1725-Pos
The E71A Mutation Alters Selective Ion Permeability in KcsA
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The mechanism of selectivity in potassium channels has been extensively studied using the prokaryotic potassium channel, KcsA. Computational studies suggest that a glutamate-aspartate H-bond behind the selectivity filter in KcsA may play a role in determining the permeation properties of the channel. However, the mutant E71A , which disrupts this H-bond interaction and abolishes pH-dependent inactivation is reported to have either no effect on K+ selectivity (1) or to increase K+ selectivity (2) as measured by reversal potentials. Using an 86Rb+ flux assay, WT KcsA exhibits strong K+ selectivity, such that there are no measurable 86Rb+ fluxes supported by Na+ and Li+. In contrast, both Na+ and Li+ support significant 86Rb+ fluxes in the E71A mutant, indicating an enhanced Na+ and Li+ permeability.
In eukaryotic inward rectifying potassium channels (Kir), the E71 equivalent residue is part of a glutamate-arginine salt bridge that, when disrupted dramatically reduces K+ selectivity. KirBac1.1 is a prokaryotic channel that serves as a structural model of eukaryotic Kirs, but contains an H-bond in the equivalent position, similar to KcsA. By patch-clamping giant liposomes, we show that KirBac1.1 is K+-selective ($P_{Na}/P_K < 0.08$) as measured by reversal potentials shifts, but, like KcsA E71A, shows significant Na+ and Li+-driven 86Rb+ fluxes. We also find that the KcsA E71A mutant, similar to WT KirBac1.1. This loss of stability in these channels may suggest that the differences observed in permeation result from a weakened interaction with ions at the selectivity filter. Studies to examine ion permeation in eukaryotic Kir channels by 86Rb+ flux are ongoing.
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2. J. F. Cordero-Morales et al., Nature Structural & Molecular Biology 13, 311 (2006).

1726-Pos
Characteristic Frequency Analysis of Inward Rectifier Kir 2.1
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INTRODUCTION: Impedance spectroscopy cannot distinguish between ion channel subtypes. We hypothesized that amplitudes of specific characteristic frequencies will correlate with the current amplitude passed by a specific ion channel subtype (characteristic frequency). We chose to test this hypothesis using the human inward rectifying potassium channel, Kir 2.1.
METHODS: IV-relationships were generated using a standard voltage step protocol (−140 to 0mV, 7mV steps) performed in whole-cell voltage clamp mode on HEK293 cells stably transfected with KCNJ2, which encodes Kir 2.1. Noise functions containing equal magnitudes of 1-15 kHz frequencies (amplitudes: 25, 50, 75 or 100mV) were inserted into each voltage step. The real component of the Fast Fourier transform (FFT) of the output signal was calculated with and without noise for each step potential. The magnitude of each frequency as a function of voltage step was correlated with the IV-relationship.
RESULTS: In the absence of noise (control), magnitudes of all frequencies correlated poorly ($|R|<0.15$) with the IV relationship. With noise, magnitudes of frequencies between 0.2-1 and 2-4 kHz demonstrated high negative ($R < -0.9$) and positive correlation ($R > 0.9$) respectively, with the IV-relationship. Two nodes of zero correlation were also found (1.39 +/- 0.10 kHz and 8.49 +/- 0.74 kHz). Increasing noise amplitude increased the absolute value of the correlation for the aforementioned frequencies without significantly changing the nodes of zero correlation.
CONCLUSIONS: These data suggest that the observed frequency response reflects current passing through Kir 2.1 channels. However it remains unknown whether any of these characteristic frequencies are unique to Kir2.1. Identifying characteristic frequencies of other ion channel subtypes could allow simultaneous measurement of multiple ionic currents.

1727-Pos
Calcium Channels Exhibit Electric Field Dependent Valve-Like Behavior
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We propose a new model characterizing the valve nature of ion channels. There are four fundamental elements that any physiological valve must possess: a tube, a one-way gating mechanism, a gate-sensitive force, and a conducted substance. Macroscopic valves (heart or veins) have the gating mechanism attached to the tube. In our model of ion channels, the gating mechanism is the hydration state of the ion. A sufficient membrane electric field at the surface of an ion channel will strip water molecules from the ion, allowing the ion to enter the channel. The electric field decays exponentially away from the membrane within several nanometers. If the ion channel extended too far from the membrane, negligible hydration stripping would occur, the hydration shell would remain around the ion, and the ion could not enter the channel. Our measurements of ion mobility in an electric field show that hydration stripping occurs at 400 V/cm, corresponding to 7 nm from the membrane. Calcium ion channels extend 4 nm externally from the membrane, and will have the ion hydration shells stripped from the ion at the channel entrance. Internally, the calcium ion channel extends 12 nm from the membrane. A stripped calcium ion will enter on the external side of the channel, but upon exiting on the internal side, will rehydrate and be unable to re-enter the channel, creating one-way flow. Thus, the calcium channels exhibit valve behavior, with the channel being the tube, the hydration shell the gating mechanism, the electric field the gate-sensitive force, and the stripped ion the conducted substance. This model can be extended to other ion channels. The macroscopic valves are thyroporetic (tube-attached gate), while the ion channel valves are thyrofluidic (conductor-attached gate).

1728-Pos
Selecting Ions by Size in a Calcium Channel: the Ryanodine Receptor Case Study
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Calcium channels not only distinguish between ions of different charge (e.g., Ca2+ vs. Na+), but also between of the same charge but of different size (e.g., Na+ vs. K+). Size selectivity in calcium channels is analyzed in the ryanodine receptor (RyR) using a recent permeation model of RyR. This model describes ion permeation as electrodiffusion and ions as charged, hard spheres. RyR is modeled as five conserved negatively charged amino acids whose terminal carboxyl groups are very flexible. The model correctly reproduces experiments where three different monovalent cations compete for the pore at many different concentrations. Size selectivity occurs both because smaller ions fit into the crowded selectivity filter better and because they can screen the protein's negative side chains more effectively.

1729-Pos
Insights from a Toy Model of Calcium Channels on Sieving Experiments and Eisenman Sequences
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A simplified model of a calcium channel is used to re-evaluate interpretations of both sieving experiments and Eisenman selectivity sequences in calcium channels. In the model channel, the carboxyl groups of the calcium channel selectivity filter are approximated as a homogeneous liquid of half-charged oxygens (two for each carboxyl group) separated from the bath by a semipermeable membrane that allows permeating ions into the selectivity filter, but does not allow the oxygens to leave the filter. Sieving experiments are usually interpreted with the logic that the physical diameter of a channel is equivalent to the largest particle that will go through that channel. However, our model has no radial geometric constraints, but still produces results that show net flux of ions quickly dropping to zero as the ions increase in diameter. These results indicate that forces like crowding of ions in the pore act on large ions that keep them from entering the channel. These forces are related to the pore diameter, but the results of the experiments should not be interpreted as indicating the pore diameter directly. We also used this simplified model to attempt to discern why the 11 Eisenman selectivity sequences are the only ones that have been observed. By altering the dielectric constant of the selectivity filter (and thereby the penalties for shedding waters of hydration), as well as oxygen concentration within the channel, we observed the circumstances under which each Eisenman sequence appears. We also observed a small number of non-Eisenman sequences.

1730-Pos
Molecular Simulation of Ompf Channel in Salts of Divalent Cations: Molecular Insight on Charge Inversion
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Extensive recent experimental and theoretical work has shown that the interaction of biologically relevant divalent cations (such as Mg2+, Ca2+) has surprising properties. One of the most fascinating and unexpected effect is the so-called charge inversion or charge reversal phenomenon: cations accumulate at the interface in excess of its own bare charge, thus inverting the effective

charge of the interface. Recently, charge inversion has been reported in the bacterial channel OmpF, in the presence of salts of divalent cations [Alcaraz et al. Biophys. J. 96 (2009) 56]. Aiming to get an insight on the atomistic mechanism of the cation interaction with the protein, we have performed extensive MD simulations of a realistic model of the OmpF WT protein in a POPC membrane in MgCl2 and explicit water. The simulations were computationally highly demanding, with half million atoms in a simulation box and production runs around 25 nsec.
The simulations were performed employing the NAMD simulation package running in 128 processor at the CESGA Supercomputing Center. Our main result is that we have observed charge inversion of certain important acidic groups. The observed charge inversion is accompanied by a change in the transport mechanism of ions inside the channel and a reversal in the selectivity of the channel. Overall, our simulations give an accurate microscopic image of this unexpected effect with potentially important biological and nanotechnological implications.

1731-Pos
Increased Salt Concentration Promotes Negative Cooperativity in OmpF Channel
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The concept of positive cooperativity appeared in the study of oxygen uptake by hemoglobin to explain that when a molecule of oxygen binds makes it easier for a second molecule to bind. Quite the reverse, negative cooperativity refers to the situation where the presence of the first molecule makes it more difficult for the second molecule to bind. We study here the effect of salt on the pH titration of the OmpF channel, paying attention to the current noise, conductance and ion selectivity that are analyzed in terms of the Hill formalism. In all cases, values lower than 1 are found, suggesting a negative cooperativity. Although OmpF porin is a trimer, it was shown by a number of different methods that each monomer is identical and functionally independent. Thus, the slowed-down channel titration is a property of each monomer. Surprisingly, we find that increasing salt concentration promotes negative cooperativity, which is seen as a salt-induced decrease of the Hill coefficient. This observation seems to exclude direct electrostatic interactions between protonation sites as the source of the phenomenon, suggesting another, more subtle mechanism(s). The binding of cations to certain acidic residues has a crucial effect at low pH because results in an inhibition of channel conductance that additionally provides an anionic selectivity to the channel. This suggests that the binding site could play a certain role in the protection of the bacteria against acidic media

1732-Pos
Anions from the Hofmeister Series: Single Molecule Detection with a Solitary Protein Nanopore
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Nanopores have emerged in recent years as versatile single molecule detectors. The sensing principle is based on transient interruptions in the ion-current of an electrolyte, induced by the entry, transport, and exit of a particular analyte from the pore. The improving the detection capability of the nanopore is essential. Recently (Rodrigues et al., 2008) we have shown that the ''salting out'' are responsible for the KCl-induced enhancement in identification of individual molecules of poly(ethylene glycol) using solitary α-hemolysin nanoscale pores. The result suggested that specific ion effects may take place. Hofmeister effects are almost ubiquitous (Lo Nostro et al., 2006). Despite the huge number of studies devoted to this issue that date back more than a century, their origin is still debated. There are only isolated studies of the phenomenon at the confined spaces. For this reason, we focused on the effect of monovalent anions on a simple bimolecular complexation reaction between poly(ethylene glycol) and α-hemolysin nanoscale pore at the single-molecule level.
We find that the type of anions used here has dramatic influence on the ''on-rate'' constant of the reaction (the difference reaches several hundred times). As a consequence of this, the transition rate and the detection limit of the nanopore based sensor is correspondingly changed. The all probed anions follow the Hofmeister ranking according to their influence on the on-rate constant ($F^- > Cl^- > Br^- > I^-$) and the solubility of the analyte ($F^- < Cl^- < Br^- < I^-$). Therefore, salting-out phenomenon is responsible for the anion-induced effect on single molecule detection with a solitary protein nanopore. These results will advance the development of devices with sensor elements based on single nanopores.
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1733-Pos
Extension of Poisson-Nernst Planck Theory of Ion Conductivity with Soft-Repulsion Potential between Ions and Protein. Sensitivity of I-V Properties of α-Hemolysin Channel on its Penetration Depth into Membrane
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A soft repulsion (SR) potential between mobile ions and protein atoms is introduced to Poisson-Nernst-Plank (PNP) theory of ion transport as an alternative to commonly used hard sphere repulsion (HR). Two sets of SR were tested: one is parameterized for all atoms of 20 essential amino-acid residues using full atomic molecular dynamic simulation (SR-MD); and another is a truncated Lennard-Jones potential (SR-LJ). The effect of different models of short-range interaction between protein atoms and mobile ions (HR, SR-MD and SR-LJ) were studied using α-hemolysin channel protein. In addition, four different methods of setting the diffusion coefficients were analyzed in order to evaluate the effect of diffusion distribution on predicted currents. Our calculations show that the diffusion distribution has a strong influence on the size of total currents whereas has significantly less effect on rectifications, reverse potentials and selectivity. Therefore, for proper modeling of these properties, the potential of mean force (PMF) may play a more important role than the diffusion distribution. SR-MD has a better approximation of PMF near the protein surface than HR and significantly improves selectivity predictions.
Additionally, we have studied the dependency of α-hemolysin I-V properties on the penetration depth of the channel into the membrane. The results show that rectification and reverse potentials are very sensitive to the penetration depth. The depth, predicted by matching calculated rectification with the experimentally determined one, is in a very good agreement with the neutron reflection experimental result. Our free energy estimation also indicates that there is a minima near the predicted depth.

1734-Pos
Monitoring Ion Channel Charge Displacements using Radio Frequencies
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Here we introduce a new technique to examine voltage-dependent ion-channel biophysics using radio frequency (RF) interrogating electric fields. The approach exposes the cell membrane to an RF electric field and measures vibrational electric current evoked by the RF field. *Xenopus* Oocytes transfected to express Shaker-B IR ion channels were used as the experimental model. A 500 kHz RF signal was applied to the membrane using extracellular bipolar metal electrodes, and RF charge displacement measurements were made during traditional two-electrode whole-cell voltage clamp. Voltage clamp was used to depolarize the oocytes and measure whole-cell K^+ current at several transmembrane potentials. The RF interrogation signal was superimposed on top of the comparatively slow (DC) voltage clamp command signal. Results show that the measured RF membrane current was a function of DC membrane potential. The RF current was separated into conduction and displacement components to examine the voltage-dependent RF conductance, G_{RF}, and capacitance, C_{RF}. Remarkably, the RF capacitance, C_{RF}, had a voltage sensitivity and half-activation voltage that correlated with the Shaker-B IR channel DC conductance measured using whole-cell voltage clamp. These data are consistent with the hypothesis that electrostatic interactions between the channel protein and K+ in the pore constrain the mobility of K+ and lead to changes in RF capacitance with membrane depolarization. The approach might offer a means to examine electrostatic interactions associated with ion channel function or to estimate voltage dependence of channel activation using extracellular RF signals. [supported by NIH R01DC04928, NSF IGERT DGE-9987616]