1707-Pos
Molecular Dynamics of Trace Amine Transport through Neuronal Membranes
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The trace amines are a family of endogenous compounds, synthesized in neurons, for whom a family of receptor proteins has been identified. Unlike neurotransmitter receptors, trace amine receptors do not appear to be expressed at the plasma membrane of cells, rather remaining in the cytosol. This requires trace amines readily cross the lipid bilayer in order to interact with the receptor and effect signal tranduction. It has previously been assumed that this occurs via passive diffusion. However, the unknown rate of passive diffusion in allowing trace amines to cross the synaptic cleft has hindered the progress of recent studies attempting to determine their physiological role (M.D. Berry, *J. Neurochem*, **90**, 257-271, (2004), A. G. Ianculescu *et al*, Endocrinology, **150**, 1991 (2009)). Molecular dynamics (MD) simulations have been carried out to determine the position dependent diffusion constant, *D(z)*,and the Potential of Mean Force (PMF) of several trace amines both inside and outside the membrane. From this data, the trace amine flux through the membrane can be calculated. Using specialized free energy simulation techniques, MD trajectories have been generated and analyzed to determine the mean force exerted on the trace amines, 2-phenylethylamine (2PE), its protonated form, 2PE+ and on 3-iodothyronomine, at distances ranging from 20 angstrom right to the middle of a symmetric sphingomyelin membrane. Preliminary results indicate a potential barrier ~13 Kcal/mol for 2PE+ and ~20Kcal/mol for 2PE. These relatively high potential barriers are consistent with a very low transmembrane flux due to passive diffusion. The contribution this information makes to the question of yet-undiscovered transporters for trace amines is discussed in the conclusions.

1708-Pos
Induction of Liposome Leakage by Photodynamic Action: Dependence on the Kind of Fluorescent Probe
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Photosensitized damage to liposome membranes was studied by using different dye-leakage assays based on fluorescence dequenching of a series of dyes upon their release from liposomes. Irradiation of liposomes with red light in the presence of a photosensitizer, trisulfonated aluminum phthalocyanine ($AlPcS_3$), resulted in the pronounced leakage of carboxyfluorescein, but rather weak leakage of sulforhodamin B and almost negligible leakage of calcein from the corresponding dye-loaded liposomes. The photosensitized liposome permeabilization was apparently associated with oxidation of lipid double bonds by singlet oxygen as evidenced by the requirement of unsaturated lipids in the membrane composition for the photosensitized liposome leakage to occur and the sensitivity of the latter to sodium azide. The fluorescence correlation spectroscopy measurements revealed marked permeability of photodynamically induced pores in liposome membranes for such photosensitizers as $AlPcS_3$ and $AlPcS_4$. It was proposed that the difference in permeability of these pores to carboxyfluorescein and calcein was associated with size restriction. Verification of this hypothesis by studying the effect of PEGs of different molecular weights is under way.

1709-Pos
Intrinsic Versus Extrinsic Voltage Sensitivity of Blocker Interaction with an Ion Channel Pore
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Many physiological and pharmacological agents act by occluding the conduction pore of ion channels. A hallmark of charged blockers is that their apparent affinity for the pore usually varies significantly with membrane voltage. Two models have been proposed to explain voltage dependence of channel block. One model - prevalent during the past three decades - assumes that the charged blocker itself directly senses the transmembrane electric field, i.e., that blocker binding is intrinsically voltage dependent. In the alternative model, the blocker does not directly interact with the electric field; instead, blocker binding acquires apparent voltage dependence solely through the concurrent movement of permeant ions across the field. Although less frequently invoked, this latter model may better explain voltage dependence of channel block by large organic compounds that are too bulky to fit into the narrow part of the pore where the electric field is steep. To date no systematic investigation has been carried out to distinguish between these voltage-dependent mechanisms of channel block. When the voltage dependence of block by organic compounds is believed to be extrinsic, it has never been demonstrated that the block can be rendered voltage independent - the most fundamental characteristic of the extrinsic mechanism. In the present study we find that a retinal cyclic nucleotide-gated (CNG) channel can be blocked via either mechanism, depending on the nature of the blocker. With this channel as a model, we systematically examine both intrinsic and extrinsic types of voltage dependence of channel block, and illustrate their electrophysiological hallmarks and analytical characteristics.

1710-Pos
Self-Consistent Calculations of the Current and Access Resistance in Open Ion Channels
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The problem of calculating the current and access resistance in open ion channels is considered. A self-consistent analytic solution is introduced for an arbitrary number of species within the Poisson-Nernst-Planck (PNP) equations formalism. The model considered is a cylindrical channel of radius *a* in the protein which allows ions to cross a membrane that is bathed by two solutions of different concentration on its left and right-hand sides. Electro-diffusion in this system is described by the Poisson equation combined with the continuity equations for the mobile ions. The PNP equations are solved in the bulk in the Boltzmann approximation in 3D, assuming spherical symmetry, and in the pore in a 1D approximation. The boundary conditions (BCs) for the potential and concentration are set at infinity. The internal BCs for the current and the gradient of the potential are set at the surfaces of two hemispheres of radius *a*. The two solutions are matched together at the internal BCs using an iterative procedure in a self-consistent way. The method allows for calculation of the currents for an arbitrary number of ions species that have different diffusion constants in the channel and in the bulk. The sizes of the ions are taken into account by introducing a ''filling factor'' as an additional fitting parameter. The method is applied to model experimental I-V characteristics of the Gramicidin A channel for various concentrations, yielding qualitative good agreement.

1711-Pos
Effect of Charge Fluctuations on Conduction in Biological Ion Channels
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How open ion channels are able to conduct ions with a throughput comparable to free diffusion, and yet remain highly selective, is an unresolved scientific conundrum of long standing. To shed new light on this problem, the effect of charge fluctuations on the conduction of open ion channels is investigated theoretically. The model considered is a cylindrical channel across the membrane bathed by two solutions of different concentration. The charge fluctuations at the channel mouth are analyzed using Brownian Dynamics simulations and shown to have the form of trichotomous noise on the timescale of nanoseconds. The channel potential with a local minimum at the selectivity site due to the fixed wall charge is calculated by solution of the 3D Poisson equation for two configurations, with one ion moving along the channel axis in the presence or absence of the fluctuating charge at the channel mouth. It is shown that narrow channels act as electrostatic amplifiers of the modulation of the potential barriers at the selectivity site, due to charge fluctuations at the channel mouths. This modulation at the selectivity site was largely neglected in earlier research. It results in a leading order contribution to the transition rates of open ion channels. The proposed model of ion permeation takes into account the dynamical effect of the charge fluctuations through the resultant shot noise, which flips the electrostatic potential at the selectivity site, causing it to fluctuate between three values at a rate corresponding to the random arrivals of ions at the channel mouth. The model is applied to calculation of the current-voltage characteristics of Gramicidin A channel for different concentrations and is shown to be in good agreement with experimental results, including the effect of current saturation at high concentrations.

1712-Pos
Free Energy Calculations along Complex Proton Transport Pathways
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Ion transport processes through protein channels is an essential component of cellular function. One especially interesting example is the ClC-ec1 antiporter, which transports proton (H^+) and chloride ions (Cl^-) in opposite directions with a stoichiometric ratio of 1:2. In this work, the multistate empirical valence bond (MS-EVB) molecular dynamics method has been applied to simulate the explicit translocation of a Grotthuss shuttling excess proton from the intracellular residue Glu203 to the extracellular residue Glu148 through a transient water chain inside the channel. The minimum energy proton transport pathway was first identified using the string method and the free energy profile, i.e., the

potential of mean force (PMF), was calculated along the path. The electrostatic coupling between the excess proton and chloride ion was also explored. These studies therefore provide a more detailed picture of the proton transport process in the ClC-ecl antiporter.

1713-Pos

Ion Selectivity in the Aspartate Transporter Glt_{PH}

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The aspartate transporter Glt_{PH} is an integral membrane protein that catalyses the movement of aspartate across lipid bilayers. Glt_{PH} utilises established ion gradients, transporting two sodium ions with each aspartate molecule. Previous studies have shown that the ion binding sites demonstrate selectivity for Na^+ over both Li^+ and K^+ ($Na^+ > Li^+ > K^+$) [1]. The sodium binding motif is similar to that of another sodium dependent leucine transporter, LeuT. Computational studies have attributed different mechanisms to ion selectivity in each of the two sodium binding sites in LeuT [2]. Selectivity in the first site results from the binding of the negatively charged carboxylate group of the substrate resulting in strong electrostatic interactions while selectivity in the second site is enforced by an almost rigid cavity of coordinating ligands held in place hydrogen bonding networks.

Using various computational techniques, we describe the thermodynamic contributions to the free energy of binding that give rise to the experimentally observed selectivity sequence $Na^+ > Li^+ > K^+$ in Glt_{PH} and compare and contrast them to those in LeuT.

[1] Boudker, O. et al. Nature 2007, 445, 387-393
[2] Noskov, S.; Roux, B. J. Mol. Biol. 2008, 377, 804-818

1714-Pos

Microscopic Mechanism of Ion Selectivity in the Nak Pump

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The sodium/potassium pump establishes the Na+ and K+ concentration gradients across the plasma membrane of animal cells and therefore plays an essential role in maintaining cell volume and secondary active transport of other solutes. The crystal structures of the Na+/K+ pump provide atomic insight into the binding of K+ ions and conformational transitions during the functional cycle. However, important details about the ion-selectivity remain to be addressed. In particular, 2 out of the 3 binding sites are shared between Na+ and K+ and it is not clear how this pump selects K+ over Na+ when in the outwardly facing conformation (E2P) or Na+ over K+ when in the inwardly facing conformation (E1). We have undertaken free energy calculations to understand the physical principles that govern the ion selectivity in Na+/K+ pump and dissected various factors that may contribute to the selectivity. We found that the pump elegantly modulates the electrostatic environment of the binding sites to achieve the corresponding selectivity. Our results are consistent with available experimental data and provide new hypothesis to test experimentally. [Supported by NIH grant GM062342].

1715-Pos

The Role of Architectural and Structural Forces in Ion Selectivity

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A novel theoretical framework is presented to clarify the role of architectural and structural forces in ion selectivity by expressing the relative free energy of bound ions in terms of a reduced local system coupled to a potential of mean force (PMF) representing the influence of the surrounding environment. The PMF is separated into two contributions. The first includes all the harsh forces keeping the ion and the coordinating ligands confined to a small microscopic region, but do not prevent the ligands from adapting to ions of different radii. The second regroups all the remaining forces that serve to dictate a precise geometry of the coordinating ligands best adapted to a given ion. In the limit where the precise geometric forces are dominant, the binding site is almost rigid and ion selectivity is controlled by the ion-ligand interactions according to the classic ''snug-fit'' mechanism of host-guest chemistry. In the limit where the precise geometric forces are negligible, the ion and ligands behave as a self-organized ''confined droplet'' that is free to fluctuate and adapt to a smaller ion. But selectivity can also occur under such conditions. In the small and crowded volume, ion selectivity is determined by the ion-ligand and ligand-ligand interactions and is controlled by the number and the chemical type of ion-coordinating ligands. The theoretical framework is used to analyze K+ binding sites in the KcsA channel and Na+ binding sites in the LeuT transporter.

1716-Pos

Mechanisms of Ion Permeation through Gramicidin a Channels

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Gramicidin A (gA) channels make an ideal system to test all-atom molecular dynamics (MD) of membrane proteins and mechanisms of ion permeation. In addition to being the most studied membrane ''protein'', gA channels are tiny, allowing for long MD runs and calculations of potential of mean force (PMF) in tractable time. The binding sites at either end of the gA channel can both hold a single cation. At low concentration, permeation occurs as a series of independent events in which one cation at a time moves across the pore. Ion permeation usually is described using the ion position z in the direction of the pore axis as a ''reaction coordinate''. But it is not known whether z is a good reaction coordinate to describe the process. A powerful tool to characterize the mechanism of ion permeation in the gA channel is the ''committor'' probability: the fraction of trajectories initiated from a given position that first commit to the left or right binding site of the channel. We evaluate the committor probability distribution function to identify the physical reaction coordinates of a K+ in gA using extensive MD calculations. At high concentration, permeation is dominated by 2-ion processes where cations are bound at either ends of the small pore. To understand the impact of double ion occupancy on the mechanism of ion permeation, we calculate the 2-ion PMF. The results show that if the first ion resides in the inner binding sites at one end of the channel, then the outer and inner binding sites for the second ion at the other end of the channel become shallow. The energetics of double occupancy is explained by considering the dipole moment fluctuation of the single-file water molecules inside channel. [Supported by NIH grant GM070971].

1717-Pos

Thermodynamically Dominant Hydration Structures of Ions and their Role in Ion-Specificity

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To understand the basis of ion-specific effects in biology, it is necessary to first understand the hydration structure and thermodynamics of ions. Based on a multi-state organization of the potential distribution theorem, we present new insights on the role of ion-water interactions and water density fluctuations at the size-scale of the ion in determining the ion-hydration structure and thermodynamics. We find that the hydration free energy of the ion depends on three quantities: 1) the hydration free energy of the ion in a specified n-coordinate state, where in the n-coordinate state n water molecules are present within the coordination volume of the ion; 2) the probability, xn, of observing that n-coordinate state around the ion; and 3) the probability, pn, of observing n water molecules in the coordination volume in the absence of the ion. Based on this development we find that only a small subset of water molecules in the first hydration shell of the ion sense the chemical type of the ion. Further, these core-water molecules tend to attenuate the interaction of the ion with the rest of the medium, and thus the higher coordination states of the ion more sensitively reflect density fluctuations of the solvent medium at the size scale of the observation volume. The relevance of this development in understanding ion-pairing and the selective binding of ions to biological molecules is discussed.

1718-Pos

On the Domain of Applicability of Currently used Force Fields for the Calculation of the Activity of Alkali Ions at Physiological Ionic Strength

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Alkali ions are present in virtually all biological processes. Their energetic properties have been so far predicted mostly by MD or MC calculations based on effective potentials derived for infinite diluted conditions (i.e. a single ion surrounding solely by water molecules) [1]. However, in physiological conditions, the concentration of K+ is sub-molar in the cytoplasm [2], and it may be by one, or even two, orders of magnitude larger near globular proteins or nucleic acids and in the active sites of of enzymes or channels [3-5]. The presence of a large ionic strength I is likely to limit the accuracy of the currently used potentials.

Here we will discuss recent calculations of the activity coefficients for K+,Na+ ions at increasing I. Such coefficients have been obtained by calculating the excess chemical potentials from thermodynamics integration [6], with several commonly used biomolecular force fields. Preliminary results show that classical force fields generally overestimate the activity coefficients of ions.

[1] M. Patra and M. Karttunen. J. Comput. Chem., 25:678-689, 2004.