1702-Pos
Measuring the Release of Fluorescein from MscL-Loaded Liposomes with Stressed Lipid Bilayers
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The bacterial mechanosensitive ion channel of large conductance (MscL) acts as an emergency release valve to protect against osmotic stress[1,2]; its pore diameter is estimated to increase by >25Å on opening[3,4]. To investigate mechanisms for controlling the gating of this channel for use in targeted drug delivery systems, we have encapsulated the fluorescent dye 5,6-carboxyfluorescein (CF) into liposomes (diam. 100 nm) via sonication and extrusion using the Liposo-Fast system, followed by incorporation of MscL protein. Functional assays of MscL by patch-clamping confirmed that the dye did not affect the protein incorporation, and unencapsulated dye was removed by column purification. Liposomes were incubated with different concentrations of the amphipath L-α-Lysophosphatidylcholine (LPC) dissolved in ~3% MeOH for 30 min to induce stress on liposomal membranes[5]. Dye release from the liposomes via MscL was monitored as increased fluorescence in the external medium. The percentage fluorescence change quantified using a BMG Omega Polarstar Reader (excitation at 485nm and emission at 535nm). Based on these results, we demonstrate cargo release by MscL by means of manipulating the curvature of liposome membranes[4].
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1703-Pos
Osmotically Challenging Single Escherichia Coli Cells: 1,2,3,Ready,Burst!
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We use high spatial and temporal resolution spinning-disk confocal microscopy, fluorescent-labeling strategies, and automated image analysis to investigate the response of the mechanosensitive channel MscL to osmotic stress in living E. coli bacteria. We establish the viability of individual cells using a red-fluorescent nucleic acid stain, propidium iodide, and correlate it with cellular levels of EGFP-tagged MscL. We demonstrate that MscL promotes integrity of the cell membrane in the face of environmental osmotic pressure. For these experiments, a micro-fluidic device with temperature control and multi-generational capability was developed to determine which cells are viable and dividing following exposure to osmotic stress, and to study the ability of cells to recover from temporally varying stress stimuli.

1704-Pos
The Role of MscS Cytoplasmic Domain As An Osmolyte Filter
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Mechanosensitive (MS) channels, inner membrane proteins of bacteria, open and close in response to mechanical stimuli such as changes in membrane tension during osmotic stress. In bacteria, these channels act as safety valves thus preventing cell rupture upon hypoosmotic shock.The MS channels of small conductance, MscS, are homoheptameric and consist of a large cytoplasmic (CP) domain that features a balloon-like, water filled chamber opening to the cytoplasm through seven side pores and a small distal pore. This CP domain is considered to be a molecular sieve, which prevents a loss of essential osmolytes and metabolites at the cytoplasmic side. In bacteria, glutamate is a predominant anion that helps to maintain potassium pools at an optimum level and also is a prevalent osmoprotectant maintaining the cell turgor. We have explored, using molecular simulations, the free energy landscape characterizing the translocation and exit of a glutamate ion through one of the side pores, to illustrate the role of the CP domain in selective filtering of glutamate. The adaptive biasing force (ABF) method is applied to the glutamate molecule to determine the free energy barrier along the chosen translocation pathway. The transport kinetics of glutamate based on the measured free energy profile, suggests the presence of an entropic barrier that slows down the passage of glutamate through the pore but lets water pass quickly. A low enthalpic barrier of ~4 k_BT is sufficiently low to prevent glutamate from clogging the pore. Analysis of the electrostatic potential of the CP domain indicates that the CP interior presents an environment relatively unfavorable for anions.

Biophysics of Ion Permeation

1705-Pos
Cholesterol Deficiency, Statins and Rhabdomyolysis
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Cholesterol's (CH) function cannot be to stiffen membranes because: 1) We synthesize CH but cannot burn it for energy; 2) Mankind has over 100 genes devoted to CH management, regulation, trucking, biosynthesis, degradation, etc. 3) Unlike CH plant sterols have branches on their sidechains. 4) Most cultured animal cells die without CH. 5) We have >2 ABC intestinal transporters that selectively block phytosterol uptake. 6) The lipid mole fraction of CH rises and falls with the $[Na^+]$ that faces the membrane. Literature evidence is here gathered that shows cells in vivo leak Na^+ due to the $\Delta\Psi$ and the blood $[Na^+]$ which is 0.1M. Although the Na^+ leakage is slow (10^{-12} cm/sec) compared to H^+ leakage (10^{-5} cm/sec), the high $[Na^+]$ makes them equivalent. Furthermore the presence of 1/3 mole% CH, as typically found in membranes facing blood, reduces the Na^+ leakage to 1/3 that found in bilayers lacking CH. FDA data suggest excess statins taken by ~200 patients in the last 8 years resulted in lethal rhabdomyolysis (RH). If cholesterol's function is to limit Na^+ leakage (Prog. Lipid Res. 40 (2001) 299), then 3 times as much ATP is needed to compensate for leaked Na^+. Muscle cells, lacking a pentose shunt cannot make much NADPH, which is essential for CH synthesis. Such a CH deficiency in muscle would provoke Na^+ leakage into cells that may burst because of osmotic swelling. A common feature of RH in autopsies is that salt is found in the muscle cells. Due to the branches on their side chains, plant sterols appear to be designed to inhibit H^+ leakage (op cit.). They are only found where the plasma membrane has a H^+ gradient. CH is only found in cells where the plasma membranes facing Na^+.

1706-Pos
Ion Channel Activity of Pentameric Phospholamban
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Phosholamban (PLN) is an integral membrane protein, which is involved in the contractility of cardiac muscles by regulating the sarco/endoplasmic CaATPase (SERCA). PLN exists in equilibrium between monomer and pentamer. Monomeric, unphosphorylated PLN inhibits SERCA, whereas PLN phosphorylation releases the inhibition and allows calcium translocation into sarcoplasmic reticulum. The pentameric PLN structure (1,2) and its possible activity as ion channel (3,4) are still a matter of debate. In order to understand if PLN pentamer can have ion channel activity, we have performed experiments by using two different biomimetic systems, namely supported nanoBLMs and traditional BLMs.
Conductivity measurements on nanoBLMs show that PLN pentamer can form a pore, which is permeable to small ions such as Na^+, Cl^- and ClO_4^-, but not to the bigger choline (Cho^+) ion (radius 3,3Å). These data are in agreement with the hypothesis of Oxenoid and Chou (1), according to which the narrowest part of the wt-PLN pentamer is about 3,6Å in diameter. Moreover, single channel recordings on BLMs reveal independent current fluctuations between a closed and a defined opening state at two distinctly different conductances. Also in previous experiments small unitary channel fluctuations with two different conductances were observed (5).
In conclusion the present results and previous works (1,5), support the view that PLN works as an ion channel.
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