different level of effectiveness and cause different biophysical changes on cells in a biofilm. The research we present here is a first step in determining antibiotic effectiveness on matched samples of planktonic and biofilm cells, and quantifying the biophysical effects of antibiotic application.

Membrane Pumps

2798-Pos Board B405
Protonation Dependent Water Permeation of Ion Binding Pocket of Na^+ Bound Na^+, K^+-ATPase
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The six key acidic amino acid residues in the ion binding pocket of the Na^+, K^+ -ATPase (NKA) proposed to drive ion binding, release and possibly determine Na^+ or K^+ selectivity by dynamically altering their protonation states during the ion transport cycle. We investigate the effect of each protonation state using molecular dynamics (MD) and density functional theory (DFT) simulations and determine the probable protonation schemes of the Na^+ bound conformation of NKA. MD simulations of all possible protonation schemes show that the bound Na^+ ions are most stable when three or four protons reside in the binding sites, and that Glu954 near site III is always protonated. Glutamic acid residues in the three binding sites act as water gates, and their deprotonation triggers water permeation to the binding sites. From DFT calculations of Na^+ binding energies, we conclude that three protons in the binding site are needed to effectively bind Na^+ from water and four are needed to release them in the next step. We speculate that the water permeation of site III provokes the protonation of Asp926 which will induce Na^+ release, and Glu327, Glu954 and Glu779 are all likely to be protonated in the Na^+ bound occluded conformation. Our data provides key insights into the role of protons in the Na^+ binding and release mechanism of NKA.

2799-Pos Board B406
Exchange of Sodium Or Potassium Ions against Protons at Cytoplasmic Side of Na,K-ATPase
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The Na,K-ATPase performs active transmembrane transport of sodium and potassium ions. In addition, exchange of sodium or potassium ions against protons has been found under appropriate conditions. To study this exchange