C3 Molecular dynamics simulations of membrane electroporation

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Duration of the experiments: 90 min
Max. number of participants: 18
Location: Computer room
Level: Basic

PREREQUISITES

No specific knowledge is required for this laboratory practice.

The aim of this laboratory practice is to get familiar with the tools for molecular dynamics, possibilities to set on models and graphical presentation of atomistic models.

THEORETICAL BACKGROUND

The application of strong electric fields to cells or tissues permeabilizes the cell membrane and produces aqueous-filled pores in the lipid bilayer (along with other chemical and structural changes of the membrane) [1]. Electroporation is witnessed when the lipid membrane is subject to transmembrane voltage (TMV) of the order of few hundred millivolts, which is induced during exposure to an electrical pulse. Such TMV is sufficient to produce an electric field within the membrane of the order of ~ 10^8 V/m. The electroporation process is believed to involve (1) charging of the membrane due to ion flow, (2) rearrangement of the molecular structure of the membrane, (3) formation of pores, which perforate the membrane and are filled by water molecules (so-called aqueous, or hydrophilic, pores), (4) an increase in ionic and molecular transport through these pores, and, under appropriate conditions, membrane integrity recovery when the external field stress is removed [2,3].

Molecular Dynamics (**MD**) simulations belong to a set of computational methods in which the dynamical behavior of an ensemble of atoms or molecules, interacting via approximations of physical pair potentials, is determined from the resolution of the equation of motions [4]. MD simulations enable one to investigate the molecular processes affecting the atomic level organization of membranes when these are submitted to voltage of magnitude similar to those applied during electropulsation [5,6]. In MD simulations, two methods can be used to increase TMV: the "external electric field method" where each atom with charge q_i is added a force $F_{e,i} = q_i \cdot E$, and the "charge imbalance method" where an excess of positive (and negative) ions is created on the two sides of the membrane mimicking capacitive charging. Both methods are equivalent to simulate electroporation [7].

EXPERIMENT

Due to the limited time and large resources needed to generate MD trajectories of membranes, the latter will be provided to the students. The simulations concern pure planar phospholipid bilayers (membrane constituents) and water described at the atomic level. A set of long trajectories spanning a few nanoseconds generated with or without a TMV induced by unbalanced ionic concentrations in the extracellular and intracellular side will be provided. The students will (1) determine the distribution of the electric potential and electric field in model membrane bilayers (2) measure the membrane capacitance, (3) visualize at the molecular level the formation of membrane pores under the influence of a TMV, and (4) measure the intrinsic conductance of such pores.

REFERENCES:

[1] Kotnik T., Rems L., Tarek M., Miklavčič D. Membrane electroporation and electropermeabilization: Mechanisms and models. *Annu Rev Biophys,* 48:63-91, 2019.

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[4] Hollingsworth S.A., Dror R.O. Molecular dynamics simulation for all. *Neuron* 99:1129-1143, 2018.

[5] Rems L., Tang X., Zhao F., Pérez-Conesa S., Testa I., Delemotte L. Identification of electroporation sites in the complex lipid organization of the plasma membrane. *eLife* 11: e74773, 2022.

[6] Kasparyan G., Hub J.S. Molecular simulations reveal the free energy landscape and transition state of membrane electroporation. *Phys Rev Lett,* 132:148401, 2024.

[7] Kasparyan G., Hub J.S. Equivalence of charge imbalance and external electric fields during free energy calculations of membrane electroporation. *J Chem Theory Comput,* 19:2676–2683, 2023.

EXPECTED RESULTS

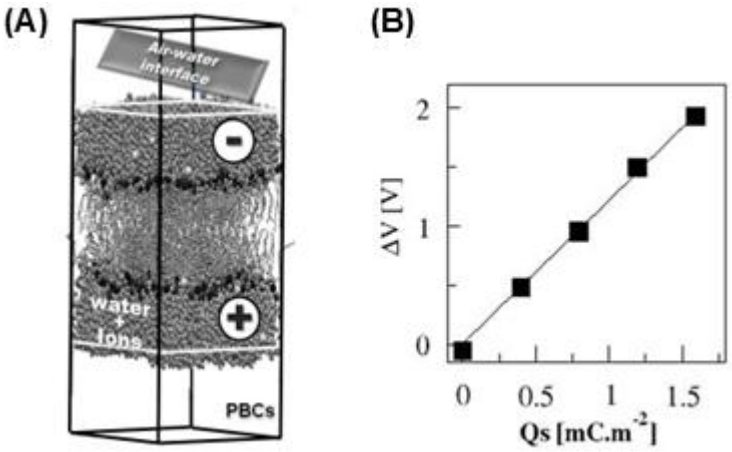

Figure 1: (A) POPC lipid bilayer prepared for MD simulation using the charge imbalance method. The bilayer is surrounded by water baths containing NaCl. The water baths are separated by a vacuum layer that prevents ions from escaping from one to the other water bath across periodic boundary conditions (PBCs). Charge imbalance Q is created by moving a certain number of Na^+ ions from upper to the lower bath. (B) The graph shows the voltage measured from simulations plotted as function of the charge imbalance. The linear behavior of the transmembrane voltage shows that the membrane behaves as a capacitor with a capacitance C = Qs/V, where Qs is the charge imbalance per unit area.

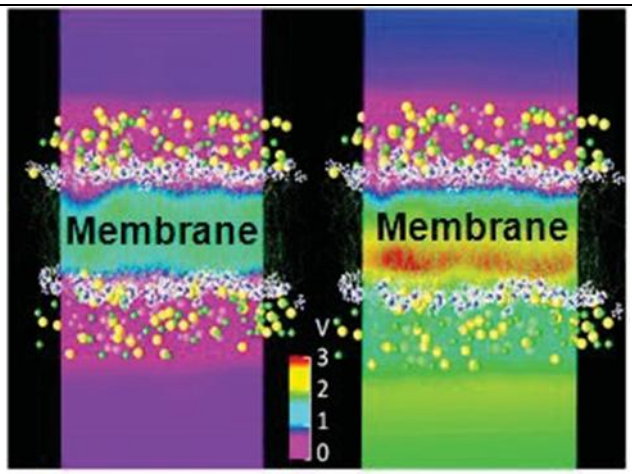

Figure 2: Electrostatic potential maps generated from the MD simulations of a POPC lipid bilayer (acyl chains, green; head groups, white) surrounded by electrolyte baths at 1 M NaCl (Na+ yellow, Cl- green, water not shown) terminated by an air/water interface. Left: net charge imbalance $Q = 0$ e (TMV=0 mV). Right: $Q = 6$ e (TMV=2 V), where e presents the elementary charge.

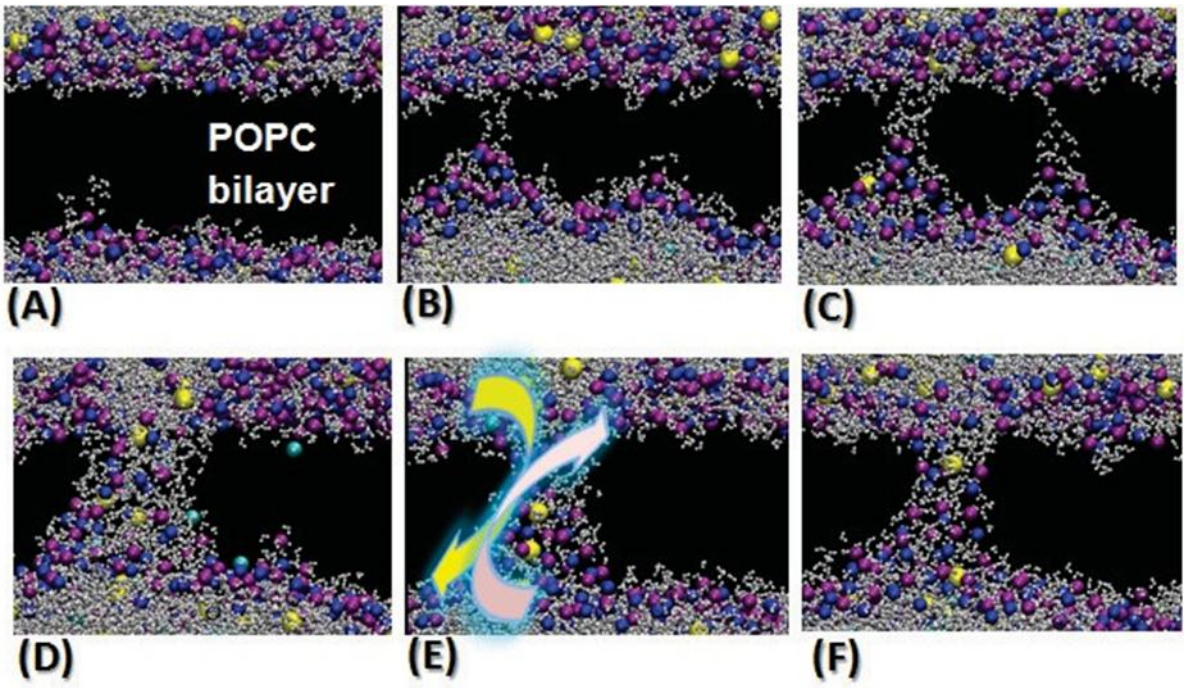

Figure 3: MD simulation of pore formation in a POPC bilayer subject to sufficiently high TMV. (A) Bilayer at equilibrium. (B-C) Formation of water wires at the initial stage of the electroporation process. (D-F) Formation at a later stage of large water pores that conduct ions (yellow and cyan spheres) across the membrane and that are stabilized by lipid head-group (magenta and blue spheres) [3].

NOTES & RESULTS