3127-Pos Board B232
Recreating Ion Channel IV Curves using Specific Frequency Components
John Rigby, Steven Poelzing.
INTRODUCTION: Impedance spectroscopy cannot distinguish between ion channel families. We hypothesized that amplitudes of specific characteristic frequencies will correlate with the current amplitude passed by a specific ion channel families. Previously, we demonstrated the feasibility of this technique using the inward rectifying potassium channel, $K_{IR}2.1$. In this study, $Na_V1.5$ is used to demonstrate that the technique is applicable to other families of ion channels.
METHODS: IV curves were generated using a standard voltage step protocol performed in whole-cell voltage clamp mode on HEK293 cells transiently transfected with SCN5A (encodes $Na_V1.5$). Noise functions containing 1-50 kHz frequencies were inserted into each voltage step. The real component of the Fast Fourier transform (FFT) was then calculated for each trace. Each frequency magnitude as a function of voltage step was correlated with the IV curve.
RESULTS: The magnitude of 22.5 and 24.5 kHz correlated well with the IV curve of $Na_V1.5$ in the presence of the noise function ($R>0.8$), and poorly in the absence of noise ($|R|<0.3$). Two nodes of zero correlation were also found (11.36 +/- .08 kHz and 36.23 +/- 4.79 kHz. For $K_{IR}2.1$, current and frequency amplitudes did not correlate well between 11 and 36 kHz, suggesting that this correlation may be unique to $Na_V1.5$. On the other hand, frequencies were identified below 10 kHz whose amplitudes highly correlate with either one or both channels.
CONCLUSIONS: These data suggest that specific frequencies exist which can re-create the shape of both $K_{IR}2.1$ and $Na_V1.5$ IV curves. Furthermore, the correlation at some frequencies is channel specific, while others are not. This methodology could be a powerful tool for assessing the behavior of multiple ionic currents simultaneously during a freely running action potential.

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Mapping the Importance of 4 factors in Creating Monovalent Ion Selectivity in Biological Molecules
Michael Thomas, Dylan Jayatilaka, Ben Corry.
The ability of macrocycles, enzymes, ion channels, transporters and DNA to differentiate between ion types is often crucial to their function. Using molecular dynamics simulations on both detailed systems and simple models we quantify the importance of four factors which affect the ion selectivity, including the number of coordinating ligands [1], their dipole moment [2], the cavity size [3] and their vibrational motion. The information resulting from our model systems is distilled into a series of 'selectivity maps' that can be used to 'read off ' the relative free energy associated with binding of different ions, and to provide an estimate of the importance of the various factors. While our maps cannot capture all elements of real systems, it's remarkable that our simple model produces differential site binding energies in line with experiment and more detailed simulations for a variety of systems. This makes our maps a very useful tool for assisting in understanding the origins of selective binding and transport. Our studies show that the various suggested mechanisms of ion selectivity can be important in various situations. The chemical nature of the coordinating ligands is essential for creating thermodynamic ion selectivity in flexible molecules, but as the binding site becomes more rigid the number of ligands and the reduction of thermal fluctuations can become important.
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[3] Doyle D et al. (1998). The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Nature***280**, 67-77

3129-Pos Board B234
Testing the Applicability of Nernst-Planck Theory in Ion Channels
Chen Song, Ben Corry, Bert de Groot.
The question of whether Nernst-Planck (NP) theory, which is a macroscopic method for calculating ion flux, is still valid in microscopic narrow ion channels has been remaining a mystery for some years. Recently, we tested the ability of the NP theory to accurately predict channel currents by combining and comparing the results with those of Brownian dynamics (BD) simulations. The extensive tests for simplified and realistic ion channels indicate that the NP theory is still applicable in narrow ion channels provided that accurate concentrations and potentials can be input into the NP equation properly, as the currents obtained from the combination of BD and NP match well with those obtained directly from BD simulations. Here, we show first results comparing NP calculations and molecular dynamics (MD) simulations that show promising agreement, further confirming the validity of the NP theory at the microscopic scale. This finding opens a door to utilizing the results of microscopic simulations in continuum theory which can provide an efficient way to calculate the ion flux in ion channels, and might stimulate further effort in this direction.

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A New Poisson-Nernst-Planck Equation (PNP-FS-IF) for Charge Inversion Near Walls
YunKyong Hyon, James E. Fonseca, Bob Eisenberg, Chun Liu.
The plasmas of biology are interacting mixtures of ions - often charged spheres - that do not behave like the ideal solutions of textbooks. Interactions are always present because of strong electrical forces. Flows are usually present. Life without flow is death. We analyze ionic solutions as complex fluids with an approach that has successfully analyzed complex systems like liquid crystals that are dominated by interactions between composite components. The finite size of ions is particularly important in biology in crowded environments like channels, active sites of enzymes, or charged surfaces. We here deal with surfaces and try to capture the essential features of charge inversion (layering) near a charged wall. Charge inversion (layering) near walls is a characteristic phenomenon resulting from the electrostatic interactions in systems with charged walls. The mathematical model is derived by the energy variational approach (*EnVarA*) – J.Chem.Phys. (2010) 133:104104 – that combines the action of conservative (Hamiltonian) systems and the dissipation of Onsager and Rayleigh. Both are written in the same laboratory coordinates after variational derivatives of variables are taken. The generalized energy and dissipation include entropic and electrostatic components, and repulsion between spheres. An interfacial electroneutrality constraint between bulk and charged wall captures some essential features of charge inversion. Taking variational derivatives yields a field theory of partial differential equations and boundary conditions that are appropriate for life's solutions – that interact and flow – as well as thermodynamic equilibrium. The new equations, PNP-FS-IF, include (1) a nonlocal contribution of finite size (FS) and (2) an interfacial constraint (IF) of electroneutrality. PNP-FS-IF produces charge inversion near walls. We compare the charge inversion seen with PNP-FS-IF and Monte-Carlo simulations.

3131-Pos Board B236
How Interactions Control Molecular Transport in Channels
Anatoly B. Kolomeisky, Karthik Uppulury.
The motion of molecules across membrane channels and pores is critically important for understanding mechanisms of many cellular processes. Here we investigate the mechanism of interactions in the molecular transport through nanopores by analyzing discrete stochastic models. According to this approach the channel transport is viewed as a set of chemical transitions between discrete binding sites along the pore. It is shown that the strength and spatial distribution of molecule/channel interactions can strongly modify the molecular fluxes. Our analysis indicates that the most optimal transport is achieved when the binding sites are near the entrance or exit from the channel depending on the sign of the interaction potential. This observation allows us to explain recent single-molecule experimental results on translocation of different polypeptides. It also agrees with available information on distribution of binding sites in many membrane channels. In addition, we studied the role of intermolecular interactions during the channel transport, and it is argued that an increase in the flux can be observed for some optimal interaction strength. The mechanisms of these phenomena are discussed.

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Investigating Co-Transport Mechanisms in the AmtB Ammonium Transporter using QM/MM Molecular Dynamics
Shihao Wang, Sefer Baday, Simon Bernèche, Guillaume Lamoureux.
AmtB from Escherichia coli is a transmembrane protein with an important role in ammonium transport, especially at low external ammonium concentrations. However, whether AmtB is a channel that permeates NH_3 or an NH_3/H^+ co-transporter is still an open question. An extensive series of hybrid Quantum Mechanical(QM)/Molecular Mechanical(MM) simulations has been performed to investigate the mechanism of ammonium transport through AmtB. Focus has been placed on the deprotonation of ammonium and the possible co-transport of H^+ and NH_3. Constraint dynamics simulations have been used to obtain the potentials of mean force for the possible NH_4^+ deprotonation paths involving water molecules and/or protein side chains. Further investigations on the transport pathways of H^+ and NH_3 have shown the details of the co-transport mechanism. The distribution of solvent and ammonia inside the pore is also analyzed

and the possible mechanisms of ammonia re-protonation and how side chains are reset back to original state are presented.

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Permeation Mechanism in the AmtB Ammonium Transporter: Putative Electrogenic Co-Transport of NH3 and H+

Shihao Wang, Sefer Baday, Esam A. Orabi, Simon Bernèche, **Guillaume Lamoureux**.

Despite the growing amount of structural information, the molecular details of the mechanism by which membrane proteins of the Amt/Rh family mediate ammonium transport remain elusive. For instance, in protein AmtB from Escherichia coli, it is not known whether NH3 is diffusing passively through the protein pore or is involved in an NH3/H+ co-transport mechanism.

Using state-of-the-art computational methods (polarizable force fields and hybrid QM/MM molecular dynamics simulations combined with free energy calculations) we investigate the thermodynamics and kinetics of various mechanisms for proton co-transport. Based on these simulations we propose a plausible NH3/H+ co-transport mechanism in which the twin-histidines dyad lining the pore plays a central role.

3134-Pos Board B239

Investigating Ammonium Transport Mechanisms in AmtB and RhCG by Molecular Dynamics Simulations

Sefer Baday, Shihao Wang, Guillaume Lamoureux, Simon Bernèche.

Membrane proteins of the ubiquitous Amt/Rh family mediate the transport of ammonium. Despite the availability of different X-ray structures that provide many insights on the ammonium permeation process, the molecular details of its mechanism remain controversial. Functional experiments on plant ammonium transporters and rhesus proteins suggest a variety of permeation mechanisms including the passive diffusion of NH3, the antiport of NH4+/H+, the transport of NH4+, or the cotransport of NH3/H+. The X-ray structures have revealed that the pores of the prokaryotic AmtB and the eukaryotic RhCG proteins share a similar architecture suggesting that they might both catalyze the diffusion of NH3. However, molecular mechanics simulations of both proteins reveal that small differences in the pore lining residues might actually alter the properties of the pore. We notably find that the pore of the AmtB transporter can stabilize water molecules at much greater extent than the pore of RhCG. The possible presence of water molecules in the pore lumen of AmtB opens the door to alternative permeation mechanisms, notably involving the co-transport of H+. We discuss the possible permeation mechanisms in both the AmtB and RhCG proteins in light of some recent functional studies, and illustrate how closely related proteins can support quite different mechanisms.

3135-Pos Board B240

A View of Hydrogen/Hydroxide Flux Across Lipid Membranes

J. Wylie Nichols, **Ronald F. Abercrombie**.

A topic emerging roughly thirty years ago and engendering an incompletely resolved controversy is addressed: the relatively high permeability and pH independence associated with H^+/OH^- passive movements across lipid membranes. The expected characteristics of simple H^+/OH^- diffusion and those of a reaction between H^+ and OH^- being attracted from opposite surfaces and condensing in an interfacial region of the membrane are considered. An interfacial H^+/OH^- reaction mechanism predicts the experimentally observed behavior of a H^+/OH^- flux that is independent of the pH measurement range. In order to obtain the correct magnitude of flux, it is assumed that H^+ and OH^- within the interfacial zone become electrostatically aligned on opposite sides of the hydrophobic membrane core. Electrostatic attraction combined with charge delocalization among a small cluster of water molecules surrounding the ions sufficiently reduce the Born energy for insertion into lipid, accounting for the experimentally determined magnitude of this flux. The pH independence associated with H^+/OH^- passive movements across membranes could have satisfied a requirement for pH homeostasis in emerging life forms and provided stability for natural selection.

3136-Pos Board B241

Single Channel Measurements of N-Acetylneuraminic Acid-Inducible Channel (NANC) in E. coli

Janhavi Giri, **John M. Tang**, Christophe Wirth, Caroline M. Peneff, Tilman Schirmer, Bob Eisenberg.

Escherichia coli can use *N*-acetylneuraminic acid (Neu5Ac) as its sole carbon source even if the general outer membrane proteins OmpF and OmpC are not expressed: NanC - a monomeric outer membrane channel - allows Neu5Ac to move into the bacterial periplasm. Recently, a high resolution structure of NanC in two different crystal forms was reported by Wirth et al., J.Mol.Biol., (2009) 394:718 (PDB codes: 2WJQ and 2WJR). Our goal is to determine appropriate 'baseline' ionic conditions to study the transport of Neu5Ac through NanC using single channels in lipid bilayers. Measurements of single channel currents showed that NanC has two modes of time dependent behavior ('gating'). In the many situations we have tested, the modes are not induced or changed by surrounding ionic conditions or voltage. Single channels of NanC at pH 7.0 have: (1) a large conductance (around 100 pS to 800 pS in 100 mM KCl to 3M KCl) that varies with the polarity of the applied voltage; (2) anion over cation selectivity ($V_{reversal}$ around +16 mV in 250 mM KCl || 1 M KCl); (3) voltage-dependent gating (channel closures above ±200 mV). Single channel conductance of NanC decreases about 50% when HEPES concentration is increased from 100 μM to 100 mM in 250 mM KCl at pH 7.4, consistent with the two HEPES binding sites observed in the crystal structure (PDB code: 2WJR). Studying alternative buffers, we found that phosphate interferes with the channel conductance, whereas TRIS could not be used because it reacts with Ag/AgCl electrodes producing artifacts even in the presence of Agar-KCl bridges. Our further studies of NanC will use no pH buffers, but low concentration (250 mM) salt solutions adjusted to neutral pH 7.0.

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Blebbistatin Protects Rodent Myocytes from Death in Primary Culture via Inhibiting Na/Ca Exchange

Yinzheng Guan, Xiaoying Zhang, Yingxin Li, Chris Szeto, Xiajie Ai, Xiongwen Chen.

Introduction: It has been long recognized that rodent myoyctes die during long-term primary culture, which limits the use of genetically altered myocytes for signaling studies. Blebbistatin (BLB), a myosin II ATPase inhibitor, has been used to protect rodent myocytes. However, the mechanisms underlying the protective effects of this drug are not clear and are the topics of this study.

Materials & Methods: Adult rat ventricular myocytes (ARVM) were isolated and cultured with or without BLB (10μM) and BDM(10mM) for 72 hours. Myocyte death was evaluated by trypan blue staining. The effects of these two drugs on myocyte contraction, intracellular Ca transient ([Ca]i, Indo-1,410/480), SR Ca content, L-type calcium and Na/Ca exchanger currents were studied acutely.

Results: 1, Both BDM (61.5 ± 6.4%) and BLB (74.0 ± 3.2%) promoted myocyte survival in culture at 72 hours (control: 7.0 ± 1.8%); 2. ARVM fractional shortening was reduced by BLB (1.7 ± 0.4%) and BDM (0.5 ± 0.1%, control: 6.5 ± 0.7%); 3, Acutely, the amplitude of $[Ca]_i$ ($\Delta[Ca]_i$) was depressed by both BDM (0.038 ± 0.005) and BLB (0.065 ± 0.008) comparing to control (0.130 ± 0.010). 4, Diastolic Ca was significantly increased by BLB (0.90 ± 0.06) but not by BDM (0.73 ± 0.06) comparing to control (0.70 ± 0.05). 5. BLB and BDM significantly reduced the SR Ca content(Δ[Ca]i in BLB vs. BDM vs. control: 0.16 ± 0.016, 0.09 ± 0.01, 0.24 ± 0.03). 5. The mechanisms of the protective effect of BDM and BLB are different in that BDM mainly reduced Ca influx through the L-type Ca channel (85% reduction) and Na/Ca exchanger (60% reduction) while BLB inhibited Na/Ca exchanger (100% inhibition) without altering the LTCC(<5% reduction).

Conclusion: These results suggest both BDM and BLB protects rodent myocytes in culture by preventing cytosolic and SR Ca overload by both common and different mechanisms: both BDM and BLB inhibit NCX while BDM, but not BLB, reduces $I_{Ca\text{-}L}$.

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On Conduction and Gating in K^+-Channels

Carmen Domene, Simone Furini.

Potassium channels can conduct passively K^+ ions with rates of up to ~ 10^8 ions per second at physiological conditions, and they are selective to these species by a factor of 10^4 over Na^+ ions. Ion conduction has been proposed to involve transitions between two main states, with two or three K^+ ions occupying the selectivity filter separated by an intervening water molecule. The largest free energy barrier of such a process was reported to be of the order of 2-3kcal mol^{-1}. Here, we present an alternative mechanism for conduction of K^+ in K^+ channels where site vacancies are involved, and we propose that coexistence of several ion permeation mechanisms is energetically possible. Conduction can be described as a more anarchic phenomenon than previously characterized by the concerted translocations of K^+-water-K^+. Experiments also suggest that local structural changes in the selectivity filter may act as the a gate referred to as C-type inactivation. An extensive computational study on KirBac, is presented which supports the existence of a physical gate or constriction in the selectivity filter of K^+ channels. Our computations identify a new selectivity filter structure, which is likely associated with C-type inactivation.