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