Supporting Information

Lipid Oxidation: Role of Membrane Phase-Separated Domains

Maria C. Oliveira[†, ‡], *Maksudbek Yusupov*[‡], *Annemie Bogaerts*[‡], *Rodrigo M. Cordeiro*[† *]

[†]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Avenida dos Estados 5001, CEP 09210-580 Santo André, SP, Brazil

[‡]Research Group PLASMANT, Department of Chemistry, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium

***Corresponding Author:** rodrigo.cordeiro@ufabc.edu.br

Table S1. Area per lipid and bilayer thickness obtained from the last 100 ns of some replicates. The bilayer thickness is represented between brackets.

Replicates	DPPC + ChL / POPCOOH + 6 POPC-ALD				
		native lipids		oxidized lipids	
	ChL	DPPC	POPC	POPCOOH	POPC-ALD
1	0.22±0.08 –	0.66±0.26 (4.47±0.03)	0.58±0.19 (3.81±0.03)	0.66±0.26 (3.22±0.03)	0.71±0.28 (3.03±0.13)
2	0.22±0.08 –	0.68±0.26 (4.27±0.05)	0.58±0.19 (3.83±0.06)	0.67±0.25 (3.20±0.03)	0.72±0.29 (3.15±0.21)
3	0.22±0.09 –	0.68±0.26 (4.43±0.03)	0.58±0.22 (3.70±0.04)	0.66±0.26 (3.17±0.03)	0.67±0.30 (3.14±0.12)

POPCOOH + 6 POPC-ALD	
Replicates	**Area per lipid (nm²) and Bilayer thickness (nm)**
1	0.71±0.01 (3.29±0.04)
2	0.71±0.01 (3.28±0.05)
3	0.71±0.01 (3.28±0.04)

Table S2. Properties of each domain calculated from the last 100 ns of simulation.

System	Thickness (nm)		Thickness mismatch (nm)
	Lo	Ld	Lo/Ld
DPPC + ChL / POPC	4.33±0.03	3.81±0.03	0.52
DPPC + ChL / POPC + 6 POPC-ALD	4.24±0.08	3.82±0.05	0.42
DPPC + ChL / POPC + 32 POPC-ALD	4.32±0.04	3.52±0.03	0.80
DPPC + ChL / POPCOOH	4.19±0.04	3.43±0.03	0.76
DPPC + ChL / POPCOOH + 6 POPC-ALD	4.27±0.03	3.21±0.03	1.06
DPPC + ChL / POPCOOH + 16 POPC-ALD	4.21±0.04	3.32±0.03	0.89
DPPC + ChL / POPCOOH + 32 POPC-ALD	4.12±0.10	3.40±0.07	0.72

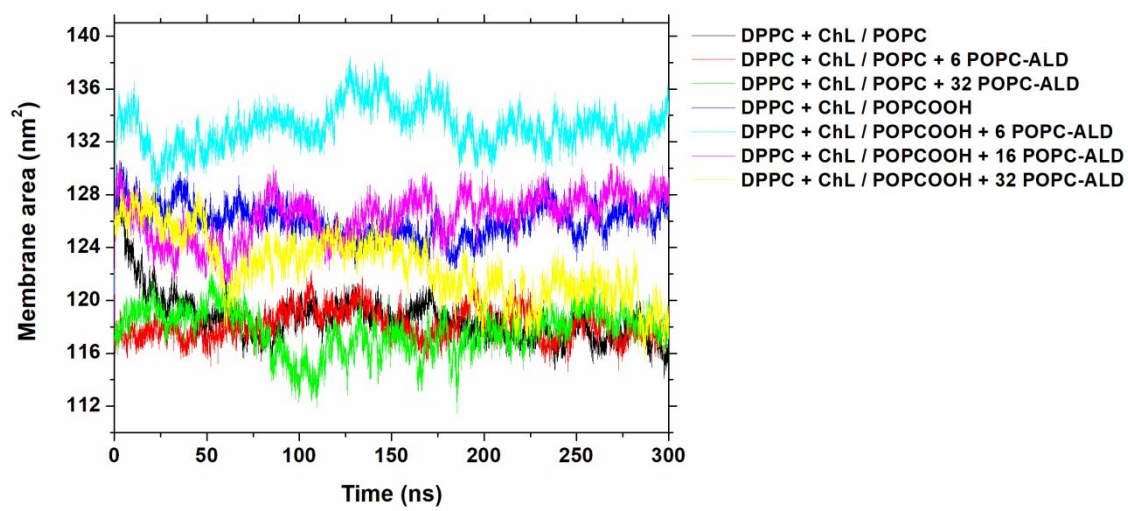

Figure S1. Membrane area along the simulation time. The values were calculated using a single model membrane for each system.

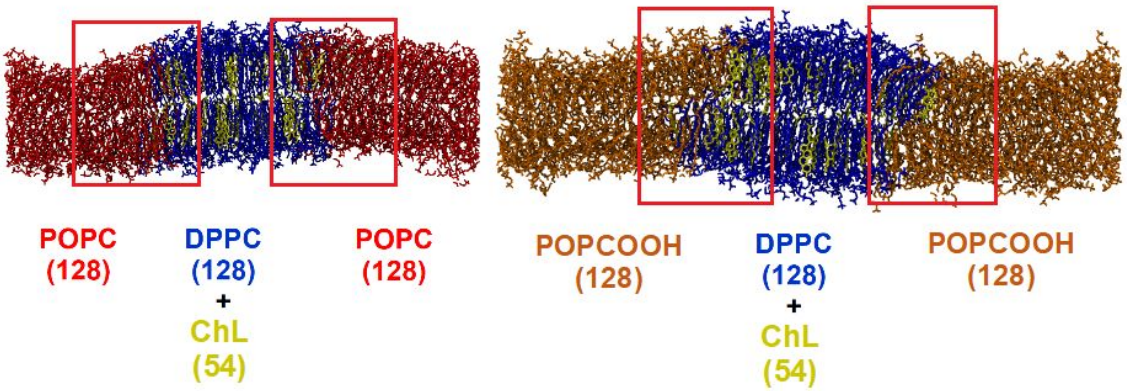

Figure S2. Snapshots of the equilibrated DPPC + ChL / POPC and DPPC + ChL / POPCOOH systems at 300 ns. The interface regions are represented in the red rectangles.

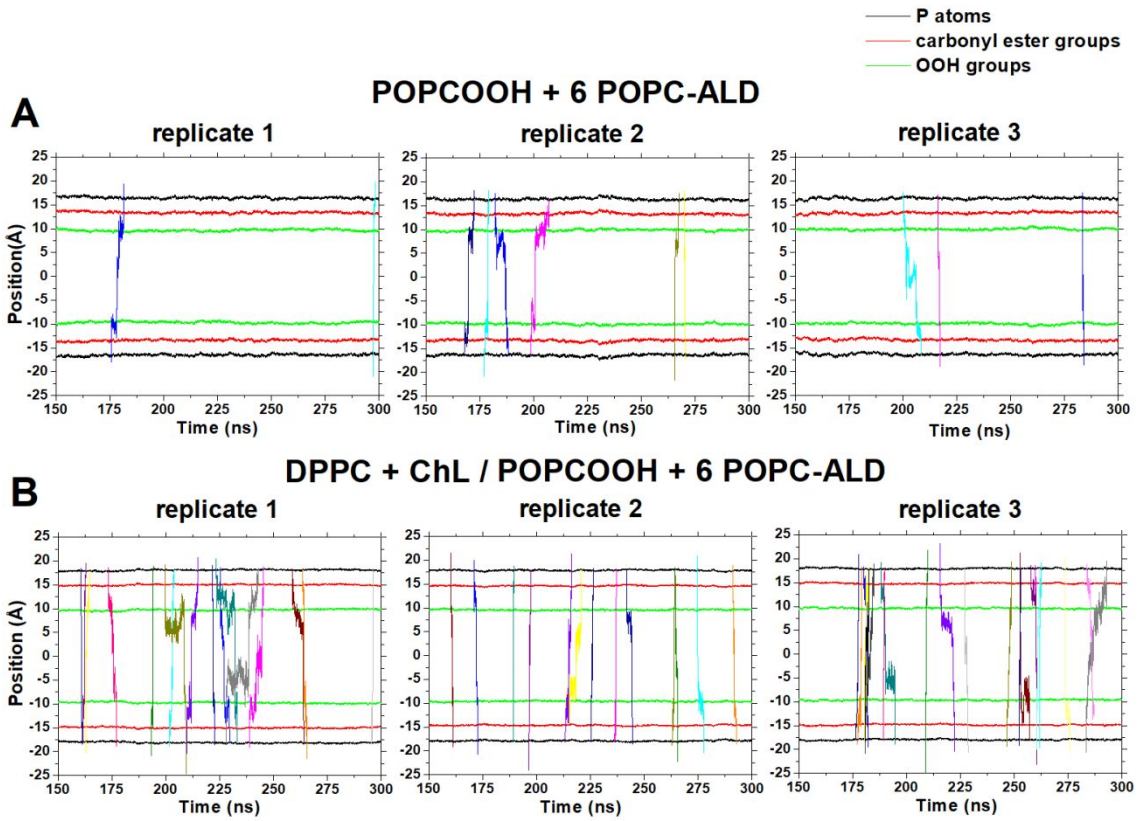

Figure S3. Permeation events for all replicates of (**A**) POPCOOH + 6 POPC-ALD and (**B**) DPPC + ChL / POPCOOH + 6 POPC-ALD systems.

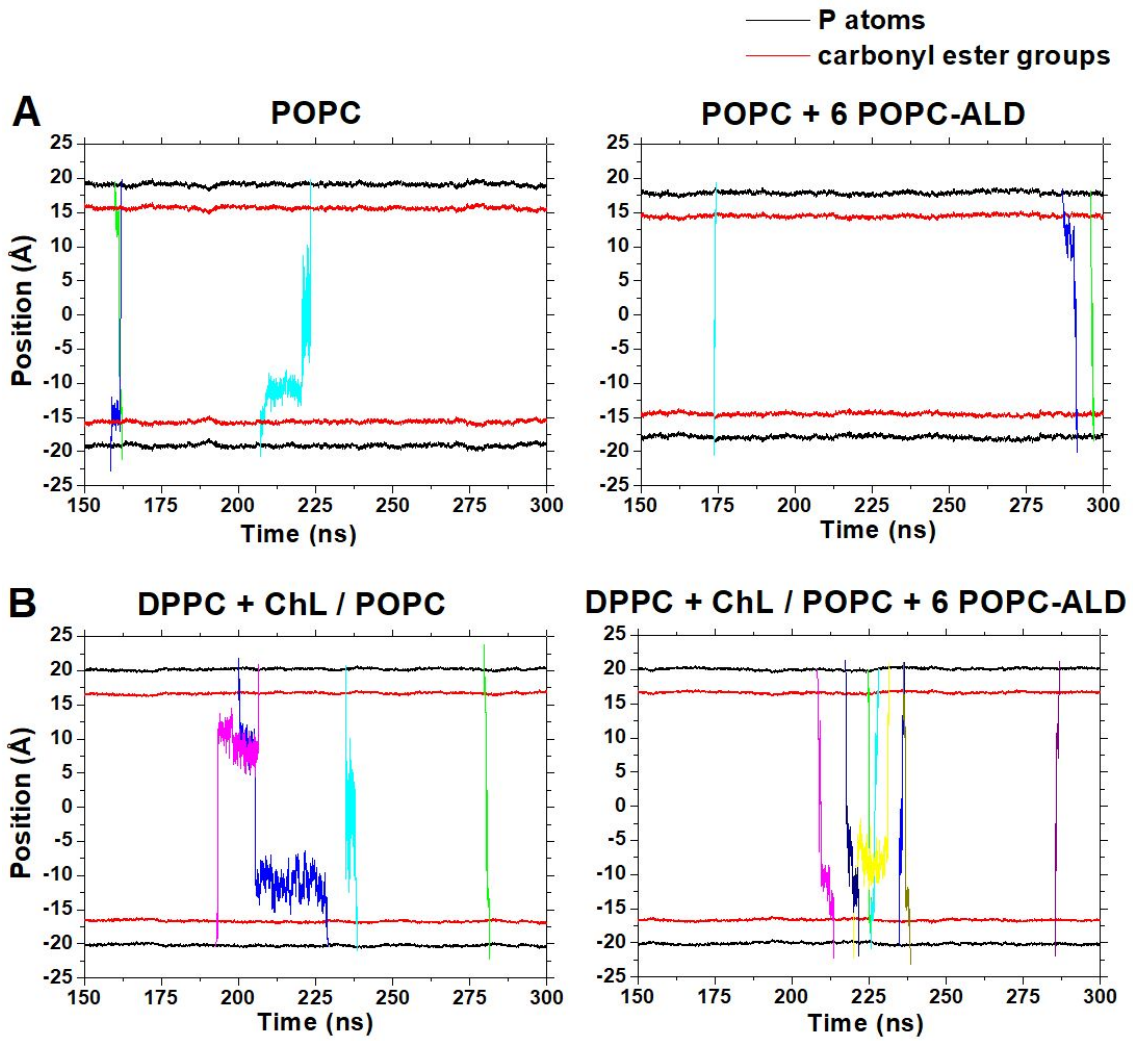

Figure S4. Number of water permeation events calculated from the last 150 ns of simulation to measure the effect caused upon addition of POPC-ALD lipid molecules to: (**A**) a homogeneous membrane composed of POPC lipid molecules, and (**B**) the POPC domains of a heterogeneous membrane.

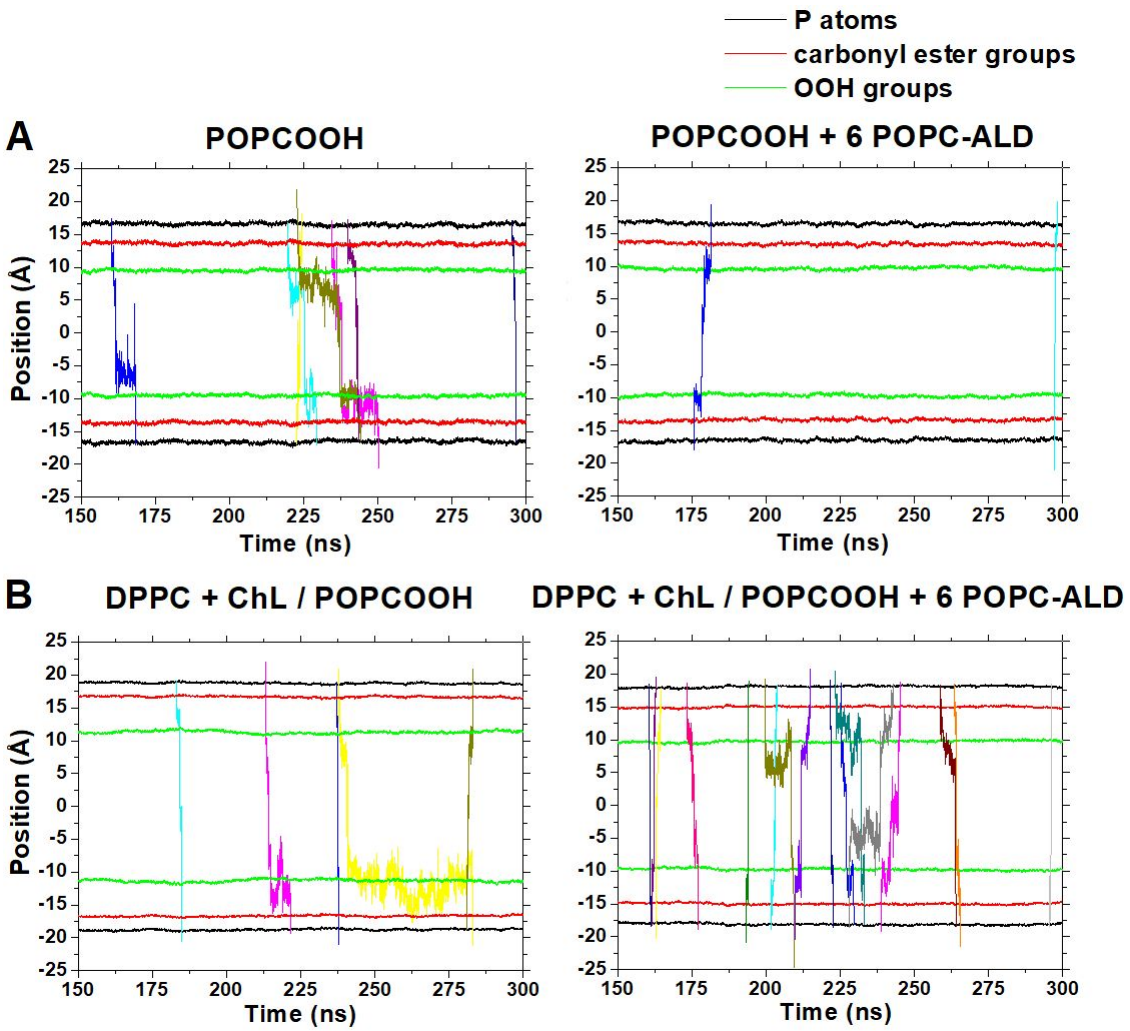

Figure S5. Number of water permeation events calculated from the last 150 ns of simulation to measure the effect caused upon addition of POPC-ALD lipid molecules to: (**A**) a homogeneous membrane composed of POPCOOH lipid molecules, and (**B**) the POPCOOH domains of a heterogeneous membrane.

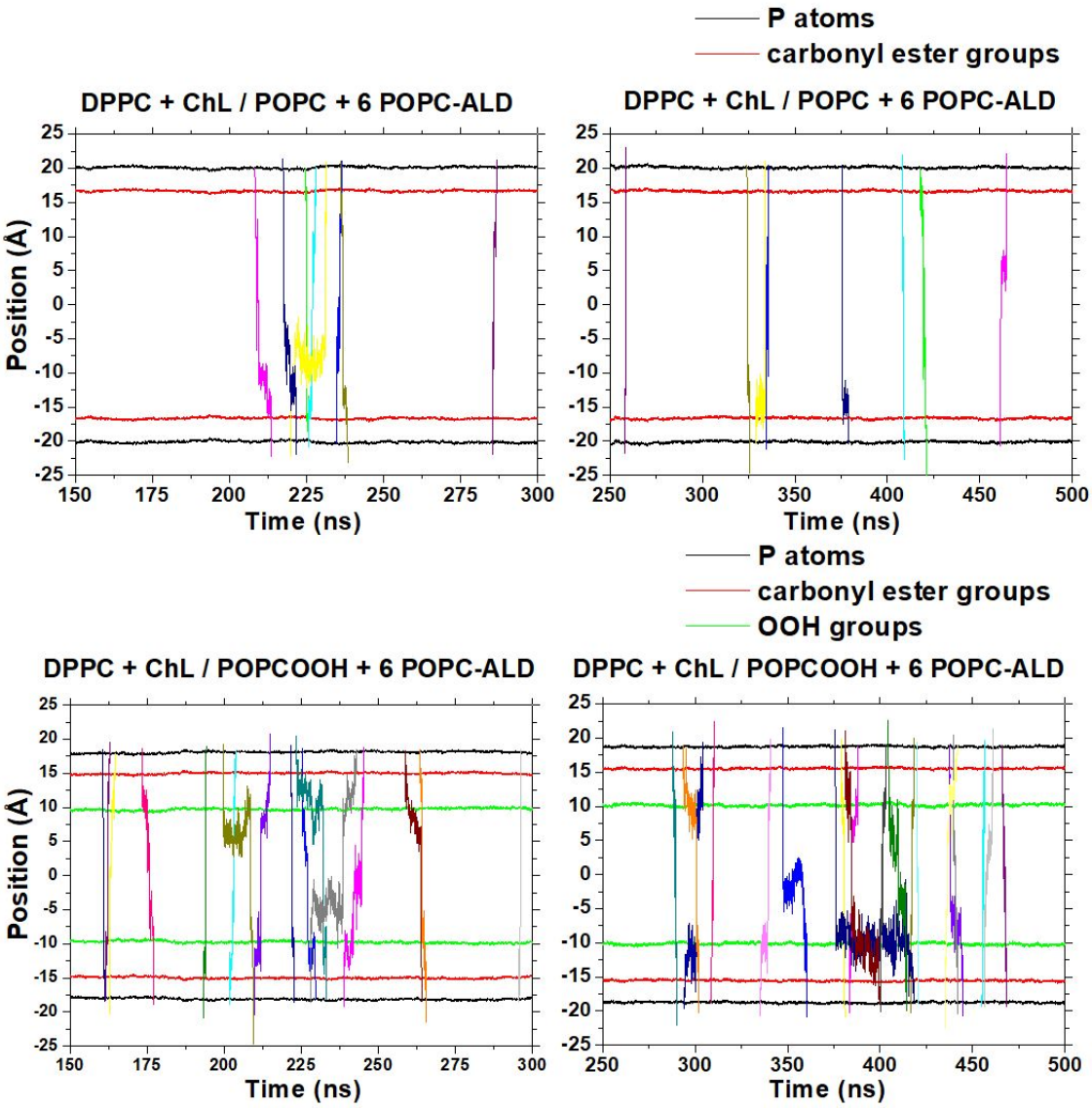

Figure S6. Number of water permeation events, calculated from the last 150 ns of simulation (on the left side) and from the last 250 ns of simulation (on the right side).

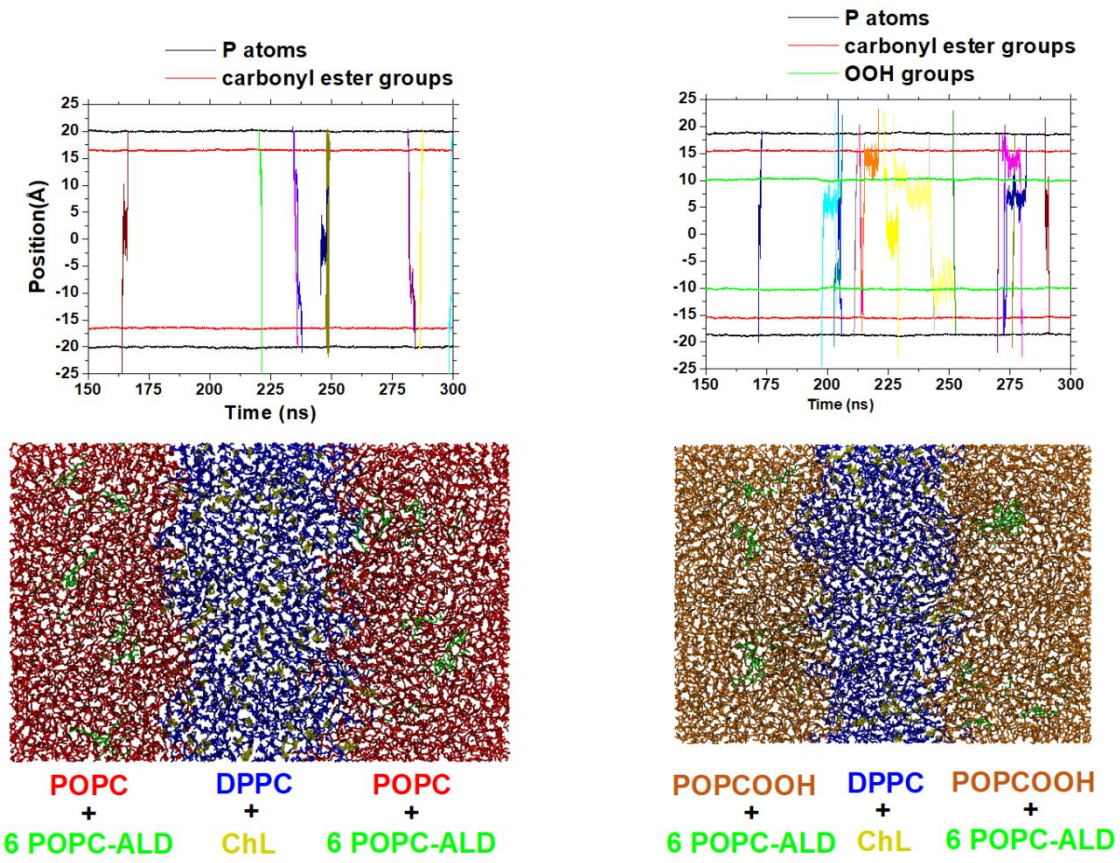

Figure S7. Number of water permeation events, calculated from the last 150 ns of simulation, by increasing the box size in the *y*-axis. The bottom images represent a top view of the model membranes at 300 ns.

Coarse-Grained Simulations

Coarse-Grained (CG) simulations were performed to obtain a phase-separation in a ternary mixture composed of DPPC/ChL/DIPC lipids. The system contains 512 lipid molecules (256 per leaflet), 128 cholesterol molecules, and 11012 water molecules (~22 water molecules per lipid molecule). The equilibration was performed with a time step of 0.03 ps for 12 μs, coupling to a heat bath at 310 K, and a pressure of 1 bar. The v-rescale thermostat and the Parrinello-Rahman barostat were used with relaxation times of 1.0 and 12.0 ps, respectively. The barostat in the lateral (*xy*) and normal (*z*) directions was coupled semi-isotropically, with a compressibility equal to 3×10^{-4} bar^{-1} in both directions. The Coulomb interactions were treated with the *Reaction-Field* (RF) method. Starting from the last configuration of the DPPC/ChL/DIPC ternary mixture after phase separation, we replaced 20% of the total lipids of the Ld domain (10% of the membrane total lipids) by lipid aldehydes, derived from either POPC lipids (POPC-ALD) or DIPC lipids (DIPC-ALD). These new systems were equilibrated further for 12 μs.

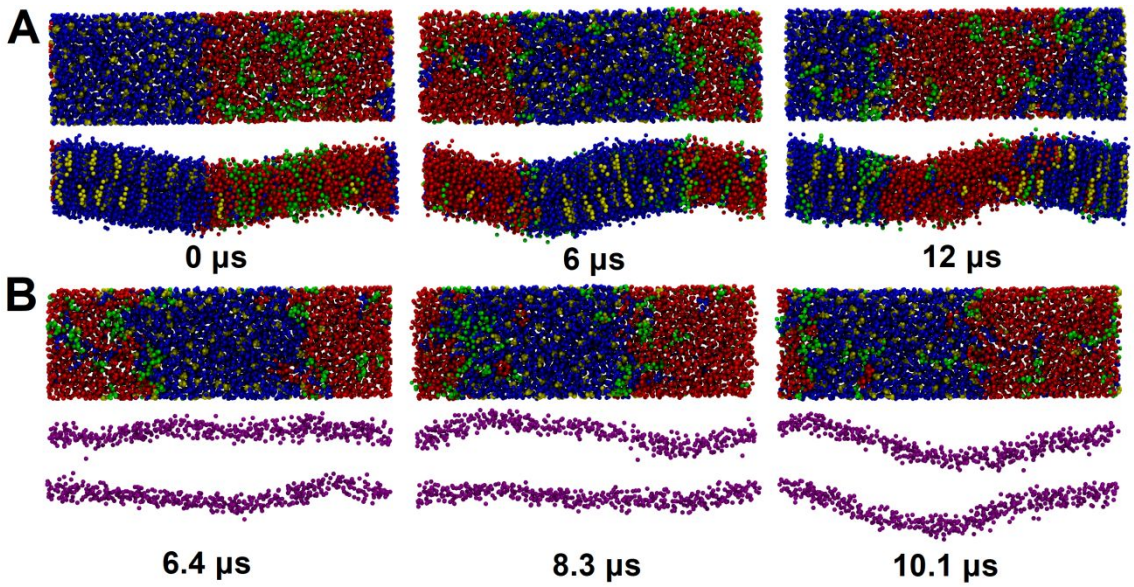

Figure S8. (**A**) Top view (upper panels) and side view (lower panels) of the CG model membrane. Blue, red, green and yellow beads represent DPPC, DIPC, POPC-ALD lipids and cholesterol molecules, respectively. Water molecules are removed for the sake of clarity. (**B**) The same model membrane. Purple beads are water molecules. Note that only the water molecules that are 5 nm away from the headgroups are represented.

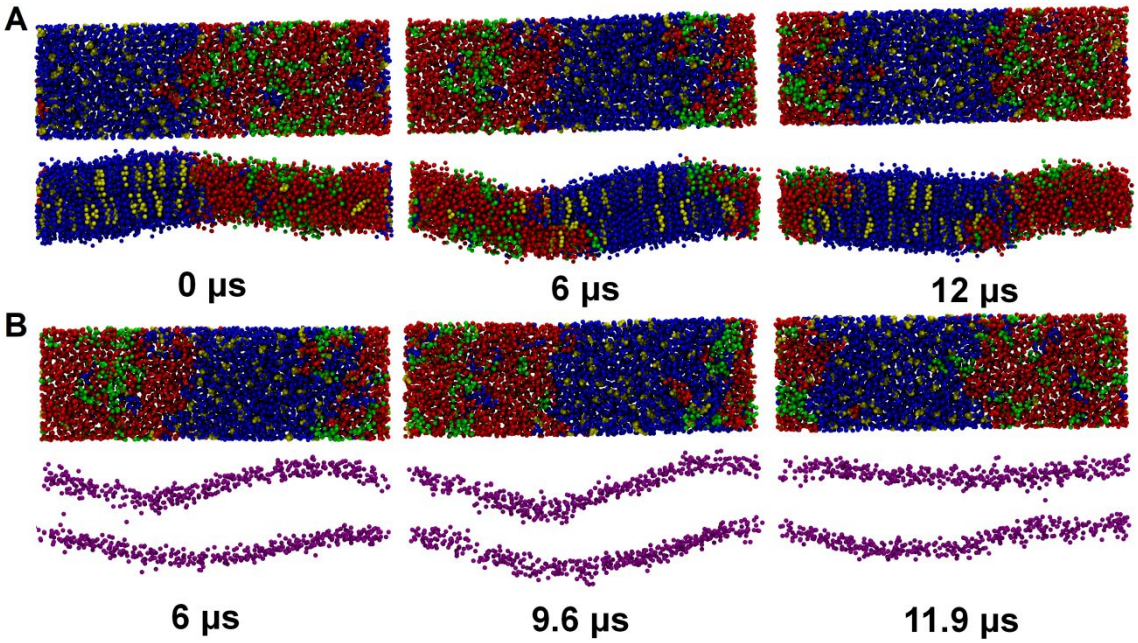

Figure S9. (**A**) Top view (upper panels) and side view (lower panels) of the CG model membrane. Blue, red, green and yellow beads represent DPPC, DIPC, DIPC-ALD lipids and cholesterol molecules, respectively. Water molecules are removed for the sake of clarity. (**B**) The same model membrane. Purple beads are water molecules. Note that only the water molecules that are 5 nm away from the headgroups are represented.

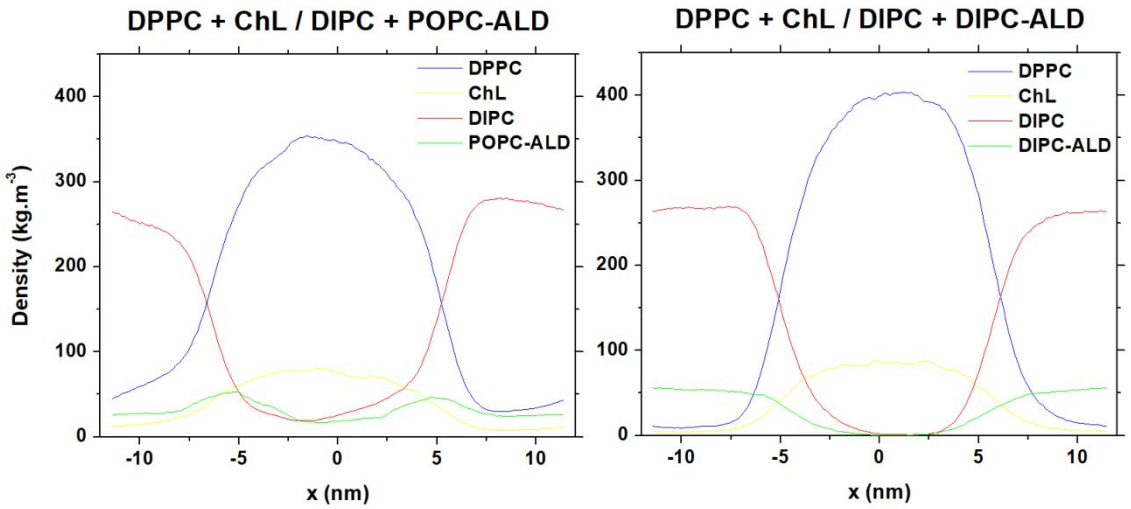

Figure S10. Density profiles of the CG model membranes in the *x*-axis, obtained from the last 6 μs of the CG MD simulations.