plate-plate rheometer by applying a cyclic sinusoidal strain of a given amplitude, and analyze the non-linear stress-strain relationship. With each cycle, the maximum stress and the linear modulus of the material decrease, and the transition from a linear to a strain-stiffening response occurs at higher strains. If the strain amplitude is increased and a new set of cycles is taken, this 'working' of the material repeats for the now higher strain amplitude. However, for each strain step, the first oscillation of the set of cycles is qualitatively different from the following ones. First, when compared to the stress-strain response of a gel that has not been previously worked at smaller strains, the two match closely. Secondly, upon unloading, this first oscillation shows a significantly increased dissipation.
Upon addition of covalent crosslinks by incubating the collagen gels with 2% glutaraldehyde solution after polymerisation, the stress-strain relationship becomes independent of the loading history. We hypothesize that the microscopic mechanism responsible for the history dependence is due to intra-fibrillar slip of adjacent collagen monomers. We present evidence from direct observation using confocal microscopy to image collagen gels under shear that supports this hypothesis.

2881-Pos
Direct Detection of Tension Recovery After Local Stretching of Cell Surface
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Cellular response to the externally applied force has a vital effect on cell proliferation, propagation and finally on its ultimate fate. Details of the molecular basis of the mechanical response are , however, little known. Here we measured mechanical and structural responses of a cell under locally applied force on an atomic force microscope (AFM) equipped with a fluorescence microscope for live cell imaging. When a colloidal AFM probe was first pressed on the cell surface and then pulled up, the tensile force sensed by the cantilever was recovered after initial relaxation. This recovery of the tensional activity was inhibited when cells were treated with cytochalasin D, the inhibitor of actin polymerization, or blebbistatin, the inhibitor of ATPase activity in myosin II, suggesting that the tension-recovering activity was driven by actin-myosin contractility. Our method allows us to investigate the dynamic processes of the mechanical maintenance of subcellular structures in a single cell.

2882-Pos
Probing the Micrheology of Mesenchymal Stem Cell Migration to Tumors
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Mesenchymal stem cells (MSCs) are excellent candidates for the development of cell-based gene delivery systems; however, extended cell culture, required for therapeutic development, alters MSC morphology, reducing MSC migration upon reinfusion. Spontaneous migration of MSCs to tumors is mediated by tumor secretion of proangiogenic chemokines. Multiple particle tracking microrheology was used to investigate the effect of tumor-secreted molecules on MSC viscoelasticity, which was correlated with MSC migration and morphology. Within 24 hours after MSC treatment with tumor-conditioned media (TCM), MSCs were elongated, with more than 5-fold difference in the length of lamellipodia. Within 24 hours, the migration of MSCs, measured using a Boyden chamber assay, toward TCM was increased 10-fold over control media. The mean squared displacements (MSDs) of 100-nm carboxylated polystyrene particles, injected into the cytoplasm of human MSCs using the Biolistic Particle Injection System, were determined with 33 ms temporal and 5 nm spatial resolution using multiple particle tracking. The frequency dependent elastic and viscous moduli were calculated from the complex shear moduli, which were determined from the Fourier transform of the time-dependent MSDs, using the frequency-dependent Stokes-Einstein equation. Pretreatment of MSCs with TCM resulted in rapid changes in cytoplasmic viscoelasticity with a 9.8-fold increase in the average elastic moduli, which increased from 35 to 344 dyn/cm2, and a 3.5-fold decrease in the average viscous moduli, which was reduced from 99 to 28 dyn/cm2, within 1 hour ($n = 6$-8 cells per group). We hypothesize that tumor-secreted molecules increase MSC mobility by altering cytoskeletal organization. Changes in MSC viscosity may be in part to reduced actin cross-linking during cytoskeletal reorganization. Increased MSC rigidity may be due to MSC elongation, which leads to the formation of polymer entanglements as the ratio of cell length to width is greatly increased.

2883-Pos
Toward Magnetic Control of Cell Polarity
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Cell polarity is involved in many aspects of cell and developmental biology. It is of fundamental importance for processes as diverse as cell motility, division, or differentiation. Cell polarization manifests itself through complex signaling and transport mechanisms by which molecules are asymmetrically localized within the cell. Whereas usual genetic and biochemical approaches are adapted to identify the elements in a transduction pathway which are necessary for the emergence of a cell polarity, they are not sufficient to know if the sole localized activity of a given effector is sufficient for the cell to acquire a polarity or to determine the kinetics of polarity formation. To address these important issues, we present a novel approach based on functionalized magnetic nanoparticles which are used to induce a localized signaling event of polarization. By doing so, we are able to monitor the cellular dynamic response to a local perturbation while preserving the complexity of the interaction feedbacks needed for the emergence of a global polarity. In our experiments, fluorescent magnetic nanoparticles (100-500 nm in size) are coated with purified constitutively active Cdc42 proteins, a key regulator of cell polarity. Once injected in the cytoplasm of live cells, these nanoparticles are manipulated using a customized magnetic setup able to exert forces on the order of 1-100 pN. We monitor at the single cell level the dynamics of nanoparticles and analyze the role of diverse factors (cytoskeleton, ER, substrate rigidity) on their intracellular mobility. Finally, we measure in different cell lines (Hela, 3T3) the effect of the local signalization on downstream effectors such as actin dynamics.

Bacteria & Motile Cells: Signal Transduction

2884-Pos
Generating Alternating Bidirectional Gradient Fields for Dynamic Measurements of Chemotactic Response
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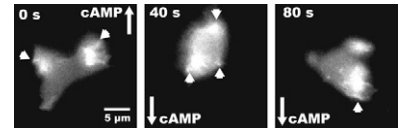
Prerequisite to a quantitative analysis of biochemical signalling networks is a well defined stimulus in space and time and a defined marker of biochemical response at the single cell level. We combine time-lapse fluorescence microscopy with a microfluidic chamber, which allows applying a defined temporal sequence of a spatially homogeneous chemical gradient over an ensemble of cells. The distribution of a chemoattractant in the flow chamber is characterized and the performance of the device found in good agreement with finite element calculation. To elucidate the dynamics of cellular response we investigate the velocity distribution of the amoeba Dictyostelium discoideum as a response to alternating cAMP gradients in opposing directions. We find pronounced directional migration at low switching frequencies, while at switching frequencies above 0.01 Hz stochastic cellular motility exhibiting seemingly non-responsive cells is observed. We demonstrate that the dynamics of intracellular polarization as displayed by the distribution of Lim-GFP is delayed with respect to the external change in gradient. We expect the microfluidic set-up to be useful for comparison of experimental data and computational systems modelling of cellular responses.



2885-Pos
The Response of Single E. Coli Cells to Changes in External Osmolarity
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The extreme concentrations of chemicals in a bacterium's cytoplasm generate a large osmotic pressure that inflates the cell. Using a number of interconnected systems, bacteria actively regulate their turgor pressure to resist changes in their local environment. In response to osmotic shock and changes in internal ion concentration, the osmosensory transporters ProP/U, BetT/U, TrkAH and KdpFABC transport external chemicals such as proline, choline and potassium into the cell, whereas the mechano-sensitive channels MscS and MscL export solutes from the cell in response to increased membrane strain. Although each has been shown to play a role in the regulation of turgor, details of how the different systems are coordinated by a cell is poorly understood. Previous measurements of osmoregulation in bacteria have been unable to directly probe the adaptation of turgor pressure, focusing instead on the activity of various transporters, or the change in cellular survival rates. Here we move beyond these limited measurements using AFM and fluorescence imaging to monitor turgor pressure and cell volume adaptation on a single cell level with a time

resolution on the order of seconds. To explore different mechanisms used by bacteria, we moved exponentially growing cells from LB medium to an iso-osmotic buffered medium and allowed the cells to adapt. We subsequently challenged the cells with varying levels of sucrose (as an external osmolyte) and potassium or proline. We measured the dependence of the adaptation time and adaptation level on different amounts of extracellular potassium or proline and the magnitude of the osmotic shock. This type of measurement allows us to uncouple the different adaptation pathways and to study them individually and in small groups to quantify their function and interactions.

2886-Pos

Nck Function in Tyrosine Kinase Signaling to the Actin Cytoskeleton

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Tyrosine kinase signaling leads to the post-translational modification of proteins and their binding partners. These modifications lead to the membrane recruitment of signaling proteins, promoting an increase in their local concentration, which results in a cellular response to the phosphorylation of tyrosine residues. Nck, an SH2/SH3 adaptor protein, functions in tyrosine kinase signaling by linking tyrosine phosphorylation on the membrane with binding partners, such as N-WASp, that function in facilitating actin nucleation and polymerization. However, quantitative and mechanistic aspects of signaling through Nck remain poorly understood. To explore the linkage of Nck to the actin cytoskeleton, our lab developed a system in which Nck SH3 domains can be aggregated on the plasma membrane following antibody application. Aggregation of Nck SH3 domains results in localized actin polymerization in the form of actin comet tails. Using the Virtual Cell, we have built a comprehensive, quantitative actin cycle model. With this model, we have produced predicted results that have been confirmed in vivo. This model predicts experimental comet tail length, actin distribution within the comet tail, and maximum actin concentration in the tail based on the number of molecules in the aggregate and the speed at which the aggregate is moving across the cell surface. We have also adapted the model to test the implications of the recent findings that binding of two N-WASp molecules to the Arp2/3 complex enhances actin nucleation and polymerization when compared with single N-WASp activation of the Arp2/3 complex. The combination of modeling and precise experimental manipulation provides unique insights into the relationship between increased local concentration of Nck and resulting localized actin polymerization.

2887-Pos

Probing the Protein-Protein Signaling Mechanism in Intact Archaeal Cells Using Time-Resolved FTIR Difference Spectroscopy

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Elucidation of the molecular mechanisms of protein-protein interaction and signal transduction remains an important goal in biophysics. Fourier Transform Infrared (FTIR) difference spectroscopy allows the study of protein structural changes at atomic resolution, however most FTIR studies are currently performed on purified proteins removed from their original environment. Here, we present evidence that FTIR spectroscopic methods can be successfully applied to detect conformational changes of individual proteins and protein complexes in the native cell membranes as well as in intact cells. The FTIR spectra of haloarchaeal blue-light phototaxis receptor sensory rhodopsin II (SRII), a seven-helical membrane protein, linked to its full-length cognate transducer HtrII was studied in isolated cell membranes. The SRII-HtrII complex exhibited a greater extent of conformational changes assigned to receptor-transducer interactions compared to earlier studies of heterologously expressed, detergent purified and reconstituted SRII-HtrII complex which is truncated in the membrane-proximal region. The difference spectra of full-length complex also reveal conformational changes which are likely to occur in the distant cytoplasmic region of the transducer that functions as a binding site for histidine kinases. Moreover, for the first time, time-resolved spectra of the SRII-HtrII complex were recorded in intact halobacterial cells (Halobacterium salinarum) using visible light to trigger the SRII signaling pathway, which ultimately controls the cell's motility. The ability to monitor time-resolved protein structural changes that occur inside living cells has the potential to significantly expand the scope of biological FTIR spectroscopy. For example, the effects of transmembrane potential and interaction of proteins downstream of the receptor-transducer complex can be investigated in a native environment.

2888-Pos

Crystal Structure and Mutational Analysis of the Periplasmic Flagellar Protein FlgA

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Bacterial motility is achieved by rotation of flagella. The bacterial flagellum is a macromolecular complex that allows bacterial cells to swim in liquid environment. The complex is composed of the helical filament, the flexible hook and the basal body embedded in bacterial inner and outer membranes penetrating peptidoglycan layer. The basal body is divided into three sub-structures, the LP-ring, the MS-ring and the rod. The LP-ring, as molecular bushing, spans between bacterial outer membrane and peptidoglycan layer. FlgH and FlgI are the subunit proteins of the LP-ring and a periplasmic flagellar protein FlgA is involved in the P-ring assembly. Previous biochemical studies indicated that FlgA might associate with FlgI after secretion into the periplasm and act as a key protein for the flagellar P-ring assembly. The atomic structure of Salmonella FlgA has been determined at 2.1 Å resolution. The over-all structure revealed that FlgA comprised of two distinct domains as previously shown by limited-proteolysis experiments. The beta-clip fold in the FlgA structure could be involve in binding to peptidoglycan. FlgA mediates the assembly of the flagellar P-ring by means of its interactions with the carbohydrate moieties of peptidoglycan. Site-directed mutagenesis to residues at the putative FlgI binding site of FlgA caused reduced ability to complement in the FlgA-deficient Salmonella strain, indicating that FlgI required the constitutive interaction with FlgA in the flagellar P-ring assembly. We discuss about fundamental functions of FlgA and propose the molecular mechanism of the flagellar P-ring assembly.

Unconventional Myosins I

2889-Pos

Cymobase - the Reference Database for Cytoskeletal and Motor Proteins

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Motor proteins are involved in processes like cellular transport, muscle contraction, and cell division. Three motors are myosin, dynein, and kinesin. They convert chemical energy (ATP) into mechanical work (movement). Protein sequences are the bases for many biochemical and cell biological experiments, as well as bioinformatical analyses.
We implemented a web application (CyMoBase) to represent all sequence related information. Since the fist publication of Pfarao (www.cymobase.org), many changes have been integrated in the database scheme and the web application. Now, it is also possible to derive information about structures and genes. Over the years, the number of data has increased considerable. Today, there are 37 proteins, 16500 sequences, 132 domains, 819 species, 599 publications and 1392 projects, with a total sequence length of over 16 million amino acids. The web application has got a new central search page including nine search modules (species names, protein classes, taxonomy, species groups, domains, sequence meta data, publications, sequence names, and genes) and eleven result tabs (sequences, publications, downloadable FASTA files, alignment viewer, phylogenetic trees, sequence stats, domain composition, complex inventory, protein inventory, molecular weights, and class composition). All search modules can be combined to filter the results.
Furthermore, we provide the gene structure of all sequences as computed by WebScipio. An other viewing option is the ''Complex Inventory'', which presents the existence or absence of sequence homologs of certain complexes. The aligned protein sequences are available via the ''FASTA Files'' result tab.
The size of the database, the kind of annotation, the possibility to use and combining different search modules, and the number of information and options offered by the web interface makes Pfarao the reference database number one for cytoskeletal and motor proteins.

2890-Pos

Regulation of Myosin Motility by D-Loop of Actin

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Subdomain 2 of actin, which contains the DNase I binding loop (D-loop, residues 38-52), slightly changes its conformation during actin polymerization and interacts with the C-terminus of the adjacent subunit in actin filament. This region is suggested to be important for actin-myosin interaction: it was found that