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# FACTORS INFLUENCING MEMBRANE PORE FORMATION: CHEMICAL REACTIONS, ELECTRIC FIELDS, AND TEMPERATURE EFFECTS

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**Annotatsiya** Biologik membranalar hujayra o‘zaro ta’sirini boshqaradi va tanlangan o‘tkazuvchanligini ta’minlaydi. O‘tish kanallari hosil bo‘lishi kimyoviy reaksiyalar, elektr maydonlari va temperatura o‘zgarishlari ta’sirida yuzaga keladi. Ushbu jarayonlarning mexanizmlarini tushunish biotexnologiya va tibbiyotda muhim ahamiyatga ega. Maqolada membrana teshiklarining hosil bo‘lishiga ta’sir etuvchi omillar haqida ma’lumot beriladi.

**Kalit so‘zlar:** Membrana o‘tish kanali, kimyoviy reaksiyalar, elektroporatsiya

**Аннотация.** Биологические мембраны регулируют клеточные взаимодействия и избирательную проницаемость. Образование пор происходит под воздействием химических реакций, электрических полей и изменений температуры. Понимание этих процессов имеет важное значение для биотехнологии и медицины. В статье рассматриваются факторы, влияющие на образование пор в мембране.

**Ключевые слова:** Образование пор, химические реакции, электропорация

**Abstract.** Biological membranes regulate cellular interactions and selective permeability. Pore formation occurs due to chemical reactions, electric fields, and



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temperature changes. Understanding these processes is crucial for biotechnology and medicine. The article discusses the factors influencing membrane pore formation.

**Keywords:** Membrane pore formation, chemical reactions, electroporation

## INTRODUCTION

The cell membrane, a critical structure that separates the internal cellular environment from the external milieu, is composed of a lipid bilayer embedded with proteins, serving as a selective barrier to maintain cellular integrity and regulate the transport of ions, molecules, and nutrients. Despite its structural stability, the formation of pores within the membrane, a process known as "membrane pore formation," is a fundamental and dynamic event that plays a pivotal role in various biological and biophysical processes [1]. Membrane pore formation is essential for numerous physiological functions, including nutrient uptake, waste removal, signal transduction, and cellular communication. It is a finely regulated process that can be triggered by various stimuli, such as chemical, mechanical, electrical, and thermal factors. Pores can form spontaneously or in response to external forces, and their size, duration, and functionality are crucial for determining the fate of the cell. The formation of transient or permanent pores can significantly alter the membrane's permeability, enabling the passage of ions, small molecules, or even larger biomolecules, depending on the pore's characteristics [2]. In biological systems, membrane pores are integral to several critical processes. For instance, the mechanism of electroporation, a technique widely used in molecular biology, relies on the temporary formation of pores to introduce foreign DNA, RNA, or other molecules into cells. This method has found applications in gene therapy, drug delivery, and cancer treatment, demonstrating the practical significance of understanding pore formation. Moreover, the controlled formation of pores is essential for the functioning of ion channels and receptors, which regulate cellular signaling and homeostasis. On the other hand, unregulated or excessive pore formation can be detrimental to cells, leading to membrane disruption, loss of cellular contents, and eventual cell death [3]. Such events are often associated with pathological conditions, including necrosis and apoptosis, where the breakdown of membrane integrity plays a central role. Therefore, understanding the factors that induce and control pore formation is of great importance, not only for fundamental cell biology but also for the development of medical and biotechnological applications.

## CHEMICAL REACTIONS IN MEMBRANE PORE FORMATION

Chemical reactions play a pivotal role in the structural and functional modulation of biological membranes, particularly influencing the formation of



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pores [4]. Among the numerous chemical processes that impact membrane dynamics, nitration, oxidation, and interactions with reactive oxygen and nitrogen species (RONS) are of primary significance. These reactions can alter the physicochemical properties of membrane lipids and proteins, thereby facilitating or impeding pore formation. This section delves into the molecular mechanisms by which these chemical reactions contribute to the initiation and stabilization of membrane pores. Nitration is a process where a nitro group (-$NO_2$) is introduced into the membrane's lipid or protein molecules, usually resulting from the interaction of reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$) with the lipid bilayer. This modification predominantly affects the tyrosine residues of membrane proteins and the unsaturated fatty acid chains of phospholipids. The nitration of these components induces several structural changes that directly influence pore formation [5].

The incorporation of nitro groups into fatty acid chains disrupts the tightly packed arrangement of the lipids, resulting in increased membrane fluidity. This disruption weakens the cohesive forces between lipid molecules, making the bilayer more susceptible to deformation and pore formation. The presence of nitro groups also increases the hydrophilicity of the membrane surface, which can facilitate the aggregation of water molecules and the stabilization of hydrophilic pores. Nitration can lead to conformational changes in membrane-associated proteins, affecting their interaction with lipids and the overall membrane stability. These disruptions can serve as nucleation sites for pore formation, especially when external forces such as electric fields or mechanical stress are applied [6].

Oxidation is another crucial chemical process that significantly affects membrane integrity. It involves the interaction of reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals (•OH) with membrane lipids and proteins. The oxidation of these membrane components can lead to structural modifications that favor pore formation. This process generates lipid hydroperoxides and aldehydes, which disrupt the regular packing of lipid molecules, thereby increasing membrane permeability [7].

## RONS AND THEIR ROLE IN PORE FORMATION

RONS encompass a broad category of highly reactive molecules, including superoxide, hydrogen peroxide, hydroxyl radicals, nitric oxide ($NO^{\bullet}$), and peroxynitrite. These species are produced as by-products of cellular metabolism and can accumulate under stress conditions, such as exposure to toxins, pathogens, or external stimuli like electric fields [8]. RONS can oxidize cholesterol molecules, leading to the formation of oxysterols, which disrupt the regular arrangement of lipids. This disruption reduces membrane stability and increases the likelihood of



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pore formation, particularly in regions with high cholesterol content. RONS can induce oxidative stress within the cell, which overwhelms the cell's antioxidant defense mechanisms. This stress results in widespread damage to membrane lipids, proteins, and even nucleic acids, leading to the formation of multiple pores. In this state, the membrane's selective permeability is lost, and ions, small molecules, and even larger entities can traverse the membrane uncontrollably [9].

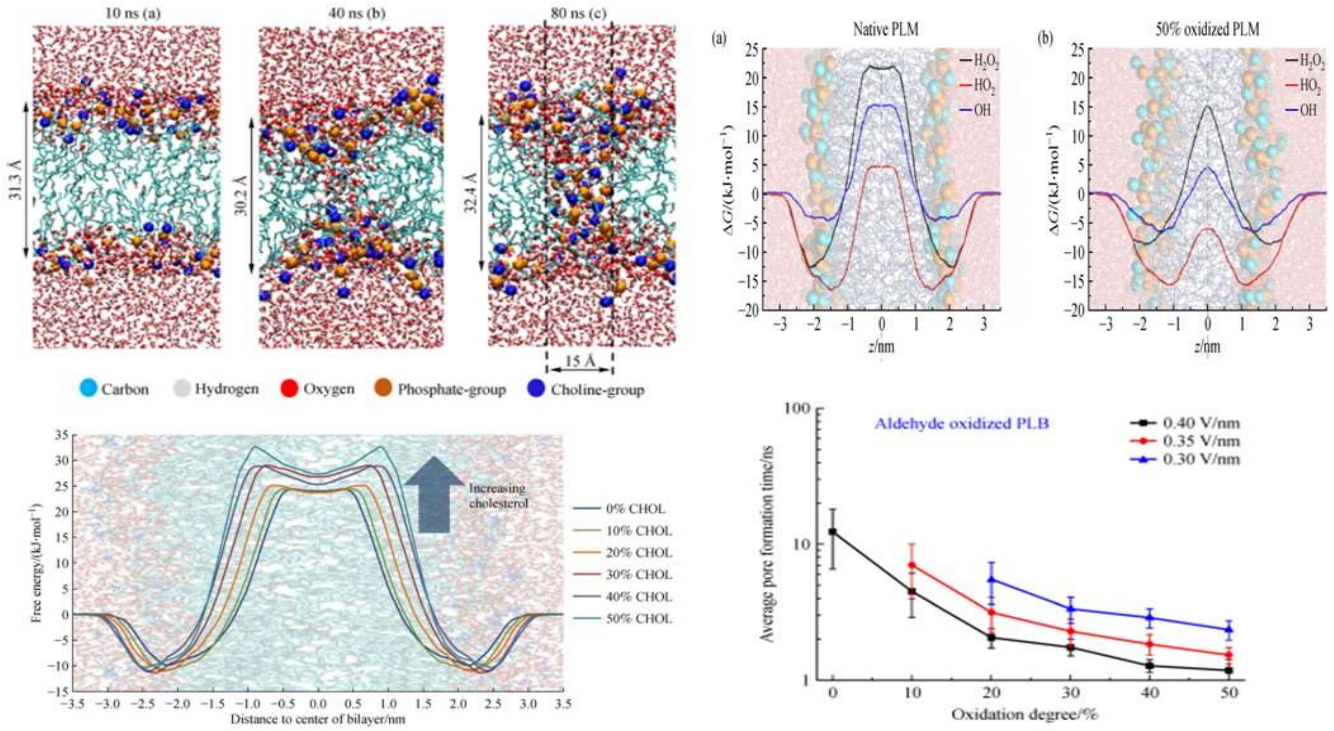



***Figure 1. Snapshot of MD simulations, at (a) 10 ns, (b) 40 ns and (c) 80 ns. Free energy profiles (FEP) of the hydrophilic (a,b) and hydrophobic (c,d) ROS and RNS. FEPs of H2O2 (upper part) for various cholesterol concentrations in the cell membrane. Average pore formation time for three different electric field values, as a function of the oxidation degree of the PLB, forlipid oxidation into aldehydes. Reprinted/adapted with permission from Ref. [10]. 2019, The Authors.***

## EFFECT OF ELECTRIC FIELD ON MEMBRANE PORE FORMATION

The application of an electric field to biological membranes, a process known as electroporation, has been extensively studied as a means of inducing pore formation. The electric field exerts a force on charged molecules and ions within and around the membrane, resulting in the reorganization of the lipid bilayer structure and the creation of transient or permanent pores. This phenomenon has significant implications for biotechnological applications, such as gene therapy, drug delivery, and cell fusion, making it a crucial area of study. Electroporation is the process by which an external electric field disrupts the integrity of the cell membrane, causing the formation of pores that can vary in size and duration. When



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a cell is exposed to an electric field, the field induces a transmembrane potential across the lipid bilayer. If the applied electric field exceeds a critical threshold, the resulting potential difference across the membrane causes the lipid molecules to rearrange, leading to the creation of hydrophilic pores. Formation of Transmembrane Potential Under the influence of an electric field, the distribution of charges within the membrane changes, resulting in polarization. This polarization generates a transmembrane potential (ΔV), which is proportional to the strength of the applied electric field (E). As ΔV increases, the membrane's structural integrity weakens, and the probability of pore formation rises [11].

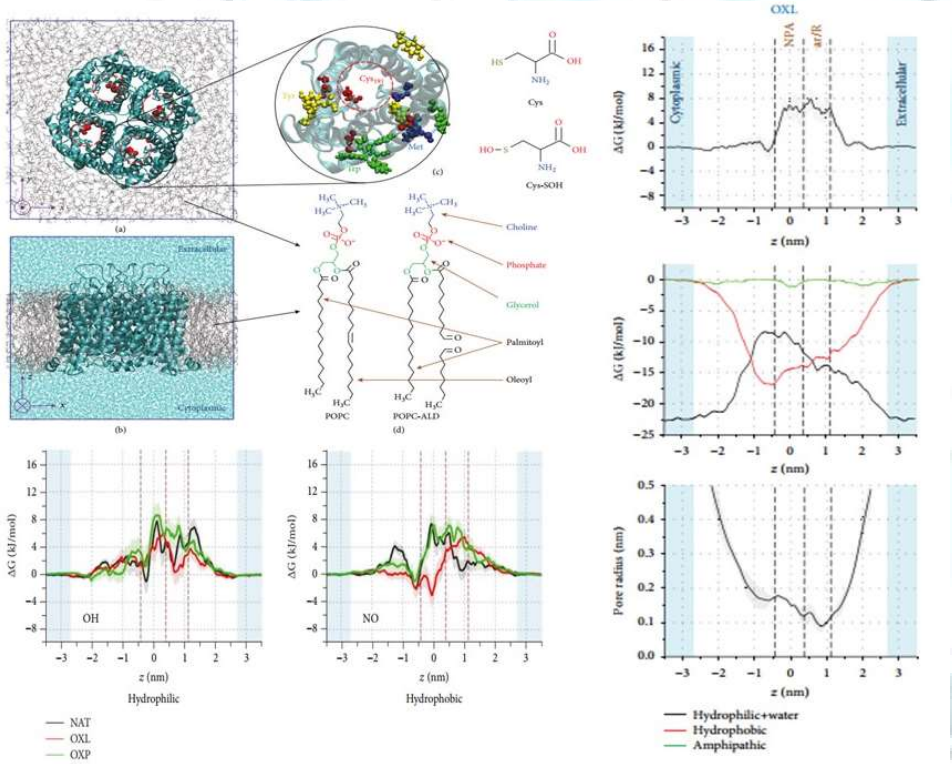



***Figure 2. Top and side view of the native aquaporin model system. Average FEPs of the hydrophilic (H2O2 and OH) and hydrophobic (NO2 and NO) RONS across AQP1 pores of the native (NAT, black curve) and oxidized (OXL and OXP, red and green curves) model* systems. *Reprinted/adapted with permission from Ref.[12]. 2019, The Authors.***

The electric field induces a force that compresses the lipid bilayer, reducing the energy barrier required for pore formation. This electrocompression causes the membrane to become thinner, allowing water molecules and charged ions to penetrate the bilayer. This leads to the formation of hydrophilic pores that can expand as the electric field strength increases. Once the initial pores are formed, they can expand under the continued influence of the electric field. The electric field attracts ions and water molecules into the pores, which stabilizes their structure. If the field strength remains above the critical threshold for an extended



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period, these pores may become permanent, leading to irreversible damage to the membrane. For instance, the formation of pores can lead to the influx of calcium ions (Ca2+), triggering signaling pathways that influence cellular functions such as apoptosis, proliferation, or differentiation. Understanding these effects is crucial for optimizing electroporation techniques in therapeutic and research settings [13].

The effect of an electric field on membrane pore formation is a complex process influenced by the field strength, membrane composition, and pulse parameters. Electroporation provides a powerful tool for controlling membrane permeability, offering numerous applications in medicine and biotechnology. By manipulating the electric field parameters, researchers can induce pore formation in a controlled manner, enabling the targeted delivery of therapeutic agents or genetic material into cells [14].

## EFFECT OF TEMPERATURE ON MEMBRANE PORE FORMATION

Temperature is a critical factor that influences the structural and functional properties of biological membranes. Changes in temperature can significantly alter the fluidity, flexibility, and permeability of the lipid bilayer, thereby affecting the process of pore formation. Understanding the role of temperature in this context is essential for deciphering how cellular membranes respond to environmental changes and stressors. When the temperature rises, the kinetic energy of lipid molecules within the membrane increases. This heightened energy results in enhanced molecular motion, which affects the packing and organization of the lipid bilayer [6]. As temperature increases, the lipid molecules transition from a more ordered, gel-like state to a disordered, liquid-crystalline state. This increased fluidity makes the membrane more pliable and reduces the energy barrier required for pore formation. The lipid tails experience greater freedom of movement, allowing them to rearrange more readily, which facilitates the initiation and expansion of pores. Higher temperatures lead to more frequent thermal fluctuations in the membrane's structure, creating transient defects. These defects act as nucleation sites where pores can form, allowing water molecules and ions to penetrate the bilayer. As the temperature continues to rise, these pores can stabilize and expand, making the membrane increasingly permeable. Elevated temperatures can also affect the conformation and mobility of membrane proteins, which may interact with lipid molecules and further promote pore formation. Proteins may undergo conformational changes that destabilize the surrounding lipid matrix, creating localized disruptions that contribute to pore initiation [3].

At low temperatures, lipid molecules exhibit reduced kinetic energy and adopt a more ordered, tightly packed configuration. The membrane transitions into a gel-like state, characterized by decreased fluidity and rigidity. This rigidity makes



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it more difficult for lipids to reorganize, thereby increasing the energy barrier required for pore formation. The absence of sufficient thermal energy at lower temperatures means fewer defects and disruptions in the lipid bilayer. As a result, the membrane maintains its integrity, and the likelihood of spontaneous pore formation is significantly reduced. Organisms living in colder environments or exposed to cold stress have adapted their membrane compositions to maintain fluidity, often incorporating unsaturated fatty acids that prevent membrane solidification. This adaptation helps preserve membrane permeability and functionality, even at low temperatures. Temperature plays a dual role in regulating membrane pore formation. High temperatures increase membrane fluidity, facilitating pore initiation and expansion, while low temperatures stabilize the membrane, hindering pore formation. Understanding these dynamics is crucial for applications in biotechnology and medicine, where temperature-controlled processes can optimize electroporation, cryopreservation, and thermal therapy [15].

## CONCLUSION

Membrane pore formation is a complex process influenced by various chemical, physical, and environmental factors. Chemical reactions, such as nitration and oxidation, alter the fluidity and stability of membrane lipids and proteins, facilitating pore formation. The application of an electric field enhances this process through electroporation, dependent on the field's strength and duration. Temperature also plays a crucial role, with higher temperatures promoting pore formation due to increased fluidity, while lower temperatures stabilize the membrane structure. Understanding these mechanisms is essential for applications in drug delivery, gene therapy, and other biomedical fields, allowing researchers to develop techniques for manipulating membrane permeability effectively.

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