difficult to investigate the fast gating properties of certain channels e.g the Ryanodine Receptor (RyR2). RyR2 channels, which play a key role in the intracellular Ca2+ induced calcium release mechanism, demonstrate a complex gating characterized by bursts of very fast open-close transitions that cannot be resolved by conventional PLB apparatus. Furthermore, the low current amplitude these channels produce in presence of the physiological ion Ca2+ , complicates the analysis.
We have developed a robust platform based on glass or quartz nanopore membranes (GNMs, 200-3000 nm radius pore size), for performing high bandwidth, low noise measurements of such ion channels in lipid bilayers. Previously, we have demonstrated incorporation of bacterial toxins [1] and porins in these small bilayers. Here we report the successful incorporation and measurement of RyR2 activity in such a system. Vesicles prepared from sarcoplasmic reticulum enriched in RyR2 channels were fused through osmotic swelling to PLBs formed on a GNM with a 3000 nm radius orifice, allowing unprecedented resolution of single RyR2 channel events at 10 kHz.
In addition, we have fused vesicles containing nystatin and ergosterol to small PLBs (< 1000nm radius) successfully. The latter method has the potential to provide a general technique for incorporation of a variety of ion channels in small GNM bilayers.
[1] White et al J. Am. Chem. Soc., 129, 11766-11775, 2007

2788-Pos
The Intracellular Loop of Orai1 Plays a Central Role in Fast Inactivation of CRAC Channels
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Store-operated Ca^{2+} entry (SOCE) due to activation of Ca^{2+}-release-activated Ca^{2+} (CRAC) channels leads to sustained elevation of cytoplasmic Ca^{2+} and activation of lymphocytes. CRAC channels consisting of four pore-forming Orai1 subunits are activated by STIM1, an endoplasmic reticulum Ca^{2+} sensor that senses intracellular store-depletion and migrates to plasma membrane proximal regions to mediate SOCE. One of the fundamental properties of CRAC channels is their Ca^{2+}-dependent fast inactivation (CDI). To identify the domains of Orai1 involved in CDI, we have mutated residues in the Orai1 intracellular loop linking transmembrane (TM) segment II to III. Mutation of four residues $V^{151}SNV^{154}$ (MutA) at the center of the loop abrogated fast inactivation leading to increased SOCE as well as higher CRAC currents. Point mutation analysis identified five key amino acids $N^{153}VHNL^{157}$ that increased SOCE in Orai1 null murine embryonic fibroblasts. Expression or direct application of a peptide comprising of the entire intracellular loop or the sequence $N^{153}VHNL^{157}$ blocked CRAC currents from both WT and MutA Orai1. A peptide incorporating the MutA mutations had no blocking effect. Concatenated Orai1 constructs with four MutA monomers exhibited high CRAC currents lacking fast inactivation. Reintroduction of a single WT monomer (MutA-MutA-MutA-WT) was sufficient to fully restore fast inactivation, suggesting that only a single intracellular loop can block the channel. These data suggest that the intracellular loop of Orai1 acts as an inactivation particle, which is stabilized in the ion permeation pathway by the $N^{153}VHNL^{157}$ residues. These results along with recent reports support a model in which the N terminus and the selectivity filter of Orai1 as well as STIM1 act in concert to regulate the movement of the intracellular loop and evoke fast inactivation.

2789-Pos
A High Throughput Microfluidic Approach Enables Fast Exchange of Solutions and Ligand Gated Ion Channel Recording from Cell Ensembles
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Electrophysiology is the preferred technique for characterizing ion channel function and kinetics. It is the most functionally pertinent assay for screening in terms of information content. High throughput pharmaceutical screens often use a population patch approach, which eliminates cell-to cell variability of single cell recordings. However, currently available population patch platforms have key shortcomings such as a) the inability for fast exchange of solutions, b) the inability to apply multiple compounds to the same ensemble of cells, and c) the inability to record fast desensitizing channels.
Here we present novel data showing that by using a microfluidic network design along with population patch recording we are able to overcome these obstacles. We validated our system using cells expressing voltage-gated channels in ensembles of up to 30 cells under voltage clamp. Moreover, these results showed that there is fast compound application (<100ms). The time course of compound application was confirmed using fluorescent indicators and biological reporters, such as GABA-A expressing cells. These data also validated our ability to record from fast desensitizing ligand gated ion channels without appreciable desensitization. We compared the time course of solution exchange with and without a protective layer technique and additionally characterized application of multiple compounds to the same ensemble of cells. In conclusion, the novel microfluidic approach allows for the fast exchange of compounds and facilitates the recording of fast activating voltage and ligand-gated channels.

2790-Pos
Components of *E. Coli* Energy-Transducing Complexes, ExbB and TolQ, Display Ion Channels
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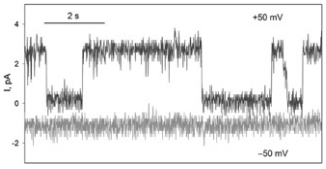
ExbBD/TonB and TolQR/TolA complexes of the cytoplasmic membrane transduce energy stored in the electrochemical proton gradient to drive cellular import of siderophores or maintain outer membrane barrier function. Both complexes are utilized for cellular import of colicins. It is unknown how they exert energy transducing and import functions.
We explore the idea that energy-transduction by ExbBD/TonB and TolQRA is coupled to ion- translocation through ion channels formed by transmembrane helices of ExbB and TolQ. Plasmid-expressed ExbB and TolQ were extracted with detergent from membranes, purified, and reconstituted into liposomes. For channel measurements proteoliposomes were fused to planar lipid membranes. Changes in protein tertiary structure upon membrane reconstitution were detected by thermal melting of alpha-helices using far-UV circular dichroism. ExbB reconstituted into liposomes, in contrast to its behavior in detergent, melted cooperatively, implying inter-helix interactions. ExbB and TolQ displayed cation-selective ion channels of small conductance (Figure). Divalent cations decreased channel conductance.
Channel formation was more prominent at pH<6. It is proposed that transmembrane ion current through ExbB and TolQ channels is transduced into conformational changes of periplasmic domains of the membrane-anchored TonB and TolA components of the complexes.



2791-Pos
Phases, Transport, and Dielectric Properties of Water Confined in Nanoscale Channels
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Biological channels formed from membrane proteins assemble into complex conduits for passage of select ions and molecules across the cell membrane. The selectivity properties of some of these channels has inspired the search for synthetic analogs that may serve as nanoscopic filters for various technical applications, including electronic devices and desalination membranes. To understand better the properties of water confined to nano-sized channels, we study the structure and dynamics of water inside long, hydrophobic channels under ambient pressure and temperature using classical molecular dynamics simulations. We find that water undergoes distinct transitions in structure and dynamic properties as the channel diameter is varied and describe the resulting anisotropic properties of the water in these confined geometries.

2792-Pos
Computational Evaluation of Nanopore Conductivity in Electroporation
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Intracellular transport of molecules hardly permeant through plasma membrane could be significantly enhanced by application of electric field, inducing membrane electroporation (EP). Electroporation has been applied to amplify the insertion of nucleic acid molecules in genetic modifications, drug transport in cancer treatment, and immune stimulation. Optimal protocol of EP should be selected with regard to the application, taking into account membrane composition and physico-chemical properties of transported molecules. Various probes have been applied to test the permeability and selectivity of the molecular transport through electropores. They are used for selecting optimal protocol and obtaining the electropore characteristics, such as the pore median radius and their density. To accomplish this task with high accuracy, an appropriate theoretical model should be implemented. In case of electropores there are two main difficulties to be addressed. First