SUPPORTING INFORMATION:

Pore Formation Coupled to Ion Transport through Lipid Membranes as Induced by Transmembrane Ionic Charge Imbalance: Atomistic Molecular Dynamics Study

Andrey A. Gurtovenko[*,1] and Ilpo Vattulainen[1,2]

[1]*Laboratory of Physics and Helsinki Institute of Physics, Helsinki University of Technology, P.O.Box 1100, FIN-02015 HUT, Finland, and* [2]*Memphys-Center of Biomembrane Physics, Physics Department, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark*

Simulation Details:

We carried out atomic-scale molecular dynamics (MD) simulations for lipid bilayers comprised of zwitterionic dimyristoylphosphatidylcholine (DMPC) molecules. Force-field parameters for DMPC were taken from the united atom force-field of Berger *et al*;[1] this force-field has been shown to correctly reproduce a variety of experimentally studied properties, such as the average area and volume per lipid. Further, we have recently used this force field for studies of lipid bilayer mixtures comprised of DMPC and cationic lipids.[2] Water was modeled using the simple point charge (SPC) model.[3] For sodium and chloride ions the default set of parameters supplied within the Gromacs force field was employed.[4]

The Lennard - Jones interactions were cut off at 1 nm. The long-range electrostatic interactions were handled using the particle-mesh Ewald (PME) method.[5,6] The simulations were performed in the NpT ensemble by separately coupling the lipid bilayers and water with salt ions to the thermostat. Temperature was set to 323 K and was kept constant using a Berendsen thermostat[7] (with a coupling time constant of 0.1 ps). Hence, the studied DMPC bilayers were in the liquid-crystalline phase (the main transition temperature of a DMPC bilayer is 297 K).[8] For completeness, we also considered the temperature dependence of pore formation, ion leakage, and sealing of a pore by conducting additional MD simulations for a system with the largest ionic charge imbalance at the physiological temperature T = 310 K. Pressure with a reference value of 1 bar was controlled by a Berendsen barostat[7] (with a coupling time constant of 1.0 ps), using a semi-isotropic pressure coupling separately in the direction of the bilayer plane and the z-direction (bilayer normal). All bond lengths of lipid molecules were constrained using the LINCS algorithm,[9] while the SETTLE scheme[10] was used to constrain the water geometry. The time step of 2 fs was used in all simulations, which were performed using the Gromacs suite.[4]

System Setup:

To model a transmembrane charge imbalance explicitly, we used a double bilayer setup, that is, two lipid bilayers in a simulation box.[11,12] For preparing initial configurations we started with two pre-equilibrated DMPC bilayers (of 128 lipids each) solvated in a box with ~ 10200 water molecules; this structure was taken from Ref. 12 (the "2B_salt system" of Ref. 12 before adding salt). The initial value for the average area per lipid for a fully hydrated, pre-equilibrated DMPC bilayer was around 0.66 nm^2. In the double bilayer system, the two DMPC bilayers separate the "inner" (between the bilayers) and the "outer" water regions (note that the terms "inner" and "outer" are used here for convenience only because periodic boundary conditions are applied in all three dimensions).

The sodium and chloride ions were then added to the two water phases, replacing randomly chosen water molecules. The number of Cl ions was the same in both water reservoirs (20 chloride ions corresponding to ~ 0.22 M), whereas the excess of Na^+ ions was created in the "inner" water phase between bilayers with respect to the "outer" bath (the condition of electroneutrality in the whole system was fulfilled). The initial structure of the double bilayer system with the largest ionic charge imbalance is shown in Figure S1.

At T = 323 K we simulated four different systems by varying the initial transmembrane charge imbalance as follows:

A. 6 Na^+ ions (60 ns simulation, pore formation within 1 ns).

B. 5 Na^+ ions (60 ns, pore formation after 5 ns and pore closure at ~ 58 ns).

C. 4 Na^+ ions (60 ns, no pore formation).

D. 1 Na^+ ions (40 ns, no pore formation).

Simulation A was also employed in another simulation where we studied the sealing of a pore. For that purpose, we used the state of simulation A at t = 50 ns as a starting point for a complementary simulation E, since at that moment there was a residual charge imbalance of 1 ion per bilayer. In simulation E, we removed the charge imbalance by hand and then followed the sealing of a pore, see the end of SI.

Additionally, we carried out one simulation (F) at T = 310 K to elucidate the main effects with regard to temperature.

The above systems consisted of more than 42,000 atoms. To test possible system size effects, we further extended our work by doubling the total number of atoms in each system (amounting to two bilayers of 256 lipids each in a box with more than 20,000 water molecules, around 84,000 atoms in total) and studied the systems for a short period of time (typically about 5 ns). Then, for example, for the system with the largest Na^+ imbalance, the pore formation coupled to the subsequent sodium transport through the pore was observed within 1.5 ns and the observations were in line with those discussed in the Communication.

Finally, pore formation in our simulations was observed in one of the two bilayers only, i.e. the other bilayer stayed intact. No multiple pore formation was observed.

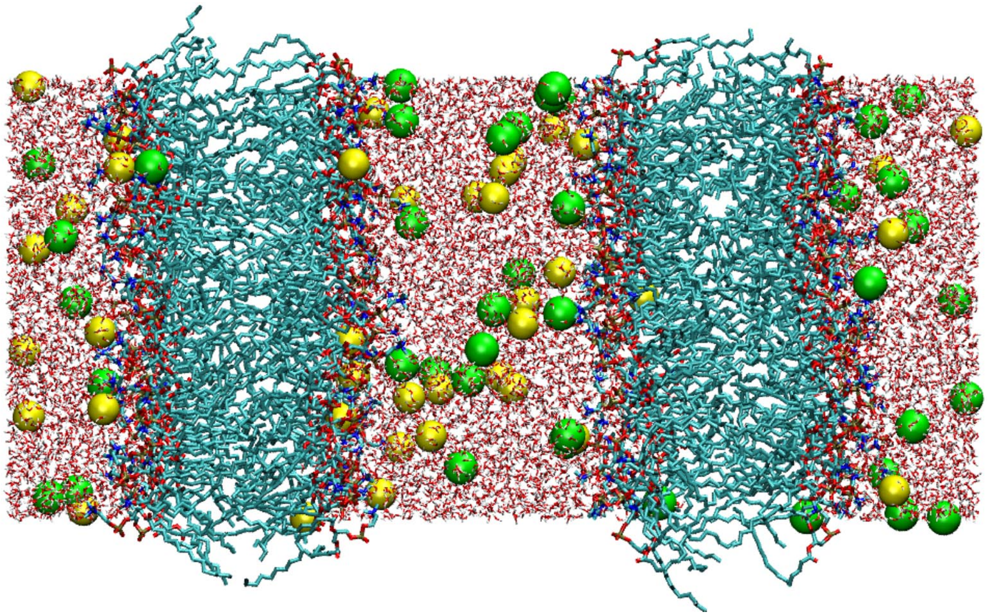

Figure S1. Initial structure of a double bilayer system with a sodium charge imbalance of 6 Na^+ ions per bilayer (i.e. 12 Na^+ ions for the whole double bilayer system): 20 Cl and 26 Na ions in the "inner" water bath between two bilayers (in the middle of the present figure) and 20 Cl and 14 Na ions in the "outer" water reservoir. Lipids are shown in cyan, water in red, sodium ions in yellow, and chloride ions in green.

Analysis:

The electrostatic potential and the electric field across a bilayer were calculated from Poisson's equation by integrating over charge densities which were determined directly from MD simulations. The field and the potential were chosen to be zero in the middle of the "inner" water phase between the two bilayers (i.e., $z = 0$ in the middle of the "inner" water bath). As in previous studies,[13] only the initial part of the trajectories prior to actual pore formation was used for calculating the electric field and the potential. Furthermore, these quantities were also averaged over the two bilayers in a simulation cell.

Thus, we used the initial 500 ps of simulation data to calculate the results presented in Figure 2 of the Communication (a charge imbalance of 6 Na^+ ions per bilayer). For the system characterized by an initial imbalance of 5 Na^+ ions per bilayer, we calculated corresponding properties over the first 4 ns of the MD simulations, since then the pore was formed after ~ 5 ns. The electrostatic potential for the system with the largest charge imbalance (6 Na^+ ions) after the permeation of 2 sodium ions was estimated from the simulation of 2 intact bilayers at the charge imbalance of 4 Na^+ ions (see "System Setup"). Correspondingly, for the system with the initial charge imbalance of 6 Na^+ ions, the residual transmembrane voltage (with one excess ion per bilayer after 10 ns, see Figure 3 of the Communication and Figure S2) was estimated from the simulation of 2 intact bilayers at the charge imbalance of 1 Na^+ ion, see "System Setup".

Remarkably, the electric field E inside the bilayer can be estimated using a simple formula for the field in a planar capacitor: $E = Ne/2\varepsilon_0 A$, where N is the ionic charge imbalance per bilayer and A is the area of the bilayer. For the system with the initial charge imbalance of 6 Na^+ ions and A = 42.88 nm^2 one then has $E \approx 1.265$ V/nm, which does coincide with the plateau value of the electric field (~ 1.27 V/nm) in Figure 2 of the Communication (3 nm < z < 4 nm). Now, using the measured value of the transmembrane potential (~ 2.85 V), one can estimate the "effective" thickness d_{eff} of our membrane capacitor: d_{eff} ~ 2.25 nm, which is comparable to the hydrophobic thickness of a DMPC bilayer (~ 2.6 nm)[14] but much smaller than the average thickness of a bilayer, d_{PP} ~ 3 nm. Great care should be taken in comparing these numbers, however, since it is not obvious how d_{eff} should be interpreted. Namely, most of the ions which define the surface charge of the model capacitor are located in bulk water (absorption of sodium ions has not completed yet since the data correspond to the early stages of the simulation) and, therefore, it is plausible to assume that the distance between model capacitor's planes d exceeds the thickness of a membrane. On the other hand, the relative permittivity of a membrane system increases from about 3 to 80 when going from the hydrophobic core to the water phase; this, in turn, will decrease d considerably. In all, the above suggests that one can think only of some "effective" thickness of a membrane capacitor which, though, can be estimated from E and the transmembrane voltage measured in MD simulations.

Due to the stochastic nature of the electric field induced by a transmembrane charge imbalance, the field and the potential possess substantial fluctuations. To characterize the fluctuations of the transmembrane potential, the corresponding trajectories used for the averaging were split into 5 pieces, from which the standard errors of mean were calculated.

To further illustrate the effects of ions on the electrostatic properties of a bilayer system, we also calculated individual contributions to the electrostatic potential due to lipids, water, and ions, see Figure S3.

The charge imbalance per bilayer presented in Figure 3 (bottom) of the Communication was calculated as follows. First, we computed the center of mass (CM) positions for both bilayers, Z_1 and Z_2. Then, sodium ions with $Z_1 < z < Z_2$ were assigned to the "inner" water bath (and to the "outer" bath otherwise). A half of the difference in numbers of Na^+ ions inside the two baths then gives us the ionic charge imbalance per bilayer. The number of water molecules in a pore, see Figure 3 (top) of the Communication, was calculated by counting the number of water molecules located within 0.5 nm from the center of a bilayer with a pore.

In Figure S2 we present a continuation of Figure 3 of the Communication for $t > 10$ ns.

Furthermore, to elucidate the role of temperature in the studied processes, in Figure S4 we present the number of water molecules in a pore and the transmembrane ionic charge imbalance at $T = 310$ K for a system with the initial ionic charge imbalance of 6 sodium ions. A comparison with Figure 3 of the Communication reveals that the time scales of pore formation and ion leakage increase by a factor of 2 - 3 compared to the case at $T = 323$ K. In other respects, the essential features remain the same.

The results indicate that the system ends up in a metastable state where the pore may or may not have a long lifetime. In part, this is related to the residual Na^+ imbalance at long times. In Figure S5 we show the number of water molecules in a pore after removing the residual charge imbalance of 1 sodium ion in the system presented in Figure 2S. The ion was removed at $t = 50$ ns, and we may observe that the pore remains open for a few nanoseconds, and is finally sealed at about 57 ns.

Finally, to better illustrate the considerable redistribution of lipid head groups towards the hydrophobic region of a membrane upon pore formation, and to emphasize their role in stabilizing the water pore, we present an additional visualization in Figure S6.

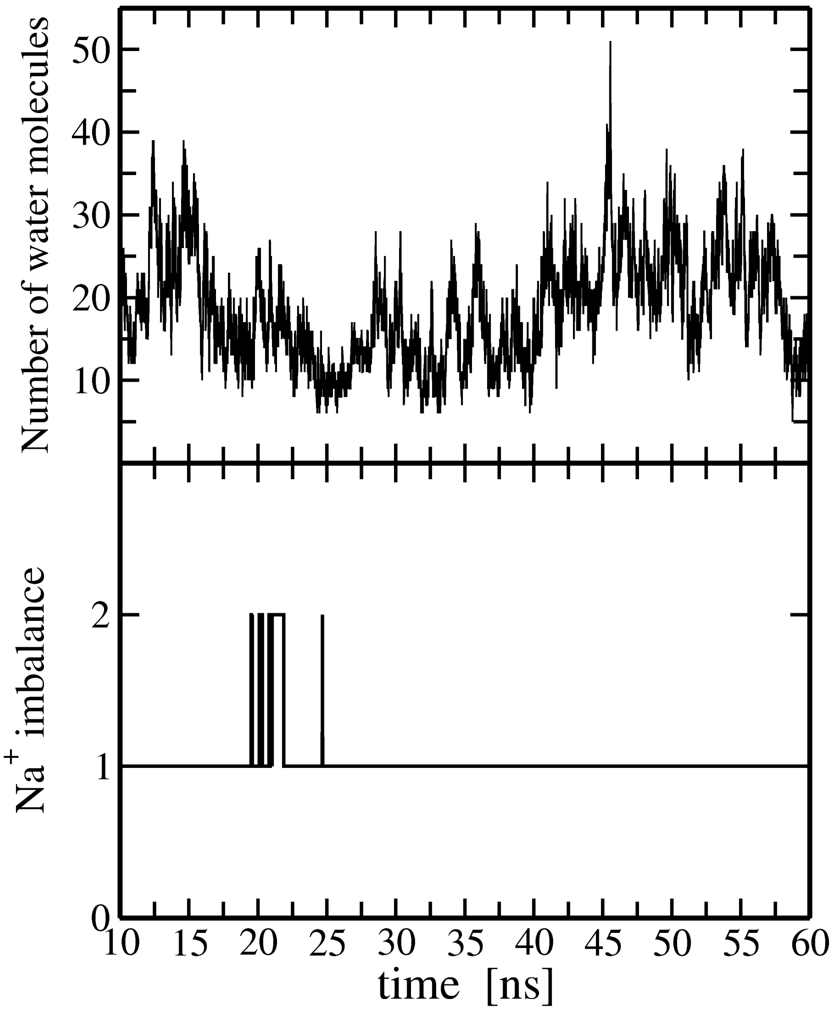

Figure S2. The number of water molecules in a pore (*top*) and the transmembrane ionic charge imbalance (*bottom*) as a function of time for $t > 10$ ns and at $T = 323$ K for the system with the initial transmembrane charge imbalance of 6 Na$^+$ ions per bilayer.

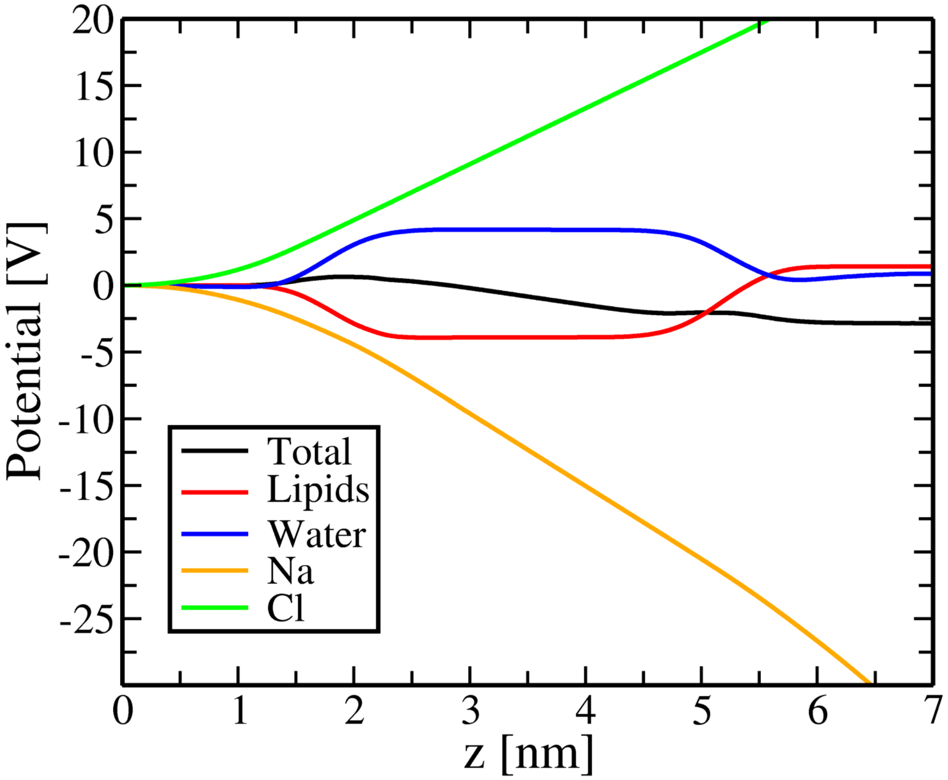

Figure S3. Componentwise contributions to the electrostatic potential across the bilayer (black) due to lipids (red), water (blue), sodium (orange) and chloride (green) ions. Shown are results for a system with the initial ionic charge imbalance of 6 Na^+ ions per bilayer at $T = 323$ K. Only the first 500 ps of the MD trajectory (prior to pore formation) was used for the calculation. $z = 0$ corresponds to the middle of the water phase between the two bilayers.

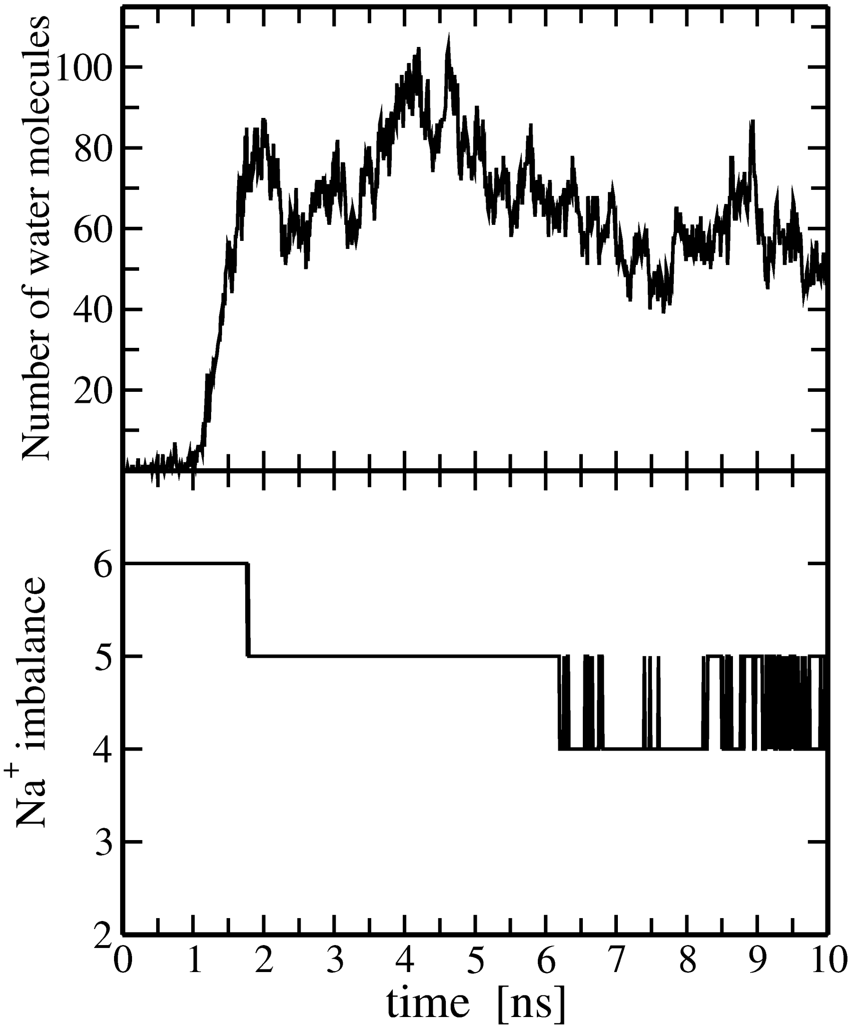

Figure S4. The number of water molecules in a pore (*top*) and the transmembrane ionic charge imbalance (*bottom*) as a function of time for the system with the initial transmembrane charge imbalance of 6 Na$^+$ ions per bilayer at physiological temperature T = 310 K.

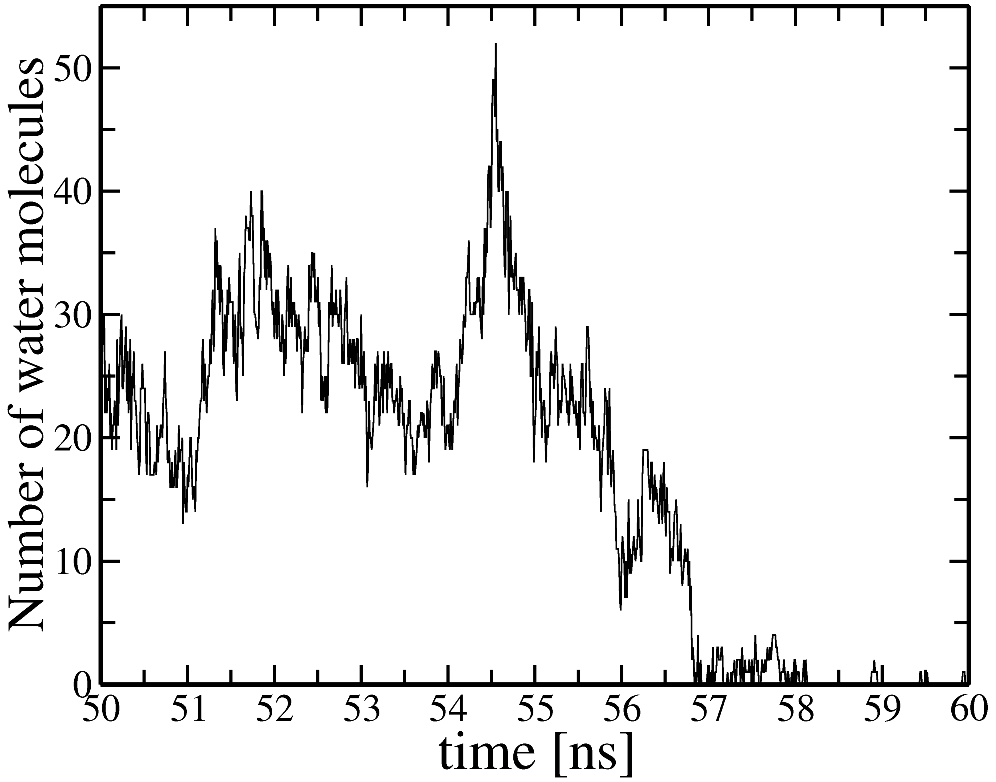

Figure S5. The number of water molecules in a pore as a function of time for the system with the initial ionic charge imbalance of 6 Na^+ ions at *T*=323 K, which was fully discharged at *t* = 50 ns.

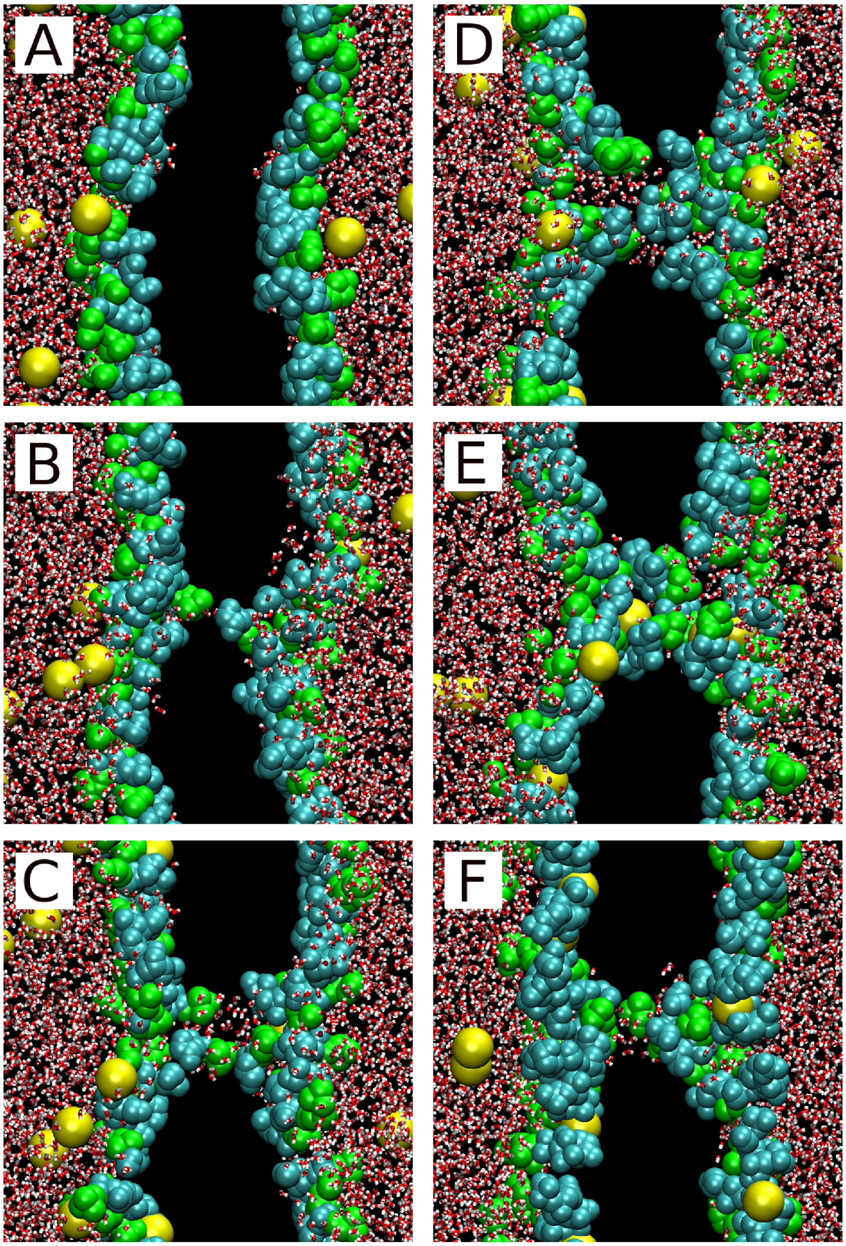

Figure S6. Pore formation coupled to transmembrane ion transport induced by the ion charge imbalance of 6 Na^+ ions at T = 323 K: (A) initial structure at 0 ps, (B) after 450 ps, (C) 650 ps, (D) 1000 ps, (E) 4380 ps, and (F) 45 ns. Water is shown in red-white, choline groups of lipid head groups in green, phosphate and glycerol groups in cyan, and Na^+ ions in yellow. Chloride ions as well as non-polar acyl chains of lipids are not shown. Excess of Na^+ is on the left-hand side.

REFERENCES

(1) Berger, O.; Edholm, O.; Jahnig, F. *Biophys. J.* **1997**, *72*, 2002.

(2) Gurtovenko, A. A.; Patra, M.; Karttunen, M.; Vattulainen, I. *Biophys. J.* **2004**, *86*, 3461.

(3) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; Hermans, J. In *Intermolecular Forces*; Pullman, B., Ed.; Reidel: Dordrecht, 1981; p 331.

(4) Lindahl, E.; Hess, B.; van der Spoel, D. *J. Mol. Model.* **2001**, *7*, 306.

(5) Darden, T.; York, D.; Pedersen, L. *J. Chem. Phys.* **1993**, *98*, 10089.

(6) Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. *J. Chem. Phys.* **1995**, *103*, 8577.

(7) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684.

(8) Cevc, G.; Marsh, D. *Phospholipid Bilayers: Physical Principles and Models*; Wiley: New York, 1987.

(9) Hess, B.; Bekker, H.; Berendsen, H. J. C.; Fraaije, J. G. E. M. *J. Comput. Chem.* **1997**, *18*, 1463.

(10) Miyamoto, S.; Kollman, P. A. *J. Comput. Chem.* **1992**, *13*, 952.

(11) Sachs, J. N.; Crozier, P. S.; Woolf, T. B. *J. Chem. Phys.* **2004**, *121*, 10847.

(12) Gurtovenko, A. A. *J. Chem. Phys.* **2005**, *122*, 244902.

(13) Tieleman, D. P. *BMC Biochemistry* **2004**, *5*, 10.

(14) Nagle, J. F.; Tristram-Nagle, S. *Biochim. Biophys. Acta* **2000**, *1469*, 159.