demonstrate the importance of both the SecA N-domain as well as its C-domain in stabilizing the interaction in vivo.

286-Pos

Increasing Chloride Conductance Through the SecY Complex By Mutagenesis or Trivalent Cations

Huan Bao, Kush Dalal, Franck Duong.
University of British Columbia, Vancouver, BC, Canada.

The SecY complex or channel is responsible for translocation of proteins across the bacterial inner membrane. The complex maintains a seal for small molecules by means of a plug domain and a hydrophobic pore, consisting of six isoleucine residues arranged in a ring. When these pore residues are mutated into asparagine, a specific conductance for small monovalent anions, like chloride, is observed. Here, we show that an enhanced chloride conductance is also observed when bulky phenylalanine residues are introduced into the pore ring. The increased conductance is accompanied by an increase in protein translocation. Chloride conductance was also observed upon addition of trivalent aluminum cations, which are suspected of binding to negatively charged residues near the lateral gate of SecY.

287-Pos

Stability of Vesicles With Incorporated Aquaporin Z Under Various Physiochemical Conditions

Jinsoo Yi[1], Jens Nording[1], Julie Valbjorn[1], Chandrasmitha Bhatt[1], Per Brandt Rasmussen[1], Anitta Krogh Jensen[1], Carlo David Montemagno[2], Jørgen Steen-Pedersen[1].
[1]Danfoss AquaZ A/S, Nordborg, Denmark, [2]University of Cincinnati, Cincinnati, OH, USA.

Stability of Vesicles with Incorporated Aquaporin Z Under Various Physiochemical Conditions

Aquaporins are integral membrane proteins that can transport large amounts of water molecules across the cell membrane by facilitated transport. One aquaporin can transport over 3 billion water molecules per second. Among many different types of aquaporins, we are using *E. coli* Aquaporin Z that is known as a highly specific water channel. This prokaryotic origin aquaporin is easy to modify genetically and it is possible to produce it in large amounts. By means of nanobiotechnology we attempt to develop aquaporin-incorporated membranes that can act as energy efficient water filtration membranes. To fabricate the aquaporin incorporated filters, it is important to characterize the aquaporin endurance against various chemical and physical conditions, in order to know what type of nanofabrication technologies can be used in the final membrane production. In our membrane concept, aquaporin incorporated vesicles are the main building blocks because thermodynamically vesicles are stable and aquaporin retains its functionality when incorporated into vesicles. In this research, we have tested aquaporin-incorporated vesicle functionality with respect to various physiochemical influences such as pH, high temperature, long time UV exposure, high pressure, oxidative stress and several solvents at various concentrations. Subsequently, we have characterized the effect of the applied physiochemical conditions by stopped flow light scatter (SFLS) to check if the water permeability of the vesicles was changed or killed by the various treatments.

288-Pos

Atomistic Model For the Outward-Facing State of Lactose Permease and Quantification of Ligand Binding

Pushkar Y. Pendse, Jeffery B. Klauda.
University of Maryland, College Park, College Park, MD, USA.

The Major Facilitator Superfamily (MFS) is an important class of membrane transporters whose members are found in unicellular organism to complex systems like the human central nervous system. Lactose Permease (LacY) of E. coli, an MFS protein that transports various sugar molecules across plasma membrane, has been studied as a prototype of secondary transport proteins. Though the structure of LacY in the cytoplasmic open state (inward-facing) has been determined, the atomic-level details of the periplasmic open structure (outward-facing) are unknown. Using a two-step hybrid simulation approach that involves Self-Guided Langevin Dynamics (SGLD) simulations with an implicit membrane followed by molecular dynamics simulations with an explicit membrane, we have obtained an opening of LacY on the periplasmic side that is consistent with the Double Electron-Electron Resonance (DEER) experiments (Smirnova et al., PNAS, 2007). The inward-facing state is stabilized by several helix-helix hydrogen bonds involving side chains of the residues N245, S41, E374, K42 and Q242 and mutations in these residues may destabilize these interactions and help crystallize the outward-facing state. We believe that this hybrid simulation approach can be extended to determine the unknown structures and mechanisms of other secondary active transporters. In addition, sugar binding to LacY has been investigated and our simulations support the alternating access model of sugar transport (same binding site accessible from either side of the membrane). More extensive studies on binding involved alchemical free energy perturbation calculations on αβ-(Galp)2 and ββ-(Galp)2 to better understand the anomeric binding effect. Our methods are tested by comparing the computed binding free energy values for NPG (p-nitrophenyl α-D-galactopyranoside) with the experimental values (Nie et al., JBC, 2006). This work will aid in understanding the effect of substrate structure and affinity to LacY.

289-Pos

Single Molecule Studies of *E-ColiF$_1$F$_o$ ATP Synthase* in Lipid Bilayers

Wei M. Ho, Richard M. Berry.
University of Oxford, Oxford, United Kingdom.

While rotation of *F_1 ATPSynthase* has been well characterised at the single molecule level over the past twelve years, direct observation of coupled rotation of *F_1F_o ATP Synthase* in energised lipid bilayers is still fraught with difficulties. One of the biggest challenges is the creation and preservation of a stable and energisable lipid bilayer, so that the proteins can be inserted and remain functional in its native environment. Such a setup must also be coupled with a high resolution microscopy technique, in order to allow direct single molecule observations. In this study, we report the use of the *droplet-on-hydrated-support bilayers* (*DHBs*) technique, by Heron et. al. , to observe both fluorescence labelled and gold labelled *EColiF$_1$F$_o$ATPase*, which have been inserted into the lipid bilayer. This was done using *Total Internal Reflection Microsopy* (*TIRF*) and a novel Dark Field Microscopy setup. The 2-dimensional diffusional constant of the protein ensemble in the lipid bilayer was characterised by tracking the position of the proteins over time. Furthermore, we have also attached the F_o portion of the protein to a functionalised substrate sitting below the bilayer and thereby inhibiting its movement. The F_1 portion is, in turn, labelled with gold beads and free to rotate above the bilayer. This configuration would allow the F_o to behave as the stator while the F_1 as the rotor, so that coupled rotation can be observed. The next part of this on going work would thus be to provide either ATP or a proton motive force to drive the rotation of the protein in hydrolysis and synthesis mode. We hope to report these findings in the coming conference.

290-Pos

Exploring Conformational Changes in the RbsABC Transporter Using EPR Spin Labeling

Michael J. Simon[1], Matthew C. Clifton[2], Mark A. Hermodson[1], Huide Zhang[1], Cynthia V. Stauffacher[1].
[1]Purdue, West Lafayette, IN, USA, [2]Fred Hutchinson Cancer Center, Seattle, WA, USA.

ATP Binding Cassette (ABC) transporters are transmembrane transporters that use the energy released by ATP hydrolysis to transport a wide array of substrates. They are found in all kingdoms of life, and are complicit in various genetic conditions, such as cystic fibrosis, macular degeneration, and multi-drug resistance. The E. coli ribose transporter (RbsABC) is a multisubunit ABC transporter complex with a periplasmic ribose binding domain, a transmembrane domain dimer, and a cytoplasmic nucleotide binding domain. The ribose transport complex has been shown to assemble and disassemble into distinct combinations of the subunits based on the presence of cofactors (ATP and analogues, ADP, orthovanadate, and magnesium), suggesting a series of steps for how the subunits associate and subsequently transport ribose.

To further explore the conformation of the subunits in response to these different sets of cofactors, cysteine mutations were introduced to allow the addition of EPR spin labels. These labeled mutants will be used to determine whether subunits are bound. Additionally, double mutants will be used to elucidate conformational state of subunits.

291-Pos

Investigation of the Mobile Regions of GM2 Activator Protein Using Continuous Wave and Pulsed Electron Paramagnetic Resonance and Fluorescence Spectroscopy

Jeff D. Carter, Gail E. Fanucci.
University of Florida, Gainesville, FL, USA.

GM2AP is an 18kDa protein that is involved in the catabolism of the ganglioside GM2. GM2AP is thought to bind GM2 in intralysosomal vesicles and present the oligosaccharide head group for hydrolytic cleavage. Mutations in GM2AP lead to an accumulation of GM2 in the lysosomes, causing the lysosomal storage diseases Tay Sachs or the AB variant of Sandhoff's disease. The crystal structure of GM2AP revealed the protein in five different crystal forms, with large differences in the diameter and area of the opening to the lipid binding cavity differences can be attributed to the flexibility of the mobile loops. Site directed spin-labeling combined with continuous wave and pulsed electron paramagnetic resonance methods has been used to investigate the intramolecular distances between the mobile loop regions of GM2AP. Distance profiles are obtained with and without the physiological ligand GM2 as other phospholipids.

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

Ying Yin, Shaoqing Yang, Fei Zhou, Linda Yu, Chang-An Yu.
Oklahoma State University, Stillwater, OK, USA.

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

Mathias Viard[1,2], Himanshu Garg[2], Robert Blumenthal[2], Yossef Raviv[1,2].
[1]SAIC-Frederick, Frederick, MD, USA, [2]CCR Nanobiology Program, Frederick, MD, USA.

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

Deri Morgan, Thomas E. DeCoursey.
Rush Medical Center, Chicago, IL, USA.

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

Clive S. Barker[1], Irina V. Meshcheryakova[1], Alla S. Kostyukova[2], Fadel A. Samatey[1].
[1]Okinawa Institute of Science and Technology, Okinawa, Japan.
[2]Robert Wood Johnson Medical School, Piscataway, NJ, USA.

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

Olga Lucia Ospina Ramirez, Jesus Perez-Gil.
Complutense University of Madrid, Madrid, Spain.

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