

molecules studied were the nucleotides, NAD and NADH (1.2nm), and synthetic anions, tetraglutamate (T-Glu, 1nm) and 1-Hydroxypyrene-3,6,8-trisulfonate (HPTS, 0.8nm). VDAC channels were reconstituted into planar phospholipid membranes bathed in 1.0 M NaCl (pH 8.0). NAD, NADH and other nucleotides decreased the conductance of VDAC for NaCl demonstrating that they could permeate into the channel. In contrast, T-Glu and HPTS did not change the single channel conductance indicating that VDAC is not permeable to these molecules. Reversal potential measurements support this interpretation by finding almost ideal selectivity of Na<sup>+</sup> over T-Glu. Both NAD and NADH increased single channel noise as they penetrated into the channel while T-Glu had no effect. HPTS increased noise but unlike NADH this was not voltage-dependent when HPTS was added asymmetrically indicating no penetration into the channel. The differences in effective size and charge cannot explain the difference in permeation characteristics. Thus VDAC must select among these based on shape and charge distribution.

### 1008-Pos Board # B55

#### WHAT DO WE REALLY KNOW ABOUT IONIC DEHYDRATION ENERGIES?

Peter C. Jordan; Brandeis University, P.O. Box 549110, Waltham, Massachusetts 02454-9110

Simulating ion interaction with channel proteins and the determination of permeation free energies (or enthalpies) requires force field parameters describing ionic interaction with various solvating groups. A well-tuned ion-water force field is a critical starting point for such work. These fields are commonly tested by comparison with absolute ionic dehydration energies; however, these energies are not thermodynamically measurable, but must be estimated on the basis of extra-thermodynamic assumptions. Recent work (Tissandier et al. *J. Phys. Chem. A*, 102:9308 [1998]) suggests that the absolute free energy (enthalpy) for the standard hydrogen electrode may have been underestimated by as much as 20 (50) kJ mol<sup>-1</sup>. This would increase all monovalent cation dehydration free energies by 20 kJ mol<sup>-1</sup> and decrease the corresponding quantity for monovalent anions by the same amount. The energy shifts would be twice as large for divalent ions. A number of experimental and theoretical approaches to determining absolute dehydration energies are contrasted. Some estimates of the effects that these uncertainties have on force field parameters are provided.

### 1009-Pos Board # B56

#### A STUDY OF MUTATIONS OF OMPF PORIN USING POISSON-NERNST-PLANCK THEORY

Trudy A van der Straaten<sup>1</sup>, John M Tang<sup>2</sup>, Robert S Eisenberg<sup>2</sup>, Umberto Ravaioli<sup>1</sup>, Narayan Aluru<sup>1</sup>, Sameer Varma<sup>1</sup>, Eric Jakobsson<sup>1</sup>; <sup>1</sup>University of Illinois, 405 N Mathews, Urbana, Illinois 61801, <sup>2</sup>Rush Medical Center

Ion (K<sup>+</sup> and Cl<sup>-</sup>) permeation through the trimer *ompF* and its mutant *G119D* has been studied using a three-dimensional (3-D) Poisson-Nernst-Planck (PNP) model. The solution of Poisson's equation over the entire domain provides a simple way to include external boundary conditions and image force effects at dielectric discontinuities. Macroscopic current flow is calculated from a continuity equation for each ion species, describing flux down an electrochemical gradient. Water, protein and membrane are treated as uniform background media with specific dielectric constants, and an appropriate diffusivity is assigned to each ionic species. Fixed charges from the OPLS force-field, with modifications to account for the titration state of the ionizable residues, were used to construct a 3-D charge distribution for each trimer. Using diffusivity ( $D = D_{K^+} = D_{Cl^-}$ ) as a single adjustable parameter the current-voltage (I-V) curves, selectivities and ion occupancies have been predicted for both channels for a range of salt concentrations. In accordance with measurement the model predicts a reduced conductance and enhanced cation selectivity for *G119D*. Recent simulations suggest that the lower conductance is largely a result

of a narrowing of the pore constriction, rather than electrostatic effects, although further work is needed to confirm this.

### 1010-Pos Board # B57

#### DYNAMIC IN-SITU CHANGES IN INTRACELLULAR Ca<sup>2+</sup> MEASURED BY CONFOCAL LASER-SCANNING MICROSCOPY IN HUMAN FIBROBLAST CELLS - THE EFFECTS OF MINERAL WATER COMPOSITE

Byunghee Yoo, Jongwon Shim, Sanghoon Han, Ihsup Chang; Pacific R&D Center/Pacific Co. Ltd., 314-1 Bora-ri Kiheung-eup, Yongin-si Kyunggi-do Korea, 449-729 Korea, Republic of

Confocal laser scanning microscopy (CLSM) is widely used to monitor intracellular calcium levels in living cells loaded with calcium-sensitive fluorophores. There are many research results reported that calcium signalling plays a central role in cell in the mediation of cell functions. Especially we are interested in the human fibroblast cells for the understanding of skin physiology. The Ca<sup>2+</sup> concentration in the human fibroblast cells has been evaluated utilizing the dye Fluo-4 and measuring directly the fluorescent response by CLSM equipped with micro perfusion pump. For the experiment, nano-clustered mineral water solutions are prepared, the mineral water solutions are used as an extracellular solution and the intracellular levels of Ca<sup>2+</sup> ions are quantitatively measured. The external mineral solutions effects on the amounts of ions transferred not on the transfer rate, and that the magnesium ions may play a key role in the Ca<sup>2+</sup> transfer in case of the human fibroblast cells. The water cluster sizes in the mineral solutions were estimated by the half height width from the 17 oxygen NMR. We think that there may be a certain kinds of relationship between water cluster size and the ion transfer mechanism.

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### 1010.1-Pos Board # B58

#### RATE THEORY FROM BROWNIAN DYNAMICS: ANALYSIS OF A SIMULATED ION CHANNEL

Matthew Hoyles; Australian National University, The Australian National University, Canberra ACT, 0200 Australia

Reaction rate theory (RRT) has been applied with success to ion channels, and in particular has provided intuitive explanations for the operation of multi-ion channels. This is despite doubts about whether the physical assumptions of RRT are valid in ion channels, and so whether the energy profiles deduced from it are useful and accurate. In practice RRT is used by fitting an abstract model of states and transition probabilities to the conductance data; the energy profile is then deduced from this model. It seems likely that a mechanism of states and transitions does occur in multi-ion channels, but from different physical causes than those which underlie RRT. To test this hypothesis, and to examine how such a mechanism might arise, we have performed Brownian dynamics simulations of a model KCSA channel, and analysed the results for evidence of states and transitions. We also construct free energy profiles of transition events in the channel, based on the electrostatic model used in the simulations, and compare these profiles with the rates of transition.

### 1011-Pos Board # B59

#### ON-THE-FLY SIMULATIONS OF ION CURRENT IN REALISTIC MODELS OF ION CHANNELS: THE KcsA POTASSIUM CHANNEL

Anton Burykin<sup>1</sup>, Claudia N. Schutz<sup>1</sup>, Jordi Villà<sup>2</sup>, Arieh Warshel<sup>1</sup>; <sup>1</sup>USC, <sup>2</sup>IMIM/UPF, Spain

A general method for simulation of ion currents in realistic models of biological channels is developed. This method provides a fast and reliable estimate of the energetics of the system by combining semimicroscopic calculations of the self-energy of each ion and an implicit treatment of the interactions between the ions as well as