## **Supporting Information**

## Distribution of Lipid Aldehydes in Phase-separated Membranes: A Molecular Dynamics Study

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**Figure S1.** Top view (left side) and side view (right side) of the CG model membranes during phase-separation. DPPC, ChL, and DIPC beads are represented as van der Waals spheres in blue, yellow and red, respectively. Water molecules are not represented for the sake of clarity. The phase-separation into DPPC + ChL (left, Lo phase) and DIPC (right, Ld phase) within 3 µs is very clear.



**Figure S2.** Side view of the CG model membrane consisting of 25% POPC-ALD and 75% POPC lipids. POPC, POPC-ALD and water beads are represented as van der Waals spheres in red, green, and purple colors, respectively. No pore formation is observed, even after 10  $\mu$ s, in line with all-atom MD simulations from literature [1].



**Figure S3.** Side view (upper panels) and top view (lower panels) of the CG model membrane consisting of 100% POPC-ALD lipids. POPC-ALD lipids are represented as van der Waals spheres in different colors: blue, dark yellow, pink, and cyan colors represent the choline, phosphate, glycerol groups, and acyl chains of POPC-ALD, respectively. Water beads are represented in purple color (see upper panel). Pore formation is observed already after 18 ns, in line with all-atom MD simulations from literature [1].



**Figure S4.** Angle distribution of the CG and all-atom (AA) models of POPC-ALD lipids. The values were calculated from the last 100 ns of the equilibrium simulations. Although the maxima of the angle distributions for the CG and AA models don't match, the angle variations are very similar. As the degrees of freedom in a CG model are lower than in an AA model, their angle distribution values will not be exactly the same, but the agreement is quite satisfactory. In the legend: GL1, C1A and D2A represent the CG beads of the oxidized chains; while C1B, C2B, C3B, and C4B represent the CG beads of the non-oxidized chains. Note that the AA models from our previous atomistic simulation were converted in CG models to perform the analysis, using the *backward* tool [2].



**Figure S5.** Two-dimensional distribution of the area per lipid for the DPPC + ChL / DIPC + POPC-ALD system (top view of a single MD frame at three different times) (left side). The right side also represents a top view of the membrane, but in this case the lipids are explicit. The box sizes in x and y-axes are the same in the right and left sides, but they are represented in different scales. The rise in the area per lipid at 12  $\mu$ s can be seen from the red circles.



**Figure S6.** Two-dimensional distribution of the area per lipid for the DPPC + ChL / DIPC + DIPC-ALD system (top view of a single MD frame at three different times) (left side). The right side also represents a top view of the membrane, but in this case the lipids are explicit. The box sizes in x and y-axes are the same in the right and left sides, but they are represented in different scales. The rise in the area per lipid at 12  $\mu$ s can be seen from the red circles.



**Figure S7.** Average bilayer thickness of the CG model membranes. The values were calculated from the last 6 µs of simulation.

## **Preparation of the Atomistic Models**

We also performed atomistic MD simulations using the same lipid composition as for the CG model membranes. All initial atomistic models were built using the Packmol software [3]. We performed these simulations with the united-atom GROMOS 53A6 force field [4]. We used well-validated models for the parameters of the lipids DPPC, POPC-ALD, and cholesterol molecules [4-6]. The parameters for the DIPC and DIPC-ALD lipids were adapted from the parameters available for the GROMOS 43A1-S3 force field [7]. We provide the topologies for all molecules in the ZIP file.

We performed the equilibration during 10 µs with a time step of 2 fs. Constant temperature was maintained at 310 K using the Nose-Hoover thermostat [8,9], with a relaxation time of 0.5 ps. Pressure was anisotropically coupled at 1 bar using the Parrinello-Rahman barostat [10], with a relaxation time of 2 ps and compressibility of  $4.5 \times 10^{-5}$  bar<sup>-1</sup>. Electrostatic interactions were explicitly calculated within a cut-off of 1.1 nm with a relative dielectric constant of 1. Beyond this cut-off, the interactions were computed by the reaction-field method [11], with an infinitely large dielectric constant. Water molecules were modeled with the *simple point charge* (SPC) model [12]. We applied periodic boundary conditions in all Cartesian directions. The covalent bond lengths were constrained using the LINCS algorithm [13].



**Figure S8.** Top view of the atomistic model membranes, where the Ld domain is composed either of (**A**) DIPC and POPC-ALD lipids or (**B**) DIPC and DIPC-ALD lipids. Water molecules are not represented for the sake of clarity. Even after 10  $\mu$ s, we observe no accumulation of POPC-ALD (**A**) and DIPC-ALD (**B**) at the interface between the Lo/Ld domains.



**Figure S9.** Density profile of the atomistic model membranes, where the Ld domain is composed either of (**A**) DIPC and POPC-ALD lipids or (**B**) DIPC and DIPC-ALD lipids. We observe many fluctuations (due to the too limited simulation time), but no clear maximum in the density profile, hence no accumulation of POPC-ALD (**A**) or DIPC-ALD (**B**) at the interface between the Lo/Ld domains.

	area per l	inid (nm²)		
	and bilayer thickness (nm) <sup>a</sup>		thickness mismatch (nm) <sup>b</sup>	
DPPC + ChL / DIPC				
	CG	atomistic	CG	atomistic
	simulation	simulation	simulation	simulation
DPPC	0.63 ± 0.01	-		
	(4.41 ± 0.07)		0.00	
DIPC	0.74 ± 0.01		0.96	_
	(3.45 ± 0.14)			
DPPC + ChL / DIPC + POPC-ALD				
DPPC	0.63 ± 0.01	$0.62 \pm 0.00$	0.97	0.76
	(4.20 ± 0.11)	(4.14 ± 0.09)		
DIPC +	0.72 ± 0.01	0.65 ± 0.02		
POPC-ALD	(3.23 ± 0.20)	(3.38 ± 0.25)		
DPPC + ChL / DIPC + DIPC-ALD				
DPPC	0.63 ± 0.01	0.63 ± 0.00	1.02	0.87
	(4.32 ± 0.11)	(4.14 ± 0.09)		
DIPC +	0.74 ± 0.01	0.64 ± 0.01		
DIPC-ALD	(3.30 ± 0.21)	(3.27 ± 0.22)		

**Table S1.** The area per lipid and bilayer thickness of each domain, as well as the thickness mismatch compared between our CG and atomistic models. The values were calculated from the last 6 µs and 100 ns of simulations for the CG and atomistic models, respectively.

<sup>a</sup>The bilayer thickness is represented between brackets. Due to the high computational cost, we selected only the most relevant systems to perform atomistic simulations: DPPC + ChL / DIPC + POPC-ALD and DPPC + ChL / DIPC + DIPC-ALD.

<sup>b</sup>The thickness mismatch was calculated as the difference between the bilayer thickness of the Ld (DIPC + POPC-ALD or DIPC + DIPC-ALD) and Lo (DPPC + ChL) domains.

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