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-aspartate salt bridge that, when disrupted dramatically reduces K⁺ selectivity. KirBac1.1 is a prokaryotic channel that serves as a structural model of eukaryotic Kirks, 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 (PNa/PK < 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.


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 KCN2, 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.

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 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 shield 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 thyroeporic (tube-attached gate), while the ion channel valves are thyrohodic (conductor-attached gate).

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 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 electrodifussion 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.

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 Eisenmann 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 oxygen 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 Eisenmann 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 Eisenmann sequence appears. We also observed a small number of non-Eisenmann sequences.

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 effects 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

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charge of the interface. Recently, charge inversion has been reported in the bacte-
rial 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 de-
manding, 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 re-
sult is that we have observed charge inversion of certain important acidic
groups. The observed charge inversion is accompanied by a change in the trans-
port 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 it makes it eas-
ier 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 independ-
ent. Thus, the slowed-down channel titration is a property of each monomer.
Surprisingly, we find that increasing salt concentration promotes negative co-
operativity, 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 con-
ductance 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 Soli-
itary Protein Nanopore


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Nanopores have emerged in recent years as versatile single molecule detect-
tors. The sensing principle is based on transient interruptions in the ion-cur-
rent 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 in-
dividual molecules of poly(ethylene glycol) using solitary z-hemolysin nano-
scale pores. The result suggested that specific ion effects may take place. Hof-
meister 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 cen-
tury, their origin is still debated. There are only isolated studies of the phe-
nomenon 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 z-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 nano-
pore 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–). There-
fore, salting-out phenomenon is responsible for the anion-induced effect on sin-
gle molecule detection with a solitary protein nanopore. These results will ad-
vance the development of devices with sensor elements based on single
nанopores.

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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
z-Hemolysin Channel on its Penetration Depth into Membrane

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A soft repulsion (SR) potential between mobile ions and protein atoms is in-
troduced to Poisson-Nernst-Plank (PNP) theory of ion transport as a alterna-
tive 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 z-hemolysin channel protein. In addition,
four different methods of setting the diffusion coefficients were analyzed in
to evaluate the effect of diffusion distribution on predicted currents. Our cal-
culations show that the diffusion distribution has a strong influence on
the size of total currents whereas has significantly less effect on rectifica-
tions, reverse potentials and selectivity. Therefore, for proper modeling of
these properties, the potential of mean force (PMF) may play a more im-
portant role than the diffusion distribution. SR-MD has a better approxima-
tion of PMF near the protein surface than HR and significantly improves selectivity
predictions.

Additionally, we have studied the dependency of z-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 exper-
imentally determined one, is in a very good agreement with the neutron reflec-
tion 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 ap-
proach exposes the cell membrane to an RF electric field and measures vibra-
tional 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 dur-
ing 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 mem-
brane potential. The RF current was separated into conduction and displace-
ment components to examine the voltage-dependent RF conductance, GRF,
and capacitance, CRF. Remarkably, the RF capacitance, CRF, 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
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