A Numerical Model of Electroporation in Bacteria

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Abstract

Electroporation is a biotechnological technique which subjects cells to strong (~kV/cm), short (~ms) electric field pulses that open pores on the cells' plasma membranes. Under the appropriate conditions, the pores mediate the passage of exogenous material into cells. If the pores reseal, the electroporated cell can survive, and the process is termed reversible electroporation. Reversible electroporation is commonly used to deliver molecules such as drugs, proteins, or DNA into cells, but the governing physical mechanism remains poorly understood. Electroporation has already been used to genetically engineer cells (e.g. bacteria) to enhance their native capabilities, allow them to perform non-natural functions, and/or enable new applications in biotechnology such as production of alternative fuels (Savage et al. 2008), enhancing oil recovery (Banat et al. 2010), and even cancer treatment (Anderson et al. 2006). However, protocols for performing DNA insertion via electroporation are often developed by a trial-and-error process that is costly and lacks real-time feedback. This limitation is due in part to the lack of fundamental understanding of the factors that lead to successful DNA insertion into a cell.

In an effort to gain insight into the factors that lead to successful DNA transfer into bacteria via electroporation ("gene electrotransformation"), here we conduct numerical simulations in COMSOL Multiphysics® software to study the effect of various experimental conditions on the response of bacterial cells to electroporation. The simulations output the pore size, quantity, and spatial distribution for a given set of electroporation conditions, e.g. electric pulse strength, shape, and duration; extracellular medium conductivity; cell shape; and cell electrical properties.

This model allows one to determine advantageous conditions for DNA insertion into any given bacterium. In addition, we use the model to test the hypothesis that a bacterium's amenability to electroporation depends on how easily it becomes polarized in an external electric field. Recent work has shown that the properties of the bacterial cell wall and soft polyelectrolyte layer can significantly affect bacterial polarizability (Dingari & Buie 2014). For example, the polarizability is particularly sensitive to pH-dependent dissociation of ionogenic groups in the soft layer. We study numerically the correlation between a bacterium's cell envelope properties and its capacity to undergo electroporation, which has not been previously considered. For a wide range of experimental conditions, we calculate both the cell's polarizability (quantified by the induced electrical dipole moment in an external electric field) and its likelihood of successful DNA electrotransformation (quantified by the number of pores with sufficient size and longevity to

permit the passage of DNA molecules). The results indicate that the success of a DNA insertion experiment depends strongly on the electrokinetic properties of the cell under consideration, as well as the medium in which it is immersed. These results will inform the design of experiments intended to insert DNA into bacteria for which electroporation-induced gene transformation had previously been thought impossible.

Reference

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Figures used in the abstract

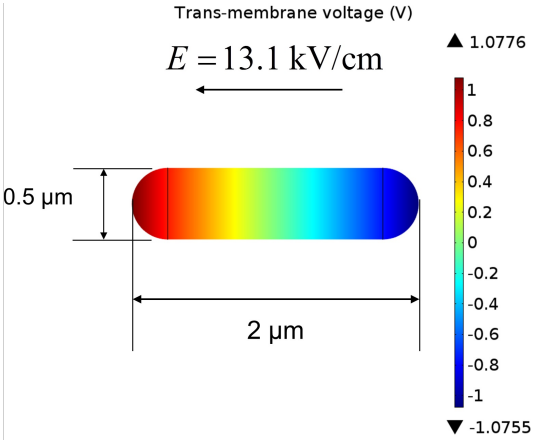

Figure 1: Induced trans-membrane voltage (TMV) distribution on the membrane of a simulated Escherichia coli bacterium, which is aligned with an external electric field with magnitude 13.1 kV/cm. The TMV is the primary controlling parameter for pore formation: the majority of pores form in regions of the membrane where the TMV exceeds 1 V.

Figure 2

Figure 3

Figure 4