statistical patterns nearly optimal for an efficient exploration of the environment. This exploratory motion is at the basis of contact formation and the establishment of appropriate synaptic connections. Filopodia and lamellipodia can also avoid obstacles and occasionally lamellipodia can displace them. From this point of view, filopodial and lamellipodial motion can be described as a random process in which errors are corrected by efficient feed-back loops. We argue that neurons not only process sensory signals, but also solve mechanical problems throughout their entire lifespan, from the early stages of embryogenesis to adulthood.

3242-Pos Board B289
Assessing the Dynamics and Mechanics of the Cell Membrane
Chilman Bae, Peter J. Butler.
The Pennsylvania State University, University Park, PA, USA.
Thermally and actively driven cell membrane fluctuations are known to be readouts for the nanomechanical interaction between the cortical cytoskeleton and the plasma membrane and the membrane. In this study, we developed a non-contact method to measure cell surface fluctuations through measurements of resistance between a microelectrode tip and the cell membrane. The system resolution was < 2 nm tested by using 2-10 Hz sinusoidal piezo stage motion with amplitudes ranging from 2 nm to 100 nm. We found that endothelial cells exhibited local membrane fluctuations of ~20 nm at a number of characteristic frequencies. To determine the role of actin in membrane fluctuation, we treated cells with 2 μM of actin depolymerizing drug, cytochalasin D, and we found that actin depolymerization increased in fluctuation amplitude up to 2 times at all frequencies. Finally, to determine role of ATP in membrane fluctuations, we treated cells with ATP depletion drug cocktail which consisted of 25nM Antimycin A + 2mg/ml 2-Deoxy-D-Glucose, and we found that ATP depletion abolished all membrane fluctuations. Therefore, actin cytoskeleton and dynamic processes facilitated by ATP may modulate membrane functions through mechanical effects on membrane fluctuations

3243-Pos Board B290
Cell Coat Mediated Cell Migration
Louis T. McLane, Anthony Kramer, Carrie Harris, Edward Park, Hang Lu, Jennifer E. Curtis.
Georgia Institute of Technology, Atlanta, GA, USA.
Cell migration depends on a sequence of adhesion and detachment events. These events arise during the cyclic migration process, which involves the integrin-dependent adhesion machinery, the acto-myosin system and the signaling pathways between them. Migration appears to rely on a delicate spatio-temporal regulation of cell adhesion to the surrounding substrate. Studies of cell migration in physiological contexts have shown that a pericellular coat, a thickened polymer matrix attached to many cell surfaces, often is required to facilitate cell migration, including that of aggressively spreading cancers. Cell motility in these systems directly depends on the formation of a large, hyaluronan-rich cell coat with an asymmetric distribution around the polarized migrating cell. Removal or alteration of the coat substantially decreases motility - to the point that such treatments have been proposed as therapies for some types of cancer. The hyaluronan biology community often speculates that cell migration requires the 'insulation' and/or the mechanical properties provided by the cell coat. However, little has been done to substantiate this claim. A bigger problem yet is that the lubricating effect of hyaluronan has been shown to oppose adhesion, which leads to a conundrum in the present context: How is it possible that inhibition of adhesion can help a cell migrate, when adhesion is absolutely necessary to gain traction and exert the force to move the cell forward? We have developed a microfluidics-based cell migration assay capable of presenting several surface gradients of fibronectin of different slopes to induce cell migration within the same device. We study the ability of these gradients to induce cell migration and their influence on the cell coat phenotype and mechanical properties using a combination of fluorescent labeling, particle exclusion assays, and optical tweezer force probe experiments.

3244-Pos Board B291
Biophysics of Tumor Cell Adhesion: From single molecules to multi-cellular interactions
Tianyi Yang, Ge Wang, **Muhammad H. Zaman**.
University of Texas at Austin, Austin, TX, USA.
Cell adhesion plays a critical role in tumor formation, invasion and metastasis. The complex processes underlying adhesion to other cells and the extra-cellular matrices are dynamic and inherently multi-scale. Unfortunately, computational and mathematical models aimed at understanding adhesion have traditionally focused on a single length-scale and have been unable to link events at the atomic and molecular scale to bulk behaviors seen in experiments. In addition, most adhesion models have been blind to the effects of matrix structure and mechanics, molecular sequence and conformations and hence can only make qualitative predictions. Using a combination of molecular dynamics to generate conformations, coarse-graining of these results for single-chain mean field theory and then further coarse graining too study processes at the bulk level, we have developed a fully multi-scale model of cell-matrix and cell-cell interactions. Our models are rooted in principles of thermodynamics, statistical and continuum mechanics and are able to capture cell-matrix and cell-cell adhesion events at a single molecular, cellular, multi-cellular and tissue level. We are also able to study the effects of soluble and insoluble ligands, functionalized nano-particles and tethered surfaces. Thus our model is able to make quantitative predictions in both in vivo and in vitro environments.

3245-Pos Board B292
Professor
Krishna Garikipati, Joseph E. Olberding, Michael Thouless, Ellen M. Arruda.
University of Michigan, Ann Arbor, MI, USA.
The free energy that drives growth, resorption and sliding of focal adhesions includes mechanical and chemical contributions. We have identified a competition among four effects that control focal adhesion dynamics: (1) work done during addition of complexes, (2) the chemical potential inherent to focal adhesions, (3) the elastic free energy associated with deformation of focal adhesions, and (4) work done on a molecular conformational change. A theoretical treatment of focal adhesion dynamics developed in the framework of rate processes driven by thermodynamics demonstrates that the mechanisms governed by these four effects allow focal adhesions to exhibit a rich variety of behavior without the need to introduce special constitutive assumptions. In this treatment, the structural unit of focal adhesions is a complex consisting of a ligand such as fibronectin, an integrin molecule, and associated plaque proteins. The binding and unbinding of these complexes causes focal adhesion growth and resorption, respectively. The reaction-limited case is considered. Our central findings are that growth, resorption and sliding are all predicted by a very simple chemo-mechanical model. Sliding requires symmetry breaking and is achieved via (1) above; (4) promotes symmetric growth, and (2) and (3) cause symmetric desorption. The role of kinetic modulation is also examined.

3246-Pos Board B293
Micromechanical Properties Of Fixed And Living Vascular Pulmonary Endothelial Cells Following Exposure To Barrier Enhancing And Barrier Disrupting Agents
Fernando M. Teran Arce, Ratnesh Lal, Joe G.N. Garcia, Steven M. Dudek.
University of Chicago, Chicago, IL, USA.
Disruption of pulmonary endothelial cell (EC) barrier function is a critical pathophysiologic event that occurs in multiple inflammatory disease processes. The actin cytoskeleton, an essential regulator of endothelial permeability, is a dynamic structure whose stimuli-induced rearrangement is linked to barrier modulation. We used atomic force microscopy (AFM) to characterize structural and mechanical changes in the F-actin cytoskeleton of cultured human pulmonary artery EC in response to both barrier-enhancing and barrier-disrupting conditions. The mechanical properties of both fixed and live cells were evaluated. Elastic modulus values in the range of 50-1000 kPa were typically measured for fixed cells, while much lower values of 1-40 kPa were characteristic of live cells. In fixed cells, a differential distribution of elasticity was observed after exposure to the barrier-enhancing compound S1P (sphingosine 1-phosphate) compared to that produced by the barrier-disrupting agonist, thrombin. After S1P, the elastic modulus was elevated primarily at the periphery, while thrombin treatment increased elasticity in the central region of the cell. These observations correspond with the distribution of F-actin in parallel-treated EC as detected by immunofluorescence. In living cells, thrombin generally increased the average elastic modulus over 60 minutes; however, the S1P response was more varied and subtle. Experiments are under way to confirm these preliminary observations. These results provide novel insights into the structural and mechanical properties that dynamically regulate pulmonary EC barrier function.

3247-Pos Board B294
Adaptive-Control Model for Neutrophil ORIENTATION in the Direction of Chemical Gradients
Daniel Irimia.
Harvard Medical School, Boston, MA, USA.
Directional movement of neutrophils in spatial chemical gradients is the result of complex intracellular signaling mechanisms that are not yet fully understood. Although many of the signaling molecules that participate in the mechanisms of gradient detection in neutrophils are already known, current models still cannot provide satisfactory explanation for the initial orientation in the direction of chemical gradients. To address these challenges, we propose a new biophysical model for neutrophil orientation in the direction of chemical

gradients, starting from unexpected experimental observations of stochastic response time of individual neutrophils after sudden exposure to spatial chemoattractant gradients. We propose that neutrophils orientation is achieved by the synergy between localized temporal sensing through expanding pseudopods and whole-cell integration of the temporal information by microtubules. In our model, microtubules play functional roles in the local positive feedback via stabilization near membranes experiencing localized temporal concentration increases, and provide global signal integration via scarcity and redistribution inside cells. Experiments using chemical inhibitors of microtubules support the hypothesis that microtubules could play a key role in cell orientation in the presence of spatial chemoattractant gradients. Modeled cells can not only detect the direction of a spatial gradient, but at the same time remain responsive to further changes in the direction of the gradient. Better understanding of neutrophil activity could have practical implications in clinical conditions of inflammation and during immune responses against bacteria and injuries.

3248-Pos Board B295

How Does The Bacterial Flagellar Motor Of Rhodobacter Sphaeroides Stop - Using A Clutch Or A Brake?

Teuta Pilizota, Mostyn T. Brown, Mark C. Leake, Richard M. Berry, Judith P. Armitage.
University of Oxford, Oxford, United Kingdom.

The bacterial flagellar motor is a rotary molecular machine ~50 nm in diameter enabling some bacterial species to swim. It is embedded in the cell envelope and connected to an extracellular helical propeller. The motor is powered by the flow of ions down an electrochemical gradient across the cytoplasmic membrane into the cell. Most of our knowledge on motor function comes from work on the *E. coli* motor, which can switch between clockwise and counterclockwise rotation, allowing the bacterial cell to change direction in response to different stimuli.

A proton-driven flagellar motor of *Rhodobacter sphaeroides* achieves the same goal as the bi-directional *E. coli* motor, that of changing cell direction in response to the external environment, but does so by stopping and rotating in only one direction.

We employed several techniques to monitor and manipulate the motor to find out how the stop is achieved. The rotation of a 0.83μm polystyrene bead attached to a truncated flagellum was monitored using back-focal-plane laser interferometry. This allowed us to observe stops in motor rotation with a high temporal (up to 0.1 ms) and angular (~1 degree) resolution. In separate experiments we tethered cells down to glass coverslips by their flagella and applied external torque with an optical trap using the cell body as a handle.

Here we characterize mechanical properties of the motor and show how the motor stops rotating - by putting the brakes on.

3249-Pos Board B296

Experimental Evidence for Conformational Spread in the Bacterial Switch Complex

Richard W. Branch, Fan Bai, Dan V. Nicolau, Teuta Pilizota, Bradley Steel, Philip K. Maini, Richard M. Berry.
University of Oxford, Oxford, United Kingdom.

The allosteric regulation of proteins has classically been understood in terms of the Monod-Wyman-Changeux (MWC) or Koshland-Nemethy-Filmer (KNF) models. These are recognized as limiting cases of a general allosteric scheme that has recently been described in a model of conformational spread. A candidate proposed for testing the model is the bacterial switch complex, an ultrasensitive multimeric protein ring responsible for controlling the direction of rotation of the bacterial flagellar motor. The complex is too large for MWC-type interactions to be applicable and cooperative binding studies have ruled out the KNF model. Here we use high-resolution back-focal-plane interferometry to resolve intermediate states of the complex predicted by conformational spread, and demonstrate detailed quantitative agreement between our measurements and simulations. Individual switch events are not instantaneous, but follow a broad distribution of switch times with mean ~ 20 ms, incomplete switches occur at a bias-dependent frequency and intervals between switches are exponentially distributed at all values of bias.

3250-Pos Board B297

Direct Observation Of [Ca2+]i Changes In Motile Sperms With 50 msec Time Resolution

Koji Matsuura, Keiji Naruse.
Cardiovascular Physiology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan.

Ejaculated motile sperms swim against flow in oviduct toward egg. For fertilization, acrosome reaction and regulation of sperm motility including hyperactivation and control of flagellar beat are important events. $[Ca^{2+}]_i$ plays a major role in all the important sperm functions that occur after ejaculation. Much work on sperm Ca^{2+} signaling has used agonists and activators rather than flow, because the small size of sperm presents inherent difficulties in direct observation of motile sperms. Indeed, the $[Ca^{2+}]_i$ in motile sperm has not been directly recorded in flow in microfluidic environment. We will report the system to record $[Ca^{2+}]_i$ in motile sperm with and without flow, and investigated the correlation between velocity of motile sperm and $[Ca^{2+}]_i$ distribution in sperm. Sperm motions in microfluidic environment and $[Ca^{2+}]_i$ changes in the motile sperms were investigated by high-time resolution confocal fluorescent microscopy with high magnification. To record $[Ca^{2+}]_i$, human sperm suspensions stained with FLUO-3AM were injected into a microfluidic channel fabricated by soft-lithography, and confocal fluorescent 4D images were reconstructed with time resolution of 50 msec/frame. $[Ca^{2+}]_i$ changes in the head, midpiece, and tail of the sperm were observed. We found a positive correlation between motile sperm velocity and maximum fluorescent intensity, corresponding to $[Ca^{2+}]_i$, in the midpiece of a sperm. Based on the studies on sperm chemotaxis, $[Ca^{2+}]_i$ is accumulated in the midpiece of sperm, and the Ca^{2+} ions in the midpieces are used for the regulation of flagellar beat mode. We can suggest that $[Ca^{2+}]_i$ elevation in the midpiece would be necessary for high-speed movement of the flagellar.

3251-Pos Board B298

Temperature-dependence Of Torque Generation Of The Na ± driven Chimeric Flagellar Motor And Visualization Of The Stator Proteins In E.coli

Akihiko Ishijima, Yuichi Inoue, Hajime Fukuoka.
Tohoku University, Sendai, Miyagi, Japan.

Bacterial flagellum is a supramolecular complex and consists of a basal body, a helical filament, and a hook. A basal body embedded in cell membrane functions as a rotary motor driven by electrochemical potential of specific ion, and rotates flagellar filament like as screw. The rotor consists of MS-ring (FliF) and C-ring (FliG, FliM, and FliN).

To learn roles of the electrostatic interaction between stator and rotor in the mechanism of torque generation, we examined the motor response over the temperature range 5-50 degree. At low temperature (23-5 degree), rotational speeds linearly decreased with decreasing temperature. With increasing temperature, however, sudden drops of speeds were observed over ~30, ~40 and ~50 degree. When the temperature returned back to 23 degreee, the speed was restored mostly in several minutes. The drop and recovery of the speed were coincided with stepwise change in the generated torque.

And, we constructed fusion proteins of rotor components and Green Fluorescent Proteins, and investigated whether rotor components are exchanged in a functional motor by FRAP analysis for a single motor labeled with GFP.

In the tethered cell that was produced each GFP fusion, a fluorescent spot was localized at the rotational center. Each GFP fusion was probably incorporated into flagellar motor as a rotor component. In order to investigate the exchange of rotor components, we carried out FRAP analysis using evanescent light. GFP-FliN or FliM-GFP recovery of fluorescence at the rotational center was observed as time passed. On the other hand, the recovery of fluorescence was not observed in the cell producing GFP-FliG. These results suggest that some rotor components assemble to motor even after functional motor is constructed.

3252-Pos Board B299

Distribution Of Traction Forces Associated With Shape Changes In Migrating Amoeboid Cells

Baldomero Alonso-Latorre, C. del Juan Alamo, Effie Bastounis, Ruedi Meili, Richard A. Firtel, Juan C. Lasheras.
University of California, San Diego, La Jolla, CA, USA.

Amoeboid motility results from the cyclic repetition of a repertoire of shape changes leading to periodic oscillations of cell area (motility cycle). This study aimed to identify the dominant shape changes and their association to the regulated activity and localization of molecular motors. For this purpose, we applied Principal Component Analysis (PCA) to time-lapse measurements of cell shape, traction forces and fluorescence from the F-actin-binding protein limEΔcoil-GFP in migrating *Dictyostelium* cells. This method provides the most significant cell shape changes of the motility cycle, together with maps of the traction forces and F-actin distribution associated with each shape change mode. It also sorts these modes according to their contribution to the variance of the cell area oscillations observed during the motility cycle. Using wild-type cells (*wt*) as reference, we investigated myosin II activity by studying myosin II null cells (*mhcA-*) and essential light chain null cells (*mlcE-*). The results revealed that *wt, mlcE-* and *mhcA-* cells implement similar shape changes during their motility cycle, although they are implemented at a slower pace in myosin mutants. The repertoire of shape changes is surprisingly reduced as only three modes are