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Review

Membrane pore formation in atomistic and coarse-grained simulations☆

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ABSTRACT

Biological cells and their organelles are protected by ultra thin membranes. These membranes accomplish a broad variety of important tasks like separating the cell content from the outer environment, they are the site for cell–cell interactions and many enzymatic reactions, and control the in- and efflux of metabolites.
For certain physiological functions e.g. in the fusion of membranes and also in a number of biotechnological applications like gene transfection the membrane integrity needs to be compromised to allow for instance for the exchange of polar molecules across the membrane barrier. Mechanisms enabling the transport of molecules across the membrane involve membrane proteins that form specific pores or act as transporters, but also so-called lipid pores induced by external fields, stress, or peptides.
Recent progress in the simulation field enabled to closely mimic pore formation as supposed to occur *in vivo* or *in vitro*. Here, we review different simulation-based approaches in the study of membrane pores with a focus on lipid pore properties such as their size and energetics, poration mechanisms based on the application of external fields, charge imbalances, or surface tension, and on pores that are induced by small molecules, peptides, and lipids. This article is part of a Special Issue entitled: Biosimulations edited by Ilpo Vattulainen and Tomasz Róg.

Contents

1. Introduction

Biological cells are the basic building blocks of skin, tissues, and other materials. Cells and their organelles are enveloped by thin membranes that separate their chemical contents from the extracellular environment. Biological membranes are supramolecular assemblies

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composed of a lipid double layer with embedded and adsorbed membrane proteins. In case of plasma membranes of eukaryotic cells this largely impenetrable central layer is stabilized by the inner cytoskeleton, and is surrounded by a carbohydrate glycocalyx at the outside. Apart from proteins, the most abundant constituent of the central layer are amphipathic phospholipids, sphingolipids, and steroids [1]; typically 20 – 50% of all lipids in plasma membranes are cholesterol molecules. Each monolayer of the membrane consists of billions of adjacent lipid molecules, which are composed of typically two fatty acyl chains (hydrophobic tails) and a hydrophilic headgroup. The two monolayers taken together, facing each other with the hydrophobic tails, serve as a barrier of 3 – 5 nm thickness, which exhibits a partial permeability to some small hydrophobic and polar molecules [2].

The lipid membrane further acts as a barrier for ions and thus enables the generation of ion composition gradients and of electrochemical gradients across the membrane by means of both ion transporters and the asymmetrical lipid distribution between the inner and the outer layer. The transmembrane potential that is maintained by the membrane is crucial e.g. for the signal transduction along nerve cells, and so is a transmembrane pH gradient essential for the ATP synthesis by the ATP synthase. However, it is known that ions may also leak through the membrane, a possible mechanism being diffusion through transiently formed water pores. These short-lived pores were discovered to develop spontaneously due to differences in ionic charges across the membrane [2–4].

Transport e.g. of metabolites is enabled by membrane-embedded channels and transporters, whose functions are partially controlled by the lipid composition. A number of transmembrane proteins, including e.g. ion channels, aquaporines, or sugar-specific channels in the outer membrane of bacteria, are involved in membrane transport. In certain cases, for instance during exocytosis, proteins promote the rearrangement of lipids to allow for the exchange of molecules between the cell interior and the outer environment [5]. The formation of a small hydrophilic pore, the so called fusion pore, as a result of lipid rearrangements is an important and inherent step during fusion [6]. But transport across the plasma membrane does not solely occur with the aid of proteins. For instance, the transient formation of hydrophilic lipid pores leads to the passive transport of water molecules, and to a lesser extent also of ions, or other hydrophilic substances across the hydrophobic core of the bilayer. Failure in the control of molecule transport typically results in cell death.

The flexibility of the membrane enables the cell to resist mechanical stress to a certain level. Nevertheless, if the force acting on the cell membrane is too strong, the membrane is prone to pore formation and rupture, i.e. pores will develop in case of high lateral tension. Consequently, membrane tension also allows to stabilize pores, which were pre-induced e.g. by external electric fields, such that pores can be kept open for several seconds [7]. The mechano-sensitivity of a cell can be investigated experimentally e.g. in aspiration experiments that allow to study membrane properties such as the membrane elasticity.

In both medicine and biotechnology exists a tremendous interest in methods that allow to transiently interrupt the membrane integrity. A transient cell opening enables e.g. the transfer of genes, drugs, antibodies, dyes, or oligonucleotides into the cell and thereby permits to trigger processes by modifying the cell interior. Thus, methods enabling the controlled increase in cell permeability to allow for the insertion of chemical compounds into the cell represent an attractive strategy and find widespread application. A large number of techniques were developed during the last decades to successfully increase the membrane permeability.

The probably most established technique is based on the application of (pulsed) external electric fields and is referred to as electroporation or electropermeabilization [8]. The application of appropriate electric pulses that vary in strength and duration permits to induce small hydrophilic pores into the membrane or tissue, and thus allows for the transient transfer of substances into the interior of the cell [9] (see Fig. 1

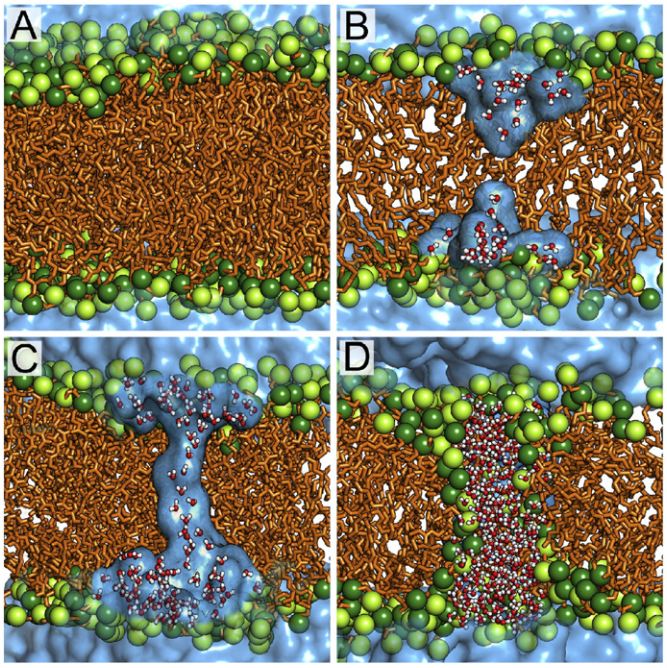

Fig. 1. Pore formation process induced by the ion charge imbalance method. *A.* An intact phospholipid bilayer before pore formation is shown. *B.* In the first step of electroporation, water molecules intrude from both sides of the membrane. Lipid tails adjacent to the pore are hidden for clarity. *C.* Formation of a water wire with hydrophobic lipid tails lining the pore wall. *D.* Expansion to a larger pore (diameter ≈1 nm) with lipids rearranged in order to stabilize the pore. Water is given in surface representation (blue), as well as in stick representation for the case of penetrating water with hydrogens and oxygens colored in white and red, respectively. Lipid tails are colored orange, phosphorus atoms of lipid headgroups dark green and nitrogen atoms light green. Structures courtesy of Kristyna Pluhackova.

for the steps involved in the formation of hydrophilic pores by electric fields). This involves in particular the efficient uptake of genetic material into selected cells [10]. In this manner, it was already feasible in the early 80s to introduce DNA into mouse lyoma cells [11]. The method has also successfully been developed and applied in the treatment of cancer in electrogenetherapy [12] or in electrochemotherapy [13–15], where the selective transfer of cytotoxic substances locally led to cell death. In addition, the destabilization of membranes by external electric fields is used to induce fusion of vesicles or cells [16]. Since pore formation is reversible under certain conditions, it was also used to porate the membrane of human erythrocytes, followed by modification of the intracellular content and membrane resealing, consequently resulting in intact red blood cells [17,18].

A different way to cure infected tissue is the treatment of these tissue cells with short peptides: Antimicrobial peptides are typically cationic and amphipathic. These small peptides are an inherent part of the innate immune system of most living organisms [19–21]. With their ability to act against pathogens in a cell-lytic manner, they exhibit an effective defense against fungi, viruses, yeasts and bacteria [19,22]. The adsorption of antimicrobial peptides onto the membrane is – under specific conditions, e.g. certain peptide concentration – thought to be followed by membrane pore formation, consequently leading to cell death [22,23]. Over the past few years, interest in small antimicrobial peptides increased with the growth of resistance against conventional antibiotics.

Membrane permeabilization has been a topic since the 70s in experiments. However, the molecular mechanisms of pore formation, by (external) electric fields, small molecules, peptides or lipids, are difficult to address in experiments for several reasons: First, the typical size of a lipid pore is in the range of a few nanometers. Next, it is formed on very short timescales ranging from nanoseconds to microseconds, and finally it is in many cases a metastable state; depending on the