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.

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Biophysics of Ion Permeation

3406-Pos Board B453

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

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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

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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

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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

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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

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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

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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

on three factors; the dipole moment of the coordinating ligands [1], the number of coordinating ligands [2] and the cavity size of the coordination site [3]. By using free energy methods in molecular dynamics simulations to study model systems as well as a range of biological molecules including channels, transporters and enzymes, we are able to determine the contribution of each of these factors to the overall ion selectivity of the molecule. Varying contributions of each give rise to the richness in ion selectivity we see.
By mapping out the importance of these factors in various cases, an estimation of the degree of selectivity of an ion selective biological molecule can be made given its structure. These results also assist in predicting the nature of a binding site of an unknown structure and have the potential to aid in the design of novel synthetic ion selective molecules.
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3412-Pos Board B459
Hydrophobic Selectivity And Electrostatic Gating In Narrow Ion Channels
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Previous studies have shown that some ion channels, such as the nicotinic acetylcholine receptors (nAChR), have a hydrophobic region lining the pore in the transmembrane domain (TM-domain) that is responsible for gating the channel, and a charged ring at the extracellular entrance of the TM-domain which is important for ion selectivity. Our recent studies show that the hydrophobic effect can also contribute to the ion selectivity, and the electrostatic effect can also play a role in the gating behavior.
1) Hydrophobic selectivity: Singe walled carbon nanotubes (SWNTs) are selected as the model of the hydrophobic pores and potential of mean force calculations are performed for Na^+, K^+ and Cl^- respectively. The results show that for the (8,8) and (9,9) SWNTs which ions can pass through under biological driving forces, the free energy difference between types of ions can exceed 2 kcal/mol. This difference arises mainly from the differing dehydration energies and this hydrophobic selectivity may complement electrostatic origins of selectivity.
2) Electrostatic gating: Preliminary electrostatic calculation results on the TM-domain of the nAChR in the closed state show that a ring of charged residues can tightly bind an ion preventing conduction. A small conformational change of the protein that increases pore radius by only 0.5 Å can reduce the binding by ~6 kcal/mol and allow permeation.

3413-Pos Board B460
The Anomalous Mole Fraction Effect in Calcium Channels: The Ryanodine Receptor Case Study
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The origin of the anomalous mole fraction effect (AMFE) in calcium channels is explored with an ion permeation model of the ryanodine receptor (RyR) calcium channel. This model predicted and experiments verified new AMFEs in RyR. In mole fraction experiments, conductance is measured in mixtures of two ion species (X and Y) as their relative amounts (mole fractions) vary. This curve can have a mimimum (an AMFE). The traditional interpretation of the AMFE is that multiple ions move through the pore in a single file. Nonlinear mole fraction curves without minima are generally interpreted as X displacing Y from the pore in proportion larger than its bath mole fraction (preferential selectivity). We find that the AMFE is also caused by preferential selectivity of X over Y if they have similar conductances. This is a prediction for any channel. Preferential selectivity causes the resistances to current flow in the baths, channel vestibules, and selectivity filter to change differently with mole fraction. This resistors-in-series model provides a fundamentally different explanation of the AMFE that does not require single filing or multiple occupancy. The success of the resistors-in-series model to predict AMFEs in RyR shows that the traditional model should be reconsidered for calcium channels.

3414-Pos Board B461
Energetics of Calcium Selectivity: A Three-Dimensional Classical Density Functional Theory Approach
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Selectivity of a calcium channel is explored with three-dimensional density functional theory of fluids (DFT). The model pore has millimolar Ca2+ affinity similar to the ryanodine receptor calcium channel. The four flexible aspartate side chains in the selectivity filter are modeled as four carboxyl groups (each as two independent, half-charged oxygen atoms) that are free to move within the selectivity filter, but cannot leave it. These oxygens coordinate the permeating ions. We examine how the ions are coordinated by computing radial correlation functions around the permeating ions. We also examine how this coordination changes in wide and narrow selectivity filters. The energetics of selectivity are computed and their components (e.g., electrostatics, excluded volume) show that the coordination of the ions by the oxygens determines the Ca2+ selectivity of the pore by the charge/space competition mechanism. In this mechanism, selectivity is determined by a balance of electrostatic and steric interactions of ions and amino acid side chains in the crowded selectivity filter. Our approach of combining three-dimensional DFT with state of the art computational techniques is unique in channel selectivity. Moreover, the DFT approach allows a natural decomposition of the energies involved in selectivity. The convolution-type calculations at the heart of the DFT are computed using a combination of fast transforms and analytical results. The software itself is built upon the PETSc framework from Argonne National Laboratory.

3415-Pos Board B462
H+ Permeation in Hv1 Voltage-gated Proton Channels
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Voltage-gated H^+ channels are structurally homologous to the voltage sensor domain of K_v, Na_v and Ca_v channels but, despite the lack an ion-selective pore domain, conduct robust H^+ current without apparent need for an accessory protein. Recent evidence indicates that although H_v1 is dimeric, each subunit contains a separate H^+ permeation pathway that can be abrogated by amino acid mutation and chemical modification. However, the molecular mechanism of H^+ permeation in H_v1 is unknown.
Previous studies suggested that H^+ permeation in voltage-gated proton channels is likely to employ a Grötthus-type H^+-hopping mechanism involving one or more protonatable amino acids. We hypothesized that residues which are required for H^+ permeation in H_v1 should be identifiable by loss of function phenotype in a mutagenesis screen. Candidate H^+-acceptor residues within the voltage sensor were selected from those conserved in H_v1 species orthologues and by examination of homology models based on H_v1 structure based on known K_v channel protein structures and refined by molecular dynamics simulations. We used site-directed mutagenesis to neutralize candidate residues by substitution to Ala or Asn. Mutated GFP-H_v1 channels were expressed in mammalian culture cells and whole-cell H^+ currents at fixed pipette pH and varying bath pH were elicited by depolarizing voltage steps. Although the apparent threshold for voltage-dependent activation of H_v1 current was altered by as much as −120 mV in certain mutants, the charge-neutralizing mutations we tested were insufficient to entirely abrogate expressed H^+ current. Mutagenesis data and molecular models were used to generate a molecular model of H^+ permeation through H_v1.

3416-Pos Board B463
Ion Transport through OmpF in Molecular Dynamics Simulations and Experiments
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The outer membrane pores F (OmpF) of E. coli bacteria is a diffusion channel which has a wide range of functions and properties of biological relevance. The temperature-dependent ion conductance in OmpF is measured in a wide range of electrolyte concentrations and compared with molecular dynamics simulations. The agreement between experiment and theory is very good. In the experiment single OmpF channels are reconstituted into planar lipid bilayers. In test studies, bulk electrolyte simulations and experiments showed that the simulations are accurate for salt concentrations up to 1 molar. Comparing the temperature dependence of the OmpF channel conductance with that of the bulk conductivity in the range from 0 to 90 degree Celsius revealed that at low salt concentrations the transport is mainly driven along the pore surfaces. Increasing the salt concentration saturates the surface charge transport and induces ion transport in the center of the nanopore. The confinement of the nanopore then favors the formation of ion pairs.