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Nanoelectropulse-induced changes in cell excitability - a molecular mechanism for membrane depolarization

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14. ABSTRACT

Molecular dynamics (MD) simulations of ion transport through lipid electropores support the hypothesis that a 5 ns electric pulse enables transport of sodium ions sufficient to depolarize an adrenal chromaffin cell. To carry out this work we converted our model systems from the old GROMOS-OPLS force fields to the newer CHARMM36 force fields and implemented changes in the ion models to improve interactions with water and with the phospholipid bilayer interface). We also initiated an investigation of the effect of transmembrane peptides on lipid bilayer systems in applied electric fields, to determine whether these peptides modify the probability of conductive defect formation.

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Abstract Molecular dynamics (MD) simulations of ion transport through lipid electropores provide theoretical support for the hypothesis that delivery of a single, 5 ns electric pulse can enable pore-mediated transport of sodium ions sufficient to depolarize the plasma membrane of an adrenal chromaffin cell, helping to explain a series of robust experimental observations. To carry out this work we converted our model systems from the old standard GROMOS-OPLS force fields to the newer and more accurate CHARMM36 force fields. For our specific studies we implemented also changes in the native ion models to improve the behavior of the calcium ion in its interactions with water (and indirectly with the atoms of the phospholipid bilayer interface). In parallel with this effort we are working with colleagues in Buenos Aires to improve the CHARMM36 Na^+ model that they are using in our joint study of ion transport through mechanically (rather than electrically) constrained lipid pores. In another parallel effort, sparked by the pursuit of the primary objectives, we have undertaken an exploratory investigation of the effect of transmembrane peptides on lipid bilayer systems in applied electric fields, to answer the questions whether these peptides increase or decrease the likelihood of conductive defect formation, and whether they increase or decrease ion conductance after permeabilizing defects are formed. This will help us understand better the role of ion channels and other membrane proteins in membrane water defect formation, and how electrical stress leads to depolarization of adrenal chromaffin cells (and other electrically active cells) and thus to activation of voltage-gated calcium channels.

Background

Nanosecond pulsed electric fields (nanosecond electric pulses – NEPs) show undimmed promise as a new modality for neuromodulation that provides short duration (low risk of damage) and precise targeting of specific cells and tissues. The goal of this project is to provide fundamental and practical information that will guide future in vitro and in vivo investigations aimed directly at using NEPs for neuromodulation. This project is associated with and carried out in collaboration with Gale Craviso, Normand Leblanc, and Indira Chatterjee at the University of Nevada, Reno under AFOSR grant FA9550-14-1-0018. The overall project employs both experimental (electrophysiology and fluorescence imaging) and computational (two-dimensional electrical modeling (numerical) of the cell and molecular dynamics simulations) approaches to achieve our goal.

My effort centered on molecular dynamics (MD) simulations of ion transport through liquid electropores with the aim of reconciling experimentally observed ion flux with rates extrapolated from the model characteristics of individual pores and pore areal density.

Objectives

1. To project from electropore kinetics and dynamics the physical (density and area) and temporal extent of membrane depolarization. We compared what is known experimentally regarding ion transport during normal depolarization and activation with the pore formation and ion transport rates that we observe in simulations to determine whether the molecular model of the lipid nanopore is consistent with the hypothesis that a single 5 ns pulse is sufficient to depolarize an adrenal chromaffin cell membrane as a result of ion permeabilization. Further, from pore formation and re-sealing times extracted from our simulations we proposed to evaluat support for our hypothesis that NEPs induce transient membrane depolarizations that can be amplified in amplitude and time by a train of NEPs.

2. To characterize lipid nanopore conductance for Na^+, K^+, Ca^{2+}, and Cl^-. Through a series of simulations, we investigated specific ion effects on bilayer and lipid nanopore physical properties, to evaluate the importance of the ion models in predicting accurately the different behaviors of Na^+, K^+, Ca^{2+}, and Cl^- in pore transport rates and interface binding.

3. To evaluate the effects of increasing the complexity of the homogeneous phospholipid bilayer systems used in previous studies. Looking beyond the general effects of including the three major univalent inorganic ions and the divalent cation calcium in lipid bilayer systems, we planned to assess the impact of various modulating factors — phospholipid composition, cholesterol, local ionic strength, temperature, intracellular and extracellular membrane physical constraints (cytoskeletal and glycocalyx-type attachments) — on specific ion conductances and bilayer physical properties. By moving our model toward a more realistic representation of the membrane, we increase its predictive value.

Accomplishments

1. Initial correlations of ion transport for individual lipid electropores with experimentally observed activation of adrenal chromaffin cells. We previously reported that a combination of our initial MD simulations of ion transport through lipid electropores with the numerical modeling results of the Reno group provides ***theoretical support for the hypothesis that delivery of a single, 5 ns pulse can enable pore-mediated transport of Na^+ sufficient to depolarize the plasma membrane of an adrenal chromaffin cell***. A more systematic and extensive set of simulations of Na^+, K^+, Ca^{2+}, and Cl^- transport through lipid electropores, with improved ion models for Na^+ and Ca^{2+}, is consistent with that initial conclusion. We have, furthermore, accumulated results with systems containing the more challenging Na^+ and Ca^{2+} ions that underscore the ***importance of the accuracy of ion models*** in their interactions with water and with the phospholipid head group interface. This is ongoing.

2. Conversion from GROMOS-OPLS to CHARMM36 force field. At the outset we determined that this project necessitated a ***conversion of the molecular models in our simulations from the widely used, but ageing, GROMOS-OPLS force fields to the newer, more accurate CHARMM36 force fields***. This is essential for maintaining the continuity of our observations into the future [1]. To confirm the validity of these changes, we compared systems constructed with the old and new force fields, using several key indices of the correspondence of the behavior of model lipid bilayer systems with experimental observations. These include area per lipid, deuterium order parameter (for atoms in the hydrocarbon tails of the phospholipids), ion binding isotherms, minimum porating electric field, and pore initiation time. For a sample of one of these comparisons, see Figure 1.

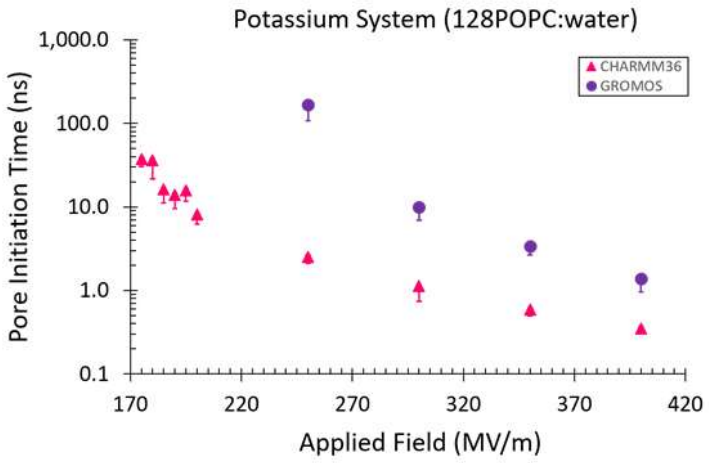

Figure 1. Pore initiation time as a function of applied electric field for GROMOS-OPLS and CHARMM36 systems containing potassium ions.

We are only now proceeding to simulate the impact of various modulating factors — phospholipid composition, cholesterol, local ionic strength, temperature, intracellular and extracellular membrane physical constraints (cytoskeletal and glycocalyx-type attachments) — on ion conductances (beginning with K^+, Ca^{2+}, and Cl^-) and bilayer physical properties.

Simulations for ion binding isotherms and lipid electropore formation require considerable computational resources (and clock hours) because of the long simulation times required for equilibration and the need for replicate simulations to generate adequate statistical confidence. This factor was not accurately estimated when project objectives were established.

This transition to the CHARMM36 force field is complete. It was delayed by unexpected problems with the native CHARMM36 calcium ion model, described below.

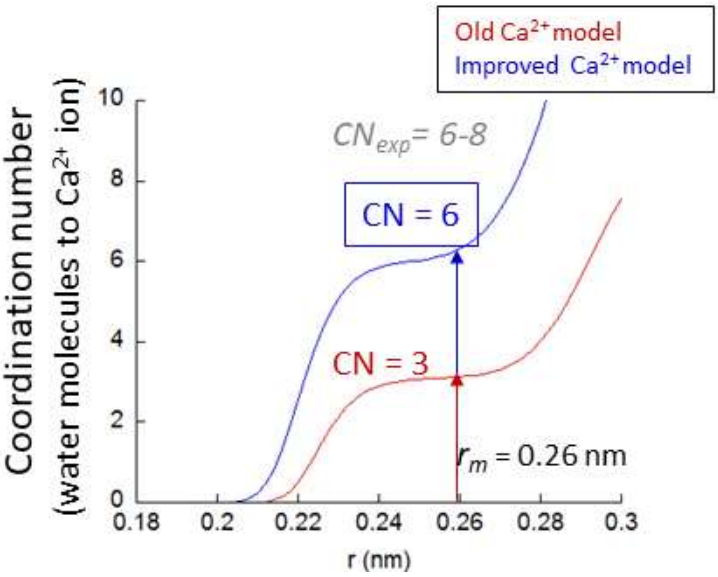

Figure 2. Calcium ion water coordination number for CHARMM36 systems containing either the ion model distributed with CHARMM36 (red line) or the modified ion model created in this work (blue line). Experimentally determined values are in the range 6 to 8.

3. Modification of CHARMM36 calcium ion model. Upon initial examination of CHARMM36 systems containing Ca^{2+}, we noticed unrealistic (and unacceptable) clustering of Ca^{2+} and Cl^- ions (i.e., undissociated $CaCl_2$ molecules) in the bulk water. We found that we were not the first to notice this deficiency in the CHARMM36 Ca^{2+} model, and we have followed an approach similar to [1] to ***improve the behavior of the calcium ion in its interactions with water (and indirectly with the atoms of the phospholipid bilayer interface)*** (Figure 2). This work is now complete, and a manuscript for publication is underway. Recently we learned that another group, leaders (like the authors of [1]) in molecular dynamics force field development, has addressed this problem in a different way [2]. None of these approaches is ***the*** correct one; we select the one most appropriate for our immediate investigations.

4. Sodium ion model (CHARMM36) and constrained pores. The CHARMM36 *sodium* ion model has deficiencies similar to those discussed above for Ca^{2+}. Although this was not part of our original plan (these problems have been reported only recently, and many workers in the field are just now responding – or are not even aware of them yet!), improving the behavior of these ion models is essential for producing reliable simulations of lipid membranes in electric fields. Our collaborators in Buenos Aires, who have been working with us for several years on ion conductance through lipid pores, have agreed to include ***improving the Na^+ model in their study of ion transport through mechanically constrained lipid pores*** (Figure 3). We will incorporate the relevant data from the Buenos Aires work, which P.I. Vernier is leading and coordinating under separate funding, into the K^+ and Ca^{2+} results that we are now generating in the Norfolk effort.

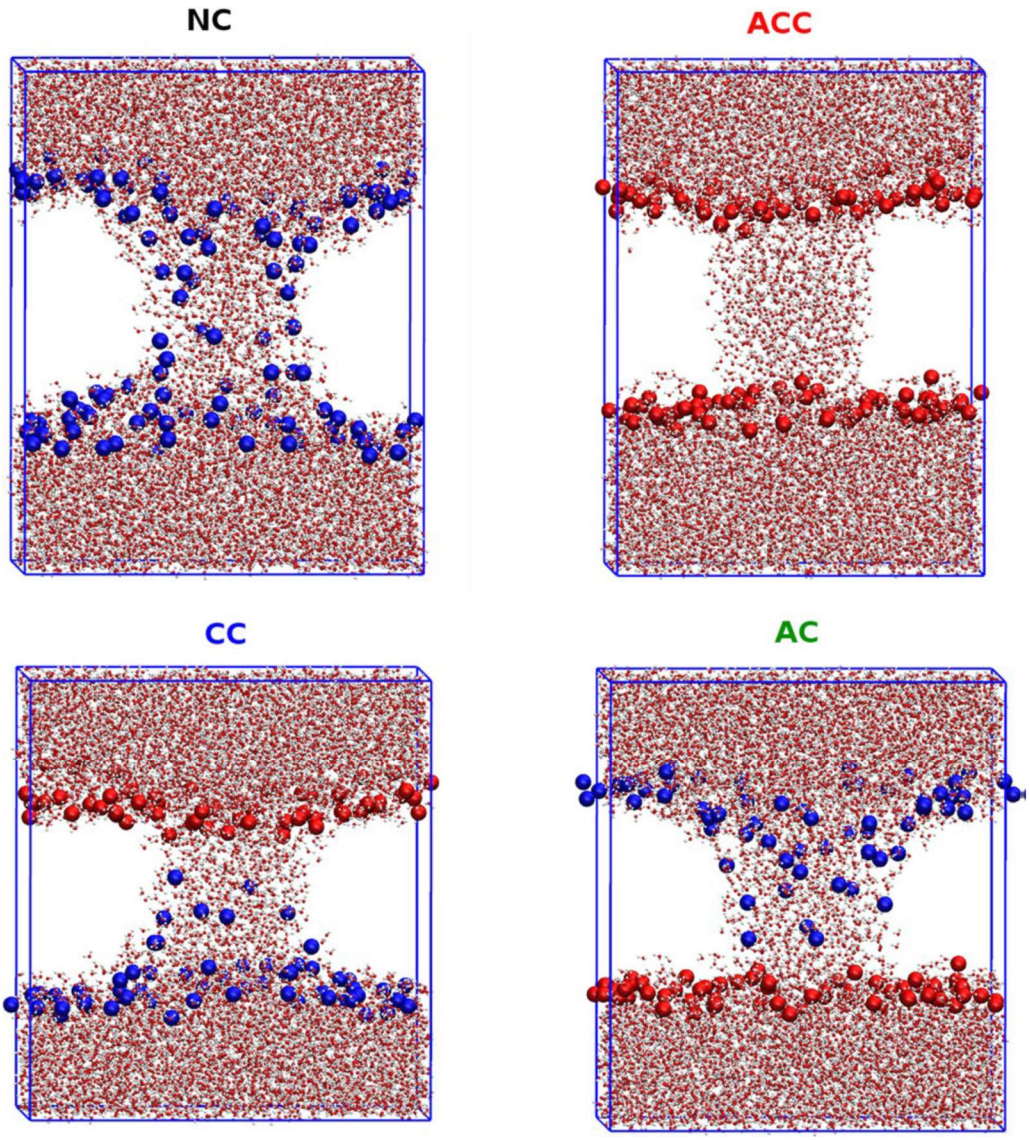

Figure 3. Mechanically constrained pores for ion transport studies [2]. NC – no constraint; ACC – anode and cathode sides constrained; CC – cathode side constrained; AC – anode side constrained.

5. Transmembrane peptides and the nanoelectroporation-driven nanoscale depolarization hypothesis. To expand our perspective of cellular responses to stress-induced membrane permeabilization, we recently initiated an ***exploratory investigation of the effect of transmembrane peptides on lipid bilayer systems in applied electric fields***. We want to know whether these peptides increase or decrease the likelihood of conductive defect formation, and whether they increase or decrease ion conductance after permeabilizing defects are formed. Answers to these questions will help us understand how electrical stress leads to depolarization of adrenal chromaffin cells (and other electrically active cells) and thus to activation of voltage-gated calcium channels. We are specifically interested in the hypothesis that ion channel gating may be a nanoscale phenomenon, at least under some conditions, that requires transport of only a few ions in the immediate vicinity of the ion channel, perhaps through a process we call nanoelectroporation-driven nanoscale depolarization. Initial results from these new studies (Figure 4) indicate the (not surprising) importance of specific amino acid residues in the bilayer interior and the (perhaps surprising) tendency of some transmembrane peptides to inhibit water defect formation in the immediate vicinity and of others to facilitate nearby water bridge construction.

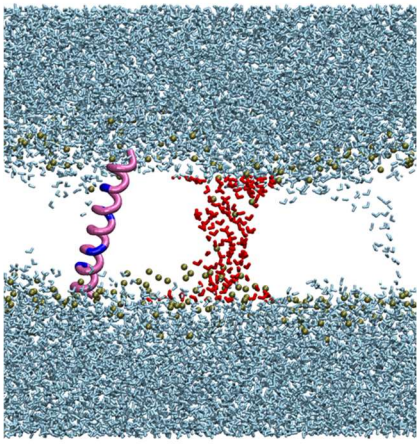

Figure 4. Lipid bilayer (POPC) with a transmembrane peptide, a lipid pore, and a water bridge.

Collaborations

As mentioned above, we actively collaborated with Gale Craviso, Normand Leblanc, and Indira Chatterjee at the University of Nevada, Reno on the research reported here. We maintained regular e-mail and telephone contact, and I traveled to Reno at least once per year to discuss various research issues, including pulse delivery and the interconnection of molecular modeling and experimental observations of ion transport through electropermeabilized membranes. In addition, I am working with M. Laura Fernández and Marcelo Risk at the University of Buenos Aires in Argentina specifically on mechanically constrained lipid pores and on optimization of sodium ion models on molecular simulations.

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