Supporting Information for:

Non-Converged Constraints Cause Artificial Temperature Gradients in Lipid Bilayer Simulations

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CONTENTS

I. SIMULATION TIMES, ANALYSIS TIMES, AND SCREENED SETTINGS OF THE COARSE-GRAINED SIMULATIONS OF THE DPPC/DLIPC/CHOL MIXTURE

Two sets of simulations were performed for the ternary lipid mixture DPPC/DLiPC/CHOL. The first set started from a random distribution of the lipids and was performed for a total simulation time of 15 µs. The second set started from the configuration after 10 µs of the simulations with one temperature coupling group of the first set and was performed for additional 5 µs. The simulation settings of all simulations of the two sets were identical (see main text for details) with an exception of the thermostat (set 1), the temperature coupling groups used for the lipids (set 1), the LINCS settings (set 1 and 2), and the time step (set 2). An overview of the screened settings and the corresponding analysis times is given in Table S1.

Most of the analyzed membrane properties were averaged over the last 2 µs of the simulations. In the first set of simulations of the ternary lipid mixture DPPC/DLiPC/CHOL, the temperature, the CHOL flip-flop, and the contacts were averaged over longer time intervals; in the second set, the temperature and the contact ratio were averaged over different time intervals. In the case of the temperature, the reason is of technical nature since the temperature of the different groups of molecules was calculated from the velocities in the GROMACS `.trr` file which were not saved with a high frequency (every 50 ns). In order to ensure a proper average, we increased the averaging time. The CHOL flip-flop was averaged over

10 µs because the flip-flop occurs quite rarely. Thus, the increased averaging time interval ensures better statistics. The contacts were averaged for the last 5 µs to enable averaging over the oscillations on the µs time scale (see Fig. 2d in the manuscript) which reflect the dynamics at the phase boundary. The contact ratios evaluated for the second set were averaged only over the last 1 µs of the 5 µs long simulations initiated from a phase-separated membrane in order to allow sufficient demixing to take place.

TABLE S1. Simulation times, screened simulation settings, and analysis times of the CG simulations of the ternary lipid mixture DPPC/DLiPC/CHOL (abbreviations: APL: area per lipid; NH: Nosé–Hoover; vr: velocity rescaling).

	set 1				set 2										
sim. time (µs)	15				5										
T coupl. gr.	1	3	1	1	1										
thermostat	vr	vr	vr	NH	vr										
`lincs_order`	4	4	8	4	4	6	8	10	12	4	6	8	10	12	12
`lincs_iter`	1	1	2	1	1	1	1	1	1	2	2	2	2	2	3
time step (fs)	20				10 / 20 / 30										30
	analysis time (µs)														
T	10				4.9										
APL	2				—										
area compress.	2				—										
order param.	2				—										
thickness	2				—										
CHOL flip-flop	10				—										
contacts	5				—										
rel. neighb.	2				—										
diff. const.	—				2										
contact ratio	—				1										

II. ADDITIONAL RESULTS FROM THE COARSE-GRAINED SIMULATIONS

A. Spatially Resolved Temperature Gradients

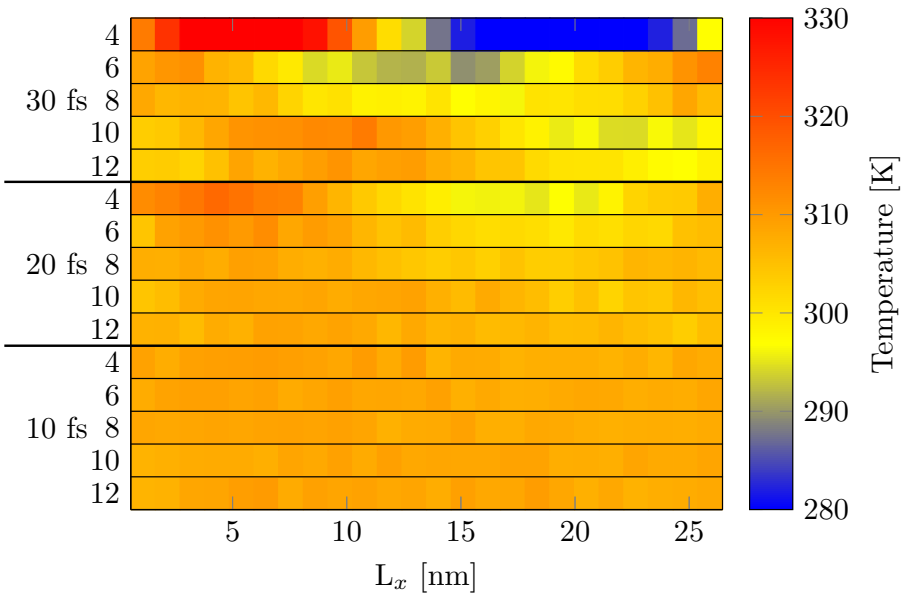

FIG. S1. Temperature along the x axis of the simulation box, chosen so as to faithfully represent the temperature of the two membrane domains. The y direction has been averaged over for clarity. Here, `lincs_iter=1` was used along with the indicated timestep (10,20,30 fs) and `lincs_order` (4,6,8,10,12). The increase in LINCS settings along with the decrease of the timestep always acts to eliminate the temperature gradients observed at the conventional settings of `lincs_iter=1`, `lincs_order=4` and 30 fs timestep. To capture the smaller lateral variations in the temperature of system, the colorbar was choosen so as to not cover the whole range: the temperature differences observed with `lincs_iter=1`, `lincs_order=4` are even more significant (≈70 K%) than indicated by the graphs.

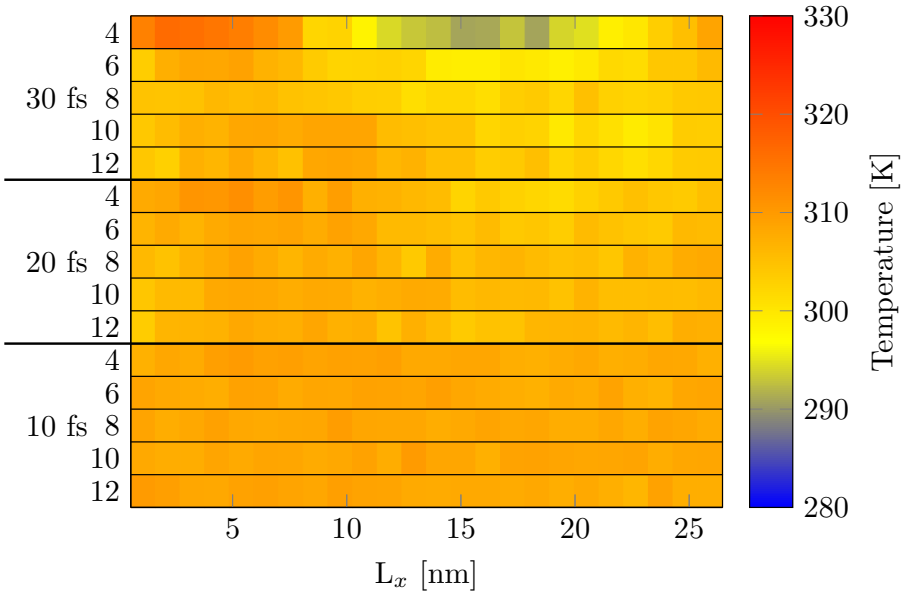

FIG. S2. Temperature along the x axis of the simulation box, chosen so as to faithfully represent the temperature of the two membrane domains. The y direction has been averaged over for clarity. Here, `lincs_iter=2` was used along with the indicated timestep (10,20,30 fs) and `lincs_order` (4,6,8,10,12). The increase in LINCS settings along with the decrease of the timestep always acts to eliminate the temperature gradients observed at the conventional settings of `lincs_iter=1`, `lincs_order=4` and 30 fs timestep.

B. Use of an Alternative Thermostat

TABLE S2. Temperatures of the ternary lipid mixture DPPC/DLiPC/CHOL simulated using one temperature coupling group for the lipids for a Nosé-Hoover thermostat analyzed for the last 10 µs of the simulation.

	membrane	DPPC	DLiPC	CHOL	water
temperature (K)	310.1 ± 0.2	305.6 ± 0.2	318.2 ± 0.3	305.6 ± 0.3	310.0 ± 0.1

C. Average Number of Contacts in Ternary Lipid Mixture

TABLE S3. Average number of contacts between the lipid types in the ternary mixture DPPC/DLiPC/CHOL analyzed for the linker beads of the phospholipids and the ROH bead of cholesterol using a cutoff distance of 0.7 nm for the last 5 µs of simulation (see also Fig. 1(d) in the main text).

	single temperature coupling group	three temperature coupling groups	`lincs_iter` = 2 `lincs_order` = 8
DPPC-DPPC	$7,196 \pm 22$	$7,038 \pm 26$	$7,060 \pm 22$
DPPC-DLiPC	640 ± 27	828 ± 46	769 ± 46
DPPC-CHOL	$3,174 \pm 19$	$2,972 \pm 8$	$3,000 \pm 16$
DLiPC-DLiPC	$4,276 \pm 36$	$4,130 \pm 37$	$4,181 \pm 26$
DLiPC-CHOL	563 ± 13	702 ± 5	670 ± 11
CHOL-CHOL	357 ± 4	313 ± 2	318 ± 2

D. Temperature Differences with Different LINCS Settings

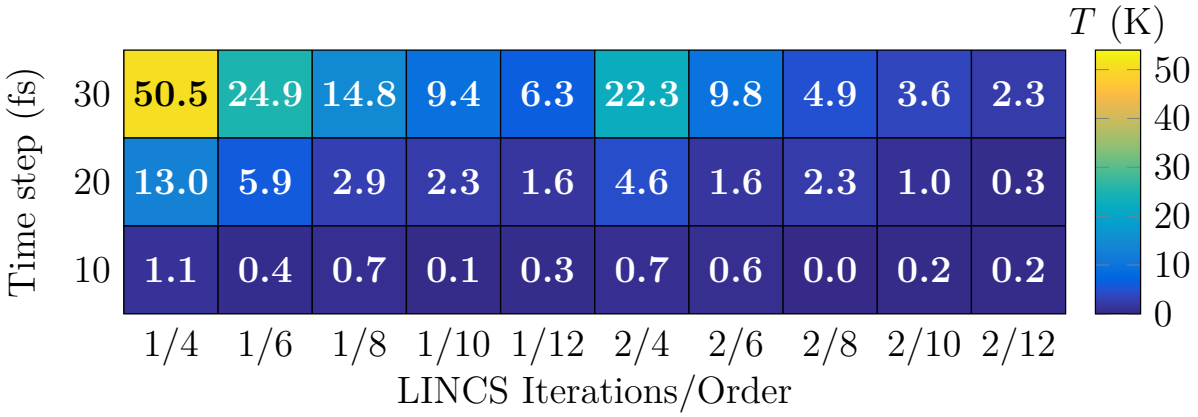

FIG. S3. Temperature difference of DPPC and DLiPC lipids with different LINCS settings in the ternary DPPC/DLiPC/CHOL mixture. This figure contains more LINCS settings than Fig. 3 shown in the main text.

E. Membrane Properties with Different LINCS Settings

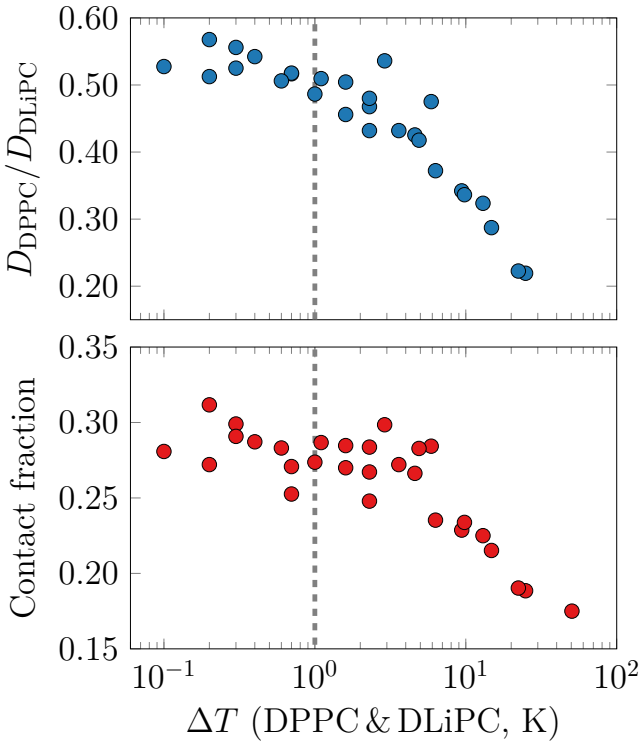

FIG. S4. The ratios of diffusion coefficients of DPPC and DLiPC (top panel) and the contact fractions (bottom panel) obtained with different LINCS settings in the ternary DPPC/DLiPC/CHOL mixture. The values are plotted as a function of the temperature difference between DPPC and DLiPC, which is also shown in Fig. S3. This figure contains more data points than Fig. 4 in the main text.