



HAL
open science

Ripple-like instability in the simulated gel phase of finite size phosphocholine bilayers

Vivien Walter, Céline Ruscher, Adrien Gola, Carlos M Marques, Olivier Benzerara, Fabrice Thalmann

► To cite this version:

Vivien Walter, Céline Ruscher, Adrien Gola, Carlos M Marques, Olivier Benzerara, et al.. Ripple-like instability in the simulated gel phase of finite size phosphocholine bilayers. *Biochimica et Biophysica Acta: Biomembranes*, 2021, 1863 (11), pp.183714. 10.1016/j.bbamem.2021.183714 . hal-03442327

HAL Id: hal-03442327

<https://hal.science/hal-03442327v1>

Submitted on 23 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Ripple-like instability in the simulated gel phase of finite size phosphocholine bilayers

Vivien Walter^{a,*}, Céline Ruscher^b, Adrien Gola^b, Carlos M. Marques^b,
Olivier Benzerara^b, Fabrice Thalmann^{b,**}

^a*Department of Chemistry, King's College London, Britannia House, 7 Trinity Street,
SE1 1DB, London, United Kingdom*

^b*Institut Charles Sadron, CNRS and University of Strasbourg, 23 rue du Loess, F-67034
Strasbourg, France*

Abstract

Atomistic molecular dynamics simulations have reached a degree of maturity that makes it possible to investigate the lipid polymorphism of model bilayers over a wide range of temperatures. However if both the fluid L_α and tilted gel $L_{\beta'}$ states are routinely obtained, the $P_{\beta'}$ ripple phase of phosphatidylcholine lipid bilayers is still unsatisfactorily described. Performing simulations of lipid bilayers made of different numbers of DPPC (1,2-dipalmitoylphosphatidylcholine) molecules ranging from 32 to 512, we demonstrate that the tilted gel phase $L_{\beta'}$ expected below the pretransition cannot be obtained for large systems (equal or larger than 94 DPPC molecules) through common simulation settings or temperature treatments. Large systems are instead found in a disordered gel phase which displays configurations, topography and energies reminiscent from the ripple phase $P_{\beta'}$ observed between the pre-transition and the main melting transition. We show how the state of the bilayers below the melting transition can be controlled and depends on thermal history and conditions of preparations. A mechanism for the observed topographic instability is suggested.

Keywords: Lipid bilayers, Molecular dynamics simulations, Phase transition

*

**

Email addresses: vivien.walter@kcl.ac.uk (Fabrice Thalmann),
fabrice.thalmann@ics-cnrs-unistra.fr (Fabrice Thalmann)

1. Introduction

Lipid membranes are fundamental components of living organisms, for their pivotal role in the structure and the biochemistry of the cells. Their properties are for a major part the consequence of the organisation of the phospholipid molecules that compose them to a large extent. This organisation is best revealed by experimenting with artificial lipid bilayers [1, 2]. Through a perfect control of the molecular composition, an extended range of geometries and the possibility to insert membrane proteins and all sort of molecules, artificial bilayers have become a standard tool in modern biophysics [3, 4].

A striking property of pure phospholipids bilayers is to exhibit a number of thermodynamic transitions upon temperature changes, meaning that they can be found in several phases including a dense structured $L_{\beta'}$ tilted gel phase at low temperature or a disordered fluid phase at higher temperature L_{α} [3, 4, 5, 6]. Decades ago, a new phase have been identified in the most common sort of phospholipid, the phosphocholines (PC lipids) [7, 8, 3]. This new phase, specific to the PC lipids and called the ripple phase $P_{\beta'}$, is characterised by important corrugation along the bilayer and the alternation between interdigitated leaflets and non-interdigitated ones. The ripple phase is observed experimentally in pure bilayers of 1,2-dipalmitoylphosphatidylcholine (DMPC) between 16 and 24°C, 1,2-dipalmitoylphosphatidylcholine (DPPC) between 34 and 41°C and 1,2-distearoylphosphatidylcholine (DSPC) between 51 and 55°C [9, 3].

This structure is sometimes presented as resulting from an alternation of gel and fluid lipid configurations [10]. There is no consensus regarding the cause [11, 12, 13, 14, 15], and the experimental structure is still subject to detailed investigations [16]. Ripple-like instability seems to be a generic feature of various different numerical lipid models [17, 18, 19].

In a recent paper, Khakbaz and Klauda investigated the phase transition of DMPC and DPPC [20]. They reported formation of a structure resembling the $P_{\beta'}$ phase for DMPC bilayers in a range of temperature, while for DPPC only a transition from the tilted to the fluid phase was observed in bilayers composed of 70 lipids. More recently, we investigated the phase transition of DPPC bilayers using Machine Learning algorithms [21]. In membranes composed of 212 lipids, we observed a transition at 315 K from a fluid phase

36 to a condensed disordered gel phase similar to the ripple phase that persists
37 well below the pre-transition temperature of 307K and the $L_{\beta'}$ tilted phase
38 was never obtained directly from a quench of the L_{α} state.

39 In this article, we provide a detailed analysis of the nature of the phase
40 of DPPC simulated with the CHARMM36 force field below its melting tem-
41 perature. We investigate the state of DPPC bilayers at 288 K, for different
42 system sizes and different thermal routes. While the $L_{\beta'}$ phase was observed
43 for small systems, we found that a disordered gel phase, reminiscent from the
44 $P_{\beta'}$ phase, occurs in larger systems. In particular, we noticed the formation
45 of corrugations whose amplitude increases with the system sizes investigated.
46 By applying different thermal treatments, we characterize the metastability
47 of DPPC and show that disordered gel is the preferred phase of simulated
48 DPPC at 288K, in the thermodynamic limit.

49 **2. Material & Methods**

50 *2.1. System Description*

51 DPPC bilayers were obtained using the CHARMM-GUI online Membrane
52 Builder [22, 23, 24, 25]. The size of the membrane was controlled *via* the
53 number of lipids, ranging from 32 to 512 and with equal amounts of lipids in
54 both leaflets. The bilayers were hydrated with water blocks of 10 nm on each
55 side to prevent any interaction of the leaflets through the PBCs along the Z-
56 axis. DPPE and DSPC bilayers made of 64 and 212 lipids were also prepared
57 using the same process and characteristics. The exact composition of each
58 system used is given in the Supplementary Materials. All initial coordinate
59 files were made available on Zenodo [26].

60 The constructed system were minimized in energy using a steepest de-
61 scent algorithm and equilibrated by running two NVT simulations at 288 K
62 for 10 ps, with respectively a 0.001 ps and a 0.002 ps step; and two NPT
63 simulations at 288 K and 1 bar, with a 0.002 ps step for respectively 100 ps
64 and 1 ns.

65 *2.2. Simulation Runs*

66 Unless specified, the following conditions and parameters were used for
67 all simulations. All simulations were performed using GROMACS 2016.4
68 [27, 28] along with the CHARMM-36 all-atom force-field [29] (June 2015
69 version). The force field parameters for the lipid molecules were provided
70 directly by CHARMM-GUI [30, 31].

71 All the molecular dynamics simulations used the leap-frog integration al-
72 gorithm [32] with a time step set to 2 fs. The temperature was controlled
73 during the simulation using a Nosé-Hoover thermostat [33, 34] with a cor-
74 relation time of $\tau_T = 0.4$ ps, and a Parrinello-Rahman semi-isotropic baro-
75 stat [35, 36] set to 1 bar in all directions was applied to the system (correlation
76 time $\tau_P = 2.0$ ps, compressibility 4.5×10^{-5} bar $^{-1}$).

77 Lipid and water molecules were separately coupled to the thermostat.
78 Following GROMACS recommendations for the CHARMM-36 all-atom force
79 field, a Verlet cut-off scheme on grid cells was used with a distance of 1.2 nm,
80 and non-bonded interactions cut-offs (Van der Waals and Coulombic) were
81 also set to 1.2 nm. Fast smooth Particle-Mesh Ewald electrostatics was
82 selected for handling the Coulombic interactions, with a grid spacing of 4 nm.
83 A standard cut-off scheme with a force-switch smooth modifier at 1.0 nm was
84 applied to the Van der Waals interactions. We did not account for long range
85 energy and pressure corrections, and constrained all the hydrogen bonds of
86 the system using the LINCS algorithm.

87 Molecular dynamics production runs lasted for a minimum of 50 ns. When
88 temperature treatments occurred, the systems were simulated for another
89 50 ns to allow for the bilayer to reach equilibrium. In all cases the analysis
90 was performed on the last 25 ns of the simulations.

91 *2.3. Analysis*

92 The areas per lipid of the systems were measured using two different
93 methods: (i) measure by projection of the bilayer onto the XY plane of the
94 box, noted A_H^p , and (ii) measure by meshing of the water-lipid interfaces of
95 each leaflet of the bilayer, written A_H^m . The projected area per lipid was
96 measured directly in Gromacs by measuring the area of the simulation box
97 in the XY plane and by dividing it by the number of lipids per leaflet. The
98 meshed area per lipid was computed using Ovito 2.9. To do so, the surface of
99 the water blocks at the water-lipid interface was meshed using a probe sphere
100 radius of 6 and a smoothing level of 30 after removing the lipid molecules.
101 The area of the meshing was then divided by the total number of lipids in
102 the bilayer.

103 The amplitude and the period of the corrugations were measured using
104 the meshing of the water-lipid interface collected for the measurement of the
105 area per lipid. The position of the vertices of the meshing were interpolated
106 using SciPy [37] on a (200,200) uniform grid of the size of the simulation
107 box, and the height of these 200×200 points were analysed. This grid was

108 directly used to generate the height maps. The amplitude of the corrugations,
109 represented by the root mean square (RMS) height of the points on the grid,
110 were calculated by subtracting the mean and then measuring the standard
111 deviation of the height $\sigma(h)$. The period of the corrugations were calculated
112 by computing the azimuthal power spectrum density (PSD) of the points of
113 the grid and determining the frequency of the highest peak of the PSD.

114 To measure the local chain tilt direction, the positions of the atoms of
115 the tails of the lipids were extracted from the simulation files using MDAnalysis
116 [38, 39]. The vectors from the first carbon atom of each tail to all the
117 carbon atom of their respective tails were computed, before calculating the
118 mean vector. The vector field was then created by binning the membrane in
119 $n \times m$ squares and averaging over time the tilt direction vectors found in each
120 square (X_i, Y_i) . As a consequence, the vector field accounts for the average
121 direction of the tails found at this location, but are not associated to fixed
122 lipid molecules.

123 The enthalpy of the systems were extracted from the simulation using
124 directly the tool provided with Gromacs, *gmx energy*. The systems used
125 strictly had the exact same number of atoms, for both lipids and water
126 molecules, to prevent changes due to the system composition. The (tilted)
127 gel - fluid phase transition enthalpy was measured using the method from [40].
128 Briefly, the total enthalpy of a system simulated at different temperatures
129 over a wide range, here 283 to 358 K, was collected. The effects of the
130 temperature increase on the enthalpy, besides any phase transition, were
131 removed from the measurement by subtracting the affine baseline measured
132 in each phase. The values were then divided by the total number of lipids in
133 the system, and the melting transition enthalpy was calculated by integrating
134 the difference between the fitted gel and fluid baseline over the range of
135 temperature at which the transition occurs (here 308 to 318 K, accounting
136 for Gromacs accuracy in setting a temperature). For the tilted to disordered
137 gel phase transition, the enthalpy was measured by bringing the two systems
138 at the exact same temperature. The difference in enthalpy measured between
139 these two systems was then divided by the total number of lipids in the
140 system.

141 **3. Results**

142 *3.1. Corrugation formation and characterization*

143 We considered DPPC bilayers prepared with CHARMM-GUI at temper-
144 ature $T = 288\text{K}$ whose size is ranging from 32 to 512 lipids. For small
145 bilayers made of 64 lipids or less, we observed the formation of a smooth L_{β}
146 tilted gel phase, in agreement with the numerous experiments and simula-
147 tions reported in the literature [41]. The geometry of these bilayers remained
148 stable over time, even after a 50 ns simulation. When larger systems (> 94)
149 lipids were concerned, we noticed the formation of a corrugation deforming
150 significantly the leaflets (*cf* Figure 1).

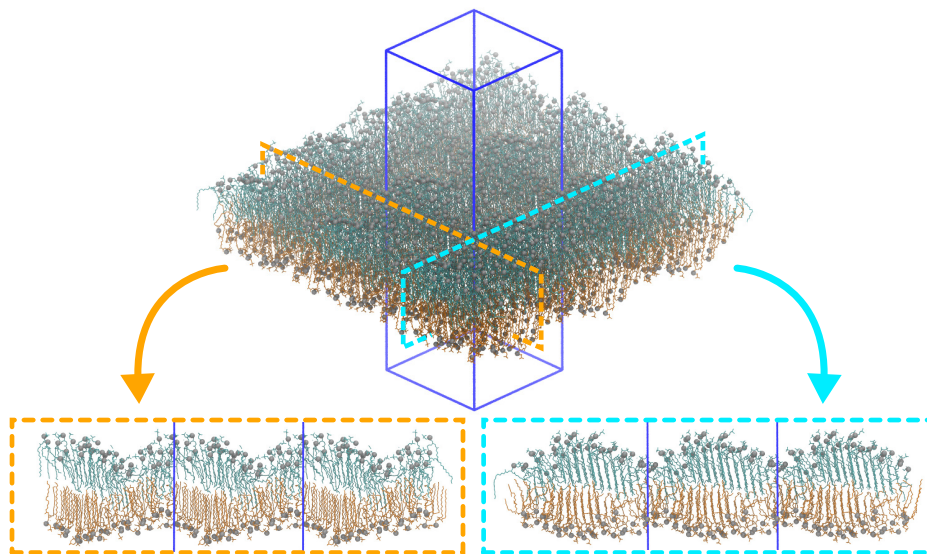


Figure 1: DPPC bilayer made of 212 lipids simulated at 288 K right after construction and equilibration. 2.5 nm thick slices of the system along each axis are shown on the bottom, highlighting the corrugations of the membrane. Top and bottom lipid leaflets are coloured respectively in cyan and orange while the phosphorus atoms are shown as silver beads. Hydrogen atoms were removed from the screenshot to improve readability. The PBC box is displayed using the blue plain lines.

151 The corrugations takes place along both simulations box axis (x and y).
152 As already observed and investigated in details for DMPC by Khakbaz and
153 Klauda [20], the molecular configurations of the lipids do not appear uniform
154 along the corrugations, and the typical stretched tails of the gel phase seem
155 to turn into the typical disordered tail configurations of the fluid phase in the

156 thin portions of the corrugations where interdigitation happens. These varia-
 157 tions in configuration can be highlighted by computing and mapping the local
 158 segment order parameter S_{mol} of each atom of each lipid (*cf* Figure 2(a)).

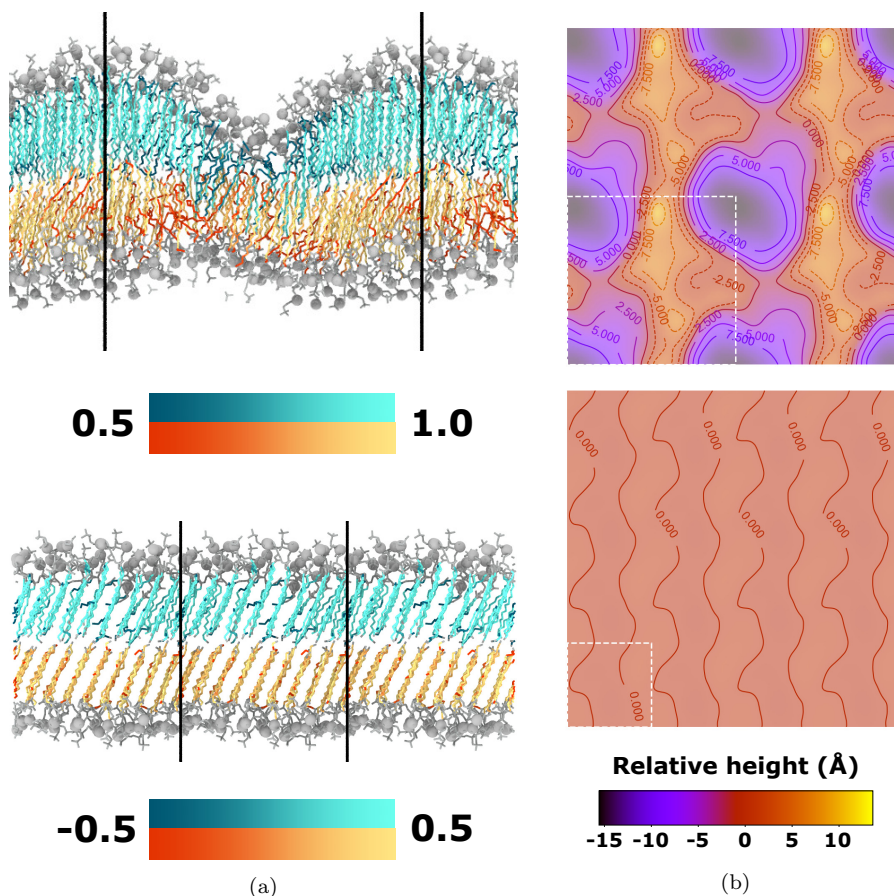


Figure 2: (Left) Topography of the upper leaflet of (Top) 256 and (Bottom) 64 lipid bilayers, highlighting the corrugations observed in the membrane. Both contour plot shares the same color code and contour line scale, in Å. The white dashed line show the system before replication. (Right) Representation of slices of the lipid membranes on the left, with a color code on the tail atoms corresponding to their order parameters.

159 We systematically characterized the corrugation and probed its evolution
 160 with system size. To quantify it in a more specific way, we investigated the
 161 topographic elevation function for each leaflet (see Figure 2(b)) from which
 162 we defined the amplitude of the corrugation as the root mean square (RMS)
 163 height h_{RMS} of the two water-lipid interfaces of the bilayers. The heights of

164 the interfaces were obtained by meshing the water surface and removing the
 165 mean height of each leaflet. In these circumstances, h_{RMS} is equal to the
 166 standard deviation of the heights $\sigma(h)$. The leaflet corrugation amplitudes
 167 are identical for both leaflets even though cross section pictures might suggest
 168 otherwise (Figure 3(a)). The corrugation amplitude increases with system
 169 size but saturates for large systems (Figure 3(b)). The longitudinal period
 170 of the corrugations always coincide with the periodic boundary conditions
 171 (PBC, see Figure 3(c)). The power spectrum densities (PSD) used to measure
 172 the periods are given in the Supplementary Materials.

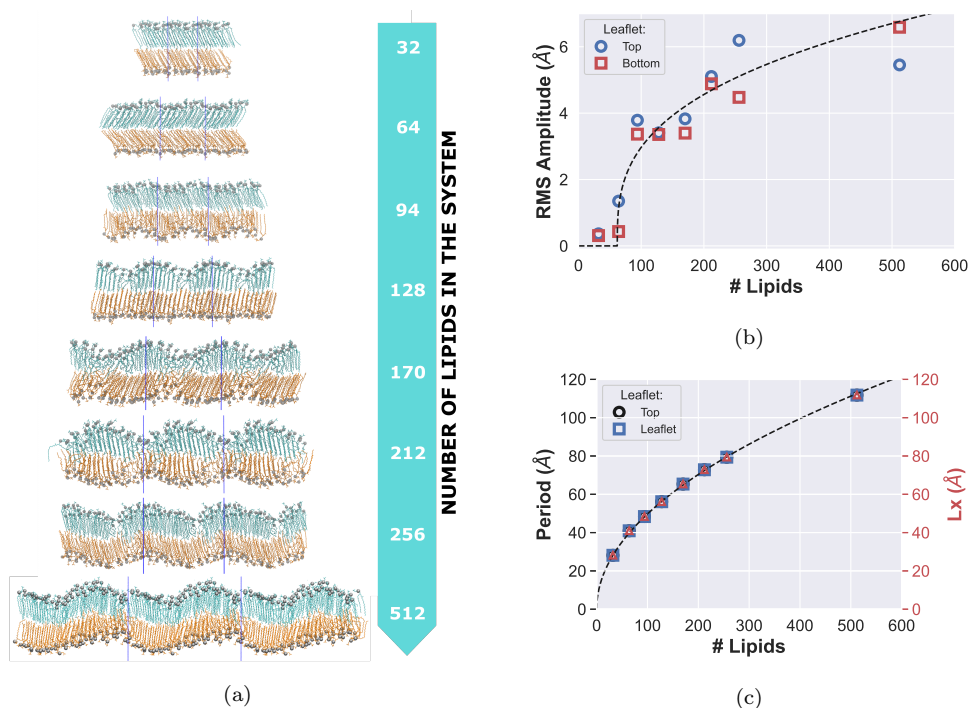


Figure 3: Effect of the size of the simulation box on the geometry of bilayers. (a) 2.5 nm thick slices of the systems made with different amount of lipid molecules and simulated at 288 K. Measurement of the (b) amplitude, or h_{RMS} and (c) period of the corrugations observed in different systems versus the number of lipids. The evolution of the size of the box is shown on top of the period. Dashed lines are respectively the cubic root and square root fits of the data.

To better quantify the nature of the corrugation, we investigated the area per lipid using two methods: i) *projection* in the (XY) plane of the box, i.e the area A_H^p of the box divided by the number of lipid; ii) *meshing* of the

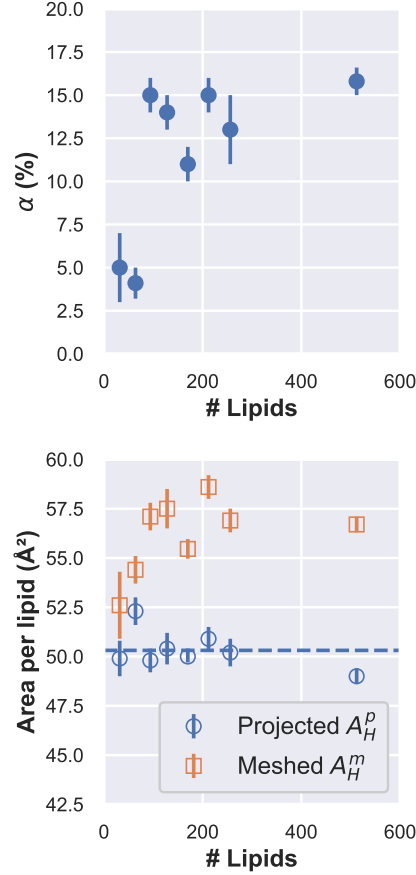


Figure 4: (Top) Differences between the area per lipid measured by surface meshing and by projection in the XY plane α (in %) and (Bottom) evolution of each type of area per lipid for different system sizes. Blue dashed line on the bottom graph is the average projected area per lipid.

water lipid interface that enables the determination of the interfacial area A_H^m . We found that the difference between interfacial and projected area per lipid

$$\alpha = \frac{A_H^m - A_H^p}{A_H^p} \quad (1)$$

173 provides an excellent characterization of the corrugated phase (Figure 4).
 174 Indeed, α is three times larger for corrugated systems ($\alpha = 14 \pm 2\%$) than

175 for tilted ones ($\alpha = 5 \pm 2\%$). The differentiation using usual methods such as
176 the area per lipid or the tail order parameter achieved less significant results
177 (*cf* Supplementary materials).

178 The local chain tilt direction in the XY plane was also determined and
179 mapped (Figure 5(a)). As expected, tilts are uniform in the $L_{\beta'}$ state and
180 present a random short-range correlation in the fluid L_{α} state. This was
181 confirmed by the distribution of the angles with respect to the X-axis, as a
182 function of the height on the leaflet. In the corrugated systems, the local
183 chain tilts displays long-range variations. The angle distribution of the cor-
184 rugated systems shows that small heights have random orientation similar to
185 the fluid, while small heights are subject to less variations.

186 We also probed the sensitivity of the corrugation to modifications of space
187 group. A simple change from cubic to rectangular simulation boxes with
188 $L_x = 2L_y$ shows commensurate corrugations periods. Moreover we carry
189 out simulations in hexagonal and monoclinic simulation boxes counting 128
190 lipids. Both of these systems were found to be corrugated, with modulation
191 vectors directed along the PBC/crystallographic directions (Figure 6).

192 To release residual stress on the bilayer configuration that could have
193 been brought by the semi-isotropic barostat, an anisotropic barostat was ap-
194 plied to the systems, with 1 bar and a $4.5 \times 10^{-5} \text{ bar}^{-1}$ compressibility set
195 along each axes, and the pressure crossed terms set to 0 for both pressure and
196 compressibility. We found that releasing residual anisotropic stress did not
197 modify the structure of the bilayers. After this simulation run, the system
198 was still found in a corrugated state, as shown in Figure 7, with a relative
199 area increase α of $11.9 \pm 0.8\%$ and a RMS corrugation amplitude of 4.89 \AA
200 comparable to the amplitude before the run (5.18 \AA). We therefore conclude
201 from this result that both tilted gel and corrugated states behave as a cohe-
202 sive, solid state on the simulated time scales. They also display significant
203 residual static stresses of 0.8 and 1.0 bars respectively in the x and the y di-
204 rections. Based on these characterizations we refer to the corrugated state as
205 the "disordered gel state", noted here L_{β}^d , to make a clear distinction between
206 these configurations and the ripple phase $P_{\beta'}$ that has only be experimentally
207 reported above the pre-transition temperature.

208 Finally one can wonder whether the appearance of the corrugation is
209 restricted to DPPC or DMPC. To answer this question, we probed the effect
210 of the tail and the head groups by considering the longer-tailed DSPC and the
211 ethanolamine-based DPPE. As shown in Figure 8, the large DSPC systems
212 were found in the disordered gel state while the DPPE systems remained in

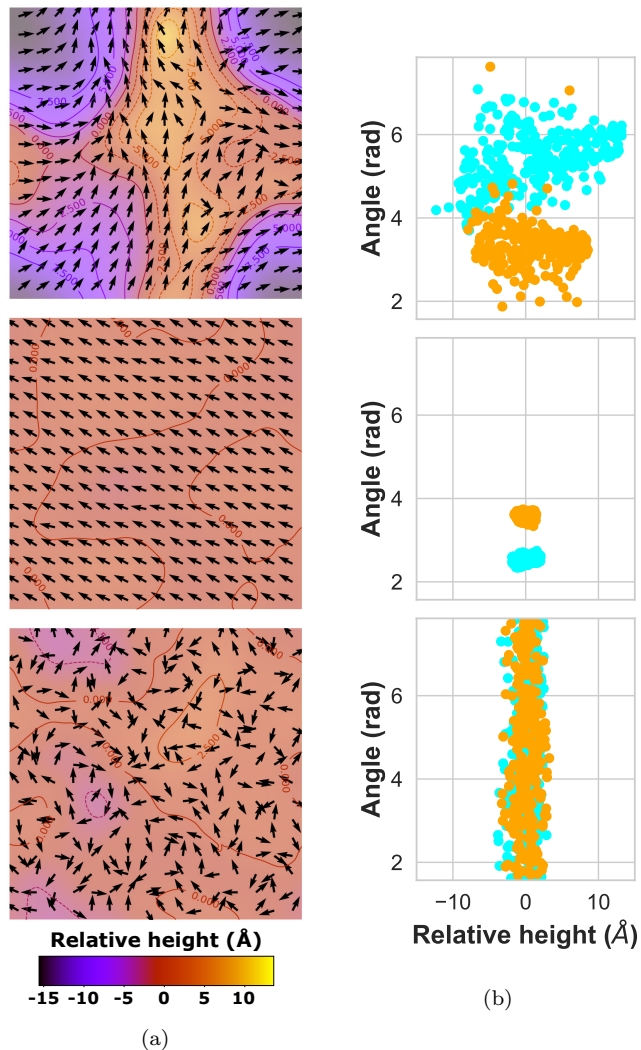
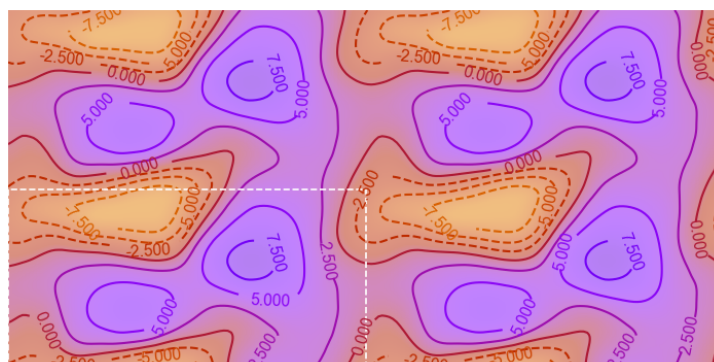
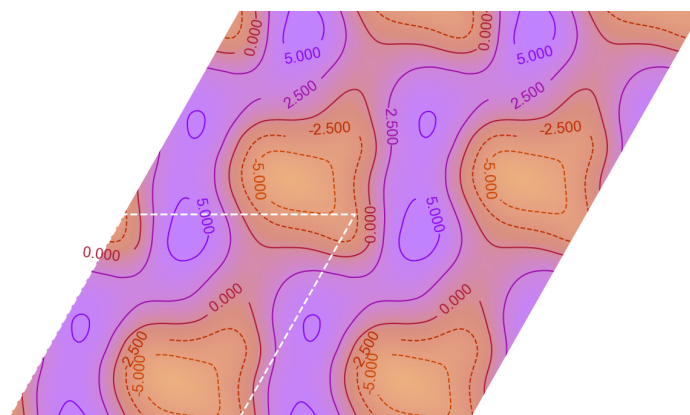


Figure 5: (a) Orientation in the XY plane of the tails of each of the 256 lipids forming a DPPC bilayer (Top) in the disordered gel phase, (Middle) in the tilted gel phase and (Bottom) in the fluid phase. Only one leaflet of the membrane is shown for each system. (b) Respective scatter distribution of the angles between the tails of the lipids and the X-axis of the membrane, as a function of the height on the leaflet. The measurement were performed on both top and bottom leaflets, respectively colored in cyan and in orange.

213 the expected homogeneous tilted gel state. The respective area differences of
 214 these systems are 12 ± 1 and 5 ± 1 %.



(a)



(b)

Figure 6: Topography of bilayers (a) made of 188 DPPC molecules in a simulation box with $L_x = 2L_y$ and (b) made of 128 DPPC molecules in an hexagonal simulation box, both simulated at 288 K.

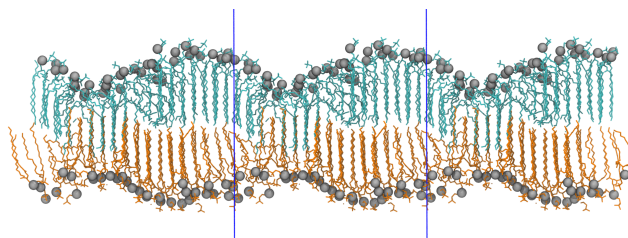


Figure 7: Slice of the 212 DPPC molecule system obtained after being simulated at 288 K for 50 ns with an anisotropic barostat.

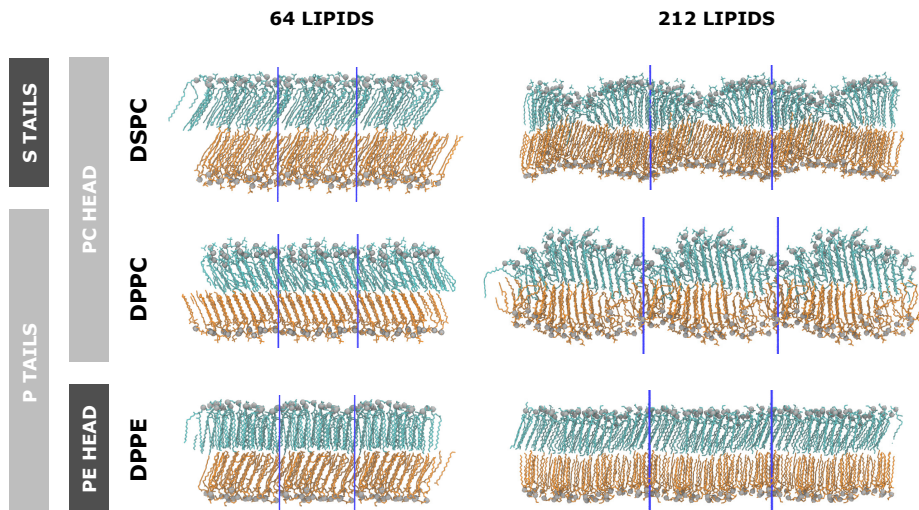


Figure 8: Profiles of bilayers made of 64 or 212 lipids, either with DPPC, DSPC or DPPE, after a 50 ns simulation at 288 K. Only the DPPE membrane remained in the tilted gel phase when constructed with a large number of lipids.

215 3.2. Influence of thermal history

216 The previous observations suggest that disordered L_{β}^d and tilted $L_{\beta'}$ gel
 217 states are two competing states whose appearance seem to be correlated to
 218 the system size for freshly thermalised systems generated with CHARMM-
 219 GUI. In what follows we probe the sensitivity of the stability of these phases
 220 to different routes of thermal treatments.

221 We focus our analysis on two system sizes: 64 lipids, which has been
 222 found in the $L_{\beta'}$ phase, and 256 lipids which has been found in the L_{β}^d .
 223 Both systems were subjected to the following thermal treatment: starting
 224 from $T = 288K$, systems were annealed at $T = 358K$ in the fluid phase.
 225 These systems are respectively named pc64-A and pc256-A, for annealing.
 226 They were then cooled down to $T = 288K$ in two different ways: either
 227 with a brutal fast cooling, named here quenched, or with a slow gradual
 228 cooling of 1K/ns that we denote gentle cooling. The quenched systems are
 229 noted pc64-AQ and pc256-AQ (annealing-quenching), and the cooled systems
 230 called pc64-AC and pc256-AC (annealing-cooling).

231 We first notice that whatever the thermal history, large systems were
 232 always found in the disordered gel state as shown in Figure 9. Gentle cooling
 233 of small systems allowed them to recover the $L_{\beta'}$ phase while quenching lead
 234 to L_{β}^d phase. As in this latter case, either phases could be obtained depending

235 on the thermal history, we conclude that the disordered gel is metastable with
236 respect to the tilted gel for small systems. Furthermore, we can note that
237 both the gently cooled and the quenched systems have a final projected area
238 per lipid A_H^p (respectively 50.1 ± 0.7 and $50.8 \pm 0.8 \text{ \AA}^2$) close to the average
239 projected area of 50 \AA^2 found for systems of all sizes, hence correcting the
240 odd value of 52.3 \AA^2 found before temperature treatment (*cf* Figure 4).

241 As large systems of 256 lipids were never spontaneously found in the $L_{\beta'}$
242 state, we decided to force them into this state by duplicating along both x and
243 y directions the 64 $L_{\beta'}$ system. The flat tilted duplicated system was found
244 to be stable on the simulation time scale, suggesting that $L_{\beta'}$ could be either
245 stable or metastable in large systems too. The nature of the relevant stable
246 thermodynamic phase for large systems remains an open question, while our
247 simulations clearly favor the disordered gel state L_{β}^d . Measurements of the
248 difference in area α can be found in the Supplementary Materials.

249 3.3. Thermodynamics of tilted and disordered states

250 Since the small systems made of 64 lipids can be prepared and controlled
251 to reach all the observed phases, we used them to compare the energetic
252 properties of the respective gel phase. Having in mind the idea of a com-
253 plex underlying potential energy landscape composed of several minima lo-
254 cated at different energy levels, we probe how far energetically the disor-
255 dered gel phase stand from the tilted gel phase minimum. Therefore we
256 performed an energy minimization using conjugate gradient to remove ther-
257 mal fluctuations on the tilted and disordered gel configurations. We found
258 $\Delta E_P = E_P(\text{disordered}) - E_P(\text{tilted}) = 64 \text{ kJ/mol}$, meaning that if both states
259 are metastable, the tilted system is the most stable one. In addition, we also
260 computed the difference in enthalpy between the disordered and the tilted
261 thermalised gel phases. To this aim, we used the same initial states, after
262 quenching or gentle cooling but without energy minimisation. Since the two
263 systems share the same atomic compositions (same number of lipid, water
264 molecules and constraints), the difference in enthalpy (kinetic and potential
265 energy according to the force field, plus the PV contribution) at a given
266 temperature should be characteristic of the enthalpy difference between the
267 two states. We found an enthalpy difference of $12 \pm 4 \text{ kJ/mol}$ at 288K. This
268 value is higher than the one reported experimentally for the calorimetric gel
269 to ripple phase pretransition, 4.6 kJ/mol [4] but obtained at a higher tem-
270 perature. We therefore repeated the measure after rising both systems at a
271 temperature close to the pretransition temperature, namely 305 K, and then

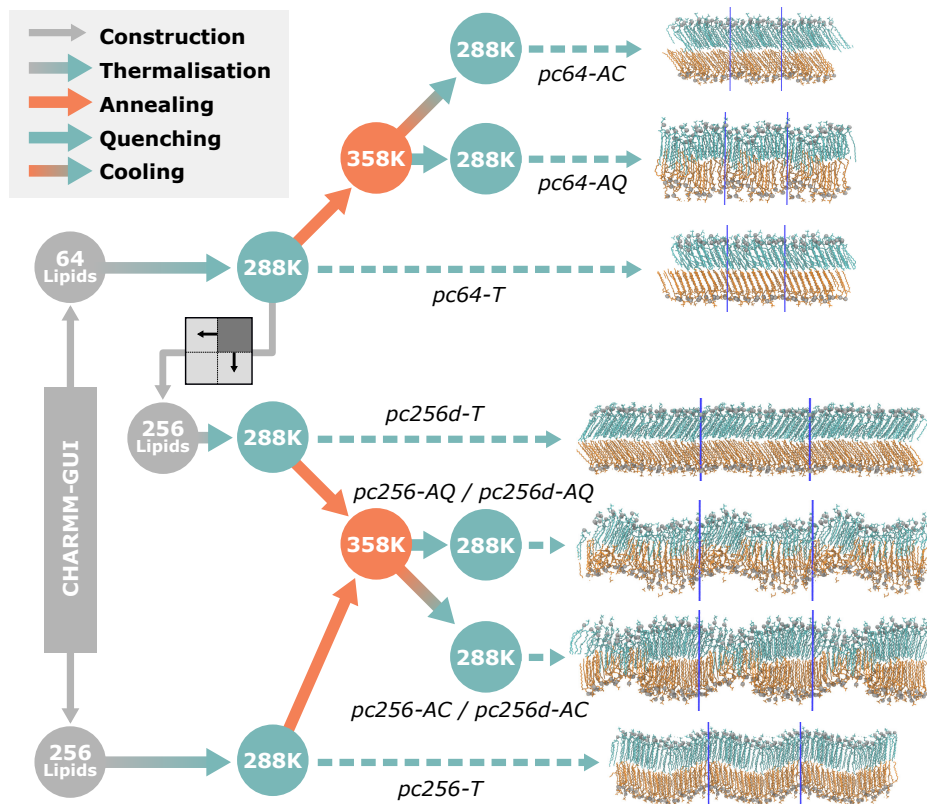


Figure 9: Synopsis of the different thermal trajectories, showing the systems obtained for different cooling rates as well as the systems they originated from. Only the results from the 256 DPPC system obtained by replication of the 64 lipid system are shown for the quenching and cooling experiments. Results for the 256 DPPC system constructed by CHARMM-GUI, as well as the intermediate systems, are shown in the Supplementary Materials.

272 found a difference in enthalpy of 3.9 ± 0.4 kJ/mol now compatible with ex-
 273 perimental measurements. It is essential to note here that, in order to obtain
 274 a disordered system configuration at 305 K, the system had first to be an-
 275 nealed at 375 K instead of 358 K, meaning that the difference in temperature
 276 required for quenching should be at least of 70 K in order to obtain the L_{β}^d
 277 phase (see Supplementary Materials).

278 The enthalpy of the $L_{\beta}^d \rightarrow L_{\alpha}$ transition was also measured in our small
 279 systems. This was performed by simulating the 64 DPPC system at temper-
 280 atures ranging from 283 to 358 K and by removing the change in enthalpy
 281 due to the change in temperature (data shown in Supplementary Materials).

282 The transition enthalpy was found equal to 27.3 kJ/mol, which is also com-
283 parable with the experimental reported values *circa* 32.2 kJ/mol [4]. We
284 can conclude that despite the approximations, and the absence of quantum
285 corrections to the bond vibrations contributions, the CHARMM36 force field
286 thermodynamic predictions seem in good agreement with experimental ob-
287 servations.

288 4. Discussion

289 A careful inspection of the corrugations shows that the topographic mod-
290 ulation is imposed by the periodicity of the simulation box. The corrugation
291 shows finite system size dependence, as its amplitude increases up to sizes of
292 the order of $L_x = 8$ nm where the modulation saturates to a value of 7 ± 1 Å.
293 Assuming a sine-like corrugation, this maximum RMS amplitude measured
294 can be converted into a peak-to-peak amplitude of 2.0 ± 0.2 nm similar to the
295 previously published values of 2.4 nm for the DPPC or even 1.8 nm in DMPC
296 bilayers [42, 16]. The saturation of the corrugation amplitude is expected,
297 given that it can only reach a fraction of the total membrane thickness. The
298 associated lateral length 8 nm can therefore be considered as a lower bound
299 of the instability characteristic longitudinal length scale.

300 We believe that the relative difference between the interfacial area and
301 the projected one, α , introduced in equation (1) can be taken as a relevant
302 order parameter for the transition between the $L_{\beta'}$ and the disordered gel
303 phases L_{β}^d . The latter being reminiscent from $P_{\beta'}$ ripple phase, α could be
304 seen as a critical parameter to investigate the existence of the ripple phase
305 and discriminate it from the tilted gel or from the fluid phase.

306 However, unlike experiments, the numerical instability occurs along two
307 orthogonal directions, or along the hexagonal axes. Non-square boxes fail to
308 select only one modulation direction. We nevertheless think that the numer-
309 ical corrugation instability is related to the experimental ripple instability,
310 as also suggested by the dependence of the presence of corrugation to the
311 chemical nature of the heads and tails of the lipids. Indeed, experiments
312 have shown that the ripple phase is specific from the phosphocholine (PC)
313 lipids [41, 43].

314 Another striking observation is the insensitivity of large systems (256
315 lipids) to thermal treatment which have been systematically ended in the dis-
316 ordered gel state. On the opposite small systems can alternate between both

317 phases. However, when thermalised at low temperatures from CHARMM-
318 GUI or slowly quenched, they end up preferentially into the tilted gel state,
319 suggesting that in the range of temperatures investigated the $L_{\beta'}$ state is
320 thermodynamically favored. By contrast the $L_{\beta'}$ state is never selected spon-
321 taneously by larger systems. For the latter, MD suggests that the disordered
322 gel state L_{β}^d is the most preferred state for all temperatures below melting.
323 Metastability and kinetic effects are certainly significant and may hide to
324 true nature of the stable phase.

325 Assuming that the difference between tilted and disordered gels has some-
326 thing to do with the pretransition, we found an enthalpy difference 3 times
327 larger than the premelting latent heat at 288K, and of the same order of mag-
328 nitude at 305K, close to the observed experimental transition. This points
329 towards the relevance of disordered gel state L_{β}^d as a ripple state analogue.
330 Moreover the higher enthalpy of the disordered gel with respect to the tilted
331 gel is consistent with a $L_{\beta'} \rightarrow L_{\beta}^d \rightarrow L_{\alpha}$ sequence of transitions as tem-
332 perature is increased.

333 The reason of the outcome of a ripple instability below the melting tem-
334 perature is nothing but obvious. Our simulations point out to a competition
335 between an homogeneous tilted state and an inhomogeneous corrugated state.
336 The transition between these states is discontinuous. The corrugated state is
337 not very tilted, and partially melted, or disordered, and interdigitated. We
338 suggest now a possible mechanism explaining the observed situation. The
339 tilted phase can be understood as the result of a frustration between lipid
340 headgroups which try to increase their exposure to water in the interface
341 region, lipid chains which try to reach an optimal packing density as a result
342 of cohesive forces, and chain stiffness for which the introduction of *gauche*
343 dihedral angles is unfavorable at low temperatures. Tilt allows lipid to op-
344 timize simultaneously those three constraints. On the other hand, melting a
345 lipid chain enables the release of the constraint acting on the chain stiffness,
346 and makes it possible to increase the hydration free-energy by reducing the
347 membrane thickness. Below melting temperature, thermodynamics makes it
348 unfavorable to melt all the lipid molecules. However, some local disordering
349 of the lipids may still be favorable, increasing the hydration of the headgroups
350 without need of spending too much energy in melting the chains.

351 Based upon those considerations, we designed a simple one dimensional
352 lipid chain model that supports the idea that in an temperature range just
353 below melting, the homogeneous tilted state is energetically unstable with
354 respect to a local corrugation of the bilayer, see Figure 10. Details of the

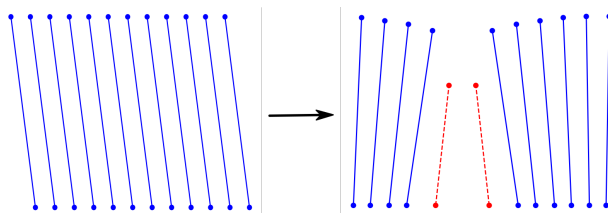


Figure 10: Schematic representation of the mechanical instability occurring in a simple one dimensional lipid chain model, where the tilted state has a higher energy than a locally disordered state. The gain in energy originates mostly from the hydration term. Blue solid rods stand for gel state, red dashed rods for fluid state.

355 parameterisation of the model and further results are presented in the Supple-
 356 mentary Materials. We conclude that the thickness modulation might indeed
 357 be caused by a subtle interplay between headgroup hydration, hydrophobic
 358 chain packing, *trans-gauche* isomerisation and tilt elasticity energy terms.

359 5. Conclusion

360 We have successfully demonstrated in this work how the size of the simu-
 361 lation box influences the ripple-like instability in a PC membrane simulated
 362 with the Charmm36 force field at low temperature, where it is usually ex-
 363 pected to be in the tilted gel $L_{\beta'}$ phase. This unexpected organisation, which
 364 we called the disordered gel phase $L_{\beta'}^d$, does not appear in small systems,
 365 which is consistent with the results from Khakbaz and Klauda [20] as well
 366 as with our results in a previously published paper [21]. The energy and
 367 geometry analyses demonstrated that this disordered gel phase has a lot in
 368 common with the $P_{\beta'}$ phase. Furthermore, this instability was not observed
 369 with PE lipids, in agreement with experimental findings. For small systems,
 370 we found ways of preparing the system in either tilted gel or disordered gel
 371 states by acting on the thermal treatment. More work is needed to deter-
 372 mine whether the Charmm36 force-field can describe a one-dimensional $P_{\beta'}$
 373 spatial thickness modulation, with the right periodicity, and at which tem-
 374 peratures. Our work suggests that simulations will have to be guided to the
 375 desired structure. Finally, we conclude that the ripple instability looks like
 376 a generic mechanism adopted by the phosphatidylcholines lipids to increase
 377 the headgroup hydration while still satisfying the packing constraints, at the
 378 expense of a mild cost in disordering/melting a small fraction of the chains.

379 **Acknowledgements**

380 V. W. warmly thanks T.E. de Oliveira for helping with simulations set-
381 up. A.G. acknowledges partial support from the Investissements d’Avenir
382 program “Développement de l’Économie Numérique” through the SMICE
383 project. The authors gratefully acknowledge support from the high per-
384 formance cluster (HPC) Equip@Meso from the University of Strasbourg,
385 through grants no. G2019A131C and G2020A140C.

386 **References**

- 387 [1] O. G. Mouritsen, *Life-as a matter of fat: the emerging science of*
388 *lipidomics*, Springer, 2005.
- 389 [2] R. Dimova, C. M. Marques (Eds.), *The Giant Vesicle Book*, 1st ed.,
390 CRC Press, Taylor and Francis, 2019.
- 391 [3] D. Marsh, *Handbook of Lipid Bilayers*, 2nd ed., CRC Press, Boca Raton,
392 2013.
- 393 [4] G. Cevc, D. Marsh, *Phospholipid Bilayers. Physical Principles and Mod-*
394 *els*, John Wiley & Sons, New-York, 1987.
- 395 [5] S. Mabrey, J. M. Sturtevant, Investigation of phase transitions in lipids
396 and lipid mixtures by high sensitivity differential scanning calorimetry,
397 *Proceedings of the National Academy of Sciences USA* 73 (1976) 3862–
398 3866.
- 399 [6] T. Heimburg, *Thermal Biophysics of Membranes*, Wiley-VCH, 2007.
- 400 [7] A. Tardieu, V. Luzzati, F. C. Reman, Structure and polymorphism
401 of the hydrocarbon chains of lipids: A study of lecithin-water phases,
402 *Journal of Molecular Biology* 75 (1973) 719–733.
- 403 [8] W. J. Sun, S. Tristram-Nagle, R. M. Suter, J. F. Nagle, Structure of the
404 ripple phase in lecithin bilayers., *Proceedings of the National Academy*
405 *of Sciences* 93 (1996) 7008–7012. doi:10.1073/pnas.93.14.7008.
- 406 [9] R. N. A. H. Lewis, N. Mak, R. N. McElhaney, A differential
407 scanning calorimetric study of the thermotropic phase behavior of

- 408 model membranes composed of phosphatidylcholines containing lin-
409 ear saturated fatty acyl chains, *Biochemistry* 26 (1987) 6118–6126.
410 doi:10.1021/bi00393a026.
- 411 [10] T. Heimburg, A model for the lipid pretransition: coupling of ripple
412 formation with the chain-melting transition, *Biophysical Journal* 78
413 (2000) 1154–1165.
- 414 [11] S. Doniach, A thermodynamic model for the monoclinic (ripple) phase
415 of hydrated phospholipid bilayers, *The Journal of Chemical Physics* 70
416 (1979) 4587–4596. doi:10.1063/1.437292.
- 417 [12] T. C. Lubensky, F. C. MacKintosh, Theory of “ripple” phases
418 of lipid bilayers, *Physical Review Letters* 71 (1993) 1565–1568.
419 doi:10.1103/physrevlett.71.1565.
- 420 [13] J.-B. Fournier, Coupling between membrane tilt-difference and dilation:
421 A new “ripple” instability and multiple crystalline inclusions phases,
422 *Europhysics Letters (EPL)* 43 (1998) 725–730. doi:10.1209/epl/i1998-
423 00424-4.
- 424 [14] C. Misbah, J. Duplat, B. Houchmandzadeh, Transition to ripple phases
425 in hydrated amphiphiles, *Physical Review Letters* 80 (1998) 4598–4601.
426 doi:10.1103/physrevlett.80.4598.
- 427 [15] K. Sengupta, V. A. Raghunathan, Y. Hatwalne, Role of tilt order in
428 the asymmetric ripple phase of phospholipid bilayers, *Physical Review*
429 *Letters* 87 (2001). doi:10.1103/physrevlett.87.055705.
- 430 [16] K. Akabori, J. F. Nagle, Structure of the dmpc lipid bilayer ripple phase,
431 *Soft Matter* 11 (2015) 918–926.
- 432 [17] A. H. de Vries, S. Yefimov, A. E. Mark, S. J. Marrink, Molecular struc-
433 ture of the lecithin ripple phase, *Proceedings of the National Academy*
434 *of Sciences* 102 (2005) 5392–5396. doi:10.1073/pnas.0408249102.
- 435 [18] O. Lenz, F. Schmid, Structure of symmetric and asymmetric “ripple”
436 phases in lipid bilayers, *Physical Review Letters* 98 (2007).

- 437 [19] A. Debnath, F. M. Thakkar, V. Kumaran, P. K. Maiti, K. G. Ayappa,
438 Laterally structured ripple and square phases with one and two dimen-
439 sional thickness modulations in a model bilayer system, *Soft Matter* 10
440 (2014) 7630–7637.
- 441 [20] P. Khakbaz, J. B. Klauda, Investigation of phase transitions of satu-
442 rated phosphocholine lipid bilayers via molecular dynamics simulations,
443 *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1860 (2018) 1489
444 – 1501.
- 445 [21] V. Walter, C. Ruscher, C. M. Marques, O. Benzerara, F. Thalmann, A
446 machine learning study of the two states model for lipid bilayer phase
447 transitions, *Phys. Chem. Chem. Phys.* (2020) –.
- 448 [22] S. Jo, T. Kim, W. Im, Automated builder and database of pro-
449 tein/membrane complexes for molecular dynamics simulations, *PLoS*
450 *ONE* 2 (2007) 880.
- 451 [23] S. Jo, T. Kim, V. G. Iyer, W. Im, Charmm-gui: A web-based graphical
452 user interface for charmm, *Journal of Computational Chemistry* 29
453 (2008) 1859–1865.
- 454 [24] S. Jo, J. B. Lim, J. B. Klauda, W. Im, Charmm-gui membrane builder
455 for mixed bilayers and its application to yeast membranes, *Biophysical*
456 *Journal* 97 (2009) 50–58.
- 457 [25] E. L. Wu, X. Cheng, S. Jo, H. Rui, H. K. Song, E. M. Davila-Contreras,
458 Y. Qi, J. Lee, V. Monje-Galvan, R. M. Venable, J. B. Klauda, W. Im,
459 Charmm-gui membrane builder toward realistic biological membrane
460 simulations, *Journal of Chemical Theory and Computation* 35 (2014)
461 1997–2004.
- 462 [26] Zenodo repo, 2021. URL: DOI: 10.5281/zenodo.4522359.
- 463 [27] H. Berendsen, D. van der Spoel, R. van Drunen, Gromacs: A message-
464 passing parallel molecular dynamics implementation, *Computer Physics*
465 *Communications* (1995).
- 466 [28] M. J. Abraham, T. Murtola, R. Schulz, S. Páll, J. C. Smith, B. Hess,
467 E. Lindahl, Gromacs: High performance molecular simulations through

- 468 multi-level parallelism from laptops to supercomputers, *SoftwareX*
469 (2015).
- 470 [29] R. B. Best, X. Zhu, J. Shim, P. E. M. Lopes, J. Mittal, M. Feig, A. D.
471 MacKerell Jr., Optimization of the additive charmm all-atom protein
472 force field targeting improved sampling of the backbone phi, psi and
473 side-chain khi1 and khi2 dihedral angles, *Journal of Chemical Theory*
474 *and Computation* 8 (2012) 3257–3273.
- 475 [30] B. R. Brooks, C. L. Brooks III, A. D. MacKerell Jr, L. Nilsson, R. J.
476 Petrella, B. Roux, Y. Won, G. Archontis, C. Bartels, S. Boresch,
477 A. Caffisch, L. Caves, Q. Cui, A. R. Dinner, M. Feig, S. Fischer, J. Gao,
478 M. Hodosecek, W. Im, K. Kuczera, T. Lazaridis, J. Ma, V. Ovchinnikov,
479 E. Paci, R. W. Pastor, C. B. Post, J. Z. Pu, M. Schaefer, B. Tidor, R. M.
480 Venable, H. L. Woodcock, X. Wu, W. Yang, D. M. York, M. Karplus,
481 Charmm: The biomolecular simulation program, *Journal of Computa-*
482 *tional Chemistry* 30 (2009) 1545–1614.
- 483 [31] J. Lee, X. Cheng, J. M. Swails, M. S. Yeom, P. K. Eastman, J. A.
484 Lemkul, S. Wei, J. Buckner, J. C. Jeong, Y. Qi, S. Jo, V. S. Pande,
485 D. A. Case, C. L. Brooks III, A. D. MacKerell Jr, J. B. Klauda, W. Im,
486 Charmm-gui input generator for namd, gromacs, amber, openmm, and
487 charmm/openmm simulations using the charmm36 additive force field,
488 *Journal of Chemical Theory and Computation* 12 (2016) 405–413.
- 489 [32] R. W. Hockney, S. P. Goel, J. W. Eastwood, Quiet high-resolution
490 computer models of a plasma, *Journal of Computational Physics* 14
491 (1974) 148–158.
- 492 [33] S. Nosé, A molecular dynamics method for simulations in the canonical
493 ensemble, *Molecular Physics* 52 (1984) 255–268.
- 494 [34] W. G. Hoover, Canonical dynamics: Equilibrium phase-space distribu-
495 tions, *Physical Review A* 31 (1985) 1695.
- 496 [35] S. Nose, M. L. Klein, Constant pressure molecular dynamics for molec-
497 ular systems, *Molecular Physics* 50 (1983) 1055–1076.
- 498 [36] M. Parinello, A. Rahman, Polymorphic transitions in single crystals: A
499 new molecular dynamics method, *Journal of Applied Physics* 52 (1998)
500 7182.

- 501 [37] P. Virtanen, R. Gommers, T. E. Oliphant, M. Haberland, T. Reddy,
502 D. Cournapeau, E. Burovski, P. Peterson, W. Weckesser, J. Bright,
503 S. J. van der Walt, M. Brett, J. Wilson, K. Jarrod Millman, N. May-
504 orov, A. R. J. Nelson, E. Jones, R. Kern, E. Larson, C. Carey, Í. Polat,
505 Y. Feng, E. W. Moore, J. VanderPlas, D. Laxalde, J. Perktold, R. Cim-
506 rman, I. Henriksen, E. A. Quintero, C. R. Harris, A. M. Archibald,
507 A. H. Ribeiro, F. Pedregosa, P. van Mulbregt, S. . . Contributors, Scipy
508 1.0: Fundamental algorithms for scientific computing in python, *Nature*
509 *Methods* 17 (2020) 261–272.
- 510 [38] N. Michaud-Agrawal, E. J. Denning, T. B. Woolf, O. Beckstein, Md-
511 analysis: A toolkit for the analysis of molecular dynamics simulations,
512 *Journal of Computational Chemistry* 32 (2011) 2319–2327.
- 513 [39] R. J. Gowers, M. Linke, J. Barnoud, T. J. E. Reddy, M. N. Melo, S. L.
514 Seyler, D. L. Dotson, J. Domanski, S. Buchoux, I. M. Kenney, O. Beck-
515 stein, Mdanalysis: A python package for the rapid analysis of molecular
516 dynamics simulations, *Proceedings of the 15th Python in Science Con-*
517 *ference* (2016) 98–105.
- 518 [40] T. E. de Oliveira, F. Leonforte, L. Nicolas-Morgantini, A.-L. Fameau,
519 B. Querleux, F. Thalmann, C. M. Marques, Fluid bilayer phase in
520 aqueous mixtures of fatty alcohol and cationic surfactant, *Phys. Rev.*
521 *Research* 2 (2020) 013075. doi:10.1103/PhysRevResearch.2.013075.
- 522 [41] B. A. Cunningham, A.-D. Brown, D. H. Wolfe, W. P. Williams, A. Brain,
523 Ripple phase formation in phosphatidylcholine: Effect of acyl chain re-
524 lative length, position, and unsaturation, *Physical Review E* 58 (1998).
- 525 [42] A. H. de Vries, S. Yefimov, A. E. Mark, S. J. Marrink, Molecular struc-
526 ture of the lecithin ripple phase, *PNAS* 102 (2005) 5392–5396.
- 527 [43] J. Katsaras, S. Tristram-Nagle, Y. Liu, R. L. Headrick, E. Fontes, P. C.
528 Mason, J. F. Nagle, Clarification of the ripple phase of lecithin bilayers
529 using fully hydrated, aligned samples, *Physical Review E* 61 (2000).