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A molecular insight into the electro-transfer of small molecules through electropores driven by electric fields☆

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ABSTRACT

The transport of chemical compounds across the plasma membrane into the cell is relevant for several biological and medical applications. One of the most efficient techniques to enhance this uptake is reversible electroporation. Nevertheless, the detailed molecular mechanism of transport of chemical species (dyes, drugs, genetic materials, …) following the application of electric pulses is not yet fully elucidated. In the past decade, molecular dynamics (MD) simulations have been conducted to model the effect of pulsed electric fields on membranes, describing several aspects of this phenomenon. Here, we first present a comprehensive review of the results obtained so far modeling the electroporation of lipid membranes, then we extend these findings to study the electrotransfer across lipid bilayers subject to microsecond pulsed electric fields of Tat_{11}, a small hydrophilic charged peptide, and of siRNA. We use in particular a MD simulation protocol that allows to characterize the transport of charged species through stable pores. Unexpectedly, our results show that for an electroporated bilayer subject to transmembrane voltages in the order of 500 mV, *i.e.* consistent with experimental conditions, both Tat_{11} and siRNA can translocate through nanoelectropores within tens of ns. We discuss these results in comparison to experiments in order to rationalize the mechanism of drug uptake by cells. This article is part of a Special Issue entitled: Biosimulations edited by Ilpo Vattulainen and Tomasz Róg.

1. Introduction

In the past decades, improvements have been achieved in developing new techniques to allow for a targeted and safe delivery of compounds to cells and tissues. Many biological and medical applications, indeed, require a local uptake of molecules to be effective. A very successful approach to enhance the permeability of cells and tissues to otherwise poorly or non-permeant species is the application of external pulsed electric fields, a process known as electroporation (EP) [1–3]. In standard EP, pulse length lies in the μs- and ms-time scale with amplitudes on the order of kV/cm; we therefore term the technique μs-msPEF (pulsed electric field). The application of such pulses to cells results in an increase of their transmembrane (TM) voltage. When the latter exceeds a specific threshold, an intense local electric field is generated at the membrane level leading to pore formation [4]. This process induces a rise in the permeability of the plasma cell membrane not only to water and ions [5], but also to charged and uncharged molecules of a wide range of sizes [6,7]. Indirect experimental evidence [8–11] suggests the presence of small, short-lived pores for which today's experimental tools are unable to provide direct observation. In the past years it was further shown that the application of ultra short (ns) intense (tens of kV/cm) electric fields (nsPEFs) induces effects similar to classical EP. This has opened new prospective of cell electro-manipulation thanks to the ability of such pulses to electroporate internal organelles as well [12–14].

Over the last thirty years, thanks to its convenience (*i.e.* ease of procedure, relatively low cost, and fast) and safety (only few side-effects have been reported [15]), μs-msPEF remains the principle non-viral method for the insertion of various poorly permeable molecules, such as dyes, disaccharides, vitamins, anticancer drugs, proteins, and genes [16], into cells and tissues. The resulting biomedical applications span from calcium electroporation [17], electrochemotherapy (ECT) [18,19], DNA vaccination [20,21] and gene regulation [22,23].

In spite of the wide use of this technique, the exact mechanisms involved at the molecular level of cell and tissue uptake are not fully apprehended. Because understanding transport dynamics in-depth is essential for its optimization, *in silico* characterization by molecular dynamics (MD) simulations, provides a powerful tool to investigate such mechanisms with atomic resolution. Thus far, two MD protocols have been proposed to mimic the effect of either μs-msPEFs or nsPEFs on planar bilayers. The response of the system to electric fields is indeed

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due to two effects: dielectric and ionic. The dielectric effect is rather fast (only a few picoseconds) and is linked to the direct polarization of the interfacial water, whereas the ionic effect is much slower and relates to membrane charging. Because the dielectric response is negligible compared to the ionic one when applying longer low intensity pulses, the effect of μs-msPEFs might be reproduced by imposing a net charge imbalance (ΔQ) across the bilayer [24–27] (Fig. 1 C). On the contrary, nsPEFs are modeled by subjecting a lipid bilayer (with no ions) to an electric field (E_{app}) perpendicular to the membrane; *i.e.* to a force proportional to the E_{app} acting on each partial charge in the system [28–30] (Fig. 1 B). Note that, because of the absence of ions, only the dielectric (fast response) effects are therefore considered. The main drawback of both currently used protocols, is the creation of unstable pores: In the electric field method, pores tend to expand beyond the dimensions of the simulation box, resulting in the rupture of the bilayer, whereas in the charge imbalance method, the TM voltage drops below a certain value once the ions have passed through the pore, resulting in pore collapse. Improvements to these protocols were proposed to stabilize the electropores and study their properties. The first one [31,32] to control pores formed with the nsPEF procedure, the second one [33] based on the method proposed in [34] to tune the μs-msPEF approach (see below for details).

It has been shown that regardless of the method used, charge imbalance or electric field, lipid bilayers electroporation takes place within identical time scales (namely few nanoseconds), provided that the TM voltages produced are the same [27,35]. While both nsPEFs and μs-msPEFs increase membrane permeability, it is not known however whether they lead to similar transport mechanisms. To answer this question, the transport of different molecules through electroporated membranes should be investigated with MD techniques using both of the proposed protocols.

However, until now, only two MD studies have been reported on the transport of molecules, specifically under nsPEFs. In 2012 Breton et al. [36] demonstrated, coupling experimental and *in silico* observations, that a single 10 ns high-voltage electric pulse can permeabilize lipid vesicles and allow the delivery of siRNA to the cytoplasm by electrophoretic drift. More recently, Salomone et al. [37] showed the possibility to use a combination of nsPEFs and a chimeric peptide (CM_{18}-Tat_{11}) as efficient transfection for pDNA administration. Insight onto the molecular mechanism of such synergic effect was possible only thanks to evidence provided by MD simulations. Following this line of research, we here extend our previous MD investigations on the translocation of Tat_{11} and siRNA to clarify some up-to-now neglected aspects of μs-msPEFs driven transport.

For completeness and bearing in mind the scope of this special issue, we start with a comprehensive review of the MD studies on membrane electroporation. The aforementioned covers over a decade of research, across numerous laboratories and has contributed substantially to our understanding of the EP phenomena at the molecular level.

2. Modeling membrane EP

As biological membranes have a very complex structure, including hundreds of different lipid species and proteins, most MD studies have focused their attention on simple lipid bilayers in their biologically relevant liquid-crystalline state. From both a theoretical and experimental perspective, zwitterionic phosphatidylcholine (PC) lipid bilayers constitute the best characterized systems [38–40] (Fig. 1). Despite their

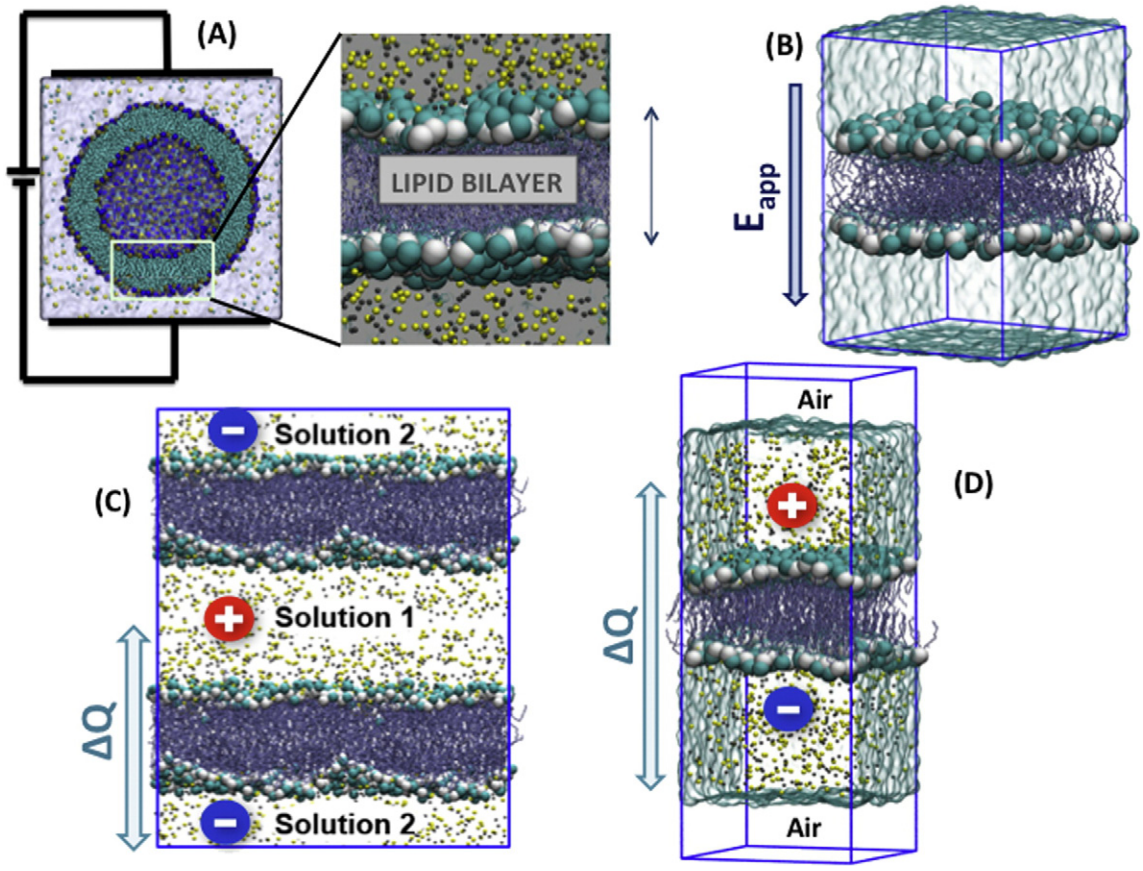

Fig. 1. Protocols for atomistic modeling of cell membranes or liposomes lipid bilayers (A) electroporation; (B) nsPEF protocol: the system is modeled in the absence of salt, and subject to an electric field E_{app} perpendicular to the bilayer (z axis); note that in some studies ions were also considered; (C) μs-msPEF protocol introduced in the double bilayer setup: A charge imbalance ΔQ is set across each bilayer and the scheme is implemented using classical 3d-PBCs. To prevent ions from migrating through the periodic boundary conditions, the simulation box (in blue) is extended in the direction perpendicular to the bilayer (z axis) to create a vacuum slab in the air/water interface protocol (D).