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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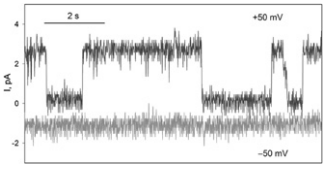
ExbBD/TonB and TolQR/TolA complexes of the cytoplasmic membrane transduce energy stored in the electrochemical proton gradient to drive cellular import of siderophores or maintain outer membrane barrier function. Both complexes are utilized for cellular import of colicins. It is unknown how they exert 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

of them is high pore dynamics and unknown shape of the pore. The other problem comes from comparable diameters of the probe molecules and electropore, both within nanometer scale. The molecular diffusion in such systems is affected by hindrance posed by the entropic barrier and molecular interactions. Therefore, a classical Nernst-Planck equation with a bulk value of the diffusion constant is very imprecise. More advanced modeling is necessary, which takes into account more physical representation of the nanopore shape, charge distribution, interactions between molecules, ions and pore walls. The comparable scale of the pores and probes should be reflected in an effective diffusion constant value. The impact of such improvements on the results, applied to a nanopore, is demonstrated by means of the Poisson-Nernst-Planck model with adjustable diffusion coefficient. The results show a significant discrepancy of the results from simplified and more advanced models.

2793-Pos
Determinant of Cation Blocking Behavior in Aquaporin-1
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The most extensively studied member of the aquaporin family, wildtype (WT) aquaporin-1 (AQP1), has been shown to effectively block cations from permeation across the cell membrane, thus maintaining the osmotic pressure of the cell. Recent experiments have suggested the essential role that the Selectivity Filter (SF) region of the channel plays in blocking cation flux. In the present study, the potential of mean forces for permeation of sodium cation (Na^+), potassium cation (K^+), and classical hydronium cation (H_3O^+) (without the possibility of Grotthuss proton shuttling) are characterized for a series of AQP1 mutants. The free energy barriers for conducting hydrated excess protons (H^+), which diffuse via the Grotthuss mechanism, are characterized as well, by utilizing the multi-state empirical valence bond (MS-EVB) method. The maximum cation conductance is calculated using the Poisson-Nerst-Planck theory. The present study reveals the key role of the SF domain in cation gating and provides insight into the subtle mechanism of proton permeation mutants of the AQP1 channel.

Muscle: Fiber & Molecular Mechanics & Structure III

2794-Pos
Loop 1's Role in a Novel Step on the ADP Release Pathway of Smooth Muscle
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Smooth muscle myosin has two N-terminal isoforms that result from alternative splicing of loop 1. Loop 1 contains a seven amino acid insert (QGPSFSY) in one isoform (SM-B) that is absent in the other (SM-A). It has been shown that the presence of the insert causes a two-fold increase in the rate of in-vitro actin sliding velocity and actin-activated ATPase activity (Rovner et al., 1997, Muscle Res Cell Motil 18:103). Based on these results and its proximity to the active site, it was hypothesized that loop 1 plays a role in modulating the release of ADP (Spudich, 1994, nature 372:515). However, little is known about the conformation of loop 1 in different nucleotide states, as it is absent in crystal structures. To further investigate the role of loop 1 in modulating ADP release we have inserted a single tryptophan residue into the interior of loop 1 in the SM-B isoform to monitor its dynamics. In combination with stopped-flow kinetics to monitor the release rate of mant-ADP from the motor domain, we have observed three steps in the ADP release mechanism, one of which is a unique transition that occurs before ADP release and following opening of the active site. Significantly, this previously undetected kinetic step appears to arise from a specific change in the state of loop 1. This is the first time a role of loop 1 in the ADP release mechanism has been directly identified and may account for the functional differences observed between two isoforms of smooth muscle myosin.

2795-Pos
A Kinetic Step Involving Loop 1 in Smooth Muscle may Dictate Isoform Specific Differences in ADP Release
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The actin sliding velocity and ADP release rates in smooth muscle myosin are modulated by the make-up of a flexible surface loop spanning the active site known as loop 1. There are only two motor domain isoforms of smooth muscle myosin and they differ in the presence (SM-B) or absence (SM-A) of a seven amino acid insert (QGPSFSY) in this loop. The presence of this insert leads to a two-fold increase in both actin sliding velocity and ADP release, although the mechanism for this difference is unknown. To investigate the role of this insert in functional differences between the SM-B and SM-A isoforms of smooth muscle myosin we have inserted a single tryptophan residue into loop 1 of both isoforms. The dynamics of loop 1 have been correlated with the kinetics of ADP release using a combination of steady-state fluorescence measurements (i.e., tryptophan emission, FRET, and acrylamide quenching) and stopped-flow kinetics. Using this approach we have already shown that the long loop SM-B isoform displays an extra step in its ADP release pathway that has not been previously observed. Here, we show that the additional transition seen in the long loop SM-B isoform is not observed in the short loop SM-A isoform upon ADP release. Furthermore, the final ADP release step is twice as slow in the short loop SM-A isoform, suggesting that the unique transition observed in the presence of the insert alters loop 1 dynamics in a way as to facilitate ADP release. This alteration of ADP release constitutes a simple and fundamental way to tune the activity of the motor at the molecular level and mechanical function at the physiological level in smooth muscle.

2796-Pos
Mutant Analysis and Computational Analysis of the Essential Light Chain and Regulatory Light Chain Interactions with Respect to Regulation of Smooth Muscle Myosin
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To understand how smooth muscle myosin's (SMM) light chains are involved in phosphorylation dependent regulation of ATP hydrolysis, we expressed single, double and triple amino acid mutants of the heavy chain, regulatory (RLC) or essential light chain (ELC) in the area of the Ca2+-binding loop of the ELC. We mutated this area because of similarities to scallop smooth myosin's Ca2+ regulatory mechanism. Since the ELC is not required to maintain the off state, we reasoned that mutations in this region would specifically alter the activity of the on (phosphorylated) state of SMM. Mutations were made to disrupt hydrogen bonding between the ELC and RLC and the heavy chain (HC) based on scallop crystal structures. All mutant ATPase activities and actin sliding velocities (ASVs) were essentially identical to wild type in the unphosphorylated. For the phosphorylated states, one class of mutant showed normal ATPase activity and ASVs; a second class showed similarly depressed ATPase activity and ASVs; and a third class with differentially depressed ATPase activity and ASVs. We also created recovery mutants that restored (and recoupled) ATPase rates with ASVs. We then used course grain discrete molecular dynamics and force constant profiling to reconcile changes in the interactions of ELC, RLC & HC in that region, with changes at the ATPase site. We created a prepowerstroke smooth muscle myosin model with an ELC Ca2+ loop from scallop that interacted weakly with the RLC and used FlexServe (http://mmb.pcb.ub.es/FlexServ/) to compute changes in flexibility between the native (1QVI) and modified model. We see that changes in the ELC RLC interaction have direct effects on lever arm flexibility and active site protein dynamics.

2797-Pos
Modulation of Actin-Myosin Interaction by N-terminal Unique Domain of Myorod of Molluscan Catch Muscle
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Myorod, a thick filament protein of molluscan smooth muscle, is an alternative product of the myosin heavy chain gene. It contains the rod domain identical to that of the rod portion of the myosin molecule and a unique N-terminal domain (NMR). We previously reported that myorod is phosphorylated within NMR at Thr141 by vertebrate smooth muscle myosin light chain kinase (Sobieszek et al., *Arch. Biochem. Biophys., 454*: 197-205, 2006). To investigate whether phosphorylation of NMR may affect the actin-myosin interaction, two peptides were synthesized with sequence corresponding to this domain. One of two peptides included a phosphorylated Thr141 (NMR-P) and the other not (NMR-unP). We found that the latter peptide interacted with rabbit and molluscan F-actin causing an aggregation and sedimentation of F-actin at low-speed centrifugation while NMR-P had no effect on the distribution of F-actin in the