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Board B542

Chemotaxis involves three complex and interrelated processes: directional sensing, cell polarization and motility. Directional sensing allows highly migrating eukaryotic cells to chemotax in extremely shallow (<2% across the cell body) gradients of the chemoattractant, cAMP in the case of *Dictyostelium discoideum*. Although directional sensing has been observed as spatially restricted responses along the plasmamembrane, our basic understanding of how cells process the gradient-controlled translocation of proteins during chemotactic movement is still largely lacking. Until now, the dynamics of the chemoattractant-receptor, cAR1, has been neglected in models describing directional sensing mechanisms. We studied in detail the dynamics of cAR1 and found that the receptors show localized differences in mobility across the cell body. In particular an agonist induced increase of mobile receptors was observed at the leading edge of cells performing chemotaxis. We showed this increase to be linked to the uncoupling of the Gα2 protein. Since this response is confined to the anterior of the cell we postulate that locally G-protein activation is enhanced. Enhancement is further facilitated by local clustering of receptors into plasmamembrane domains of ~230nm in size which in turn exhibit a diffusion constant of D=0.01μm^2/s.

Bacterial Sensing, Motors, & Motility

3240-Pos The Mechanochemistry of Mycoplasma Motility

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Board B543

Mycoplasma moves by gliding on solid substrates. Their locomotion has remained mysterious, for it involves no familiar motility related proteins. Located at the neck of the pear-shaped cell, the motility organelle consists of about 400 protein 'legs' spiking from a protein core structure. Each leg binds and unbinds from the substrate at its bulbous distal end. The remainder of the leg presumably acts as an asymmetric spring. As the legs 'row' to and fro, this asymmetry produces a net average driving force that propels the cell forwards.

In the model, the cell is connected to the motility organelle by a molecular motor that cycles through extension and contraction. The interactions between the substrate and the legs can be averaged out to be equivalent to an effective friction coefficient. Because of the asymmetric elasticity in the legs, the effective friction is rendered asymmetric in the direction of motion. The cell thus achieves a net motion through the differential displacements during the cycle of motor extension and contraction.

The model explains most of the experimental data. For example, the velocity of the cell increases nearly ten-fold over a ~30°C temperature range. The net velocity decreases linearly as the external load force increases, and the stall force is nearly independent of temperature. Finally, the result explains the cross-species comparison that longer legs correlate with larger velocity.

3241-Pos Modulation of the Rotational Direction and Speed of the Flagellar Motor by High-pressure

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Flagellated bacteria sense a variety of environmental factors and swim toward their favorable environment by rotating their flagellar filaments. The flagellar motor rotates exclusively in the counter-clockwise direction in the absence of switch-inducing protein CheY. Here, we show that high pressure also induces switching in the rotational direction of flagellar motors. *E. coli* cells that lack CheY proteins, were tethered by their flagellum to the observation window of high-pressure chamber. The rotational motion of the cell body was monitored by a high-pressure microscope, which could be available up to 2000 bar. At less than 800 bar, all cells rotated in the counter-clockwise direction and their speed were not affected. At more than 1000 bar, some cells started to rotate in the clockwise direction, and the rotational speed in both directions decreased steeply with pressure. After decompression, most of the cells recovered the normal activity in the rotational direction and speed. The high pressure is a direct and reversible stimulus for changing the motor function. The pressure-induced reversal in the rotational direction seems to modify the structure of the flagellar motor, as if the rotational direction of the flagellar motor is controlled by association with activated CheY molecules.

3242-Pos

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WITHDRAWN

3243-Pos Elasticity Of Cytoskeleton And Motion Of Magnetotactic Bacteria In AC Magnetic Field

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Board B546

Due to the coupling of the magnetosomes to cytoskeleton of magnetotactic bacteria it is possible to observe their complex dynamics in rotating magnetic fields of low frequency [1].

Here we illustrate that bacterium in unidirectional AC magnetic field orients perpendicularly to the field if the frequency is enough high. The physical reason is simple - the mean energy of the bacterium oriented perpendicularly to the field is less than that of the bacterium along the field due to finite coupling strength of the magnetic moment to its body. There are several mechanisms which determine the rigidity of this coupling. One is due to magnetodipolar interactions of the magnetosomes in the chain characterized by coercitive force . Other is due to flexibility of the protein filaments. Solution of the problem of magnetoelasticity of semiflexible filament [2] for the energy gives , where is determined by the ratio of the persistence length of the filament to its length, and Langevin field strength (is the angle the bacterium with magnetic moment makes with the field). Since is small then for a such magnetotactic bacterium as Magnetospirillum gryphiswaldense, taking the persistence length of actin, is by three orders of magnitude less than . Thus bacterium orients perpendicularly to the applied AC field due to the flexibility of the cytoskeletal proteins.

Experimental data of this flexibility are obtained by observation of the kinetics of the bacterium orientation in an external AC field and its comparison with the numerical simulation by Brownian dynamics.

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3244-Pos Swimming Trajectories of Caulobacter Crescentus Near a Surface

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Board B547

Flagellated bacteria tend to follow circular trajectories when swimming near a solid surface. *V. alginolyticus* follows circular trajectories when swimming backward with the flagellum ahead, but straight trajectories when swimming forward. In contrast, *E. coli* follows circular trajectories even though it swims forward only. We attempt to understand the difference in the swimming trajectories between forward and backward swimming cells near a surface by studying a single-flagellated bacterium, *Caulobacter crescentus*. We observed the circular trajectories for backward and straight trajectories for forward swimming *Caulobacter* cells, a phenomenon similar to that of *V. alginolyticus*. We measured the distance of the swimming cells from the surface using total internal reflection fluorescence (TIRF) microscopy. We found that the backward swimming cells follow circular trajectories simply because they stay close to the surface for some time, whereas the forward swimming cells departed promptly after they hit the surface. While staying in circular trajectory, the distance between a backward swimming cell and the glass surface varies over time. The average distance to the surface and the radius of the circular trajectory both increased with decreasing ionic strength of the medium, indicative of an effect caused by electrostatic interaction. In addition, we assessed the roles of hydrodynamic interaction, van der Waals force, Brownian motion, and the flexibility of flagellar hook in the swimming trajectory. In light of all factors considered, we propose that flexibility of the flagellar hook is the key to understanding the difference between forward and backward swimming.

3245-Pos Preconditioning Water for Bacteria Detection Using a Natural Material

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Board B548

Presently, there is a need to develop rapid detection of waterborne pathogens that afflict a majority of the World's population. In particular, developing countries where unsanitary water storage is an issue. Recently, there has been extensive research and development of biosensors, which would be a valuable tool to detect the presence of microorganisms such as bacteria in contaminated holding tanks. However, most biosensors still have poor sensitivity for low bacteria concentrations. We have developed a methodology to concentrate bacteria for rapid detection and to separate bacteria from the water to produce potentially potable drinking water. We use an economically viable and environmentally sustainable "green technology" utilizing a natural compound known as cactus mucilage, extracted from *Opuntia ficus-indica*, originally from Mexico. Cactus mucilage has been shown to be an effective flocculant that separates particulates, microbes and heavy metals from contaminated water. Experiments performed on *Escherichia coli, Bacillus globigii* and *Bacillus cereus* bacteria suspended in water have shown improved settling rates with the addition of different fractions of mucilage. Experiments have also shown that in the bacillus columns, the reaction was immediate and flocculation was complete in approximately five to ten minutes, with removal rates of up to 97%. Additionally, the bacteria, combined with the mucilage, tend to form a precipitate at the bottom of the column that can be removed with coarse filtration. Cactus mucilage is an ideal material for water treatment because it is a natural substance of low cost and ease of use, which can be readily obtained and processed. The use of this type of green chemistry can assist in the concentration of bacteria allowing it to reach the sensitivity range of most biosensors and for obtaining viable drinking water.

3246-Pos Optical Trapping and Its Application on Bacteria Adhesion

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We built an infrared laser tweezers setup and determined the force profile both near the center of the trap and at the far edge, using two independent methods in each region of the trap. The trap force is close to being linear with displacement within about 500 nm to the center of the trap. After reaching a peak trap force, it then decays gradually until about two microns away from the center of the trap. This result is consistent with the theoretical prediction of Arthur Ashkin.

Using this laser trap, we were able to trap a swarmer cell of Caulobacter crescentus, and keep it aligned along the path of the laser, creating the best view of the rotation of the cell body driven by its single flagellum. We also used the trap to bring a bacterium close to the glass surface in order to facilitate its adhesion to the glass surface within a few seconds of association, thereby studying the physical mechanism of such a process of attachment. These laser tweezers based manipulation operations prove very useful in probing the interactions between bacteria and surfaces at the single cell level.

3247-Pos Can Bacteria Sense Surfaces?

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Board B550

The overwhelming majority of prokaryotes living in close proximity to solids makes a compelling argument for the existence of surface sensing mechanisms in bacteria, analogous to the sense of touch in higher organisms. There has been evidence that some free-swimming bacteria can sense spatial gradients, but few prior studies have observed how attached organisms respond to the steep chemical gradient near substrate surfaces. We report experimental evidence which suggests that the non-motile organism *Staphylococcus aureus* possesses primitive biochemical sensory abilities, by which attached cells "feel" their substrate and localize substrate-specific biomolecules toward the interface region.

Our organism of study was a wild strain of *S. aureus* known to produce cell wall adhesins such as fibronectin binding protein. Using tips baited with fibronectin, we performed fluid atomic force microscopy (AFM) on living S. aureus cells attached to three different substrates. The substrates included inert clean glass, commercial cover glass, and fibronectin-coated glass.

Operated in force volume mode, the AFM cantilever was scanned across the attached bacteria and the fibronectin-coated tip was repeatedly brought into and out of contact with different regions of the cells, "fishing" for a reaction with biological molecules expressed on the cells. An attractive force was often observed upon separating the tip from a bacterium and its neighboring surfaces. This attraction manifested itself as one or more discrete sawtooth signatures in the force-separation profile, indicative of the extension of a protein. The stretched lengths and forces at which the bonded proteins ruptured were consistent and reproducible for a specific substrate but varied significantly between the three surfaces, both in magnitude and frequency of occurrence. The substrate influence on several aspects of the cells' physiological response suggests that complex mechanisms are involved in bacterial surface recognition.

3248-Pos Photoacoustic Study of the Photoactivation of the BLUF Domain of AppA Protein from *Rb. sphaeroides*

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Board B551

Here we present a photoacoustic study of the signaling mechanism in the BLUF domain of the anti-repressor protein AppA from *Rb. sphaeroides*. Two BLUF domain constructs AppAWT126 and AppAWT133 were studied. Also to understand the role of the individual amino acid residues in the photoactivation of BLUF domain, AppA126 constructs with altered amino acid residue Y21F and Q63E were also investigated. Our data show that the photoexcitation of the flavin cofactor in AppAWT126 construct is associated with a small increase in volume of ~ 20 mL/mol and enthalpy change of ~ 14 kcal/mol occurring within first 50 ns. No additional volume and enthalpy changes were observed on the longer timescale between 50 ns and 5 μs. Somewhat smaller volume and enthalpy changes were determined in AppAWT133 construct. The flavine excitation in the Q63E mutant does not results in measurable volume and enthalpy changes in agreement with the fact that mutation of Q63 locks the protein in the light state conformation. Excitation of photoinactive Y21F mutant leads to a small volume decrease of ~ - 2 mL/mol likely due to the increased triplet yield formation in this mutant. Presented results show that the photoactivation of the BLUF domain is not associated with significant structural changes what is in agreement with the structural data obtained by NMR spectroscopy and X-ray crystallography.

3249-Pos Studying dynamics of Min proteins in vitro

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Board B552

In Escherichia coli, the location of the site for cell division is regulated by the Min proteins, which a remarkable pole-to-pole oscillation in the cell. It was shown that these oscillations only depend on the action of MinD, which attaches to the cell membrane in an ATP-dependent manner and MinE, which expels MinD from the membrane by inducing its intrinsic ATPase activity. Several mathematical models suggest a self-organization process by which

these oscillations occur, but a direct experimental evidence for this hypothesis is still missing.

We expressed and purified both proteins and developed a simplified in vitro system that enables us to test the assumptions and predictions made by the theory.

Protein Structure Prediction

3250-Pos Predicting The Error Of Template-Based Protein Structure Modeling By Suboptimal Alignment Stability

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Board B553

Error in template-based protein tertiary structure modeling is unavoidable, but is not explicitly shown in most current prediction algorithms. The error estimation in structure prediction provides crucial information for experimental biologists who use predicted models for design and interpretation of experiments. Here we propose a method to estimate errors in predicted structures based on the stability of the optimal alignment compared with a set of suboptimal alignments. The stability of the optimal alignment is numerated by an index named the SuboPtimal Alignment Diversity (SPAD). SPAD is shown to have good correlation to actual prediction errors, both alignment shift errors and the root mean square deviation (RMSD) of predicted models to the native structures. This discovered correlation can be described by a linear regression function. Using this function, we have predicted the error of our CASP7 structure predictions by SPAD and the result matches the actual error well on the whole structure as well as at specific residues. We have compared SPAD with several other prediction quality measures such as the sequence identity between a target sequence and a template sequence, the statistical distant dependent atomic contact potential (DOPE), PRSS Z-score and the residue conservation, the gap ratio and the mutation score in the profile. Generally, SPAD is shown to have better correlation to actual prediction error, which means it is one of the best error predictors among these measures.

3251-Pos Study of Helical Kinks in Membrane protein crystal structures, and assessing the Computational Accuracy of Prediction using Molecular Dynamics simulations

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Board B554

The structural features of helical transmembrane proteins, such as helical kinks, bends, tilts, and rotational orientations are important in modulation of their function. These structural features modulate the activation dynamics of membrane proteins. In particular, the helical kinks caused by breaking of the backbone hydrogen bonds lead to hinge bending flexibility in these helices. Biophysical measurements such as spin labeling and EPR experiments and computational simulations have shown the flexibility of helical motion modulated by hinge bending region. Prolines and vestigial prolines in the structure predominantly cause the helical kinks. It is possible to have multiple helical kinks in one helix in the TM proteins and this could contribute to achieving functional diversity for a given structural topology. There are several other residues besides prolines that are also known to cause kinks because of the hydrogen bond between the side chains and the main chain of the helix at residues like Ser, Thr, Asn, Gln.

In this study, we have used the crystal structures of all helical membrane proteins (about 390 helices of length 19 to 35 residues from MPtopo database) to analyze the position and the extent of the helical kinks in transmembrane proteins. We found that most of the helical kinks are present at prolines or at vestigial prolines. However there are kinks present at other residues such as Gly, Met, Ser, Thr, and Cys. These results will be presented. We have also performed molecular dynamics simulations, starting from a canonical helix for the 390 TM helices. MD simulation results show that we can reproduce about 80% of the proline kinks, only 56% of the vesitigal proline kinks and 37% of the non-proline helical kinks.

3252-Pos An all-atom structural model for human factor VIIIa: A Molecular Dynamics simulation Study

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Board B555

Activated Human Factor VIIIa is a 1424 residue length five-domain blood coagulation protein cofactor that is critical for activation of factor X by IXa in the intrinsic pathway. Despite its central role in blood clotting, a full structure of FVIIIa is not known. In our efforts to provide specific details of protein-protein interactions among VIIIa, IXa and FX, we developed an all-atom based structural model for VIIIa based on its structural homology with recently reported partial structure of bovine Va (1SDD) and ceruloplasmin (1KCW). The homology model of A1A2 (A1- P740) heavy chain and A3-C1-C2 (E1675- Y2332) of light-chain is further refined by explicit water based MD simulations using AMBER FF99SB force-field. The dynamic refinement of ~240,000 atom system (comprising of 1400 AA residues, protein bound copper and calcium ions together with counter ions and PBC waters) for over a total period of 80 nano-seconds yielded a stable assembly of fVIIIa structure whose details provide a new look at the exisiting hypothesis of light-chain interactions with membrane surface. We present a structure-function correlation of known genetic mutations and site-specific mutagenesis data with our dynamic solution structure of FVIIIa.