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1719-Pos
Voltage Profile along the Permeation Pathway of an Open Channel
Jin Chen[1], Jorge E. Contreras[1], Albert Y. Lau[2], Vishwanath Jogini[2], Benoît Roux[2], Miguel Holmgren[1].
[1]NINDS, Bethesda, MD, USA, [2]Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL, USA.
In ion channels, the transmembrane potential plays a critical role in ion conduction by acting as a driving force for permeant ions. At the microscopic level, the transmembrane potential is thought to decay non linearly across the ion permeation pathway because of the irregular three-dimensional shape of the channel pore. To experimentally explore the voltage profile of an open channel, we studied the voltage dependence of chemical modification of cysteines substituted along the permeation pathway of cyclic nucleotide-gated (CNG) channels. Because ion conduction through these channels is not sensitive to voltage at maximal open probabilities, nor they desensitize or inactivate when exposed to ligand, CNG channels are an ideal model to these studies. Our functional observations indicate that most of the voltage drop across the permeation pathway occurs along the selectivity filter region of CNG channels. The experimental data are in good agreement with continuum electrostatic calculations using a homology model of an open CNG channel. The focusing of the transmembrane potential across the selectivity filter indicates that the electromotive driving force is coupled with the movement of the permeant ions in the filter, maximizing the efficiency of this process.

1720-Pos
Microscopic Mechanism of Ion Permeation through K^+ Channel
Takashi Sumikama[1], Iwao Ohmine[2], Shigetoshi Oiki[3].
[1]Institute for Molecular Science, Okazaki, Aichi, Japan, [2]Kyoto University, Kyoto, Kyoto, Japan, [3]Fukui University, Yoshida, Fukui, Japan.
One of the most basic roles of ion channel is the passive transport of ions through the hydrophilic pore enabling the dehydration of ions. Since the determination of the x-ray crystallographic structure of the K^+ channel, many theoretical studies on the ion permeation have been performed. However, the microscopic mechanism of ion permeation, the essence of ion channel, has not been clarified yet.
We study the passive transport of ions through the K^+ channel, Kv1.2, by the molecular dynamics simulation in which the electric field is applied. A number of ion permeation is successfully observed. The number of permeated ion, i.e., the channel conductance is mostly proportional to the ion concentration of the bulk. The number of ions in the central cavity also depends on the ion concentration. Although the ions and water molecules are transported alternately, surprisingly, the other manner of ion permeation is also observed. That is, ions can permeate without intervening water molecule at high concentration, on the other hand, a permeating ion is accompanied by two water molecules at low concentration. Therefore, the microscopic mechanism of ion permeation depends on the ion concentration, and the physiological fact that an ion permeates per a water molecule is considered to be the average of these two cases. We also discuss the role of the central cavity by comparing the ion permeation in the channel with that in the model channel.

1721-Pos
Multi-Ion Mechanism of Potassium Channel Rejection of Na and Li Ions
Ilsoo Kim, Toby W. Allen.
University of California, Davis, CA, USA.
Ion channels catalyze rapid and selective ion movement across cell membranes to control electrical and chemical activity in the body. Potassium channels have the remarkable ability to pass K ions at near diffusion-limited rates, while exquisitely blocking Na ions. The mechanisms of channel selectivity, based on simulation and experimental studies of KcsA blocking by Na and Li ions, will be discussed. Through free energy perturbation and potential of mean force calculations, we find that Na and Li can bind deep into the S4 site of the selectivity filter, coordinated by a plane of four carbonyl oxygen atoms, rather than the usual eight-ligand cage of K. However, we demonstrate that a different multiple-ion mechanism is required for Li or Na ion entry into the filter from the aqueous cavity, involving large energetic barriers that are not encountered by K. We also revisit calculations of the thermodynamic stability of these ions in other sites of the filter, within the framework of a multiple-ion free energy calculation, with some surprising results. We conclude that, under physiological conditions, the rejection of intracellular Na or Li from KcsA occurs upon entry to the filter and is not due to reduced thermodynamic stability at any site inside the filter.

1722-Pos
Exploring the Permeation Mechanism of Valinomycin Across Lipid Membranes
Borislava Bekker, Toby W. Allen.
University of California, Davis, CA, USA.
Valinomycin is a potassium specific ionophore used to transport ions down an electrochemical gradient across lipid membranes. Its small size, high selectivity, and strong antibiotic activity make it an interesting target for molecular dynamics simulations. At the same time its conformational flexibility, which strongly depends on the polarity of its environment, poses a challenge. This work was undertaken in order to elucidate the mechanism of valinomycin mediated potassium transport across a lipid bilayer. We have explored several advanced sampling techniques, but chose to perform multi-dimensional free energy calculations that explore conformational space while computing the potential of mean force for membrane translocation. By computing free energy surfaces with and without a bound K^+ ion and calculating the free energetics of the ion binding process, we can describe a cycle that reveals the overall permeation mechanism. Our results using implicit and explicit solvent and membrane models will be discussed.

1723-Pos
Investigating Ion Channels using Chemical Synthesis
Francis Valiyaveetil.
Oregon Health And Science University, Portland, OR, USA.
Chemical synthesis is a powerful method for precise modification of the structural and electronic properties of proteins. The difficulties in the synthesis and purification of peptides containing transmembrane segments have presented obstacles to the chemical synthesis of integral membrane proteins. We will present a modular strategy for the semi-synthesis of integral membrane proteins in which solid phase peptide synthesis is limited to the region of interest, while the rest of the protein is obtained by recombinant means. This modular strategy considerably simplifies the synthesis and purification steps that have previously hindered the chemical synthesis of integral membrane proteins. We will discuss a sandwich-intein fusion strategy and a sumo-fusion and proteolysis approach for obtaining the membrane spanning peptides required for the semi-synthesis. We will demonstrate the feasibility of the modular approach by the semi-synthesis of the K^+ channel, KcsA and the non-selective cation channel NaK. The use of chemical synthesis in functional investigations of the KcsA and the NaK channels will also be presented.

1724-Pos
[K^+] Induced Conformational Dynamics of the Selectivity Filter of KcsA Monitored by Solid-State NMR
Manasi Bhate, Benjamin Wylie, Lin Tian, Ann McDermott.
Columbia University, New York, NY, USA.
A solid-state NMR study of the selectivity filter of the prokaryotic potassium channel KcsA in a lipid bilayer is presented. The selectivity filter is highly conserved in both bacterial and mammalian channels and chelates K^+ very specifically. The selectivity filter is known to exist in many different conformations depending on the identity and local concentration of the permeant ion. Transitions between these different conformations have not been quantitatively characterized in a native bilayer environment. We have used 2D and 3D heteronuclear correlation spectra to site-specifically assign residues in full-length KcsA reconstituted into a lipid bilayer. We report two distinct conformations of the selectivity filter of KcsA in the presence of K^+ and Na^+. We report significant changes in the chemical shifts of key residues in the filter as the permeant ion is changed from K^+ to Na^+. Chemical shift analyses using the SPARTA database indicate that the observed conformations are consistent with a K^+-bound and a Na^+-bound state. Solid-state NMR characterization of both the K^+ and the Na^+ bound state is important for ensuing studies of channel dynamics, for which, these conformations can be considered limiting structures. Simultaneous detection of both conformers at low ambient K^+ suggests that the K^+ and the Na^+ bound states are in slow exchange on the NMR timescale ($<500\ s^{-1}$).