preparation of *E. coli* DAGK in lysophospholipids, LMPC and LMPG, allows the protein to be catalytically active as observed using a spectrophotometric assay. We also found that the ^{15}N-TROSY-HSQC spectrum quality of these samples is comparable to that of a sample prepared in dodecylphosphocholine (DPC), which is the detergent used to determine the structure of *E. coli* DAGK in the absence of the substrates. In addition, we noticed that in the two lysophospholipid conditions, even though addition of substrates does not alter peak dispersion on the ^{15}N-TROSY-HSQC spectrum of DAGK significantly, we can map the catalytic site by monitoring the peaks that shifts as the substrates are titrated. Altogether, our data indicate that the use of lysophospholipids in sample preparation allows us to acquire structural information of DAGK in its active conformation with the substrates bound at the catalytic site. This work is supported by NIH grant R01 GM47485.

1004-Plat
Mixing and Matching Detergents for Membrane Protein NMR Structure Determination
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One major obstacle to membrane protein structure determination is the selection of a detergent micelle that mimics the native lipid bilayer. Currently, detergents are selected by exhaustive screening because the effects of protein-detergent interactions on protein structure are poorly understood. In this study, the structure and dynamics of an integral membrane protein in different detergents is investigated by nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) spectroscopy, and small angle X-ray scattering (SAXS). The results suggest that matching of the micelle dimensions to the protein's hydrophobic surface avoids exchange processes that reduce the completeness of the NMR observations. Based on these dimensions, several mixed micelles were designed that improved the completeness of NMR observations. These findings provide a basis for the rational design of mixed micelles that may advance membrane protein structure determination by NMR.

1005-Plat
Structure and Dynamics of TM Domains of Human Glycine Receptor in LPPG Micelles
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The structure and backbone dynamics of the entire four transmembrane domains of human glycine receptor α1 subunit (GlyRTM1234) were studied in LPPG micelle. Using triple-resonance multi-dimensional NMR methods, over 80% residues were assigned. The chemical shift index clearly showed that most residues in the TM region were in helical conformation, consistent with a four-helix-bundle TM protein. We have collected sufficient amount of distance, H-bond, and dihedral angle restraints from NOE and chemical shift data, as well as long-range distance restraints from paramagnetic relaxation enhancement measurements. The structure model, determined using the available restraints, showed a kink between Trp72 and Cys76 in the beginning of TM3 domain. Moreover, the end of TM2 domain, S53 to S56, is more flexible compared to the rest of TM2. In contrast, part of TM23 loop, V63 to V66, showed a helical secondary structure. Backbone dynamics measurements indicated the existence of significant internal motions of TM1234 in LPPG micelles. The relaxation data also resulted in an overall rotational correlation time of ~33.4 ns, estimated based on the non-flexible helical residues. This overall tumbling time corresponds to a 66.8 kDa protein-LPPG complex with ~98 LPPG molecules per GlyRTM1234. The size is confirmed by the dynamic light scattering measurement. We suggest that the flexible end of TM2 and the beginning of TM3 can better coordinate the coupling between the TM2-TM3 loop and the extracellular domain. This coupling is thought to be important for mediating channel gating. In addition, it is confirmed that the early termination of TM2 helix in GlyR is an intrinsic property and is independent of the presence or absence of other TM domains (Funded by NIH R37GM049202 & R01GM069766).

1006-Plat
Conformational Cycle Of A Bacterial Homolog Of Human Neurotransmitter Sodium Symporters
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Neurotransmitter:sodium symporters (NSS) control the magnitude and duration of synaptic signaling through active reuptake of specific neurotransmitters. While it is known that the transmembrane sodium gradient supplies the energy for transport, the coupling mechanism to substrate translocation from its primary binding site (S1) is poorly understood. Conformational rearrangements of extracellular and cytoplasmic regions of the protein are thought to regulate alternating access to S1, and substrate binding at a second site (S2) located near the extracellular region of the transporter, to act as a symport coupler (Shi et al 2008 Mol Cell 30, 667). We used spin labeling and EPR spectroscopy to investigate the conformational dynamics of a highly homologous bacterial member of the NSS family, the leucine transporter LeuT, for which the crystal structure is known (Yamashita et al 2005 *Nature* 437, 215). Changes in global and local structural constraints derived from the EPR analysis and induced by sodium and leucine binding were then correlated to conformational changes in the LeuT structure in proteoliposomes. Sodium binding was found to increase the distance between the probes as assessed from global rearrangements measured in the extracellular region of LeuT. In contrast, subsequent leucine binding in the presence of sodium was found to decrease the distance between the probes. Consistent with these observations, sodium binding increases spin label mobility and water accessibility at positions within the S2 site. Furthermore, leucine binding increases spin label order and decreases water accessibility. These results suggest a model in which sodium binding to LeuT primes the transporter for substrate binding in S1 by increasing the population of an ''outward-facing'' conformation, exposing the substrate permeation pathway. With a filled S1, the extracellular pathway constricts, consistent with the occluded state observed in the LeuT crystal structure.

1007-Plat
X-ray Footprinting Studies on Photoactivation of Bovine Rhodopsin
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Rhodopsin, the visual G-protein coupled receptor (GPCR) in the rod cells of the vertebrate retina, is fundamental to vision. The light-activated intermediate of rhodopsin, Meta-II, initiates a signalling cascade that culminates in an electrical impulse in the visual cortex of the brain. The molecular details of agonist-induced structural change that are likely to be conserved among the members of the GPCR super family are not fully understood. We used X-ray Footprinting to study the conformational change in rhodopsin in solution upon photoactivation. Purified rhodopsin is exposed in tens of milliseconds with high flux focused X-rays. The hydroxyl radicals that are generated by photolysis of water react with the solvent accessible side chains and form stable modification products. The peptic fragments are analyzed by mass spectrometry to quantify the extent and identify the sites of oxidation. Monitoring the changes in the radiolytic modification as function of the exposure time provides information that is directly correlated with the solvent accessibilities of individual peptide or side chain residues within the protein. The difference in solvent accessibilities between dark state and light activated Meta-II state shows conformational changes near the retinal binding site, but not a large structural change as predicted by some models of GPCR activation. Labeling was also observed in the trans-membrane helical regions, this was also unexpected. We introduce a novel O^{18} labeling method to determine if transmembrane labeling arises from exchange with bulk water or is mediated by activation of bound, conserved water molecules in the GPCR structure. This is a novel approach that can probe the details of bound water structure and dynamics that function in a number of ion channels and receptors.

Platform M: Cell & Bacterial Mechanics Motility

1008-Plat
Physical Description of Mitotic Spindle Orientation During Cell Division
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During cell division, the duplicated chromosomes are physically separated by the action of the mitotic spindle. The mitotic spindle is a dynamic structure of the cytoskeleton, which consists of two microtubule asters. Its orientation defines the axis along which the cell divides. Recent experiments on dividing cells, which adhere to patterned substrates, show that the spindle orientation depends on the spatial distribution of cell adhesion sites. In the present work we show that

the experimentally observed spindle orientation can be understood as the result of the action of cortical force generators acting on the spindle microtubules. We assume that the local activity of force generators is controlled by the spatial distribution of cell adhesion sites determined by the particular geometry of the adhesive substrate. We develop a simple physical description of the spindle mechanics, which allows us to calculate the torque acting on the spindle, as well as the energy profile and the angular distribution of spindle orientation. Our model accounts for the preferred spindle orientation, as well as the full shape of the angular distributions of spindle orientation observed in a large variety of pattern geometries. Remarkably, it also describes the transition from symmetric to asymmetric spindle orientation, observed for certain changes of the shape of the adhesive patterns. We conclude that, on the basis of a few simple assumptions, we can provide a quantitative description of the spindle orientation of adherent cells. M. Théry, A. Jiménez-Dalmaroni, et al., Nature 447, 493 (2007).

1009-Plat

Taking Control of the Bacterial Flagellar Motor

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The bacterial flagellar motor is a fairly complex machine, requiring 40-50 genes for its expression, assembly and control. Furthermore, it is imbedded in the multiple layers of the bacterial membrane. That explains why, unlike many other molecular motors, it has not been studied in vitro. As spectacular studies of linear motors (like kinesin, myosin and dynein) have clearly demonstrated, an in vitro system provides the essential control over experimental parameters to achieve the precise study of the motor's physical and chemical characteristics. Here, we report significant progress towards the development of a unique in vitro system to study quantitatively the bacterial flagellar motor.

Our system consists of a filamentous Escherichia coli bacterium partly introduced inside a micropipette. Femtosecond laser pulses (60 fs and ~ 15 nJ/pulse) are then tightly-focused on the part of the bacterium that is located inside the micropipette. This vaporizes a submicrometer-sized hole in the wall of the bacterium, thereby granting us access to the inside of the cell and the control over the proton-motive force (pmf). Using a patch-clamp amplifier, we applied an external voltage between the inside and the outside of the micropipette. If the hole in the bacterium is open, that voltage should translate into a membrane potential powering the motors outside of the micropipette. As we varied the applied potential, variations in the motor's rotation speed were observed. For these preliminary results, the rotation speed was observed directly using video microscopy of fluorescently labeled filaments. That system opens numerous possibilities to study the flagellar motor and other membrane components.

1010-Plat

How Molecular Motors Shape The Flagellar Beat

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Cilia and eukaryotic flagella are slender cellular appendages whose regular beating propels cells and microorganisms through aqueous media. The beat is an oscillating pattern of propagating bends generated by dynein motor proteins. A key open question is how the activity of the motors is coordinated in space and time. To elucidate the nature of this coordination we inferred the mechanical properties of the motors by analyzing the shape of beating sperm: Steadily beating bull sperm were imaged and their shapes were measured with high precision using a Fourier averaging technique. Comparing our experimental data with wave forms calculated for different scenarios of motor coordination we found that only the scenario of interdoublet sliding regulating motor activity gives rise to satisfactory fits. We propose that the microscopic origin of such ''sliding control'' is the load dependent detachment rate of motors. Agreement between observed and calculated wave forms was obtained only if significant sliding between microtubules occurred at the base. This suggests a novel mechanism by which changes in basal compliance could reverse the direction of beat propagation. We conclude that the flagellar beat patterns are determined by an interplay of the basal properties of the axoneme and the mechanical feedback of dynein motors.

1011-Plat

Mechanics Of Neutrophil Motility On Compliant Gels Measured With Traction Force Microscopy

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Traction force microscopy (TFM) allows imaging of the traction field exerted by a cell during adhesion and spreading on an elastic hydrogel. We used a combination of TFM and microfluidics to measure the traction forces and motility of human neutrophils under both chemokinesis and chemotaxis in response to formyl-met-leu-phe (fmlp). Using polyacyrlamide gels functionalized with intercellular adhesion molecule-1 (ICAM-1), we show that neutrophil traction stresses can be measured across a broad range of gel stiffnesses, from 6 to 20 kPa. We found neutrophil directed motion is caused by a rearward squeezing uropodial stress, and the cell motion is always counter to this motion; this is true both in chemokinesis as well as chemotaxis. During turning, the orientation of the rearward stress precedes turning. Cells exert larger forces in chemotaxis (r.m.s. force of ~ 100pN) than in chemokinesis (~ 50 pN). On surfaces of different compliance, cells move with a greater force and a higher chemotatic index on stiffer substrates; these changes occur without a change in neutrophil speed. In the same magnitude gradient, cells move directedly and with greater force if the mean concentration of chemoattractant is closer to the K_D of receptor binding. Blocking with an antibody against β_2-integrin (TS1/18) completely eliminates traction forces and directed motion. RhoA has been implicated as signal transduction agent in the cell that is responsible for rearward contractile stress and the direction of neutrophil motion; we show here that inhibition of a GTPase down stream of RhoA (ROCK) with a pharmacological agent reduces directional motion and force generation, and leads to abnormal morphology in which rearward contraction is compromised. Taken together, neutrophil directed motion and force generation result from an interplay between substrate adhesiveness and uropodial contractility through RhoA.

1012-Plat

Mechanics in neuronal development

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Nervous tissue consists of several different types of cells, blood vessels, and extracellular matrix. All these building blocks differ in their mechanical properties. Particularly during growth and migration, the local mechanical environment of neurons may thus change dramatically. The softness of radial glial cells, along which neurons preferentially grow, and the neuronal preference for soft substrates strongly point towards a role of mechanics in neuronal guidance. Here we show how neurons detect and avoid stiff substrates and how their mechanoresponsiveness is used to guide their axons along distinct pathways.

In vitro, neurons continuously probe the mechanical properties of their environment. Growth cones visibly deformed substrates with a stiffness commensurate with their own compliance. To understand the growth cones' sensing of stiff substrates, we investigated their precise temporal response to well-defined mechanical stress. Externally applied stress exceeding the threshold of ~300 pN/μm^2 caused a calcium influx through mechanosensitive, stretch-activated ion channels in the growth cone membrane that triggered neurite retraction. Subsequently, neuronal processes re-extend, thereby enabling exploration into new directions.

When *Xenopus* eye primordia were cultured on polyacrylamide gels of different compliance, the morphology of the outgrowing retinal ganglion cell axons dramatically depended on the mechanical properties of their substrate. If the axons grew either on soft or on stiff surfaces, they spread over a wide area to explore different directions. In contrast, if they grew on substrates of intermediate compliance, they fasciculated and grew into one common direction, resembling an optic nerve. The concerted growth along pioneering axons depended not only on the substrates' compliance but also on that of the axons themselves. Hence, neurons may actively use mechanics as previously unknown guidance cue during growth and migration. This knowledge may ultimately help in finding new implants that promote axonal regeneration in the injured nervous system.