

agreement with x-ray measurements of lipid membrane interactions in the presence of buffers and monovalent salt solutions. [1] Parsegian V. A., Van der Waals Forces, *Cambridge University Press*, 2006, [2] Koerner et al., *Biophys. J.* 101, 2011, [3] Szymanski and Petracek, *J. Chem. Phys.* 143, 2011.

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Origins of Non-Selective Ion Transport across Lipid Bilayers

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The theoretical description of charge transport across biological membranes has remained largely unchanged since the 1960's. However, it is widely accepted that the barriers for unassisted ion permeation via a solubility-diffusion mechanism are too high, and too selective to explain experimental observations, leading to a favoring of a non-selective transient pore process. We demonstrate that an ion-induced defect mechanism, intermediate between these two processes, can yield reduced free energy barriers with little influence of hydration free energy or the membrane dipole potential. We report experimental and computational data for a range of alkali metal, halide and charged amino acid analog molecules. Our simulations reveal that membrane perturbations are central in explaining the shape and magnitude of the free energy barriers, the similarity for ions of different size, charge and chemistry, and for obtaining fluxes consistent with experiment. We will discuss results that suggest a transition from ion-induced defect to the solubility-diffusion mechanism in thicker bilayers, and for larger hydrophobic ions and ionophores. We explore the deformable membrane description to predict a greater common energetics for a range of ions, poly-ions and zwitterions and discuss the consequences for membrane interactions with charged peptides and proteins, and the roles of lipid components in membrane ion transport.

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Ion Hydration Stripping Kinetics Support Thyrofluidic Ion Channel Gating

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The thyrofluidic model of ion channel entry gating has the membrane electric field at the ion channel pore entrance responsible for stripping ion hydration shells, selectively admitting ions into the pore for transport. Capillary electrophoresis studies have shown that full hydration stripping occurs at field strengths corresponding to distances of 6.5 to 8 nanometers from a membrane. This work considers the rate at which hydration stripping occurs relative to the rate of ion migration near the membrane.

The free energy barrier for one water molecule to escape the first (inner) hydration shell of a Na⁺ ion has been calculated to be 9.5 kJ/mole, equivalent to a field strength of 118.75 V/cm. Mean lifetime of attachment of one water molecule bound to a Na⁺ ion has been calculated to be 5 ps. A linear model of the energy and time to strip 5 of the 6 water molecules from the first shell yields 47.5 kJ/mol (594 V/cm) and 25 ps. Modeling single molecule dissociation to have exponential decay yields 101.7 kJ/mol (1,270 V/cm) and 197 ps. Electric field dependent ion velocity changes occur between 200 and 600 V/cm, consistent with the linear model.

The Einstein-Smoluchowski equation shows ions with radius 0.1 nm diffuse at 3.2 nm/ns. As a result, Na⁺ will diffuse 0.08 nm (linear) or 0.63 nm (exponential) after reaching a field of 594 or 1,270 V/cm, respectively, for complete hydration stripping, the latter occurring 7 nm from the membrane. To date, all known kosmotropic ion channel pore entrances sit within 2 nm of the membrane. Thus, the membrane electric field will strip the hydration shells from Na⁺ long before the ion can reach the pore entrance. As such, electric field hydration stripping kinetics are compatible with thyrofluidic channel gating.

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Hydrophobization of Glass Pipettes for use in Tip-Dip Experiments

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Tip-dip electrophysiological experiments are used to study single-channel recordings and channel kinetics. Although an effective technique, tip-dip, there is an argument that ion flow could be due not only to ion channels on the membrane but also to transport through the glass-lipid interface, and even through the very thin glass tip. Another problem with this technique arises from the difficulty to obtain seals with large resistance that are stable and last for long periods of time. In this study we present an experimental protocol which has the purpose of reducing the above described problems. Borosilicate glass pipettes are coated with poly(dimethylsiloxane)(PDMS) for various periods of time.

PDMS is absorbed on the surface of the glass and later on, via a chemical reaction, coats the surface, rendering it hydrophobic. This hydrophobic surfaces yield better seals in tip-dip electrophysiological experiments. Here we compare seals made with and without the PDMS treatment and discuss their differences. We also considered the effect that variables in the protocol, such as time of exposure to PDMS or temperature of treatment, have on the hydrophobization process. Hence we present an experimental protocol of pipette hydrophobization that improves seal resistance in tip-dip electrophysiological experiments decreasing the problems faced by this technique.

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Modulation of Conductance and Ion Selectivity of OmpF Porin by La³⁺ Ions

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The effect of lanthanum, a high-valence rare-earth metal, known as blocker and modulator of Ca²⁺, K⁺, TRP and other channels, has been studied in a large, weakly selective biological pore, the bacterial porin OmpF. We show that millimolar concentrations of lanthanum chloride have a dramatic impact on OmpF conductance and selectivity when this channel is reconstituted into planar lipid bilayers. Lanthanum arises as a modulator of ion flow through OmpF channel, reducing the conductance for positive but not for negative applied voltages, thus inducing ion current rectification. In addition, small amounts of LaCl₃ change the selectivity of the pore from highly cationic to almost non-selective and even reverse it, turning OmpF into an anion-selective pore. The conductance inhibition for positive voltages is reversible and it is observed only when LaCl₃ is added on the same side of the protein addition. We also found that the channel rectification properties can be easily modulated by regulating the pH of the bathing solutions and that in high acidity solutions the current rectification can be totally cancelled.

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Biophysics of Polynucleotide Interactions with OmpF Nanopore Forming Protein, Possible Tool for DNA Sequencing

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OmpF ion channel porin is a water filled trimer found in the outer membrane of *Escherichia coli*. The crystal structure of channel shows constriction zone with a diameter of about 0.6 nm halfway down the nanopore barrel. In this study, single molecule OmpF protein was reconstituted into artificial bilayer and its channel activity was investigated by means of voltage clamp technique. The results showed that the presence of polynucleotides caused different pattern of ion current and gating in the channel at different potential differences and polarities. It seems due to the effect of the applied pd on the amino acids side chain in the channel lumen, the potential gradient and its direction play vital role in producing unique signature for certain polynucleotides. The current pattern could not directly be related to each strand by single factor analysis and different approaches and variants should be applied simultaneously to distinguish polynucleotides from each other. Although we restricted our studies to 10 nucleotide strands, results indicate that it can well be applied to longer ones. Furthermore, it was found that introduced polyT, polyG, polyC to the cis side at micro molar range have more effect than lower ones. Further to the investigation of polynucleotide effect with nano channel, we hope to use the result of the current study to address gene trans-location into bacteria.

Ligand-gated Channels II

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Analysis of Oligomer Assembly for the GluA2 Amino Terminal Domain

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For GluA2, an ionotropic glutamate receptor that mediates excitatory synaptic transmission at CNS synapses, the reported monomer-dimer K_d of the amino-terminal domain (ATD) varies widely, ranging from 1.8 nM to 4 μM. 1-3 To investigate causes of discrepancies in the literature, several protein constructs, diverse in glycosylation and overall size, were created and the dimer K_d analyzed by analytical ultracentrifugation with absorbance and interference optics (AUC). Sedimentation velocity (SV) K_ds varied from 2 to 11 nM, with size and glycosylation having little effect. At the highest protein concentration examined (33 μM) we did not detect formation of tetramers or larger oligomer species. Steady state fluorescence anisotropy titrations for DyLight405 labelled