The functional significance for the mobility of loop regions of GM2AP was probed using crosslinking agents to tether the two mobile loops via disulfide crosslinking to cysteine residues which were incorporated into each of the mobile loops of GM2AP. The tethered GM2AP constructs were functionally evaluated using fluorescence spectroscopy. Danysl-DHPE a fluorescently labeled lipid substrate was utilized to analyze the ability of the crosslinked GM2AP to extract the fluorescent lipid from large unilamellar vesicles containing POPC:dansyl DHPE. A blue shift in the wavelength of maximum emission for the dansyl-DHPE extracted by GM2AP allowed the amount and kinetics of lipid extraction to be assayed. The tethered constructs were also assayed for their ability to bind and sediment with a lipid membrane. Results indicate that tethering of the mobile loops mitigate the ability of GM2AP to extract its lipid ligand substrate.

292-Pos

Intact Protein Component of Cytochrome bc1 Complex Is Not Essential For the Superoxide Generation

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In addition to its main functions of the electron transfer and proton translocation, the cytochrome bc1 complex also catalyzes generation of superoxide upon oxidation of ubiquinol in the presence of molecular oxygen. The mechanism of superoxide generation by bc1 remains elusive. The superoxide generating activity seems to inversely proportional to the electron transfer activity. Complexes with less complexity in subunit structure tend to have higher superoxide generating activity. The maximum superoxide generating activity is observed when the complex is inhibited by antimycin. When the complex is treated with proteinase K, the electron transfer activity decreased and the superoxide generating activity increased as the incubating time increased. The maximum activity is obtained when the protein components of the complex is completely digested, indicating that intact proteins play little role in superoxide generation. It is speculated that the hydrophobic environment and the availability of a high potential electron acceptor from the complex is responsible for the activity. This speculation is confirmed by the detection of superoxide formation upon oxidation of ubiquinol by a high potential oxidant such as cytochrome c or ferricyanide in the presence of phospholipid vesicles or micellar solution of detergents. Little superoxide formation was observed when ubiquinol is oxidized under the hydrophilic conditions. This work was supported in part by a grant from NIH (GM30721).

293-Pos

Alteration of Membrane Protein Function Through the Photo-Activation of the Hydrophobic Probe Iodonaphtylazide

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Iodonaphtylazide (INA) has been developed 30 years ago to determine the penetration of proteins into biological membranes. Due to its very high partition coefficient into lipidic bilayers and the sensitivity of its detection, ^{125}INA has long been used to label and identify membrane proteins, to study membrane dynamics and fusion and to detect protein-membrane interactions. The labeling of membrane proteins is mediated by the azido moiety of INA that can be activated by near UV light. Upon excitation, a nitrene radical is formed leading to the covalent binding of membrane proteins in the surroundings. Besides labeling, this binding results in specific alterations of the hydrophobic domains of proteins. When applied to enveloped viruses, the treatment resulted in a complete loss of infectivity. While the overall integrity of the virus is preserved, the ability of the viral envelope glycoprotein to promote full fusion is impaired. In the case of influenza, hemifusion was not affected by the treatment indicating a blockage at the late stage of fusion. We also tested the effect of hydrophobic labeling on the function of cellular transmembrane receptors. The lateral mobility of chemokine receptors, which are G coupled receptors, was reduced and CXCR4 lost its ability to signal in response to external stimuli. However, the activity of a tyrosine kinase receptor (IGF1) was increased. The activity of a multi drug resistance transporter MRP1 was blocked by the hydrophobic treatment. Overall, photo-activation of INA in various cell lines, including those over-expressing the multi-drug resistance transporters MRP1 or Pgp, leads to apoptosis.

294-Pos

Simultaneous Measurement of Phagosome and Plasma Membrane Potentials in Human Neutrophils By Di-8-Anepps and SEER

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Neutrophils are the first line of defense against invading bacteria. Neutrophils engulf the invaders into an internal vacuole, the phagosome. Accompanying phagocytosis is the respiratory burst, in which NADPH oxidase produces reactive oxygen species by transporting electrons from cytosolic NADPH across the membrane to either intra-phagosomal or extracellular oxygen. It is well known that the plasma membrane depolarizes during the respiratory burst but very little is known about the membrane potential of the phagosome. Here we monitor the membrane potential of phagosomes as well as the plasma membrane during the phagocytosis of opsonized zymosan.
Neutrophils were isolated from whole blood and plated on glass coverslips. The cells were loaded with 5 μM di-8-ANEPPS for 30 minutes before excess dye was washed away. The cells were stimulated by addition of 2 mg/ml serum opsonized zymozan (OPZ) and were visualized using a Leica SP2 confocal microscope. SEER imaging was performed by simultaneously acquiring two images at 488 nm and 545 nm and collecting at emission ranges 470-560 nm and 570-700 nm respectively.The neutrophil plasma membrane depolarized rapidly coinciding with phagocytosis of the first OPZ particle. The potential generally decreased somewhat, but the plasma membrane potential generally remained positive to 0 mV for many minutes, during which time several phagocytotic events were typically observed. DPI produced repolarization, confirming that the depolarization was due to the electrogenic activity of NADPH oxidase. The membrane potential of each phagosome was highest upon formation and decreased within several minutes, often falling to negative voltages, while the plasma membrane remained depolarized. The phagosome membrane potentials were independent of, and at their peak often exceeded the plasma membrane potential. This study is the first to monitor the phagosome membrane potential in living cells.

295-Pos

Flio Is Not Required For Motility in *Salmonella* If Its Cytoplasmic Domain and Flip Mutant Suppressors Are Expressed

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The propeller-like flagella found in bacteria, and used for motility, possess a specialized secretion apparatus, which is imbedded in the cell membrane for their formation. Its components are highly conserved not just amongst flagellar systems, but also to the Type III secretion apparatus used by some bacteria in conjunction with virulence-associated needle complexes. The flagellar secretion system of *Salmonella typhimurium* consists of 6 integral membrane proteins: FlhA, FlhB, FliO, FliP, FliQ, and FliR. However, for the virulence-associated needle complexes of *S. typhimurium* and the flagellum of the bacterium *Aquifex aeolicus* a homolog of FliO is apparently absent. In this study we showed that deleting the *fliO* gene from the chromosome of a motile strain of *Salmonella*resulted in a drastic decrease of motility. However, incubation of the *fliO* mutant strain in motility agar, gave rise to mutants containing suppressors that help to restore partial motility. One class of the suppressor mutation was found in the *fliP* gene. Using truncation and site-directed mutagenesis analysis of the FliO protein, it was shown that expression of FliO cytoplasmic domain in cells with the *fliO* gene deleted can also partially restore the motility. When the FliO cytoplasmic domain was expressed in the FliP suppressor mutant strains an additive effect was observed, and near wild-type levels of motility were regained. The FliO cytoplasmic domain was purified and studied using circular dichroism spectroscopy. Based on secondary structure prediction it should contain beta-structure and alpha-helices, however, we showed that this domain is disordered and its structure is a mixture of beta-sheet and random coil. We assume that the FliO cytoplasmic domain becomes structured while interacting with its binding partners.

296-Pos

Surface Activity of Surfactant Protein SP-B and SP-C in Different Lipid Environments

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Pulmonary surfactant is a mixture of lipids and proteins, essential to reduce the surface tension at the air-liquid interface in the alveoli of mammalian lungs and so stabilizing the respiratory surface. Lack of an operative surfactant is associated with severe respiratory pathologies and supplementation with exogenous surfactants has been widely approached as a potential therapeutic intervention. However, the optimal lipid and protein composition of exogenous surfactants has not been properly established, and clinical surfactants currently in use differ substantially in terms of their lipid and protein moieties. In the present study we have compared the surface activity of native SP-B and /or SP-C, purified from porcine lungs, in the Captive Bubble Surfactometer (CBS), once reconstituted into two different synthetic lipid mixtures: DPPC/POPC/POPG/Chol (50:25:15:10) (lipidS), a mixture mimicking lipid composition in natural

surfactant, and DPPC/POPG/Palmitic Acid (68:22:9) (lipidTA), a system widely used as a basis for clinical surfactants. We found similar equilibrium surface tensions after 5 min of adsorption of all the samples, regardless the protein and lipid system, although SP-B exhibited somehow slower initial adsorption in lipidTA. Significant differences were found in SP-B activity under quasi static compression-expansion cycling for the two lipid systems tested. In lipidTA, SP-B allowed reaching tensions near 2mN/m, whereas in lipidS surface tension did not fall below 20mN/m. However, SP-B-containing samples produced similarly low tensions, within the two lipid compositions, once cycled dynamically at physiologically relevant compression-expansion rates. Analysis of film stability under mechanical perturbations showed that SP-B introduces a significant resistance of the films to relaxation, which is particularly remarkable in lipidS samples. This stability was maximal in the simultaneous presence of SP-B and SP-C.

297-Pos

The NHERF2 Dependent Dynamic Ca2+/LPA Regulation of NHE3 Mobility and Interaction At the Epithelial Brush Border

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Na+/H+ exchange 3 (NHE3) plays an essential role in NaCl absorption intestine and kidney. NHE3 rapidly cycles between the plasma membrane and recycling endosomal compartment under basal conditions. Those regulations require PDZ domain containing NHERF proteins. Especially NHERF2 is required both in Ca2+ inhibitory and LPA stimulatory regulations in NHE3 activity. In this study, using the FRET technique, the dynamic binding between NHERF2 and NHE3 at the brush border was investigated in the presence of Ca2+ or LPA. Zeiss 510 Meta confocal microscopy was used to perform FRET (acceptor photobleaching) between NHE3-YFP and CFP-NHERF2 on the apical brush border in polarized epithelial kidney OK cells.
We observed that NHERF2 and NHE3 exhibited 10-20% FRET signaling at the microvilli and not at the juxtanuclear region under basal conditions. As a negative control, there was no FRET signaling between CFP-NHERF2 and YFP-GPI. With treatment of Ca2+ ionophore, A23187(0.5μM) or LPA(100μM), FRET signaling was transiently abolished within one minute for A23187 and within 30min for LPA and recovered at 1 hr later.
The dynamic interactions between NHE3 and NHERF2 by LPA and Ca2+ in OK cell microvilli were quantified by FRET. We conclude that the dissociation of NHEFR2 from NHE3 at the microvillus leads to NHE3 activity inhibition by A23187 by increasing the NHE3 endocytosis and leads to stimulation of NHE3 activity by LPA by increasing NHE3 translocation to the brush border.

Protein Assemblies

298-Pos

Structural Survey of Large Protein Complexes in Desulfovibrio Vulgaris

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Large protein complexes purified by a tagless strategy have been analyzed for an unbiased survey of the stable, most abundant multi-protein complexes in Desulfovibrio vulgaris Hildenborough (DvH) that are larger than Mr ~400 k. The quaternary structures were determined for 8 out of 16 complexes by single-particle reconstruction of negatively stained specimens. The success rate of getting structure was about 10 times greater than that of previous ''proteomic'' screens. For the remaining complexes, the subunit compositions and stoichiometries were analyzed by biochemical methods. Our results show that the structures of large protein complexes vary to a great extent from one microorganism to another. None of the complexes except for GroEL and the ribosome could not be modeled from the previously known homologous structures due to organism dependent variation of quaternary structures. This result indicates that the interaction interfaces within large, macromolecular complexes are much more variable than has generally been appreciated. As a consequence, the quaternary structures for homologous proteins may not be sufficient to understand their role in another cell of interest. The diversity of subunit stoichiometries and quaternary structures of multiprotein complexes that has been observed in our experiments with DvH is relevant to understanding how different bacteria optimize the kinetics and performance of their respective biochemical networks. It is further anticipated that imaging the spatial locations of such complexes, through the analysis of tomographic reconstructions may also be important for accurate computational modeling of such networks. While templates for some multi-protein complexes such as the ribosome or GroEL could be derived from previously determined structures, it is quite clear that single-particle electron microscopy should be used to establish the sizes and shapes of the actual complexes that exist in a new organism of interest to prepare valid templates.

299-Pos

Ranolazine Preserves the Integrity of Mitochondrial Supercomplexes

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Mitochondrial respiratory complexes are known to exist in multi-complex assemblies (respirasomes). These respirasomes and their constituents are known to be damaged during ischemia reperfusion (IR) injury. In the present study we examined if ranolazine, a late sodium current blocker, and also a partial fatty acid oxidation inhibitor, preserves these assemblies after cardiac IR injury. Guinea pig hearts (n 6) were isolated and perfused with Krebs Ringer buffer (KR) and exposed to one of the following three protocols: 1) KR perfusion for 30 min, (time control), 2) 30 min global ischemia, or 3) ranolazine (10 μM) perfusion for 10 min just before 30 min global ischemia. Mitochondria were isolated by differential centrifugation and then subjected to blue native Polyacrylamide Gel Electrophoresis (BN-PAGE) to examine for damage to the respirasomes. We observed that there is a loss of protein bands after electrophoresis at 720 kDa and at 250 kDa in the untreated ischemic group. These bands were restored in the ranolazine treated group. These proteins will be subject to identification. Our results indicate that cardiac ischemia causes a loss of integrity of respiratory complexes, which is restored partially by ranolazine. A candidate for ranolazine's protective effect is cardiolipin, which stabilizes the respiratory chain supercomplexes, and which may be less oxidized after ranolazine treatment.

300-Pos

A Biophysical Investigation of the Non-Classical Release Complex of Fibroblast Growth Factor-1

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Fibroblast Growth Factor-1 (FGF-1) is a potent angiogenic agent that is released via the non-classical protein secretion pathway. Angiogenesis, the process of formation of new blood vessels, is vital to the formation of tumors, and is also responsible for tumor metastasis, as cancer cells travel from one part of the body to another through the newly formed vessels. Export of FGF-1 is based on the Cu2+ -dependent structure of multi-protein complexes, which involves the S100A13, a Ca2+ binding protein belonging to the family of S100 protein. The goal of this study is to characterize the structure of the FGF-1/S100A13 Data will be presented analyzing the interaction between FGF-1 and an S100A13 peptide that has been designed to mimic the binding region of FGF-1 on S100A13. The binding interaction was characterized using various biophysical techniques including ITC, DSC, proteolytic digestion, and multi-dimensional NMR spectroscopy. Characterization of the binding FGF-1/S100A13 interface is expected to shed light on the molecular mechanism(s) underlying the non-classical secretion of FGF-1.

301-Pos

The Monomerization of a Dimeric, Calcium-Binding Protein Involved in the Non-Classical Export of Fibroblast Growth Factor 1

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The non-classical secretion of fibroblast growth factor 1 (FGF1) is a poorly understood process. FGF1 is known to interact with the calcium-binding protein S100A13, which escorts FGF1 to the cytoplasmic surface of the cell membrane. The dimeric, highly alpha-helical structure of S100A13 has been well characterized. In addition to binding to Ca^{2+}, S100A13 has been shown to bind to Cu^{2+}. Binding of Cu^{2+} to S100A13 is believed to be crucial for the formation of the FGF1 release complex. In order to gain a better understanding of the structural forces involved in the organization of the multi-protein FGF1 release pathway, we have embarked on the determination of the 3D structure of the FGF1 release complex in solution using multi-dimensional NMR spectroscopy techniques. As a first step toward achieving this objective, we have designed an S100A13 monomer through site-specific mutations at the S100A13 dimeric interface. Results on the characterization of the S100A13 monomer using ITC, DSC, CD spectroscopy, and multi-dimensional NMR spectroscopy will be presented.

302-Pos

Reconstructing the Neisseria Type IV Pilus System in E.coli

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Type IV Pili, long, thread-like structures found on the surface of many species of bacteria, are important virulence factors involved in motility, DNA/phage uptake, biofilm formation, and adhesion. Energy for the system is supplied by a set of cytoplasmic, hexameric ATPases which interact with proteins within the bacterial membrane to traffic pilin monomers to and from the pilus. The