Chapter 3

The effect of Sodium and Chloride Ions on the stability of transmembrane pores
3.1 Introduction

3.1.1 Passive ion permeation

The presence of ions is crucial for the function and stability of all biological membranes. It is also well known that under the appropriate conditions ions permeate the membrane wall. In biological systems channels and transporters mediate the electrophysiological characteristics of membranes by selectively allowing ions to permeate through the hydrophobic lipid matrix. However, there is strong evidence to suggest that the trans-membrane potential is also influenced by the passive permeability of ions especially in the biological membranes of electrically active tissues [1]. Although the electrical properties of lipid bilayers have been studied extensively, the exact mechanism of passive ion translocation is not yet understood. It has been previously suggested that water pores, which appear in the membrane as meta-stable defects, can provide a pathway for the translocation of ions [2, 3, 4]. In this way ions may maintain their hydration shell while at the same time diffuse rapidly through the hydrophobic region of the interior of the membrane [5]. The passive transport of ionic species through lipid bilayers could also help explain one issue concerning the origin of cellular life [6]. In the early stages of life biological systems were necessarily much simpler than those found today. Nevertheless, proto-cells would still require the transport of ions and other small polar molecules across membranes and this would need to occur in the absence of any specific carriers or channels. The mechanism of passive ion permeation depends on the ability of the membrane to form and stabilize water pores of a certain radius and life time.

The formation and evolution of pores in model lipid bilayer systems has been investigated by various techniques such as electroporation [7], pipette aspiration [8] and osmotic swelling [9]. The pores that form are typically small and transient. A theoretical model that has been proposed to try to explain their behavior is based on the equation:

\[ E(r) = 2\pi\gamma r - \pi r^3\Gamma \]  (3.1)

Here \( E \) is the energy of a trans-membrane water pore in a lipid bilayer, \( r \) is the radius of the pore, \( \gamma \) is the pore line tension and \( \Gamma \) the surface tension of the membrane.
Information about the theory of transient water pores has already been given in section 2.1.3 of the thesis. The stability of these water pores, which reflects also the stability of the lipid bilayer, is influenced by various parameters. It has been found, for example, that it is possible to stabilize water pores by applying a small surface tension [10]. Another important factor that affects the stability of such pores is the absorption of ionic species at the membrane water interface.

**Ion and lipid interactions**

The binding of ions to lipid bilayers has been the focus of a number of previous experimental and theoretical studies. It is generally believed that ions penetrate deep into the surface of the membrane and alter its dynamical and structural properties [11, 12]. There is evidence that ion binding induces a more rigid packing of the acyl chains compared to a pure membrane and stabilizes the gel state of lipid bilayers. Furthermore, it is possible that strong ion-lipid interactions induce partial dehydration and immobilization of the polar lipid head-groups. Therefore, the composition and concentration of the electrolyte should be an important parameter for the stability of a membrane. In micropipette aspiration experiments, the critical tension needed to rupture membrane vesicles, has been measured [13]. Indeed, increasing the ion concentration or the affinity of the ions to the lipids increases the critical tension of the membrane thus making the membrane more stable. Recently simulation studies have investigated lipid bilayer systems in an electrolyte solution containing sodium chloride ions [14, 15, 16]. It was found that sodium ions bind to the lipid molecules and penetrate deep into the head-group region of the bilayer. The binding of the ions was seen to induce changes in the structure and dynamics of the lipid bilayer in line with expectations.

To study the translocation of ions through trans-membrane water pores we have performed molecular dynamics simulations of a DPPC bilayer containing sodium chloride ions in a buffer with different ionic strength (0M, 0.1M, 0.2M and 0.6M). The influence of sodium chloride on the stability and structure of a pre-formed water pore was investigated. Furthermore the transport of the ions through the pore was analyzed and the permeation rates were estimated.
3.2 Methods

3.2.1 Simulation details

The model of the dipalmitoyl-phosphatidyl-choline (DPPC) bilayer used in this study was similar to that used in a previous study of the stability of transient water pores in a pure DPPC lipid bilayer [17, see also Chapter 2]. The water was described by the simple point charge model (SPC) [18]. The MD simulations were performed using the GROMACS package version 3.05 [19]. Periodic boundary conditions were applied and the temperature was coupled to 323K using a Berendsen thermostat [20]. The long range electrostatic interactions were evaluated using the PME method. In all simulations the pressure in the direction normal to that of the membrane was kept constant at 1bar [20]. For the simulations performed under surface tension, the pressure in the x and y plane was varied between -10bar to -50bar. In this way a tension of around 9mN/m-30mN/m was imposed on the surface of the bilayer. A 5fs time step was used [21]. Bond lengths were constrained using the LINCS algorithm [22]. The system consisted of 128 DPPC lipids and 5909-6009 water molecules. The simulations were performed starting from two different conformations. One was an equilibrated bilayer and the other was a bilayer with a pre-formed transient pore. Both conformations were simulated under a range of different surface tension conditions applied to the membrane and with different ionic strengths of the solution (Table 3.1). The ionic strength was varied by adding an appropriate number of sodium and chloride ions. This was achieved by randomly substituting water molecules with Na+ or Cl- ions. In total 20(0.1M), 40(0.2M) and 120(0.6M) sodium and chloride atoms have been inserted into the water phase.

In Chapter 2 it has been shown that hydrophilic pores in a pure DPPC lipid bilayer can be stabilized by the application of membrane tension that is in the order of 9-18mN/m. Tension increases slightly the size of the pores as well as increasing their life time. By applying membrane tension in the pure bilayer, the transient pores could be kept open for as long as 50 to 100ns of simulation time. These results were however, obtained from simulations in which a 1.4nm cutoff plus a reaction field correction was used to evaluate the long range electrostatic interactions [23]. To demonstrate that the current simulations would be consistent with the previous results, simulations of a pure DPPC bilayer were performed using the PME method to calculate the electrostatic interactions.
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and compared with the results obtained previously. No significant differences were observed. Using tensions in the range of 9-15mN/m pores remained open for as long as 50ns. To study the effect of salt on the stability of these transient pores, the water channels have been simulated under similar conditions to that described previously for a pure bilayer except for the use of PME and the inclusion of sodium chloride ions in the solutions.

In order to estimate the size of the pore, the number of water molecules in the interior of the pore has been calculated as a function of time. This was achieved by defining a region corresponding to the hydrophobic interior of the membrane. This was taken as a slice through the membrane extending 0.8nm either side of the center of the membrane. The number of water molecules in this region were counted and averaged over periods of 1ns. This provides a rough estimate of the size of the water pore. It should be noted that using this approach the water molecules located at the openings of the pore were not counted.

The Potential of Mean Force (PMF) of the ions (Na+ and Cl-) in the simulation box has been calculated using the following equation:

\[ \Delta G = k_b T \ln \left( \frac{P_0}{P_z} \right) \]  

(3.2)

Where \( k_b \) is the Boltzmann constant, \( T \) the temperature, \( P_0 \) is the density of the ion in bulk and \( P_z \) the density of the ion as a function of their position in the direction normal to that of the lipid bilayer (z axis).
Table 3.1 Overview of the simulations of a DPPC bilayer under different conditions

<table>
<thead>
<tr>
<th>Label</th>
<th>Lateral pressure (bar)</th>
<th>Surface tension (mN/m)</th>
<th>Ions (M)</th>
<th>Simulation time (ns)</th>
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<tr>
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<tr>
<td>Pure DPPC bilayer with a pre-formed pore</td>
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<td></td>
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</tr>
<tr>
<td>A</td>
<td>10</td>
<td>9</td>
<td>0</td>
<td>79</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>15</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>19</td>
<td>0</td>
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<tr>
<td>D</td>
<td>35</td>
<td>20</td>
<td>0</td>
<td>17&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>DPPC bilayer with a pre-formed pore and NaCl ions</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>9</td>
<td>0.1</td>
<td>75&lt;sup&gt;2&lt;/sup&gt;, 39&lt;sup&gt;2&lt;/sup&gt;, 20&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
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<td>9</td>
<td>0.2</td>
<td>20&lt;sup&gt;2&lt;/sup&gt;, 39&lt;sup&gt;2&lt;/sup&gt;, 14&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
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<tr>
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<td>79</td>
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<tr>
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<tr>
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<td>26</td>
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</tr>
<tr>
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<td>31</td>
<td>0.2</td>
<td>10&lt;sup&gt;1&lt;/sup&gt;</td>
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<tr>
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<td>Equilibrated DPPC bilayer with NaCl ions</td>
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</tr>
<tr>
<td>N</td>
<td>1</td>
<td>0</td>
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</tr>
<tr>
<td>O</td>
<td>1</td>
<td>0</td>
<td>0.2</td>
<td>65</td>
</tr>
</tbody>
</table>

<sup>1</sup> membrane ruptures

<sup>2</sup> pore closes
Table 3.2 Dependence of the life time of the pore on the concentration of the ions

<table>
<thead>
<tr>
<th>Ion concentration (M)</th>
<th>Simulations</th>
<th>Pore lifetime (ns)</th>
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<td>0.0</td>
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<td>&gt;79</td>
<td>&gt;79</td>
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<tr>
<td>0.1</td>
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<td>10</td>
<td>21±9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>32</td>
<td></td>
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<tr>
<td></td>
<td>3</td>
<td>20</td>
<td></td>
</tr>
<tr>
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<td>1</td>
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<td></td>
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<tr>
<td></td>
<td>3</td>
<td>26</td>
<td></td>
</tr>
<tr>
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<td>6±0.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.5</td>
<td></td>
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<td></td>
<td>3</td>
<td>5</td>
<td></td>
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</tbody>
</table>

3.3 Results

3.3.1 Simulations under low tension

Pore closure

To study the influence of the sodium chloride ions on the stability of a water pore, molecular dynamics simulations of a DPPC lipid bilayer containing a pre-formed pore with and without ions have been performed. Without ions, the pore can be stabilized by a tension of 9-15 mN/m (simulations A and B). When there is no tension on the bilayer, the pore remains stable for around 10ns before spontaneous closure is observed. In the presence of ions, however, the quantitative behavior is changed.

In Figure 3.1 illustrations of a pre-formed water pore under a tension of 9mN/m and in the presence of sodium chloride (0.1M) ions are presented (simulation E). Initially the
ions were placed randomly in the water phase with some very close to the membrane and to the openings of the pore. In the presence of ions we observe a rapid closure of the pore followed by the relaxation and equilibration of the bilayer. As seen in Figure 3.1 the size of the channel has decreased significantly after only 5 ns. The lipids lining the pore move slowly to the interface. After around 10ns the pore collapses.

In Figure 3.2 the average number of water molecules and lipid head-group atoms in the interior of the pore are plotted versus the simulation time (see Methods). According to the criteria used, there are initially around 90 water molecules and 7 lipid head-groups in the center of the pore. These numbers fall rapidly after the addition of ions to the system. As one can see both water and head-group molecules are expelled from the interior of the membrane almost at the same time during pore closure. This synchronized movement of water and lipid head-groups away from the interior of the membrane has been observed in almost all the simulations where pore closure occurs (Table 3.1).

**Ion concentration dependence**

The rate of pore closure has also been investigated in bilayers as a function of ion concentration. Without the ions the pore stays open for at least 79ns (simulation A). To estimate the lifetime of the pore at a certain ion concentration each system has been simulated for three times under the same membrane tension (9mN/m). The starting structure for these runs was identical except for the initial ion distribution in the water phase. The results are given in Table 3.2. In general, we observe a decrease in the lifetime of the pore as the concentration of ions is increased. Increasing the ion concentration from 0.1M to 0.2M, results in the reduction of the lifetime of the pore from around 21 to 15ns. While the significance of this change could be debated, a further increase of the ion concentration to 0.6M results in a very rapid pore closure (~6ns).
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3.3.2 Simulations at the critical tension

Pore stabilization

Rapid pore closure occurred in all of the systems containing Na+ and Cl- ions investigated. This was despite the application of tension in the range between 9-16mN/m. However, a slight increase in the tension (23mN/m) results in the stabilization of the pore. The lifetime of the pore is increased from around 10ns to at least 50ns (end of the simulation H). The number of water molecules and lipid head-groups in the interior of the stabilized pore has been calculated as described above. The results are plotted in Figure 3.3. Although the fluctuations in the size of the pore are large, the average structure remains quite stable. On average the stabilized pore contains 91 ± 24 water molecules. If we assume that the shape of the pore in the center of the bilayer is cylindrical, we can estimate the radius of the pore based on the number of water molecules in the pore and the average density of water. In this way the minimum radius of the pore was estimated to be around 0.73nm. When the tension on the membrane was increased to 26mN/m the pore apparently was de-stabilized.

To investigate the behavior of the pore under high tension a series of three independent simulations of the pore under a tension of ~26mN/m and ion concentration of 0.2M have been conducted. The time evolution of the size of these pores is

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**Figure 3.1**: Time series of the process of pore closure in the presence of sodium chloride ions (0.2 M). The lipid head-groups are represented as gray spheres and the tails as black sticks. The water molecules are shown in white while the Na+ and Cl- ions are small and large spheres respectively colored in white.
described in Figure 3.5. In simulation I the pore initially expanded. The amount of water molecules in the interior gradually increased from ~100 to around 200. However, after approximately 30ns the expansion of the pore stops. The size of the pore then gradually shrank to its initial value and remained there for the rest of the simulation. In the second simulation, J, however, the pore continued to expand until the membrane ruptured. In the third simulation, K, the pore expanded initially but after approximately 15ns apparently stabilized. At this point the interior of the pore contained around 414±60 water molecules and 9±1 lipid head groups.

**Figure 3.2:** The number of water molecules (filled square) and the number of lipid head-group atoms (filled circles) in the central region of the pore as a function of time during the process of pore closure in a DPPC bilayer containing sodium chloride ions (0.2M). The tension of the bilayer is around 9mN/m.
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Figure 3.3 The number of water molecules inside the stabilized pore (tension~23mN/m) as a function of time.

Figure 3.4 A snapshot of the side and top view of the large pore (simulation K). The lipid tails and the head-groups are represented as black sticks and spheres respectively. The chloride (large spheres) and sodium (small spheres) ions are in white. The water molecules are shown only in the side view of the pore in white sticks.
A side view and a top view of the large pore are plotted in Figure 3.4. Note that a number of sodium and chloride ions are in the interior of the pore. Overall, the simulations indicate that at this tension (26mN/m), which is very close to the critical tension, the fluctuations of the size of the pore are very high. Therefore, the pore can be trapped in intermediate stages with average radii that range between 1nm (medium pore) to 1.6nm (large pore). However, in the simulation where the tension is increased to more than 31mN/m there is a clear de-stabilization of the pore (simulation L). Rapid expansion of the pore (~10ns) leading to the rupture of the lipid bilayer is observed.

From the results in Table 3.1 it is possible to estimate the critical tension of the bilayer with and without sodium chloride (0.2M). The tension above which rupture of the membrane starts to occur in the pure bilayer is around 15mN/m. The pore at the critical tension has an average radius of 1nm, containing around 190 water molecules. However, in the presence of the sodium and chloride ions the critical tension increases to 23mN/m, indicating that the stability of the bilayer has increased. An important parameter that reflects the stability of the water pore is the line tension $\gamma$ (equation 3.1). At the critical tension the line tension of the pore can be estimated using the equation $\gamma = r^{'2} \Gamma^{'0}$ (see chapter 2 of this thesis). In a pure DPPC bilayer the line tension was estimated to be around 1.5 * 10^{-11}N while in the presence of NaCl (0.2M) this increases to around 3.1* 10^{-11}N.
Figure 3.5: The number of water molecules (top) and lipid head-groups (bottom) in the pore as a function of time. The different graphs are for simulation I (solid thin line), simulation J (long dashed line) and simulation K (solid thick line). Simulations I, J, and K are described in Table 3.1.
3.3.3 The effect of the ions on the structure of the membrane and the pore

In Figure 3.6 the distribution of molecules in the simulation box for three different systems (no pore, small pore and large pore) of a DPPC bilayer in the presence of sodium chloride (0.2M) has been plotted.

**Equilibrated bilayer**

Figure 3.6a shows an equilibrated bilayer without a pore in the membrane. The tension of the membrane is set to zero. In this case the sodium ions (black area) are concentrated close to the membrane. The peak in the distribution is located only 1.5nm away from the center of the membrane. The sodium ions primarily interact with the phosphate groups of the lipids. In contrast the chloride ions (stripped area) are distributed throughout the water phase. In general, the ion distribution was found to be asymmetric with a greater concentration of ions at one monolayer than the other. Although the differences are not large this could indicate cooperative binding or simply that the system is not completely equilibrated. However, independent of the precise concentration, the general effect of the ions on the structure of the bilayer is apparent. The presence of ions gives rise to a decrease in the area per lipid from around 0.639nm$^2$ in the pure bilayer to 0.596nm$^2$ in the bilayer with the ions and an increase in the order of the lipid tails. These results are consistent with recent simulation studies of PC lipid bilayers containing sodium chloride ions [14].

**Bilayer with a small pore**

Figure 3.6b shows the density profiles for a membrane containing a pore equilibrated with an applied tension of 23mN/m (see Table 3.1, simulation H). On average the pore contains 91 water molecules and 7 lipid head groups. Again, the sodium ions are distributed closer to the interior of the bilayer than the chloride ions. However, the distributions of the sodium ions are non zero in the middle of the pore indicating that the ions associated with the pore penetrate the membrane interior.
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Figure 3.6: Density profiles of various components of the system without a pore at zero tension (a), with a small pore under a tension of 23mN/m (b) and a large pore under a tension of 26mN/m (c). The gray filled area represents the density distribution of the lipids, the black area is for the Na+ cations while the stripped represents the Cl- anions. The distribution of the water molecules (black solid line), phosphorus atoms (black dashed line) and nitrogen atoms (white thick line) are also shown.
Bilayer with a large pore

In Figure 3.6c the density profiles for a membrane containing a pore equilibrated with an applied tension of 26mN/m are shown (simulation K). The pore is large, containing around 414 water molecules and 9 lipid head-groups. The distribution of the water molecules and the lipid atoms in the system is quite different from the previous two cases. In the presence of the large pore most of the lipid molecules are involved in the formation of the pore. Thus, the bilayer becomes highly curved containing a significant amount of water molecules (see also Figure 3.4). However, despite the fact that there is increased penetration of water in the membrane the distribution of the ions in the system does not change significantly. In all three systems there is an asymmetric distribution of ions at the two interfaces.

In Figure 3.7 the potential of mean force of the ions, calculated based on their partial densities inside the simulation box is shown. Note, the center of the membrane (x-axis) is set to zero and the bilayer extends around 1.7 nm either side of the center. In the absence of a pore in the bilayer the chloride ions show a weak interaction with the interface. In the presence of the pore the chloride ions need to overcome an energetic barrier in the order of 3 kJ/mol near to the lipid interface in order to move into the pore. In contrast the sodium ions are strongly attracted to the interface but they must overcome a barrier of ~4kJ/mol to cross the pore.

3.3.4 Ion flux

In order to investigate the flux of ions through the lipid bilayer the mobility of the ions in the simulation box has been analyzed in all the simulations. When there is no pore present in the membrane or the lifetime of the pore was short (10-30ns) no ion permeated the membrane. However, when the pore was enlarged under tension, ions did permeate the membrane. Ion translocation in the simulations depends on the type of the ion and on the size of the pore. For example, in simulations I and J (see Figure 3.5) the pore slowly expands over 20-50ns. Thus the ions permeate a pore that has a different size over time. Therefore, to estimate the permeation rates for the sodium and chloride ions three types of pores have been distinguished. A small pore that contains on average 90 to 100 water molecules, a medium size pore (100 to 200 water
molecules) and a large pore (>200 water molecules). The results are summarized in Table 3.3.

In the case of small pores only one sodium cation permeated the bilayer ($J_{Na^+} \sim 0.007 \frac{Na}{ns*nm^2}$). No chloride ions permeated the bilayer. The permeation rate for the sodium ions in the presence of a medium size pore was similar to that of the small pore ($J_{Na^+} \sim 0.005 \frac{Na}{ns*nm^2}$). In contrast, 3-4 chloride anions permeated the medium size pore increasing the permeation rate of chloride anions to $J_{Cl^-} \sim 0.01-0.02 \frac{Cl}{ns*nm^2}$. When the pore was large many ions permeated the membrane. For instance, in simulation K (see Table 3.3) 57 chloride and 7 sodium ions permeated the membrane during the 70ns simulated. This corresponded to a flux of $J_{Cl^-} \sim 0.1 \frac{Cl}{ns*nm^2}$ and $J_{Na^+} \sim 0.01 \frac{Na}{ns*nm^2}$.

In Figure 3.8, the coordinates of one chloride and one sodium ion that permeate the membrane have been plotted as a function of time. These are typical examples of the permeation of the ions through the large pore. Interestingly, the time required for the chloride ions to pass through the pore was found to be much shorter than of the sodium ions. In general, the chloride ions spend on average only 760ps inside the pore. In contrast the sodium ions require around 11ns before they finally cross to the other side of the pore.

### Table 3.3 Estimation of the permeation rates for the sodium and chloride ions

<table>
<thead>
<tr>
<th>Size of the pore</th>
<th>Simulation H</th>
<th>Simulation I</th>
<th>Simulation J</th>
<th>Simulation K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small (&lt;100 (H_2O))</td>
<td>0 (Na/ns*nm^2)</td>
<td>0.007 (Na/ns*nm^2)</td>
<td>0 (Cl/ns*nm^2)</td>
<td>0 (Cl/ns*nm^2)</td>
</tr>
<tr>
<td>Medium (100-200 (H_2O))</td>
<td>0 (Na/ns*nm^2)</td>
<td>0.01 (Cl/ns*nm^2)</td>
<td>0.005 (Na/ns*nm^2)</td>
<td>0.02 (Cl/ns*nm^2)</td>
</tr>
<tr>
<td>Large (&gt;200 (H_2O))</td>
<td>0.01 ± 0.01 (Na/ns*nm^2)</td>
<td>0.1 (Cl/ns*nm^2)</td>
<td>0.01 ± 0.01 (Cl/ns*nm^2)</td>
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</tbody>
</table>

*The error bars are given only for Simulation K; the number of flux events in the other cases is too small to show statistically meaningful conclusions.*
Figure 3.7: Potential of Mean Force (PMF) for the sodium (thick lines) and chloride (thin lines) ions. The PMF of the ions have been calculated for the system of the equilibrated bilayer in simulation O (dashed lines) and a system that contains a large pore in simulation K (solid lines).

Figure 3.8: Plots of the coordinates of a typical example of one chloride (left) and one sodium (right) ion along the axis normal to that of the membrane as a function of time.
3.4 Discussion

The simulations indicate that water pores in lipid membranes are destabilized in the presence of sodium chloride making the membrane more resistant to rupturing. This finding is in agreement with experimental measurements [13]. From the simulations a molecular picture emerges why this is the case. The effect on pore stability has its origin in the fact that sodium ions absorb strongly to the interfacial region of the lipid bilayer. The sodium ions penetrate to the level of the carbonyl groups and bind strongly to the phosphate and carbonyl groups, thereby bridging neighboring lipid molecules. This bridging effect has also been observed in simulations by others [14, 15]. A direct consequence of the increase in the effective head-group interactions is a decrease of the surface area and an increase in the line tension. In terms of the lipid shape model [24] the lipids, in the presence of sodium ions, form an inverted cone. Inverted type lipids are known to destabilize phases of positive curvature. They will therefore tend to destabilize pores which are characterized by a net positive curvature.

Although pores are found to be less stable in the presence of (sodium) ions, the pores can be stabilized by applying tension to the system. Experimentally, pores can not only be stabilized by tension [10], but also by a variety of other means, including externally applied electric fields [7], the addition of positively curved lipids [25] or by the addition of anti-microbial agents [26, 27]. Stabilized pores are of biological as well as technological interest due to their ability to transport molecules across the membrane. For instance, small pores formed by anti-microbial peptides destroy trans-membrane ion gradients and can lead to cell death. On the other hand large stable pores generated by electroporation can be used to facilitate DNA uptake by cells [28, 29].

The simulations reveal a surprising difference in the permeability of sodium versus chloride through such stabilized pores. The flux of ions observed indicates that the pore is selective for anions. The chloride anions are able to permeate the pore much faster than the sodium cations. The underlying reason for the anion selectivity appears to be the binding of the sodium ions in the interfacial region. Although the solubility of sodium ions in the pore is higher than that of chloride ions (according to Figure 3.7 the difference is about 7kJ/mol), the sodium ions experience a large friction while permeating the pore. Whereas the sodium ions bind to and diffuse slowly along the interface, the chloride ions do not bind to the interface. Rather, the chloride ions pass
through the middle of the pore and diffuse through the pore at a rate similar to that in bulk water. The resulting chloride/sodium ratio of the permeation rate is about a factor of 10 for the largest pore studied. For the smaller pores the statistics of permeation are very limited. Nevertheless, it appears that the ratio of chloride to sodium drops. Anionic selective pores have been observed in lipid bilayer systems. These large pores are formed and stabilized by specific cationic lipids [30]. The permeability ratio \( (P_{\text{Cl}}/P_K) \) of the ions through these pores is found to be around 4-7. Another example of an anionic selective membrane pore is the one formed by the Magainin family of anti-microbial peptides. The channel which is composed by three to six peptide molecules exhibits an anionic selectivity \( (P_{\text{Cl}}/P_K=3) \) [31, 32].

The permeation behavior observed in the simulations is summarized in Figure 3.9. Without pores, the ion flux is expected to be extremely low. Even in the case where the ions retain their hydration shell, the free energy barrier of dissolving into the membrane interior is estimated to be of the order of 150kJ/mol [33]. When medium pores (r~0.8-1.0nm) are present, ions do manage to permeate the membrane. The flux of chloride ions is almost equal to that of sodium. In the situation where large pores (r~1.6nm) are present, that is pores which have an interior region resembling bulk water, the flux of chloride is greatly enhanced while the flux of sodium remains at a similar level to that observed for medium pores. One could speculate that in the presence of small pores (0.5nm), in the absence of bulk water in the pore center, chloride ions might be blocked from entering the pore, whereas sodium ions might still permeate via the interfacial route.

Analysis of experimental data on the permeation rates of halide ions through PC liposomes with different acyl chain lengths has shown that halide ion translocation occurs probably via a mechanism involving solubility and diffusion and not via a water pore [34]. In contrast there is evidence to suggest that the permeation of potassium and protons does occur through transient water pores [35]. The results presented here could explain why in the presence of small transient pores chloride anions are blocked and not able to permeate the pore whereas sodium cations still manage to permeate by diffusing on the pore walls. In electroporation experiments of lipid vesicles filled with 0.2 NaCl it is found that the sodium ions interact strongly with the walls of the pore [36].
Finally we can speculate about the flux of other ions, such as proton, potassium and calcium, through water pores similar to those present in our simulations. The transport of protons through lipid bilayers is anomalously high compared to other cations. The permeability coefficient for protons is 3-5 orders of magnitude higher than that of Na+ [35]. Protons are able to propagate along thin water wires [38, 39]. A water pore of minimal size already satisfies such a criterion and would therefore allow for fast proton translocation. This small pore would be also sufficient for the sodium cations translocation. However, the sodium cations would diffuse much slower in the pore than the protons. The permeation rate of potassium is expected to be similar to that of sodium (in the order of $10^{-12}$ cm/s). Divalent calcium, however, binds to the interface even more strongly than sodium [40]. As a result, diffusion across the pores will be even slower than in the case of sodium. Moreover, the line tension of the membrane will increase, making pores less likely to form.
Figure 3.9: A cartoon of ion flux across membranes without pores (upper picture), and in the presence of either a small (middle) or a large (lower) pore. A schematic free energy profile for the ions (sodium: dashed, chloride: solid) is shown to the right of each picture. Without the pores, fluxes are low due to the low solubility of ions in the membrane interior. When small pores are present, sodium can pass through the pore/membrane interface whilst chloride ions are expelled. For large pores, chloride ions can pass through the middle.
3.5 Conclusions

It is found that water pores are de-stabilized in the presence of sodium and chloride ions. The line tension of the pore seems to increase compared to the value of the pure bilayer. The critical tension of the bilayer is increased as well making the membrane more resistant to rupturing. It is believed that this effect of the specific ions on the stability of the water pore has its origin on the strong ion-lipid head-groups interactions. Although the pores are highly unstable it is possible to stabilize them under an applied surface tension that ranges between 30-35mN/m. The translocation of the ions through the stabilized pores is also investigated. The simulations suggest that the sodium and chloride ions permeate the water pores via a different mechanism.

References


The effect of Sodium and Chloride ions on the stability of trans-membrane pores


