exocytosis. We analyze these experimental protocols, derive simple formulas for special cases, and distinguish carefully between the Ca2+ current cooperativity, defined as the exponent in the relationship between exocytosis rate and the Ca2+ current magnitude, and the underlying Ca2+ channel cooperativity, defined as the average number of channels involved in the release of a single vesicle. Further, we use 3D computational modeling of buffered Ca2+ diffusion to analyze the distinct Ca2+ cooperativity measures, and demonstrate the role of endogenous Ca2+ buffers on such measures. We show that buffers can either increase or decrease the calcium current cooperativity of exocytosis, depending on their concentration and calcium-binding properties, and the distance between channel and vesicle.

Supported by the National Science Foundation grants DMS-0817703 (V.M.) and DMS-0613179 (R.B.), and the NIDDK/NIH Intramural Research Program (A.S.)

Biophysics of Ion Permeation

3406-Pos Board B453

Toward Controlling The Ion Selectivity By Manipulating Individual Subunits Among Four In A Tetrameric K^+ Channel

Qiulin Tan, Ji Wook Shim, **Li-Qun Gu**.
University of Missouri, Columbia, MO, USA.

The ion permeation in all the K^+ channels is governed by a selectivity filter that is assembled by backbone carbonyls from four identical conservative sequences, Thr-X-Gly-Tyr-Gly. Varying any part of this sequence for all four subunits often disables the K^+ selectivity. However, it is unclear how the selectivity is altered with an individual subunit among four. Understanding of this mechanism will uncover the contribution of each individual subunit to the overall ion selectivity, i.e. functional stoichiometry. So far this research has been limited due to difficulty in obtaining hetero-tetrameric channel proteins. We are studying this mechanism with a unique model K^+ channel, chlorella virus-encoded Kcv. We have found that the wild-type and tagged Kcv (with an extension of eight asparagines at the N-terminal) can be co-synthesized *in vitro* and self-assembled into various homo- and hetero-tetramers, as visualized through electrophoresis. **Most notably, when purified directly from the SDS gel, each hetero-tetramer exhibited perfect K^+ channel functions in the lipid bilayer** (this is difficult to achieve for other membrane proteins). Using this protein as the background, we obtained all types of hetero-tetramers containing different numbers of the mutant Kcv at the selectivity filter (G65C). The electrophysiology test revealed that the proteins with up to two mutant subunits in the tetramer still retain the K+ selectivity, but the selectivity is disabled for tetramers containing more than two mutant subunits. (*FEBS Letters* 581 (2007) 1027-1034)

3407-Pos Board B454

New Insights Into Selectivity of Potassium Channels Using Small Cation Blockers

Ameer N. Thompson[1], Ilsoo Kim[2], Timothy Panosian[3], Tina Iverson[3], Toby Allen[2], Crina Nimigean[1].
[1]Weill Cornell Medical College, New York, NY, USA, [2]University of California, Davis, Davis, CA, USA, [3]Vanderbilt University, Nashville, TN, USA.

KcsA channel pores are blocked by intracellular Na+ and Li+ ions. We are investigating Na+/Li+ binding locations using electrophysiology, X-ray crystallography, and molecular dynamics simulations. We found that intracellular Li+ blocks KcsA channels with low, voltage-dependent affinity and competes with K+ for the blocking site. Its movement to the blocking site is not coupled with movement of permeant ions in the field. In contrast, Na+ blocks with less affinity and larger voltage dependence. We proposed that both small cations block in the hydrated vestibule with Na+ binding deeper in the pore at a site requiring partial dehydration while Li+ resides lower, remaining fully hydrated. Molecular dynamics calculations indicated low affinity binding for Na+/Li+ in the cavity but also predicted a high affinity binding-site in the S4 site, not ''in-cage'' where K+ ions bind but ''in-plane'' coordinated by Thr75 carbonyl oxygens. In search for all potential Li+ binding-sites we crystallized KcsA in the presence of Li+. Consistent with the MD results, we found three potential binding sites, one of which is in the S4 site of the selectivity filter in the plane of the Thr75 carbonyls. This suggests that Li+ and Na+ may be favored to bind in the S4 site but that they need to overcome a large energy barrier to get there. MD simulations unveil such barriers through free energy calculations involving multiple ion mechanisms for the smaller ions. We are now investigating experimentally the existence of a high-affinity binding-site inside the selectivity filter for both Na+ and Li+.

3408-Pos Board B455

Development of a Drude Polarizable Force Field for Ion-water and Ion-NMA Interactions and Application to Selectivity in Ion Channels

Haibo Yu[1], Troy W. Whitfield[2], Sergei Yu. Noskov[3], Christopher L. Mazzanti[4], Roger E. Koeppe II,[4], Olaf S. Andersen[5], Benoit Roux[1,2].
[1]Department of Chemistry and Molecular Biology, University of Chicago, Chicago, IL, USA, [2]Biosciences Division, Argonne National Laboratory, Argonne, IL, USA, [3]Institute for Biocomplexity and Informatics, Department of Biological Sciences, University of Calgary, Calgary, AB, Canada, [4]Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, USA, [5]Department of Physiology and Biophysics, Weill Medical College of Cornell University, New York, NY, USA.

A challenge in modeling ions in biomolecular systems is the description of interactions involving ions in a heterogeneous environment, where explicit representation of polarization often becomes important. As a first step towards meeting this challenge, a Drude polarizable force field for ion-water and ion-N-methylacetamide (NMA: a model compound for peptide bond) is developed. For the first time, the alkali and halide ion interactions with liquid NMA has been characterized experimentally. By measuring the solubilities in liquid NMA, we derive the solvation free energies of KCl and NaCl in liquid NMA. Good agreements are found for both the structural and thermodynamic properties in the gas phase and in the condensed phase. As an application, the developed polarizable model is used to study ion selectivity in a reduced binding site model of the site S2 in KcsA. The results confirm the previous finding that both the number and type of ligands play an important role in K+ selective ion channels.

3409-Pos Board B456

Cation Blocking Mechanisms of the KcsA Potassium Channel Explored with All-atom Free Energy Simulations

Ilsoo Kim[1], Ameer Thompson[2], Crina Nimigean[2], **Toby W. Allen**[1].
[1]Department of Chemistry, University of California, Davis, CA, USA, [2]Department of Physiology and Biophysics, Department of Anesthesiology, Weill Medical College of Cornell University, New York, NY, USA.

We have carried out free energy simulations of multiple ion configurations in the KcsA potassium channel to understand experimentally observed Na and Li blocking and offer predictions that are supported by electrophysiological assays and X-ray crystallographic structures. Through free energy perturbation and potential of mean force calculations we find that Na and Li can bind either inside the aqueous cavity of the channel or deep into the S4 site, coordinated by a plane of 4 carbonyl oxygens rather than the usual 8-ligand cage of K. We have found good evidence to support this prediction with the existence of at least two distinct binding sites for Na and Li suggested by the experiments. We demonstrate that a different multiple-ion mechanism is required for Li and Na ion permeation, involving large energetic barriers that are not encountered by K. These studies shed light on how small monovalent cations block the KcsA channel and provide new insight into the selectivity mechanisms of potassium channels.

3410-Pos Board B457

Electrostatic Determinants of Membrane Ion Permeability

Igor Vorobyov, Borislava Bekker, Toby W. Allen.
University of California Davis, Department of Chemistry, Davis, CA, USA.

Ion channels facilitate the passage of charged molecules across cell membranes by eliminating energetic costs thought to be associated with dehydration inside a low-dielectric membrane core. However, recent atomistic simulation studies have uncovered a different picture of charge-lipid interactions with reduced barriers due to membrane deformations. Having a correct description of the origins and magnitudes of these energetic barriers is essential to describe ion permeation, as well as to understand processes that involve the interaction of charged peptides or protein domains with membranes. Here we seek energetic decompositions to unveil the mechanisms of assisted or unassisted permeation and explore the roles of membrane electronic polarizability, dipole potential and composition (including charged lipids). We find that while electronic polarizability has some considerable effects on ion solvation free energies in non-polar solvents, as well as solvent interfacial potentials, a polarizable lipid model reveals only small effects on ions in the membrane. We show that the full membrane dipole potential is not seen by ions and explore the role of the membrane electrostatics on ions inside ion channel proteins.

3411-Pos Board B458

Mapping the Common Origins of Ion Selectivity in Biological Molecules

Michael Thomas, Dylan Jayatilaka, Ben Corry.
University of Western Australia, Perth, Australia.

Numerous biological molecules selectively bind or transport particular ions. In biological systems, the discrimination between sodium and potassium is particularly important. We demonstrate that selectivity of group I ions is dependent