

Electronic Supplementary Information to *Freezing of Stressed Bilayers and Vesicles*

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This document contains the explanation of how the simulation data was prepared, from initial configurations, to equilibration, to production. Further analysis is also presented, along with a brief explanation of the analysis methods.

SIMULATION SETUP

The simulations were prepared by pre-equilibrating for 100 ns a symmetric DPPC bilayer at 330 K, a temperature well above the phase transition of even the most asymmetric bilayer. Following this, lipids were removed randomly from one of the leaflets to get to the wanted asymmetries, and these asymmetric bilayers were further pre-equilibrated for another 100 ns. From the last 10 ns of these runs, initial configurations were chosen at random for the actual production runs. The production runs, three at each temperature for each asymmetry, making 18 in total, lasted for 1 μ s each, and only the last 200 ns of the trajectories were used analysis. The water sometimes froze in the $T = 273$ K simulations. In this case, the data was discarded and a new run started. Listed in Table 1, are all the parameters used in the simulations. In this example the temperature is fixed at 273 K, and it is the only parameter that varies between simulations.

DATA, ANALYSIS & DISCUSSION

To compute the tension per leaflet we define the cumulative surface tension as $\gamma(z) = \frac{1}{2} \int_{-\infty}^z (P_{xx} + P_{yy} - 2P_{zz}) dz$, where P is the pressure tensor averaged over slabs spanning the xy -plane, the plane on which the membrane rests. One should note that while in the xy -plane the tensor can be highly anisotropic due to lipid ordering, $P_{xx} + P_{yy}$ as the trace of a tensor (P_{zz} being a constant as required by mechanical equilibrium) is coordinate system independent. By definition, $\gamma(z)$ will tend to the surface tension at infinity, which in the semi-isotropic pressure ensemble is a vanishing quantity. Under these conditions we may set the equimolar surface anywhere, so we choose the point where the normalized densities (*i.e.* if one leaflet has half the amount of lipids of the other, its density would be scaled by two) of the opposing leaflets are equal and evaluate $\gamma(z)$ there. This value γ^* , usually close to the maximum of $\gamma(z)$, is defined as the surface tension of each leaflet, the inner leaflet taking a positive value of γ^* and the outer $-\gamma^*$.

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comm-grps	DPPC W
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nstvout	5000
nstfout	0
nstlog	1000
nstenergy	100
nstxtcout	1000
xtc_precision	100
xtc-grps	
energygrps	DPPC W
nstlist	10
ns_type	grid
pbcr	xyz
rlist	1.2
coulombtype	Shift
rcoulomb.switch	0.0
rcoulomb	1.2
epsilon_r	15
vdw.type	Shift
rvdw.switch	0.9
rvdw	1.2
DispCorr	No
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tc-grps	DPPC W
tau_t	1.0 1.0
ref_t	273 273
Pcoupl	berendsen
Pcoupltype	semiisotropic
tau_p	0.2 0.2
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ref_p	1.0 1.0
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unconstrained_start	no
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lincs_warnangle	30

TABLE I. Parameters used in MD simulations

We define P_{xx} , P_{yy} as the eigenvalues of the local 2×2 tensor (*i.e.* z -component removed). In Figs. 1–6, pressure profiles ($\frac{1}{2}(P_{xx} + P_{yy})$) are shown for each run (each figure has three graphs, and each of these corresponds to a different run), followed by Figs. 7–12, where the asymmetries of the pressure profiles ($\frac{1}{2}(P_{xx} - P_{yy})$) are

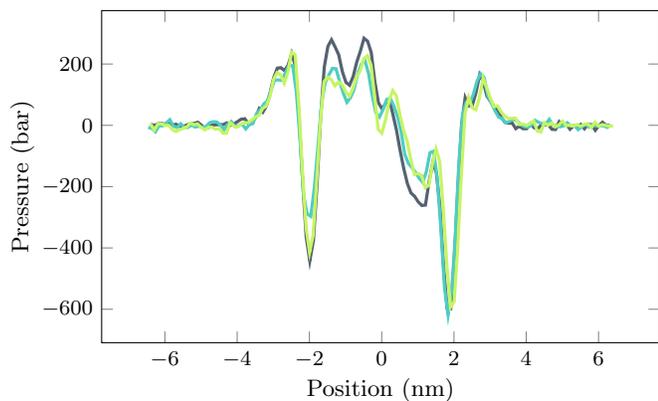


FIG. 1. Pressure profiles ($\frac{1}{2}(P_{xx} + P_{yy})$) for asymmetry 0.8, 273 K.

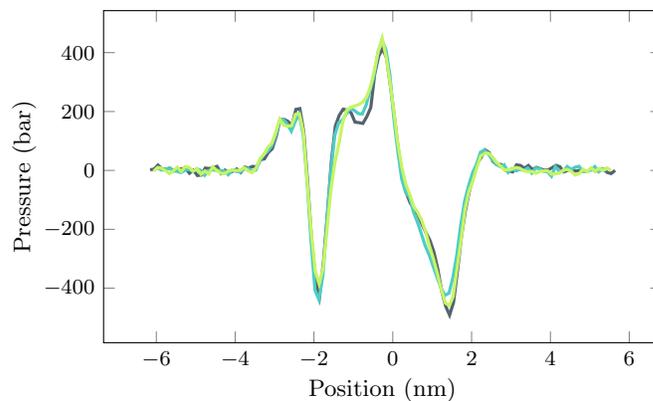


FIG. 4. Pressure profiles ($\frac{1}{2}(P_{xx} + P_{yy})$) for asymmetry 0.5, 273 K.

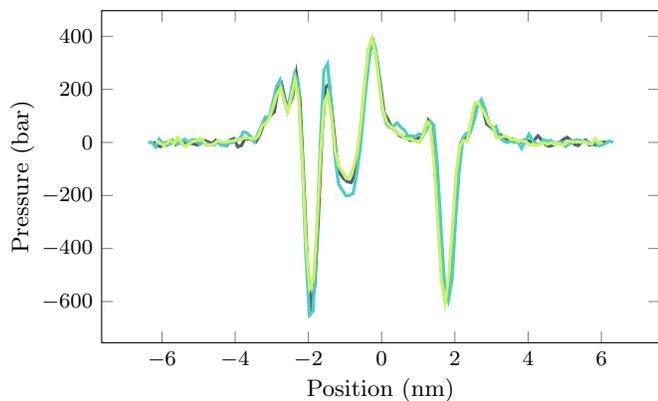


FIG. 2. Pressure profiles ($\frac{1}{2}(P_{xx} + P_{yy})$) for asymmetry 0.8, 295 K.

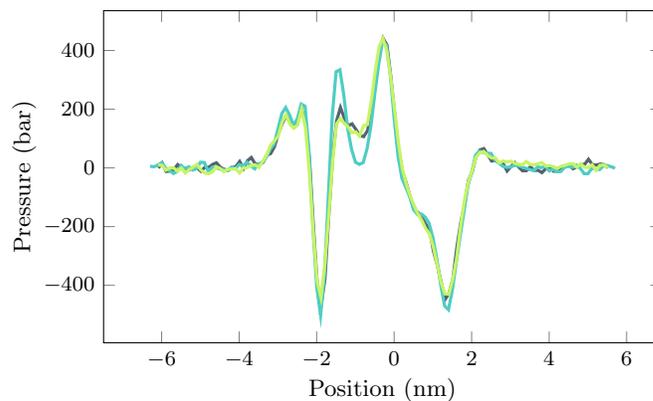


FIG. 5. Pressure profiles ($\frac{1}{2}(P_{xx} + P_{yy})$) for asymmetry 0.5, 295 K.

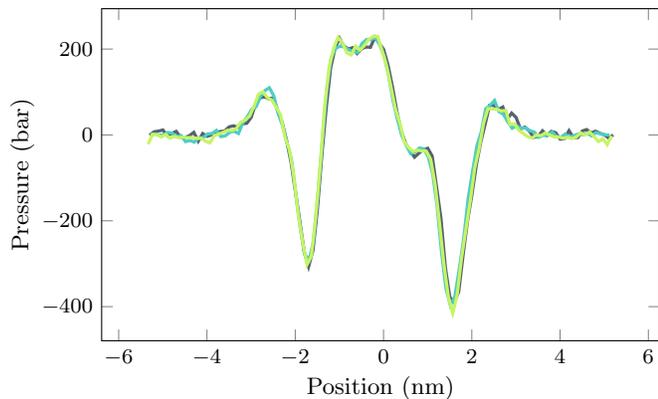


FIG. 3. Pressure profiles ($\frac{1}{2}(P_{xx} + P_{yy})$) for asymmetry 0.8, 323 K.

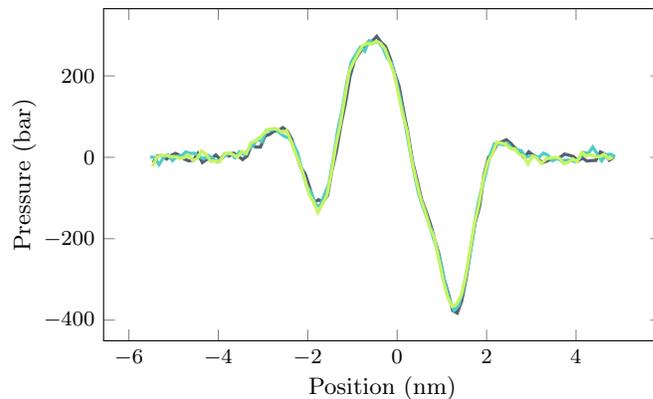


FIG. 6. Pressure profiles ($\frac{1}{2}(P_{xx} + P_{yy})$) for asymmetry 0.5, 323 K.

plotted. This asymmetry arises from lipid ordering and therefore provides a simple measure of the portion of the leaflets that are in the gel phase; a value 0 meaning no ordering, *i.e.* the fluid phase. The sign does not matter, as it is merely a definition of direction; One could plot the angle between the eigenvectors and would find it nearly

constant. These plots, especially the latter set, should be compared to the results presented in the paper, as one can readily verify that indeed the system with an asymmetry of 0.8 has fluid state in both leaflets at 323 K (Fig. 9), gel state in one leaflet and a fluid state in the other at 295 K (Fig. 8), and finally at 273 K the

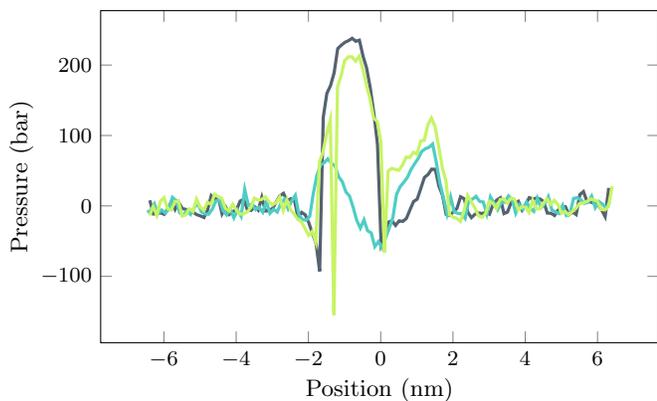


FIG. 7. Pressure asymmetries ($\frac{1}{2}(P_{xx} - P_{yy})$) for asymmetricity 0.8, 273 K.

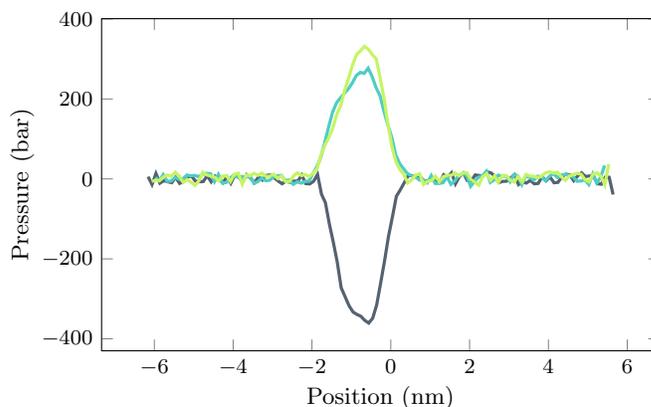


FIG. 10. Pressure asymmetries ($\frac{1}{2}(P_{xx} - P_{yy})$) for asymmetricity 0.5, 273 K.

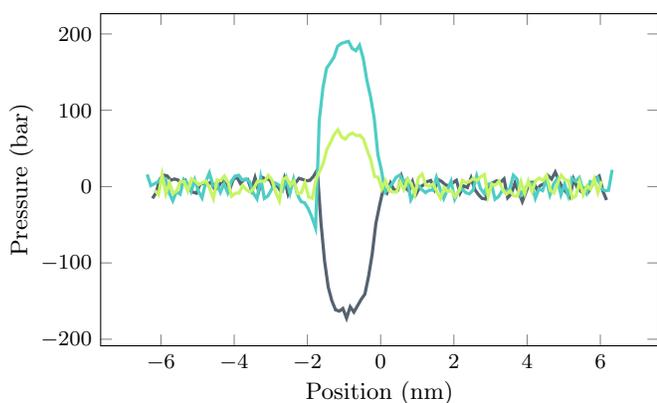


FIG. 8. Pressure asymmetries ($\frac{1}{2}(P_{xx} - P_{yy})$) for asymmetricity 0.8, 295 K.

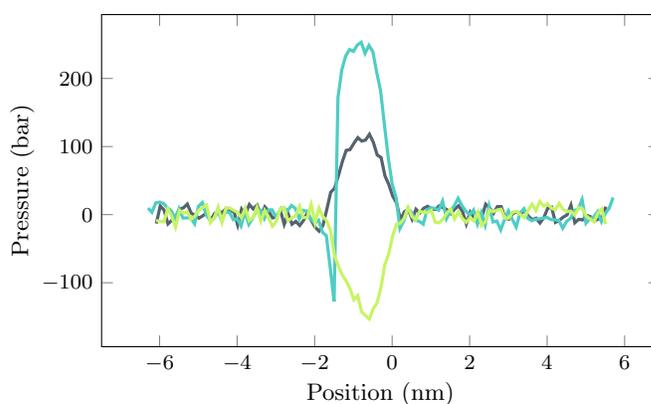


FIG. 11. Pressure asymmetries ($\frac{1}{2}(P_{xx} - P_{yy})$) for asymmetricity 0.5, 295 K.

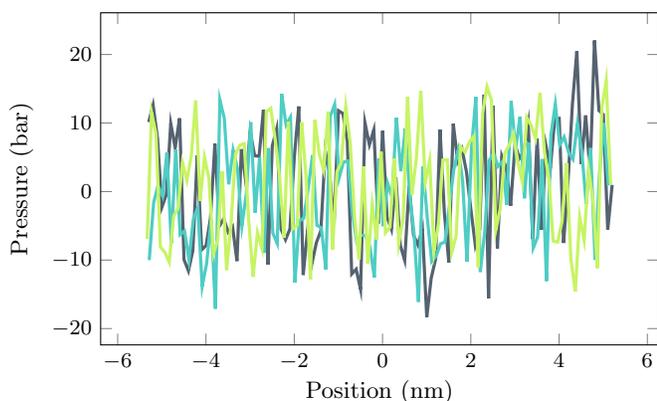


FIG. 9. Pressure asymmetries ($\frac{1}{2}(P_{xx} - P_{yy})$) for asymmetricity 0.8, 323 K.

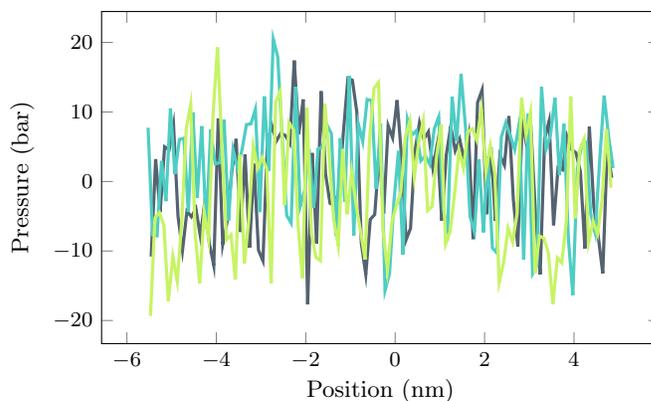


FIG. 12. Pressure asymmetries ($\frac{1}{2}(P_{xx} - P_{yy})$) for asymmetricity 0.5, 323 K.

gel state partially appears even on the less dense leaflet (Fig. 7). Similarly it can be seen that for asymmetricity 0.5, there is never ordering in the less dense leaflet and that indeed at 323 K both leaflets are in the fluid state.

While the pressure profile data is not shown here, we

took the fluid bilayer at 323 K and quenched it to a temperature of 317 K, and one of the leaflets spontaneously froze (in a 1 μ s simulation). When we tried to revert the process by changing the temperature of the the frozen system suddenly back to 323 K (again for 1 μ s), it re-

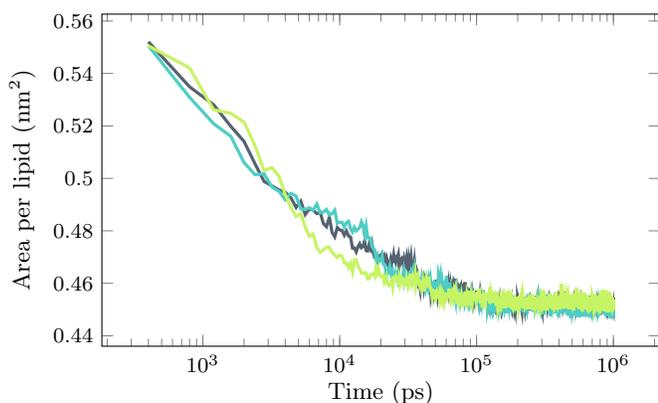


FIG. 13. Areas per lipid for asymmetry 0.8, 273 K.

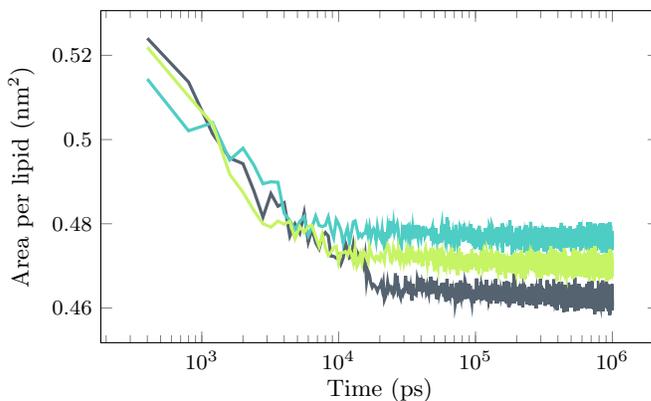


FIG. 16. Areas per lipid for asymmetry 0.5, 273 K.

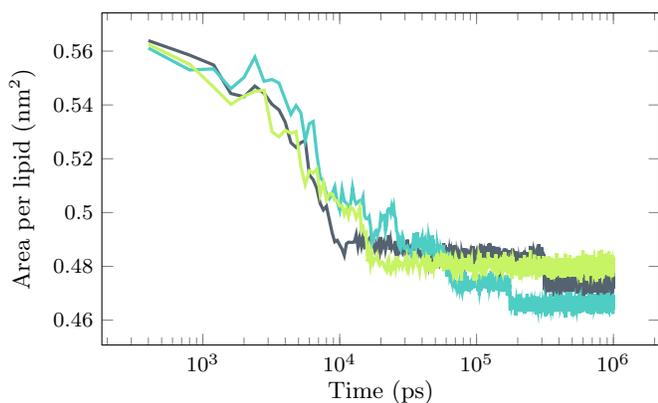


FIG. 14. Areas per lipid for asymmetry 0.8, 295 K.

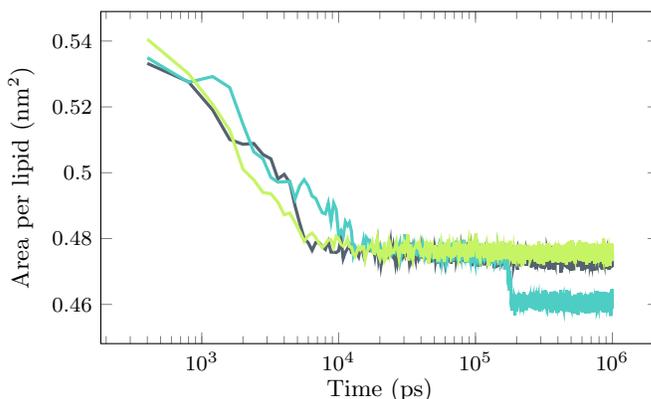


FIG. 17. Areas per lipid for asymmetry 0.5, 295 K.

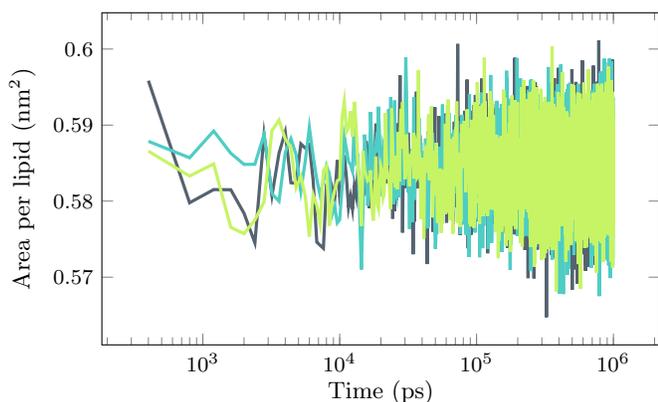


FIG. 15. Areas per lipid for asymmetry 0.8, 323 K.

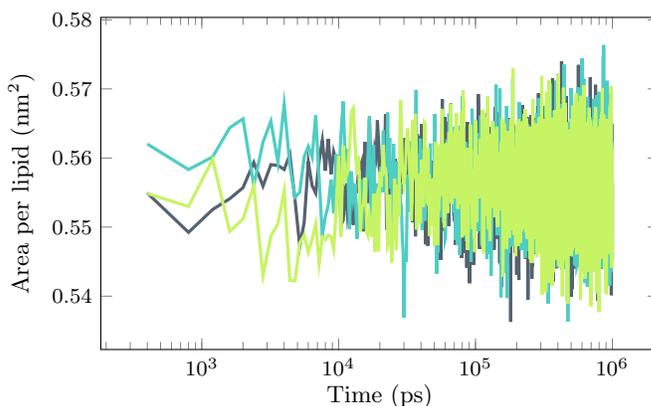


FIG. 18. Areas per lipid for asymmetry 0.5, 323 K.

mained in the gel state. This is an example of hysteresis and indicates that the phase transition temperature is not very far from these temperatures.

As the freezing of the leaflet with more lipids is a rather radical process, it can easily be seen in graphs of the total area of the leaflet. Therefore in Figs. 13–18 we have plotted the area per lipid of the outer leaflet. While it may appear that the systems have not equilibrated,

note that the x -axis is logarithmic, and that no (sudden) changes in area have occurred in the latter half of the simulation (and certainly not during the analysis period of the final 200 ns). Thus the (meta)stable states are in fact very stable. Indeed, if the area could shrink, it would so as to decrease the free energy associated with surface tension. Note, however, that because there is an asymmetric composition of lipids, it would be favourable

to flip-flop and in principle some of the changes that are apparent in the figures might be steps towards rupture or poration, and thus further away from the phenomenon that we want to model. Comparing these figures to the pressure profiles earlier, one can to some extent reason about their shapes.

Finally, shown in the snapshots of molecular dynamics simulations (Figs. 19–22) is the state of each leaflet of a bilayer of asymmetry 0.8, *i.e.* 256 lipids on the outer leaflet, 205 on the inner. The theoretical model, as explained in the paper, suggests that both the leaflets should be in the fluid state at a high temperature. It is indeed obvious from Fig. 19 that the outer leaflet has ordering typical of the fluid state, and from Fig. 20 that the same is true for the inner layer. In these figures taken from the top (or bottom) of the system one can even see that the tails are not ordered, again pointing to the fact

that both the leaflets are in the fluid state, albeit the outer leaflet is clearly more compressed. The red square represents the size of the simulation box.

As the temperature is lowered (Figs. 21, 22), the ordering of the lipids becomes apparent. In the outer leaflet (Fig. 21), all the lipids turn into their gel state: It is difficult to see the lipid tails as they are pointing almost directly away from the camera, and the packing resembles that found in lattices. This is what would be expected of a system below its freezing point. More interestingly, however, as depicted in Fig. 22, the inner leaflet does not fully freeze. This, too, was predicted by the theoretical framework. One can see (horizontal) stripes of both fluid and gel state lipids (where the tails of the lipids can be seen, they are disordered, *i.e.* in a fluid state), which means the states coexist and phase separate justifying the high ω_{gf} used in the model.

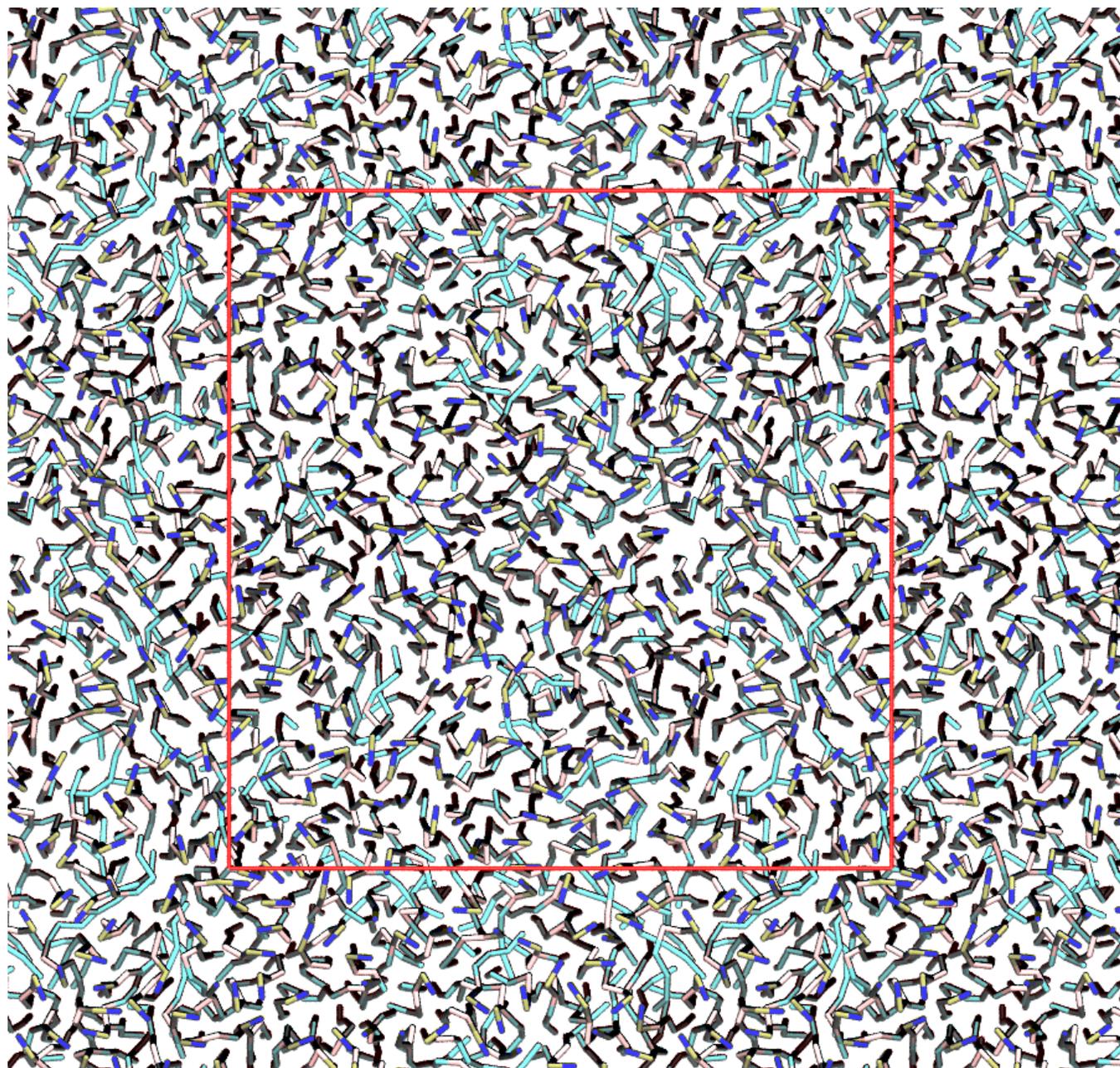


FIG. 19. Outer leaflet at $T = 323$ K

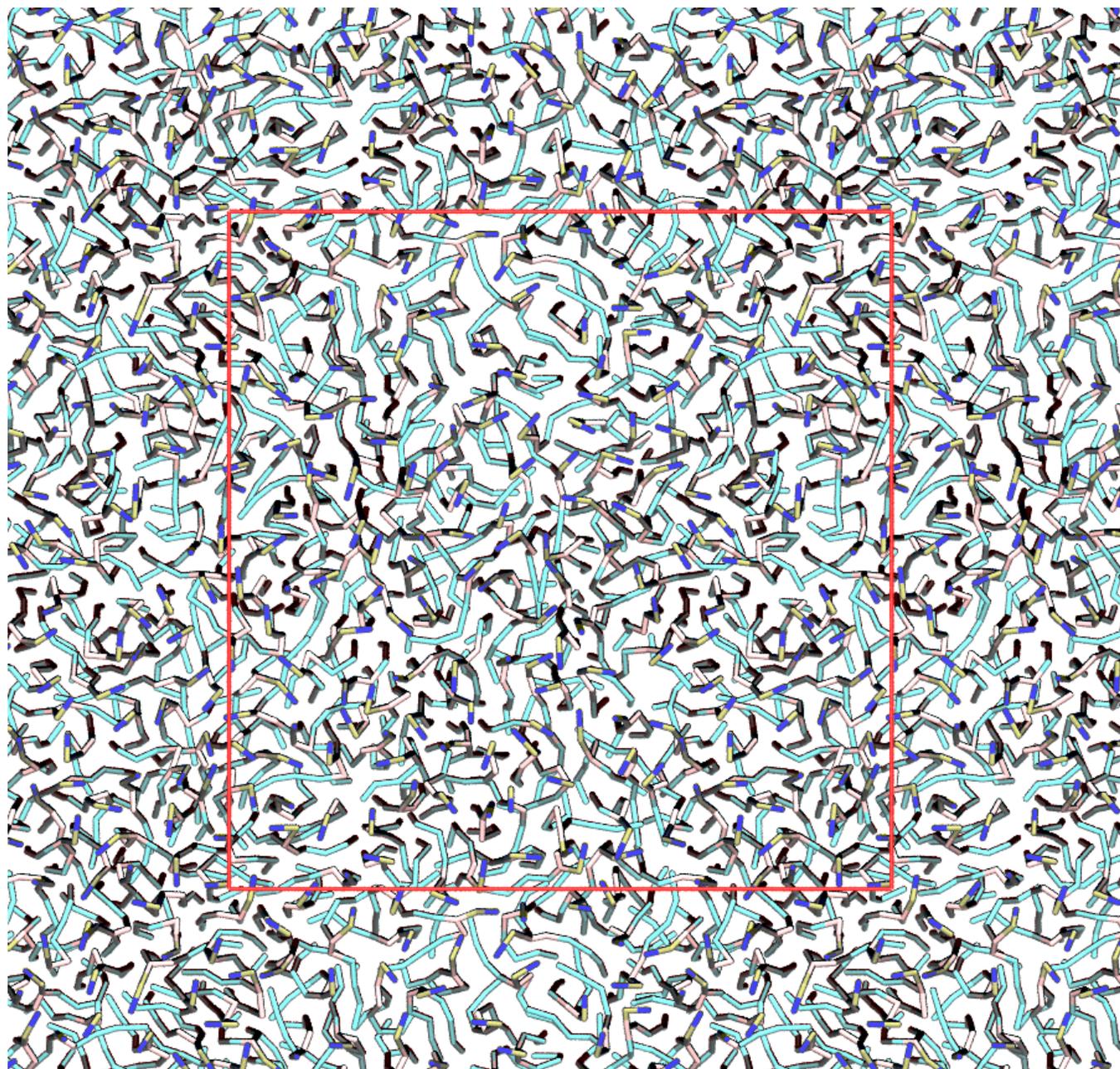


FIG. 20. Inner leaflet at $T = 323$ K

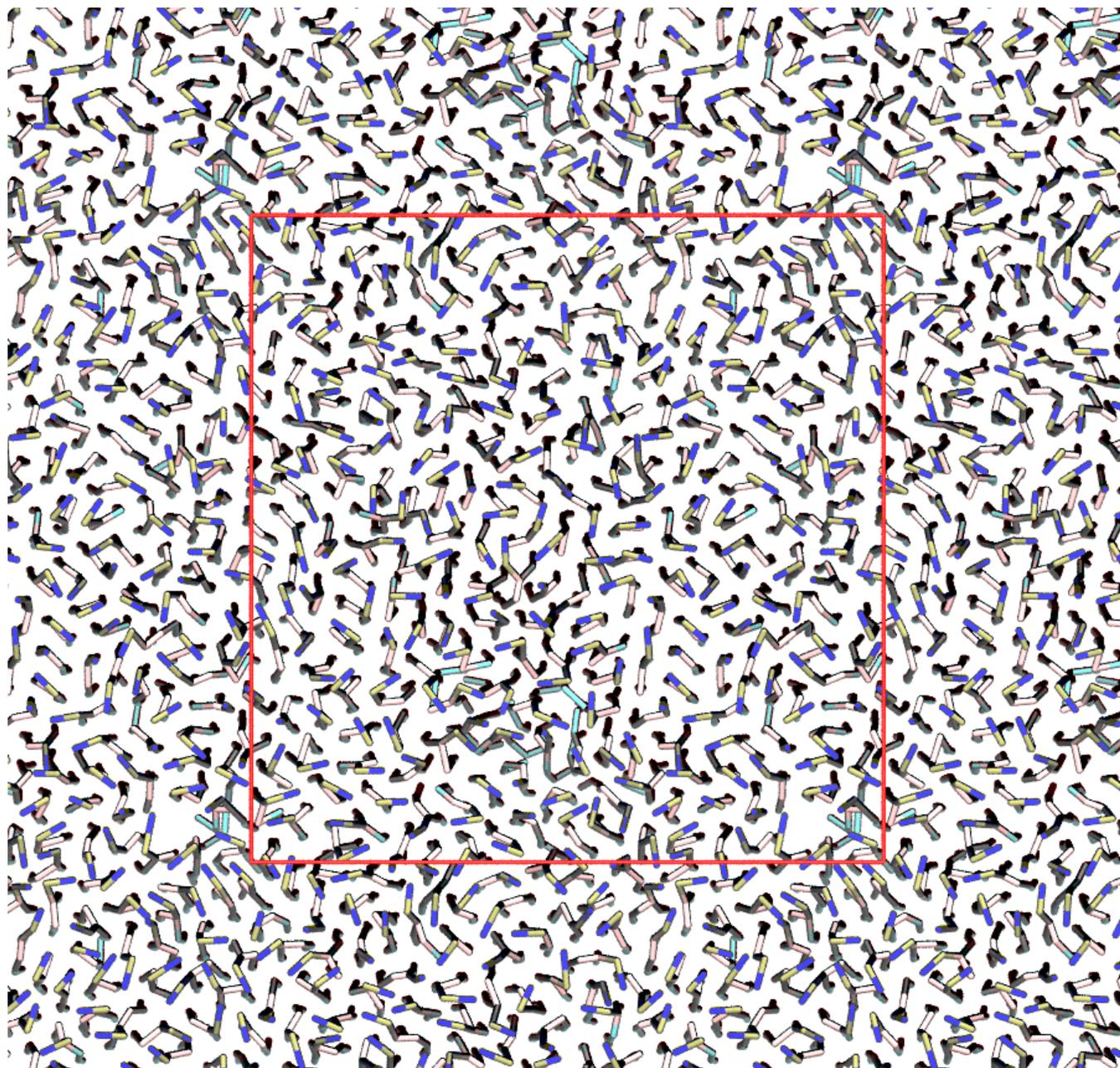


FIG. 21. Outer leaflet at $T = 273$ K

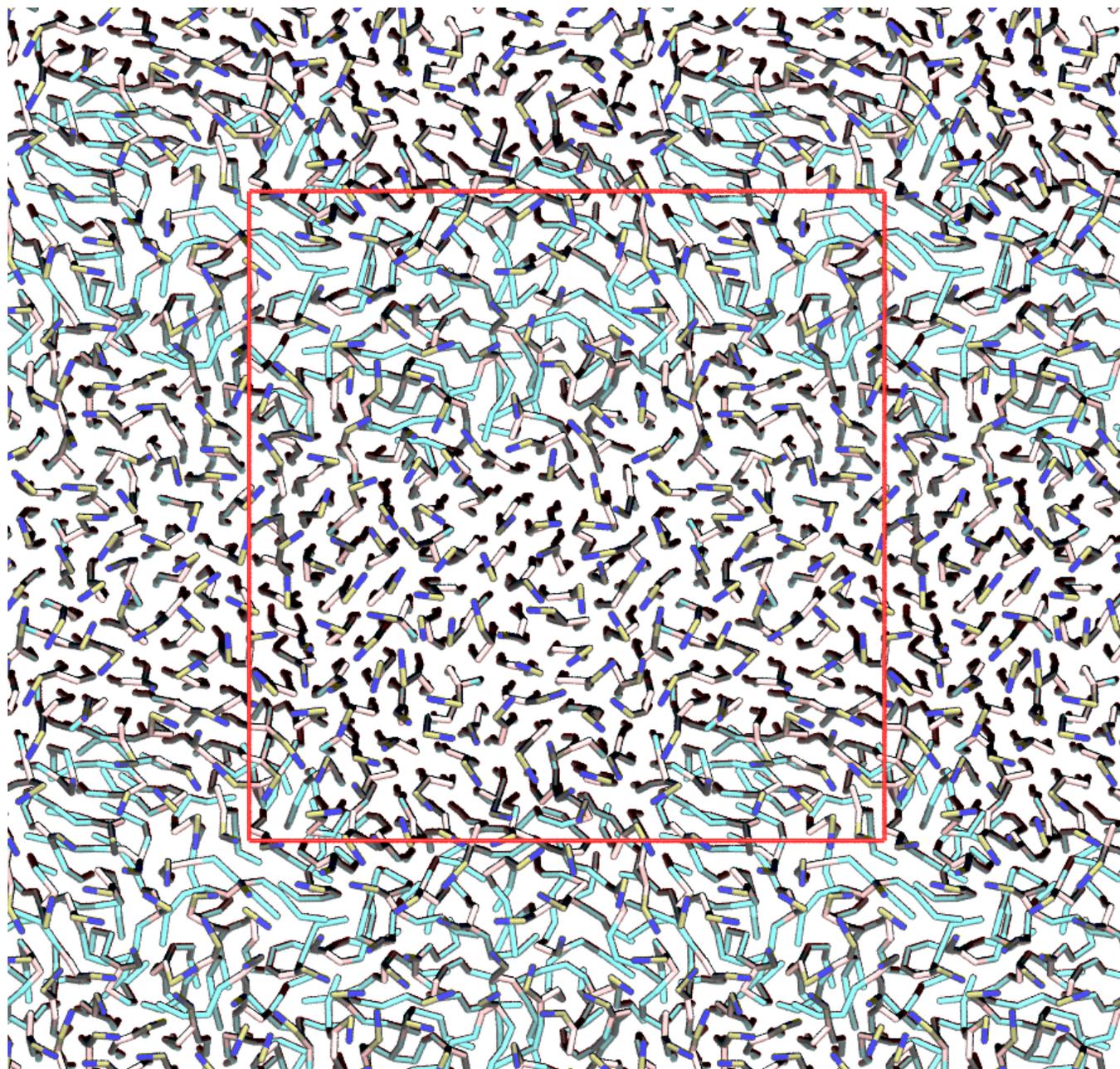


FIG. 22. Inner leaflet at $T = 273$ K