Electroporation of Skin Stratum Corneum Lipid Bilayer and Molecular Mechanism of Drug Transport: A Molecular Dynamics Study

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Supporting Information

S1. Electroporation of small bilayer (S0)

Table S1. Poration time in small bilayer system (S0)

System	Electric Field (V/nm)	Simulation Time (ns)	R1 (ns)	R2 (ns)	R3 (ns)	R4 (ns)	Average time (ns)
S0	0.3	200	-	-	-	-	-
S0	0.4	200	-	-	-	-	-
S0	0.5	200	-	-	-	-	-
S0	0.6	200	-	-	-	-	-
S0	0.7	200	-	-	-	-	-
S0	0.75	200	-	-	-	-	-
S0	0.775	200	-	-	-	-	-
S0	0.8	100	85.22	88.16	79.35	-	84.24 ± 2.11
S0	0.85	100	75.15	68.26	75.64	81.37	75.10 ± 2.32
S0	0.90	100	20.23	25.32	28.39	36.75	27.67 ± 2.99
S0	1.0	100	8.35	12.73	14.97	6.81	10.71 ± 1.64
S1	0.8	100	58.29	48.62	53.58	42.91	50.84 ± 2.86
S1	0.85	100	18.65	12.97	20.46	15.68	16.94 ± 1.43
S1	0.9	100	9.54	10.34	5.76	6.22	7.96 ± 1.01
S1	1.0	100	3.54	3.93	6.22	4.24	3.41 ± 0.45

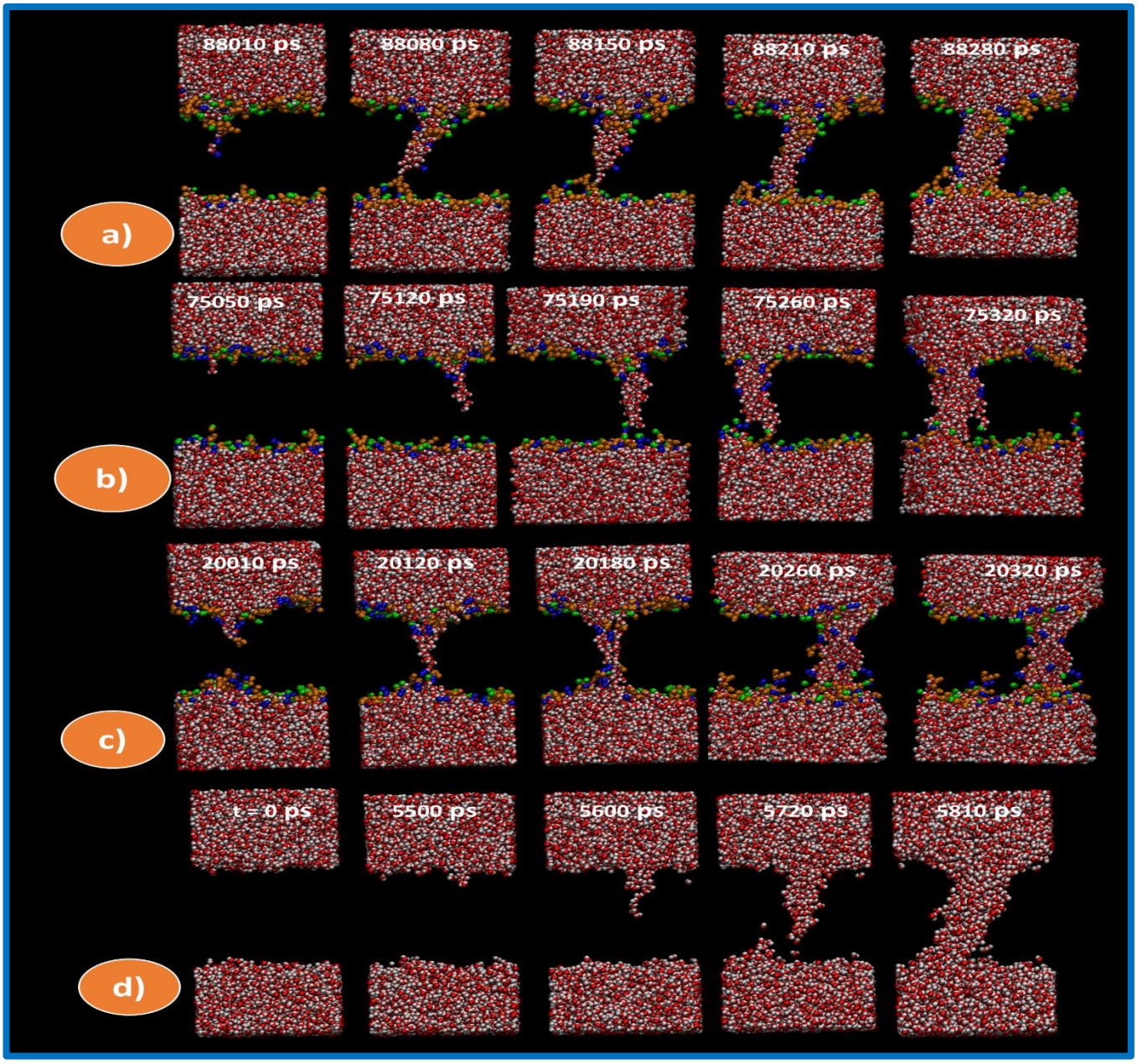

Figure S1. Front view of small lipid bilayer (S0) of 154 lipids during one of the simulation at applied electric field of a) 0.8 V/nm, b) 0.85 V/nm, c) 0.9 V/nm and d) 1.0 V/nm. To show the clear pore formation, the lipid chains have been removed. The headgroups of CER, CHOL and FFA are shown in orange, green and blue color respectively. The potential is positive at the top leaflet of the bilayer relative to the bottom leaflet. All snapshots were made using VMD software.[1] The time given in each snapshots do represent only the kinetics of the defects developed in the bilayer after application of electric field. The electroporation process is stochastic in nature and exact value of time represented here are not exactly reproducible. Four independent simulations were run for each external electric field and snapshots of one of them are shown here.

S2. Effect of Bilayer Size of Electroporation Dynamics

Table S2. System size effect on pore initiation time

Electric Field (V/nm)	S0 (ns)	S1 (ns)	S2 (ns)
0.8	84.24 ± 2.11	50.84 ± 2.86	-
0.85	75.10 ± 2.32	16.94 ± 1.43	-
0.90	27.67 ± 2.99	7.96 ± 1.01	2.85 ± 0.39
1.0	10.71 ± 1.64	3.41 ± 0.45	1.28 ± 0.21

In order to check whether bilayer size affects the poration time, two bigger systems, namely S1 (616 lipids) and S2 (2464 lipids) were simulated at different porating electric field (Table 1 and Table S1). The snapshots of system S1, on application of porating filed of 0.8 V/nm, 0.9 V/nm and 1.0 V/nm, are shown in Figure S2, Figure S3 and Figure S4, respectively. The snapshots of much bigger system, S2 (2464 lipids), on application of porating field of 1.0 V/nm, are also shown in Figure S5. In bigger system S1, the poration time decreased with increased in poration electric field. Also, the poration time changed significantly in all four simulations for an applied electric field (Table S1). In small bilayer system S0, single water pore formation was observed at each applied electric field and in each of the simulation replicas. But, in system, S1, multiple pores have been observed at higher electric field of 0.9 and 1.0 V/nm in some of the simulations (Figure S6). Pliquett et al.[2] carried out electroporation experiments on skin at higher voltage pulse and shown that the transport of negatively charged fluorescent molecules were highly localized. Also, the size and number of these localized regions increased with increase in the applied voltage across the skin. Having multiple pore in bigger bilayer system correlates with this experimental finding.[2] Tieleman et al.[3] also shown that in bigger DPPC bilayer (2304 lipids) multiple pores formed independently with sizes of up to 10 nm within nanosecond time scale at electric field of 0.5 V/nm.

The poration time, at different porating electric field for system S0, S1 and S2, is shown in Table S2. It is interesting to note that the poration time, for a given poration electric field, decreased significantly in bigger size bilayer (poration time S2 < S1 < S0). Our observation are in line with some of the results reported on electroporation of phospholipid bilayer system. Tieleman et al.[3] performed MD simulation of smaller (256 lipids) and bigger (2304 lipids) DOPC bilayer in the presence of external electric field of 0.5 V/nm (threshold ~ 0.33 V/nm). It was reported that, the poration time for smaller (256 lipids) and bigger bilayer (2304 lipids) system was 5.7 ns and 2.5 ns respectively.[3]

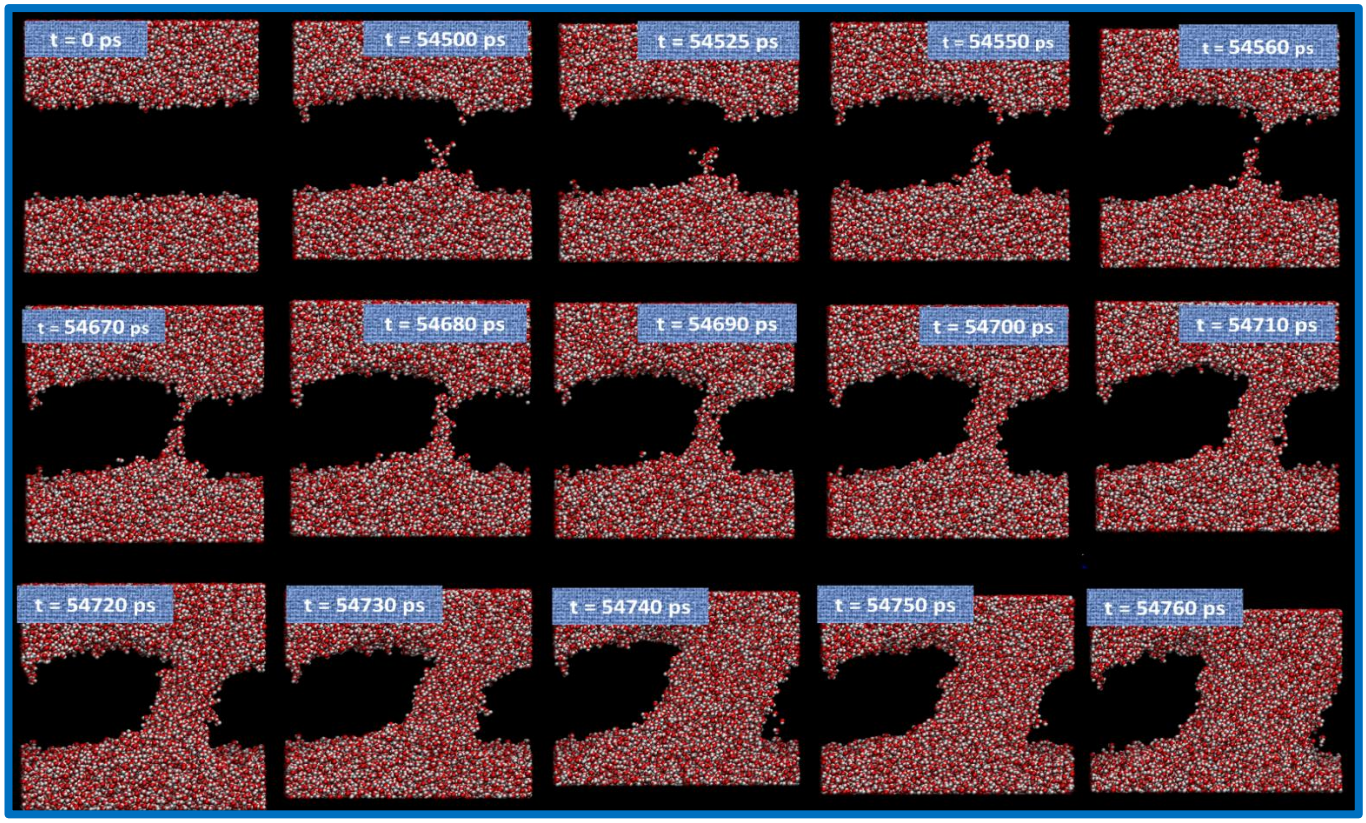

Figure S2. Front view of big skin SC lipid bilayer (S1) of 616 lipids during one of the simulation at applied electric field of 0.8 V/nm. To show the clear pore formation, the lipid molecules have been removed and only water molecules are shown here. The pore formation starts at ~54500 ps. The simulation box was periodic in all three direction, so that effectively an infinite stack of bilayers was simulated, but only the central simulation box is shown here. The potential is positive at the top leaflet of the bilayer relative to the bottom leaflet. All snapshots were made using VMD software.[1]

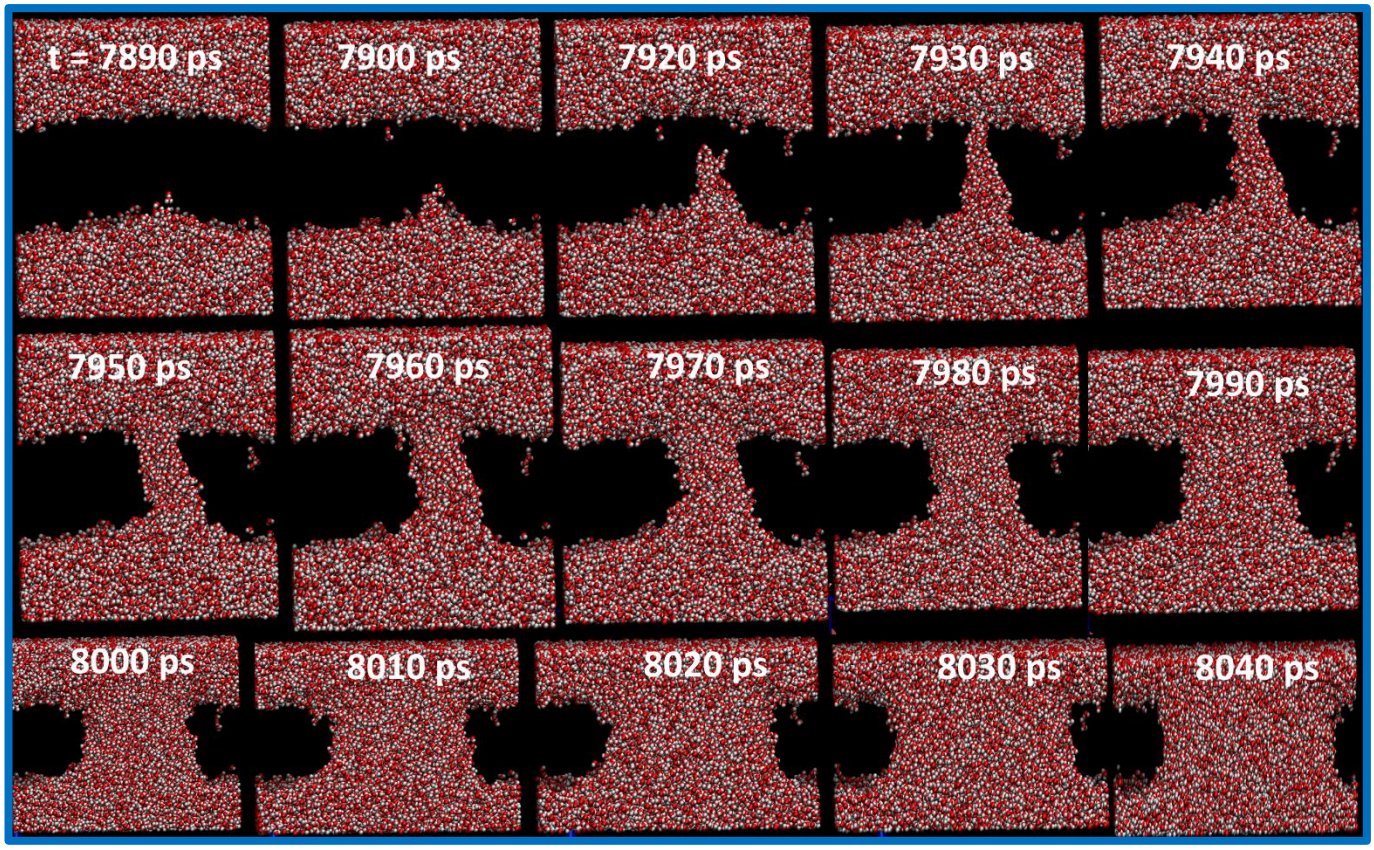

Figure S3. Front view of big skin SC lipid bilayer (S1) of 616 lipids during one of the simulation at applied electric field of 0.9 V/nm. To show the clear pore formation, the lipid molecules have been removed and only water molecules are shown here. The pore formation starts at ~7890 ps. The simulation box was periodic in all three direction, so that effectively an infinite stack of bilayers was simulated, but only the central simulation box is shown here. The potential is positive at the top leaflet of the bilayer relative to the bottom leaflet. All snapshots were made using VMD software.[1] The time given in each snapshots do represent only the kinetics of the defects developed in the bilayer after application of electric field. The electroporation process is stochastic in nature and exact value of time represented here are not exactly reproducible. Four independent simulations were run for each applied electric field and snapshots of one of them are shown here.

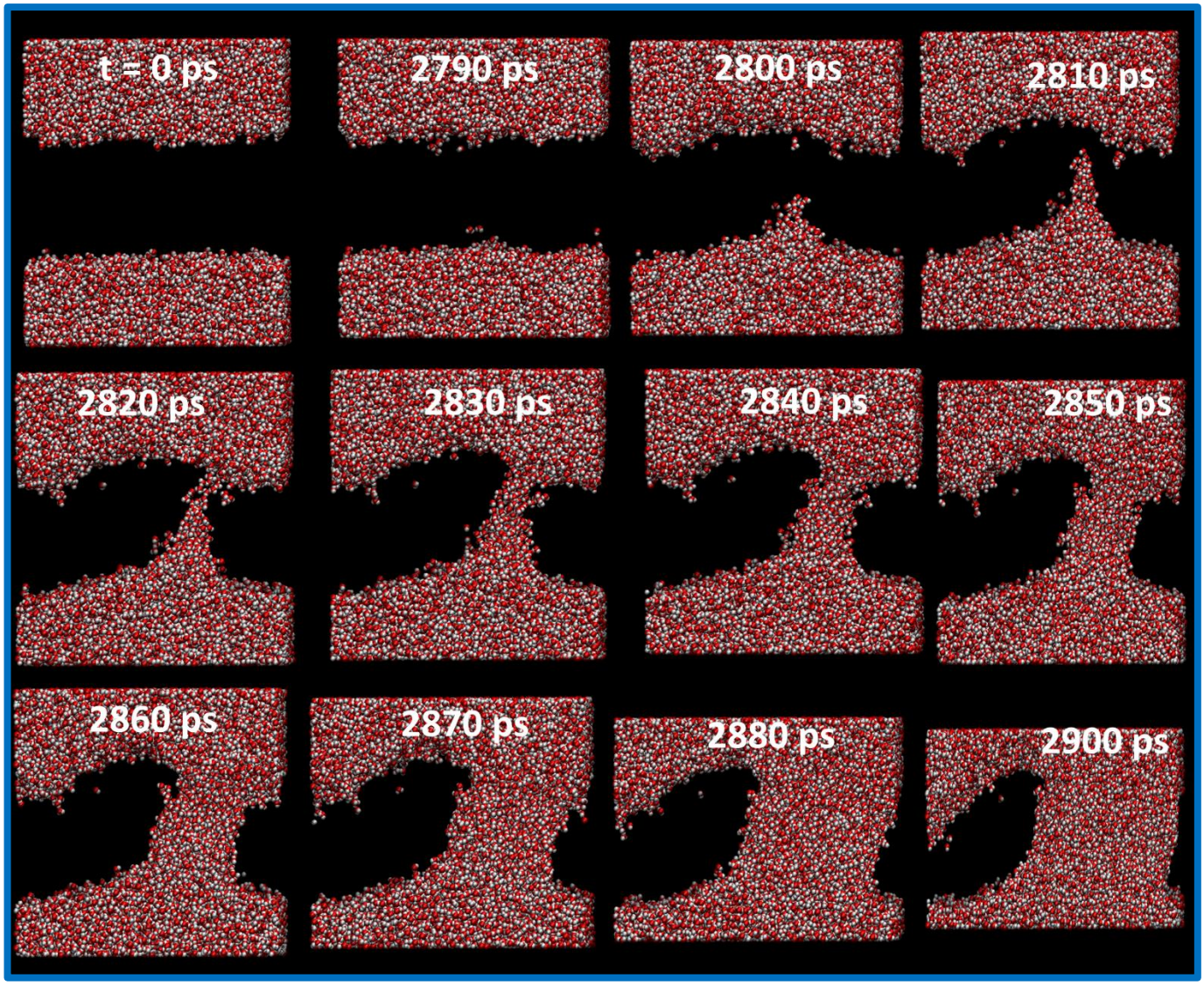

Figure S4. Front view of big skin SC lipid bilayer (S1) of 616 lipids during one of the simulation at applied electric field of 1.0 V/nm. To show the clear pore formation, the lipid molecules have been removed and only water molecules are shown here. The pore formation starts at ~2790 ps. The simulation box was periodic in all three direction, so that effectively an infinite stack of bilayers was simulated, but only the central simulation box is shown here. The potential is positive at the top leaflet of the bilayer relative to the bottom leaflet. All snapshots were made using VMD software.[1] The time given in each snapshots do represent only the kinetics of the defects developed in the bilayer after application of electric field. The electroporation process is stochastic in nature and exact value of time represented here are not exactly reproducible. Four independent simulations were run for each applied electric field and snapshots of one of them are shown here.

S3. Electroporation of ultra-bilayer system (S2)

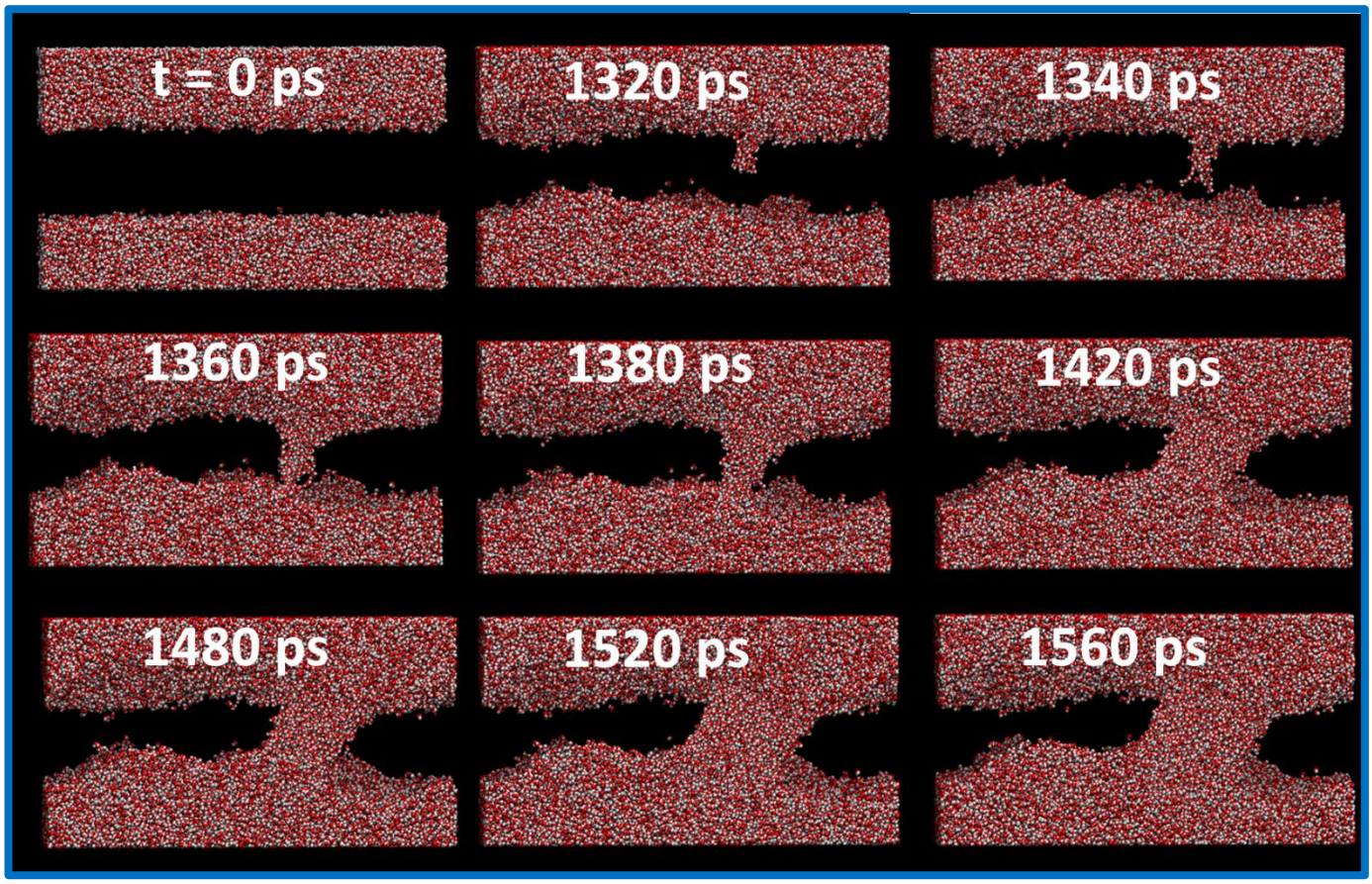

Figure S5. Front view of ultra-skin SC lipid bilayer (S2) of 2464 lipids during one of the simulation at applied electric field of 1.0 V/nm. To show the clear pore formation, the lipid molecules have been removed and only water molecules are shown here. The pore formation starts at ~1300 ps. The simulation box was periodic in all three direction, so that effectively an infinite stack of bilayers was simulated, but only the central simulation box is shown here. The potential is positive at the top leaflet of the bilayer relative to the bottom leaflet. All snapshots were made using VMD software.[1] The time given in each snapshots do represent only the kinetics of the defects developed in the bilayer after application of electric field. The electroporation process is stochastic in nature and exact value of time represented here are not exactly reproducible. Four independent simulations were run for each applied electric field and snapshots of one of them are shown here.

S4. Multi pore formation in big bilayer system during Electroporation

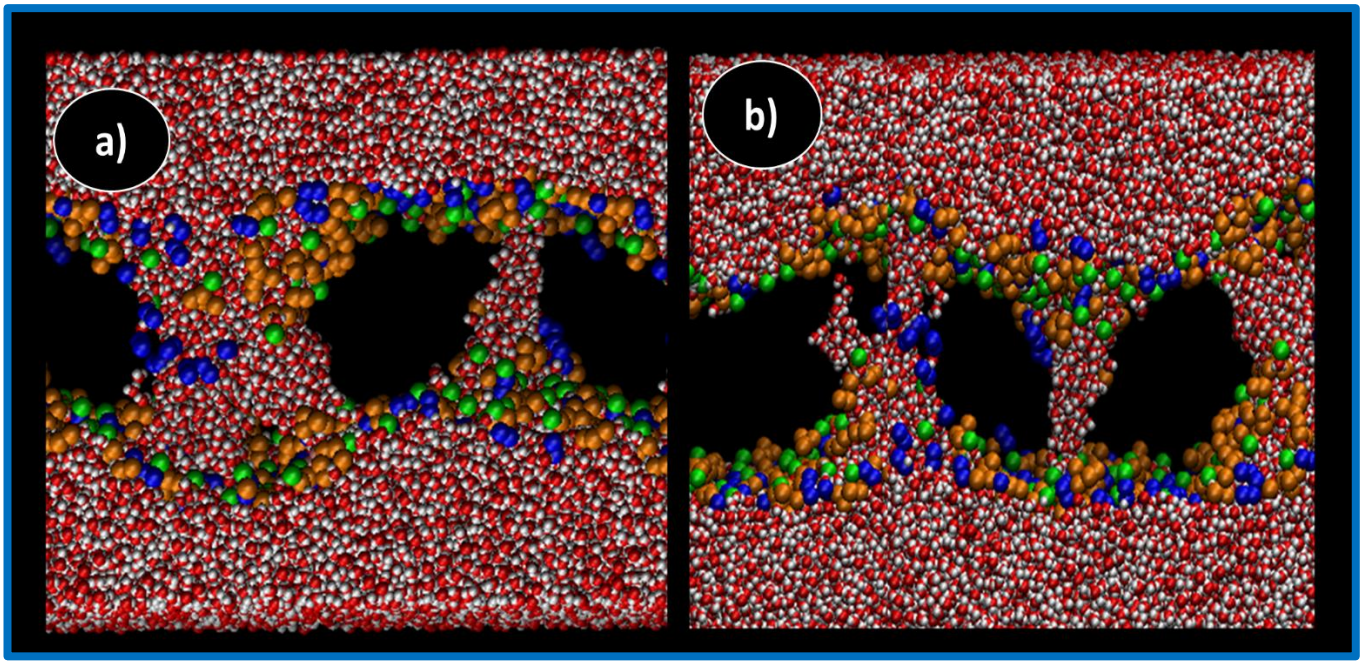

Figure S6. Front view of big skin SC lipid bilayer (S1) of 616 lipids during one of the simulation at applied electric field of a) 0.9 V/nm and b) 1.0 V/nm. To show the clear pore formation, the lipid chains have been removed and lipid headgroups and water molecules are shown here. Both water and headgroups are shown in VDW style of VMD software.[1] The headgroups of CER, CHOL and FFA are shown in orange, green and blue color respectively. The simulation box was periodic in all three direction, so that effectively an infinite stack of bilayers was simulated, but only the central simulation box is shown here. The potential is positive at the top leaflet of the bilayer relative to the bottom leaflet. All snapshots were made using VMD software.[1]

S5. Pore Radius Calculation

The pore radius changed along the bilayer normal z. The pore size was not equal in both x and y direction. To calculate the pore size of the bilayer, the bilayer was projected on the x and y plane separately and it was divided in several small bins (0.2 nm) as shown in Figure S7. The small white box shows the 0.2 nm width bin. The maximum and minimum coordinate of water molecule in each x and y direction was calculated. The difference of maximum and minimum coordinate in each x and y direction gives the pore size R_x and R_y in x and y direction respectively. The pore radius ($R = \sqrt{R_x R_y}$) calculated using geometric mean of R_x and R_y.

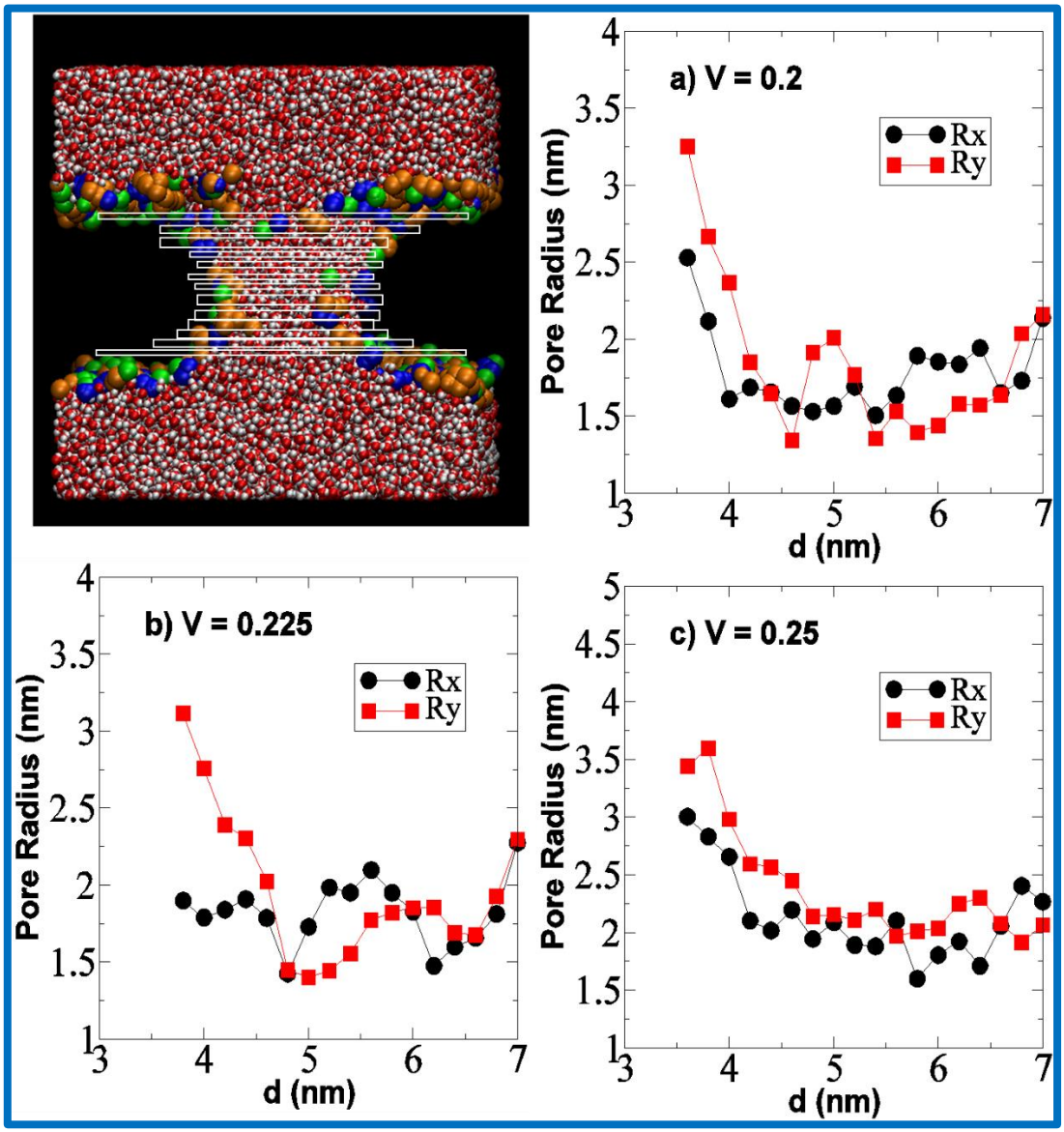

Figure S7. Calculation of the pore size of bilayer along the bilayer normal. The bilayer projected in x and y plane and divided into small bins of 0.2 nm each. Evolution of the pore size along the bilayer normal z in bilayer system (S1). The bilayer system was ran for three different sustaining electric field.

S6. Sustain Electric field after electroporation

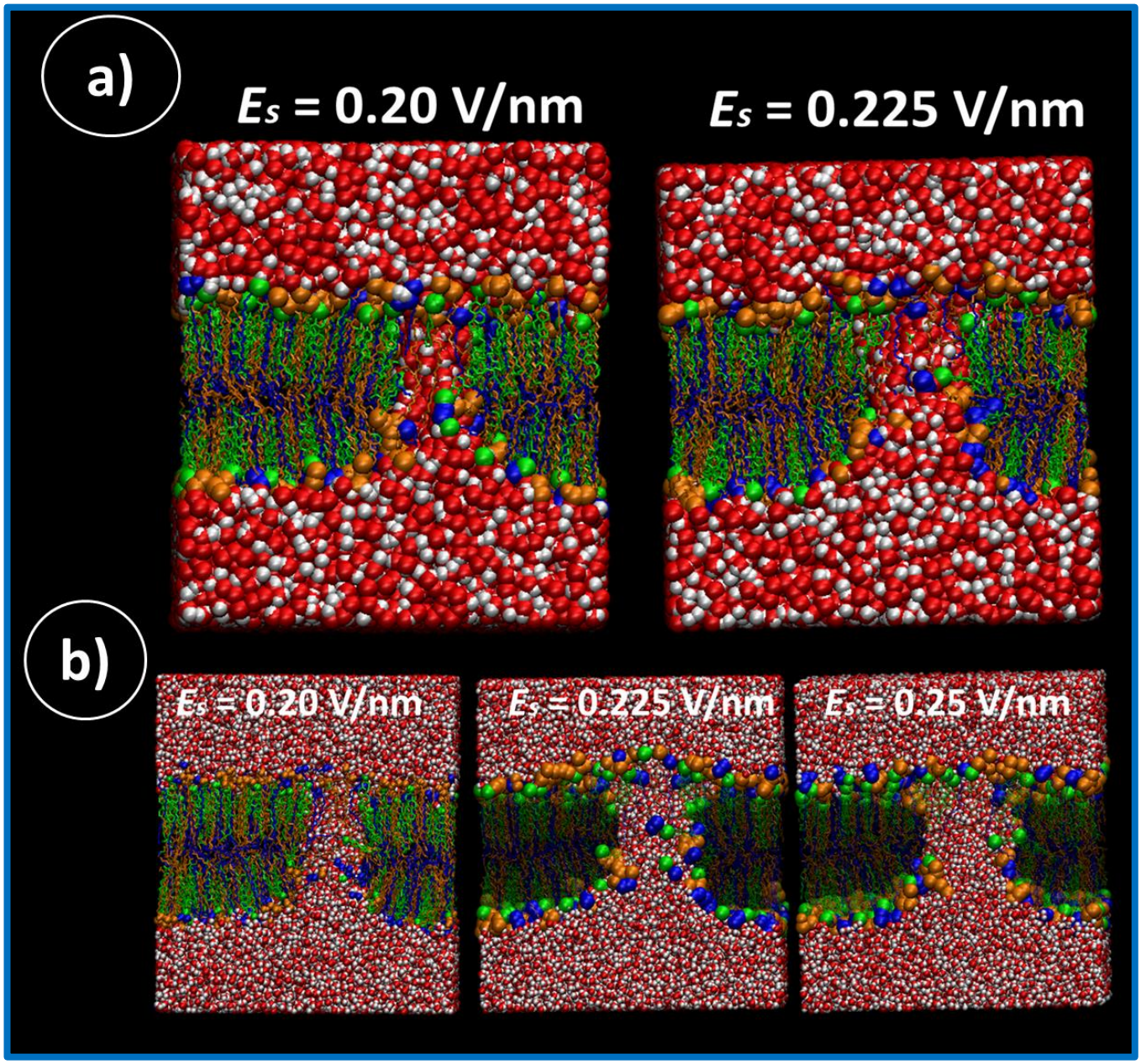

Figure S8. Front view of big lipid bilayer (S1) of 616 lipids during one of the electroporation simulation at lower sustain electric field of 0.2, 0.225 and 0.25 V/nm. Both water and headgroups are shown in "*VDW*" style of VMD software.[1] The chains of lipid molecules are shown in "*line*" style of VMD software.[1] The CER, CHOL and FFA are shown in orange, green and blue color respectively. The potential is positive at the top leaflet of the bilayer relative to the bottom leaflet. All snapshots were made using VMD software.[1] Four independent simulations were run for each applied electric field (0.2, 0.225 and 0.25 V/nm) and snapshots of the system, in which pore remained stable, is shown here. The initial pore formed bilayer configuration for these simulation were taken from the electroporation simulations of system S1 at electric field of a) 0.85 V/nm and b) 0.9 V/nm.

S7. Effect of Bilayer Composition on Electroporation of the bilayer.

Table S3. Effect of Cholesterol and Free fatty acid on poration time and threshold electric field.

System	Electric Field (V/nm)	Simulation Time (ns)	R1 (ns)	R2 (ns)	R3 (ns)	R4 (ns)	Average time (ns)
S3	0.6	200	-	-	-	-	-
S3	0.7	200	-	-	-	-	-
S3	0.8	200	-	-	-	-	-
S3	0.9	200	-	-	-	-	-
S3	1.0	200	-	-	-	-	-
S3	1.1	100	48.22	41.28	44.22	48.42	45.54 ± 1.33
S3	1.2	100	8.45	10.28	7.45	8.84	8.76 ± 0.86
S4	0.7	200	-	-	-	-	-
S4	0.75	200	-	-	-	-	-
S4	0.80	200	-	-	-	-	-
S4	0.85	100	90.24	85.46	92.15	89.45	89.32 ± 1.22
S4	0.90	100	48.61	42.78	51.23	41.63	46.06 ± 1.99
S4	0.95	100	24.46	32.15	29.63	31.25	29.37 ± 1.49
S4	1.0	100	13.89	14.86	18.24	17.57	16.14 ± 0.91
S5	0.6	100	-	-	-	-	-
S5	0.65	100	91.65	98.54	95.26	95.14	95.14 ± 1.22
S5	0.7	100	32.57	28.14	30.15	31.59	30.61± 0.83
S5	0.8	100	20.15	18.15	18.53	17.63	18.62 ± 0.47
S5	0.85	100	15.64	13.52	13.41	12.47	13.76 ± 0.58
S5	0.9	100	8.56	7.55	9.11	7.41	8.16 ± 0.35
S5	0.95	100	5.22	4.16	4.55	4.31	4.56 ± 0.21
S5	1.0	100	2.81	3.25	2.11	2.01	2.55 ± 0.26

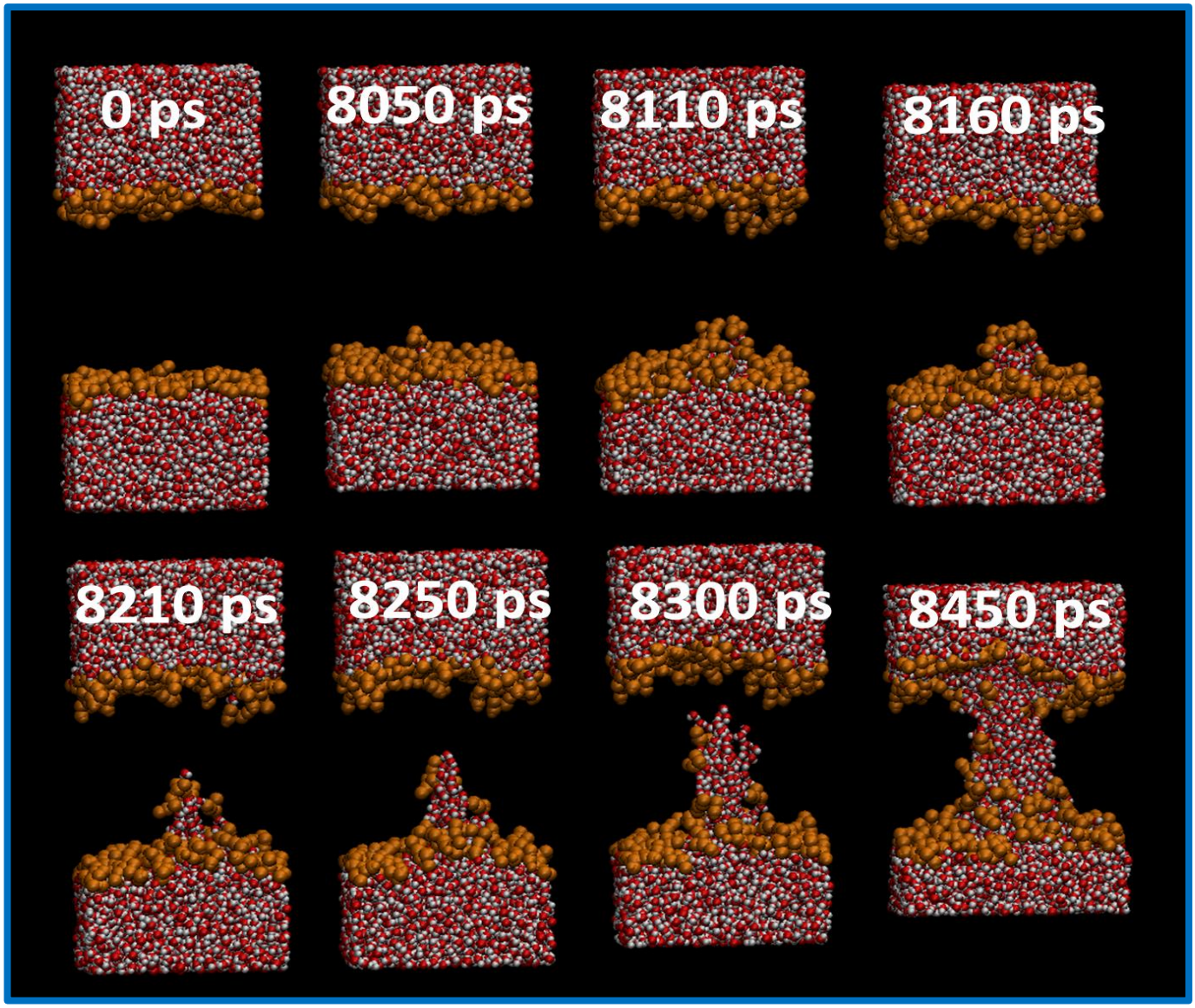

Figure S9. Front view of small CER lipid bilayer (S3) of 128 lipids during one of the electroporation simulation at applied electric field of 1.2 V/nm. Both water and headgroups are shown in "*VDW*" style of VMD software.[1] The chains of lipid molecules are not shown for the purpose of clarity. The CER headgroups are shown in orange color. The pore formation starts at ~44000 ps. The simulation box was periodic in all three direction, so that effectively an infinite stack of bilayers was simulated, but only the central simulation box is shown here. The potential is positive at the top leaflet of the bilayer relative to the bottom leaflet. All snapshots were made using VMD software.[1] The time given in each snapshots do represent only the kinetics of the defects developed in the bilayer after application of electric field. The electroporation process is stochastic in nature and exact value of time represented here are not exactly reproducible. Four independent simulations were run for each applied electric field and snapshots of one of them are shown here.

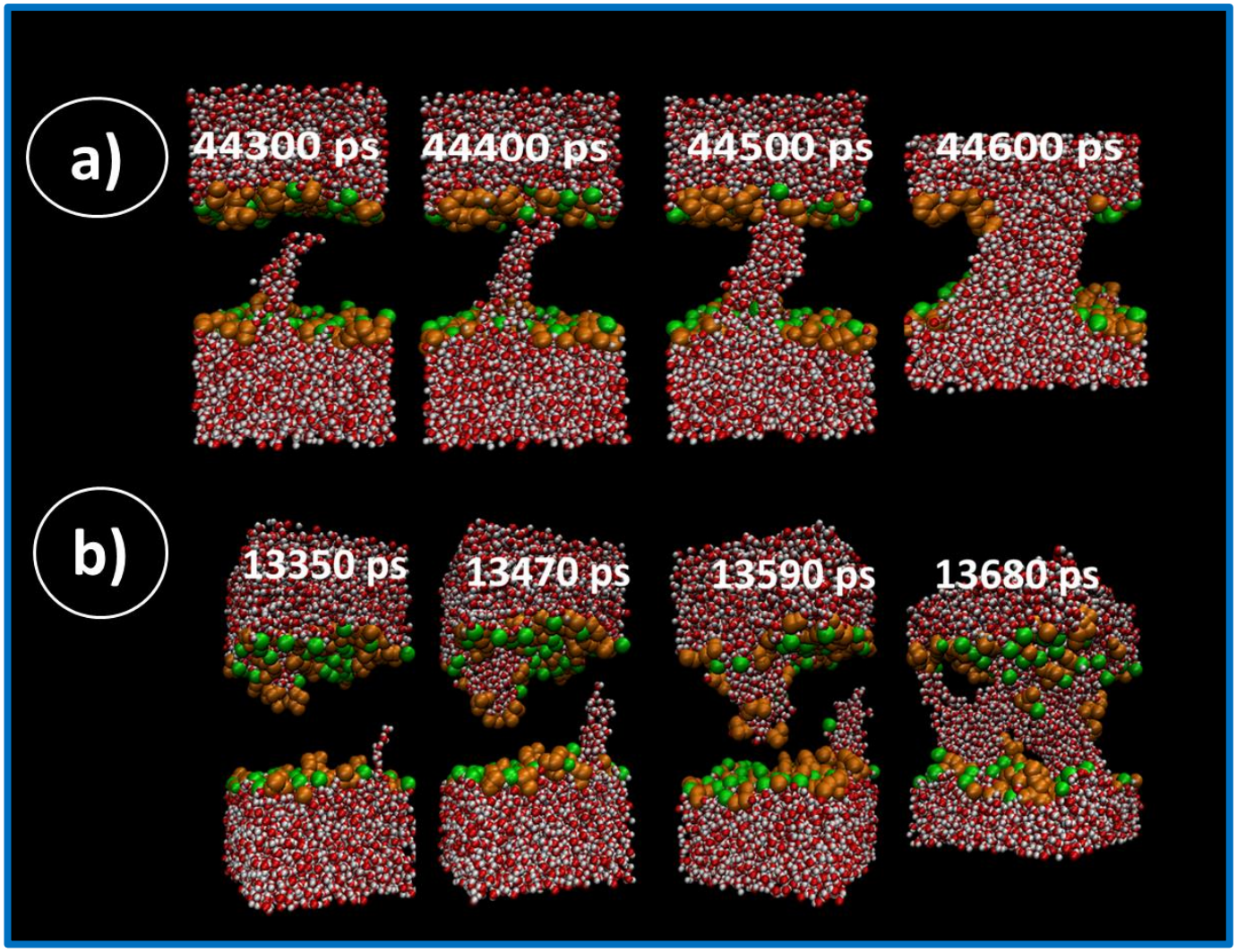

Figure S10. Front view of small lipid bilayer (S4) of 128 lipids (CER: CHOL in 1:1 ratio) during one of the electroporation simulation at applied electric field of a) 0.9 V/nm and b) 1.0 V/nm. Both water and headgroups are shown in "*VDW*" style of VMD software.[1] The chains of lipid molecules are not shown for the purpose of clarity. The CER and CHOL headgroups are shown in orange and green color respectively. The potential is positive at the top leaflet of the bilayer relative to the bottom leaflet. All snapshots were made using VMD software.[1] The time given in each snapshots do represent only the kinetics of the defects developed in the bilayer after application of electric field. The electroporation process is stochastic in nature and exact value of time represented here are not exactly reproducible. Four independent simulations were run for each applied electric field and snapshots of one of them are shown here.

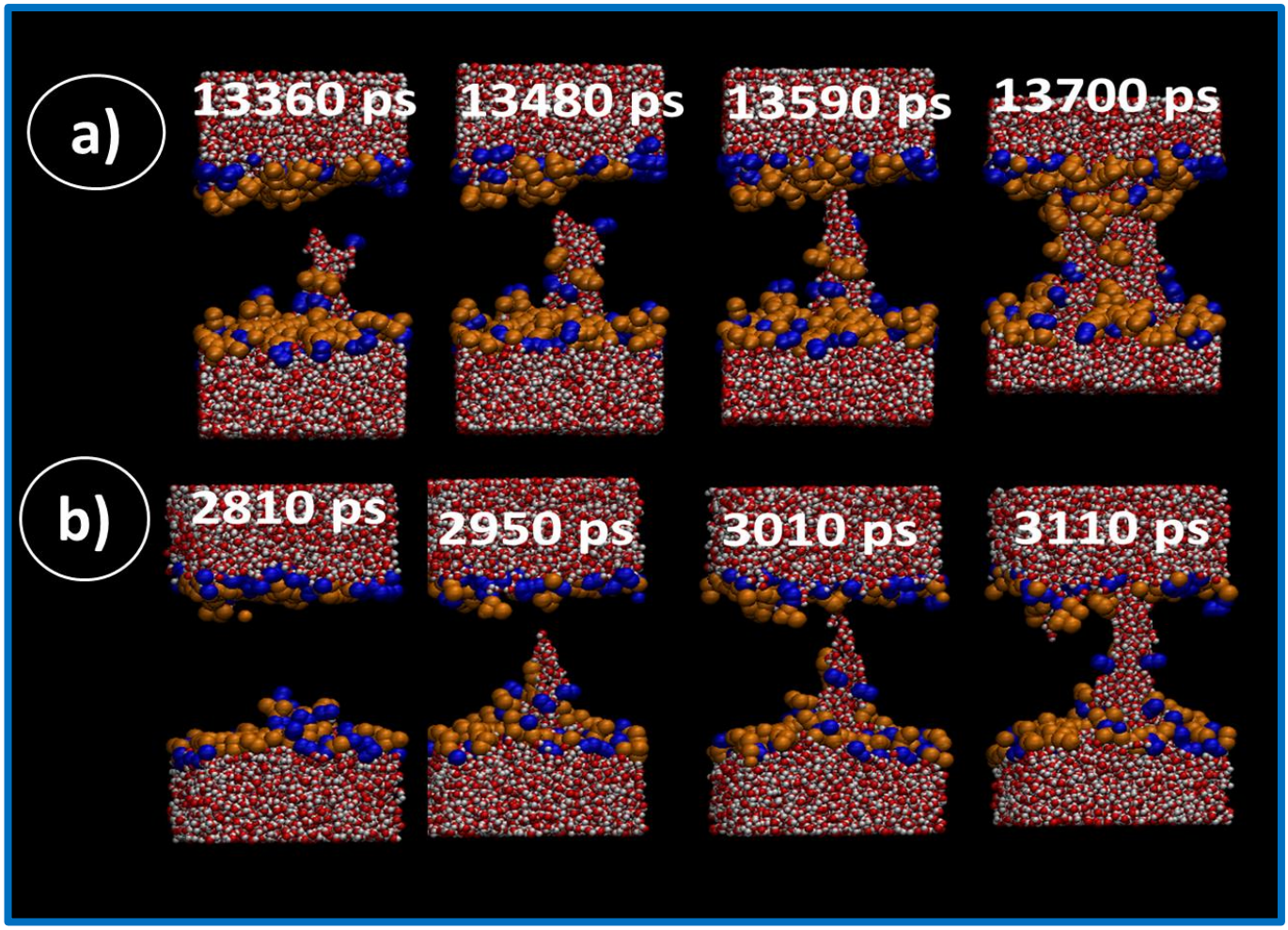

Figure S11. Front view of small lipid bilayer (S5) of 168 lipids (CER: FFA in 1:1 ratio) during one of the electroporation simulation at applied electric field of a) 0.85 V/nm and b) 1.0 V/nm. Both water and headgroups are shown in "*VDW*" style of VMD software.[1] The chains of lipid molecules are not shown for the purpose of clarity. The CER and FFA headgroups are shown in orange and blue color respectively. The potential is positive at the top leaflet of the bilayer relative to the bottom leaflet. All snapshots were made using VMD software.[1] The time given in each snapshots do represent only the kinetics of the defects developed in the bilayer after application of electric field. The electroporation process is stochastic in nature and exact value of time represented here are not exactly reproducible. Four independent simulations were run for each applied electric field and snapshots of one of them are shown here.

S8. Port Growth Rate Calculation

The pore growth rate was determined based on the change in the pore radius in given time interval. The snapshots of each bilayer was taken at two different simulation time. The evolution of the pore size along the bilayer normal z, calculated using the procedure mention in section S10. Here, *RxE* Pore radius in x direction in the last frame, *RyE* Pore radius in y direction in the last frame, *RE* Pore radius in the last frame, *RxS* Pore radius in x direction in the initial frame, *RyS* Pore radius in y direction in the initial frame, *RS* Pore radius in the initial frame. The growth rate has been calculated using the following relationship.

$$G = \frac{(RE - RS)}{\Delta t}$$

Where, G is growth rate of the bilayer pore and Δt is difference in time frame.

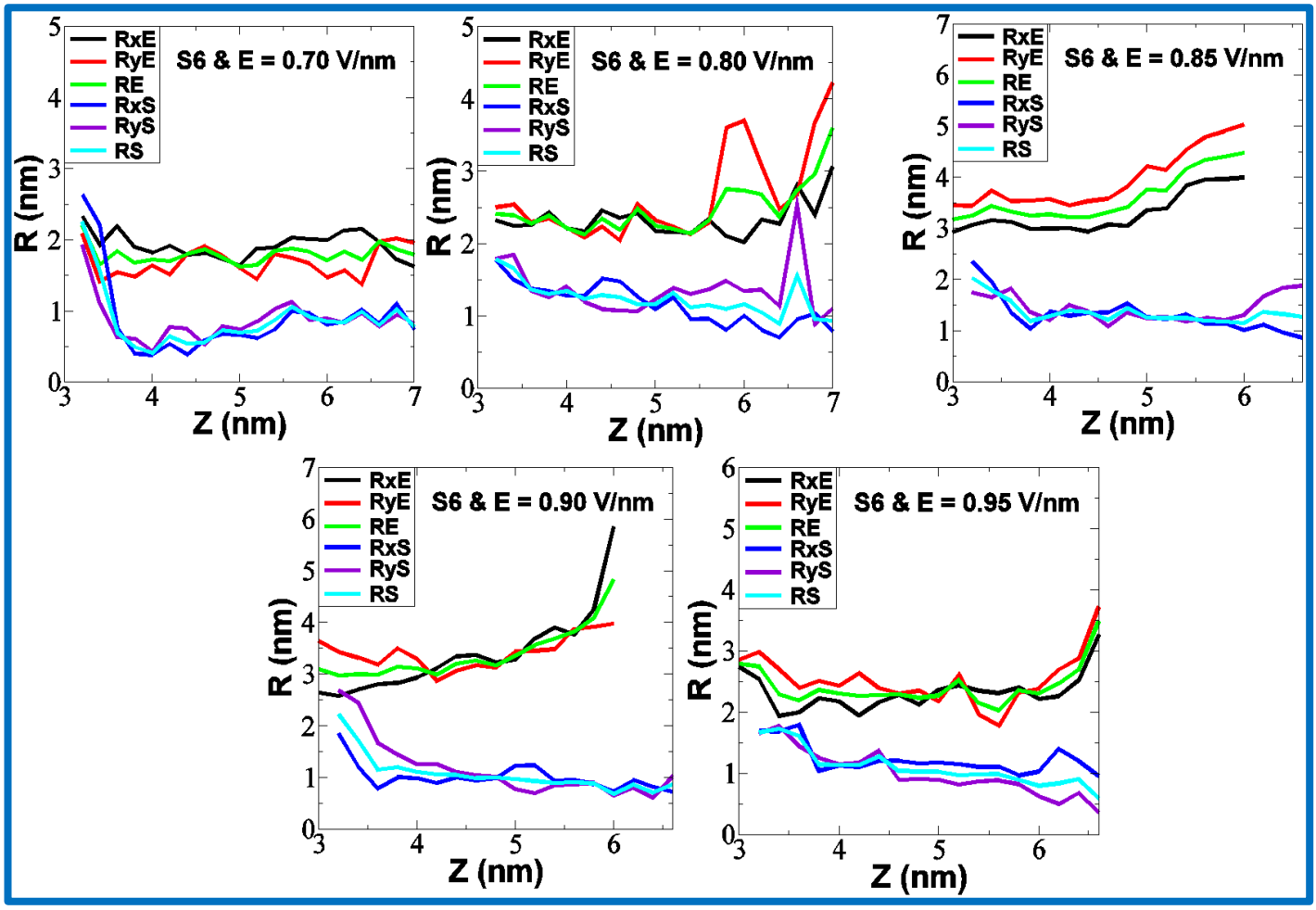

Figure S12. Evolution of the pore size along the bilayer normal (in each system S5) at different applied electric field.

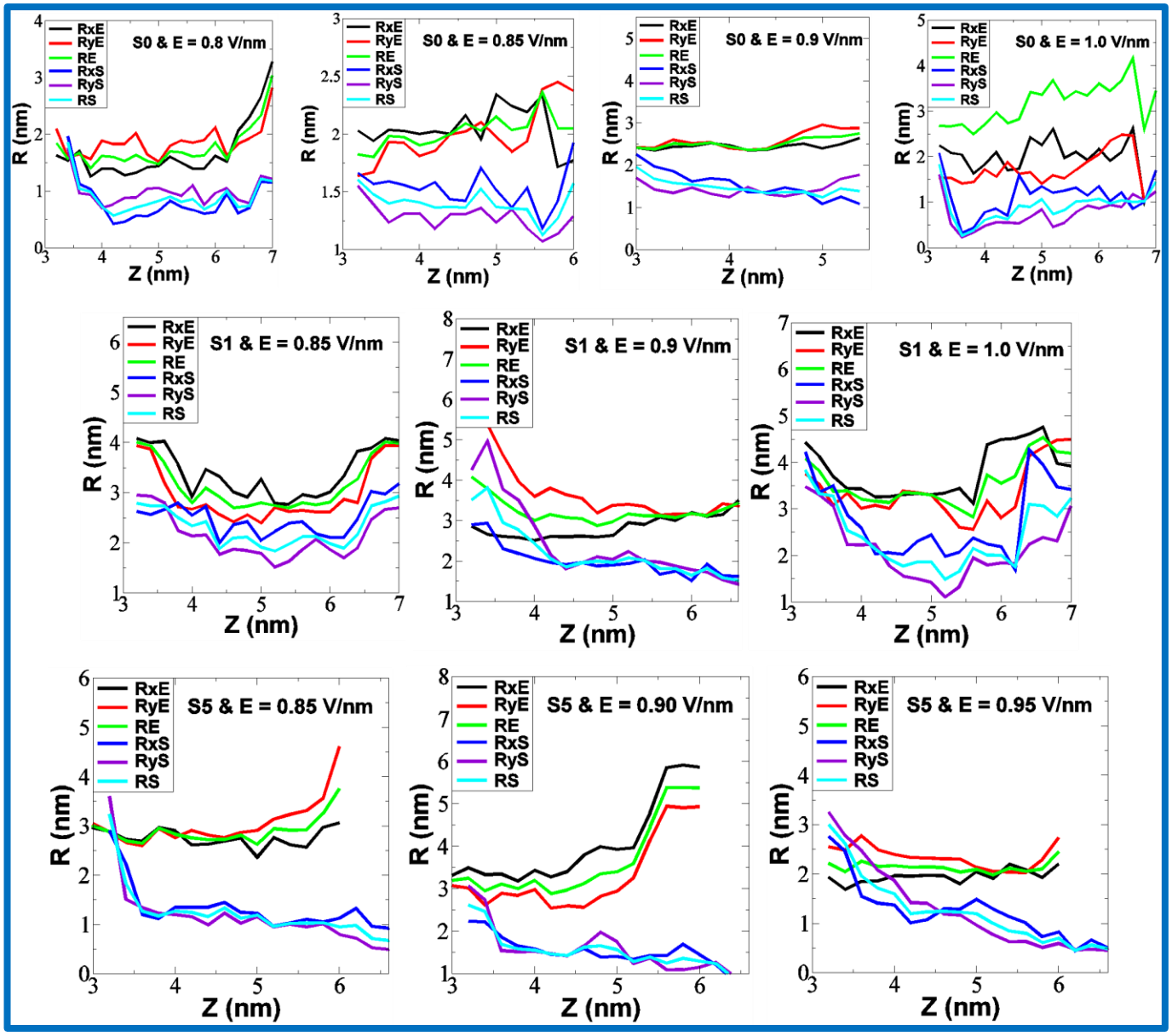

Figure S13. Evolution of the pore size along the bilayer normal (in each system S0, S1 and S4) at different applied electric field.

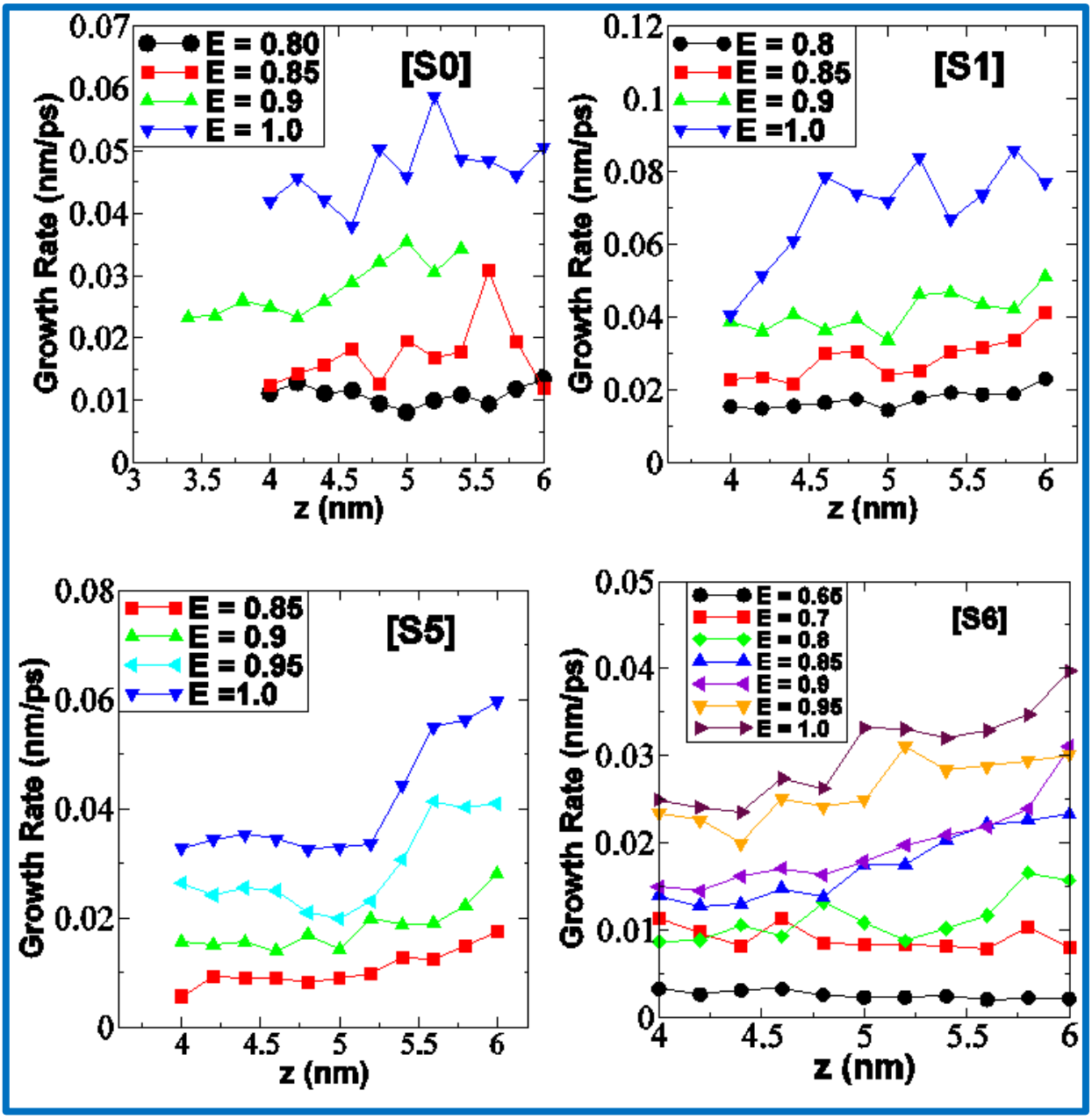

Figure S14. Evolution of the growth rate along the bilayer normal (in each system S0, S1, S4 and S5) at different applied electric field.

References

1. Humphrey, W.; Dalke, A.; Schulten, K. VMD: visual molecular dynamics. *J. Mol. Graph.* **1996**, *14*, 33-38.
2. Pliquett, U.F.; Zewert, T.E.; Chen, T.; Langer, R.; Weaver, J.C. Imaging of fluorescent molecule and small ion transport through human stratum corneum during high voltage pulsing: localized transport regions are involved. *Biophysical chemistry* **1996**, *58*,185-204.
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