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1 **Induction of non-lamellar phases in archaeal lipids at high temperature**
2 **and high hydrostatic pressure by apolar polyisoprenoids**

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4

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9

10

11

12 **Abstract**

13 It is now well established that cell membranes are much more than a barrier that separate the
14 cytoplasm from the outside world. Regarding membrane's lipids and their self-assembling, the
15 system is highly complex, for example, the cell membrane needs to adopt different curvatures
16 to be functional. This is possible thanks to the presence of non-lamellar-forming lipids, which
17 tend to curve the membrane. Here, we present the effect of squalane, an apolar isoprenoid
18 molecule, on an archaea-like lipid membrane. The presence of this molecule provokes negative
19 membrane curvature and forces lipids to self-assemble under inverted cubic and inverted
20 hexagonal phases. Such non-lamellar phases are highly stable under a broad range of external
21 extreme conditions, e.g. temperatures and high hydrostatic pressures, confirming that such
22 apolar lipids could be included in the architecture of membranes arising from cells living under
23 extreme environments.

24 Introduction

25 Since Singer and Nicolson¹ first defined the membrane as a mosaic of proteins and lipids in a
26 lamellar fluid liquid-crystalline (L_{α}) phase, our view of the membrane ultrastructure has become
27 more sophisticated, recognizing the existence of membrane domains of specific lipid
28 composition in natural membranes. Each composition corresponds to a particular phase, which
29 play important roles in cell membrane function and cell physiology as they have very specific
30 physicochemical properties, such as different lateral motions, rotations, fluidity, and phase
31 structuration, and promote the formation of lamellar or non-lamellar phase^{2,3}.

32 Lamellar, cubic and inverted hexagonal phases possess different bending fluctuations and
33 intrinsic curvatures⁴, which are mostly defined by the shape of the lipids present in the
34 membrane. This has been rationalized using the critical geometric packing parameter (P)
35 concept, which defines P as $v(a \cdot l)^{-1}$ where v is the molecular volume, l is the molecular length
36 and a is the molecular area at the lipid-water interface^{5,6}. For $P < 1$, molecules are inverted cones
37 and tend to produce positive curvatures, i.e. the hydrocarbon chains are closest to each other.
38 For $P \approx 0$, lipids have a cylindrical shape and adopt zero curvature, giving rise to lamellar phases.
39 For $P > 1$, lipids present conical shapes and adopt negative curvatures and self-assemble under
40 the so-called inverted phases, such as the cubic (Q) and hexagonal (H_{II}) phases, simply to release
41 their curvature frustration (Figure 1). Changes between lamellar and non-lamellar former lipid
42 compositions tune the flexibility and curvature of the membrane⁷ which is used by cell to tune
43 its membrane functionality by adjusting its membrane lipid compositions to adapt itself and
44 maintain essential functions under different environmental conditions.

45 Archaeal phospholipids present unique characteristics: a *sn*-glycerol-1-phosphate (G-1-P)
46 backbone, ether linkages, and isoprenoid hydrocarbon chains⁸. The methyl groups along the acyl
47 chains are responsible for the fact that both phospholipid's rotamers are nearly energetically
48 equivalent⁹, thus allowing archaeal lipids to occur in the L_{α} phase for a broad range of

49 temperature (-120°C to 120°C)^{9,10}. Isoprenoid chains pack densely, increase membrane viscosity
50 and decrease its water permeability¹¹⁻¹³. The presence of ether bounds, isoprenoid hydrocarbon
51 chains and transmembrane lipids is thought to be the reason of the incredibly high resistance of
52 archaeal cell membranes to extreme conditions¹⁴. For example, some Archaea have their
53 optimal growth conditions at low pH, high salinity, high temperature (HT) and/or high
54 hydrostatic pressure (HHP), including a highly stable and selective permeable cell membrane.
55 Intriguingly, liposomes made from archaeal lipids present highly irregular shapes¹⁵ and indeed,
56 extracellular membrane vesicles are highly produced by Archaea¹⁶. All these findings indicate
57 that non-lamellar-forming lipids may play a particular important role in the Archaea domain.

58 The geometric packing parameter predicts that most archaeal phospholipids will have zero or
59 positive curvatures which will not favour the formation of the non-lamellar phases indispensable
60 for biological activity. This is for example the case for the membrane lipids of *Thermococcus*
61 *barophilus* which have phosphatidyl inositol polar headgroup and derivatives of this polar
62 headgroup. Recently, Cario and colleagues¹⁷ proposed a novel archaeal membrane architecture
63 for the hyperthermophile and piezophile Archaeon *T. barophilus* in order to explain its ability to
64 thrive at temperatures close to 100°C¹⁸. According to this hypothesis, apolar isoprenoid
65 molecules would populate the hydrophobic space between the two bilayer leaflets, thus
66 modifying the membrane properties, decreasing permeability to proton and water and
67 membrane rigidity¹⁹. In addition, the presence of the interleaving molecules within the plane of
68 the membrane is proposed to be implied in modifying the phases transition in the membrane.
69 Indeed, the partitioning of non-polar molecules, such as alkanes, aromatic compounds and
70 isoprenoid chains into the hydrocarbon region, increases the tendency of the lipid layer to adopt
71 a negative interfacial curvature²⁰⁻²². Therefore, lipids reduce their hydrocarbon packing stress
72 self-assembling within inverted non-lamellar phases. For example, the addition of 5% alkane to
73 a mixture of PC/PE-lipids reduces the L_α-H_{II} transition by 55°C²³. To date, the effect of apolar
74 lipids on archaeal membranes has not been studied.

75 Using Small Angle X-ray Scattering (SAXS), we have determined the lipid phases from a mixture
76 of archaeal lipids in presence of different molar percentages (mol%) of the polyisoprenoid
77 squalane at different temperatures and pressures. Here we show that in absence of the apolar
78 intercalant, the archaeal lipids cannot form non-lamellar phases, and that the presence of the
79 apolar molecule imposes highly stable non-lamellar type II Q and H phases.

80

81 **Experimental section**

82 **I. Chemicals**

83 The high purity (>99%) synthetic archaeal lipids 1,2-di-O-phytanyl-*sn*-glycero-3-phosphocholine
84 (DoPhPC) and 1,2-di-O-phytanyl-*sn*-glycero-3-phosphoethanolamine (DoPhPE) were purchased
85 from Avanti Polar Lipids in the lyophilized form and utilized without further purification.
86 2,6,10,15,19,23-Hexamethyltetracosane (squalane) was bought from Alfa Aesar.

87 **II. Small angle X-ray diffraction**

88 SAXS experiments were performed on beamline I22²⁴ at the Diamond Light Source (United
89 Kingdom). DoPhPC and DoPhPE were used in a proportion 9:1 molar. Phospholipids with
90 contents of 20% (w/v water) and the adequate quantity of squalane (1%, 2.5%, 5% and 10%
91 molar) were placed inside a pressure chamber²⁵. The pressure and temperature dependent
92 experiment was carried out at six different temperatures (10°C, 25°C, 40°C, 55°C, 70°C and 85°C)
93 in pressure jumps of 50 bar from 0 bar to 1000 bar.

94 The X-ray energy was 18 keV, the momentum transfer is defined as $q = 4\pi \sin(\Theta) / \lambda$, where 2Θ
95 is the scattering angle. The small-angle regime was $0.005 < q < 0.35 \text{ \AA}^{-1}$. The type of phase can
96 be distinguished by the characteristic SAXS peak ratios²⁶: for a lamellar phase they are
97 equidistant, for a hexagonal phase they are: 1, $\sqrt{3}$, 2, $\sqrt{7}$..., for a cubic phase Pn3m they are: $\sqrt{2}$,
98 $\sqrt{3}$, 2, $\sqrt{6}$, $\sqrt{8}$, 3... and for a cubic phase Im3m they are: $\sqrt{2}$, 2, $\sqrt{6}$, $\sqrt{8}$, 3... The lattice parameter

99 a is defined as $a_{\text{lam}} = 2\pi/q_n$ and $a_{\text{hex}} = (2\pi/q_n)^{2/3}$ for a lamellar and a hexagonal phase,
100 respectively. To determine the lattice parameter for the cubic phase, the experimentally
101 obtained peak position s , where $s = 2 \cdot \sin(\Theta)/\lambda = q/2\pi$, is plotted vs $(h^2 + k^2 + l^2)^{1/2}$, where
102 h, k, l are the Miller indices by which the peaks are indexed. The plot should intercept through
103 the origin and be linear with a slope of $1/a_{\text{cubic}}$.

104

105 **Results and discussion**

106 **I. Archaeal phospholipids self-organize under lamellar phases**

107 Lipid phases in excess of water can be identified by the relative Bragg peak positions in the SAXS
108 patterns²⁶. Collected data points and phase diagrams are shown in Figure 2. In absence of the
109 apolar molecule, only lamellar phases can be identified in the SAXS spectra (Salvador Castell,
110 unpublished results). At 10°C and ambient pressure, the lattice parameter of the lamellar phase
111 is $62.7 \pm 0.6 \text{ \AA}$. As expected, the lamellar phase appears sensitive to HT (Figure S1). The lattice
112 parameter increases at least to the highest temperature tested of 85°C. However, starting from
113 70°C the sample loses its correlation between bilayers and becomes so disordered that only the
114 first diffraction order can be fitted with accuracy. The lattice parameter increases from $62.7 \pm$
115 0.6 \AA at 10°C to $73.2 \pm 1 \text{ \AA}$. The lamellar phase appears little sensitive to hydrostatic pressure.
116 Indeed, the lattice parameter varies only within the error bars of the measure. In contrast to
117 other lipid systems in which hydrostatic pressure exert a structuring impact on the bilayers²⁷,
118 we see no such effect on DoPhPC:DoPhPE-based membranes. In fact, at the highest combined
119 pressures and temperatures (85°C and >750 bar), no diffraction peaks are visible anymore.

120

121 **II. Addition of 1 mol% squalane induces the formation of inverted non-lamellar**
122 **phases**

123 Upon addition of squalane, even at the smallest quantity of 1 mol%, we observed the presence
124 of non-lamellar phases of type II, in co-existence or not with lamellar phases, which suggests
125 that the presence of squalane increases the negative membrane curvature (Salvador Castell,
126 unpublished results). At all temperatures, we observe a cubic phase, in coexistence with a
127 lamellar phase up to 40°C and a hexagonal phase above. In contrast to the lipid system without
128 squalane, the system with 1mol% squalane is both pressure and temperature sensitive. The a_{lam}
129 increases as a function of HHP from $67.6 \pm 0.3 \text{ \AA}$ at ambient pressure to $69.8 \pm 0.3 \text{ \AA}$ at 1000 bar
130 at 10°C for example, but also as a function of temperature where it reaches $69.6 \pm 0.5 \text{ \AA}$ at 40°C
131 and 1 bar (Figure S2). Temperature is known to favour the negative intrinsic curvature of the
132 lipid self-assembly. At temperatures of 55°C and above, a mixture of a Pn3m cubic phase and an
133 inverted hexagonal phase coexist. At 85°C, three different Pn3m phases with very slightly
134 different lattice values coexist, which is indicative that they have most likely different hydration
135 levels (Figure 3). Note the substantial lattice parameters of the cubic phases ($a_{Pn3m} = 254 \pm 2 \text{ \AA}$
136 and $a_{Im3m} = 332 \pm 3 \text{ \AA}$) which reflect the high potential of squalane to reduce chain packing
137 frustration. Values for the lattice parameter for bacterial lipid cubic phases are substantially
138 smaller, such as 105 Å for monoolein (MO)^{28,29} or 122 Å for 1,2-dioleoyl-sn-glycero-3-
139 phosphoethanolamine (DOPE)³⁰. However, under certain conditions this lattice parameter can
140 reach values higher than those measured here, an example is the case of a diphytanoyl PC-
141 cholesterol mixture, in which the Q_{II} phase has a a_{cubic} of 510 Å³¹. At 85°C, we observe the
142 replacement of the Pn3m by the Im3m phase (Figure 3). Thus remarkably, HHP leads to the
143 replacement of a Pn3m cubic phase with a lower a_{cubic} and higher negative curvature to a Im3m's
144 cubic phase with a higher a_{cubic} and lower negative membrane curvature. In accordance with
145 geometric considerations for those coexisting phases^{32,33}, we found that the ratio between their
146 lattice parameters is 1.28. The hexagonal phase has a lattice parameter ranging from 87.9 ± 0.5

147 Å at 55°C to 80.1 ± 0.5 Å at 85°C under ambient pressure. This a_{hex} is highly sensitive to pressure,
148 ranging from 82.2 ± 0.5 Å at 1 bar to 90.1 ± 0.5 Å at 1000 bar at 70°C for example. This is in
149 accordance with the fact that non-lamellar self-assemblies easily swell at HHP³⁴. In addition, such
150 a_{hex} is much higher than the a_{hex} usually obtained for familiar lipids which lie at 52.9 Å at 97°C
151 for MO³⁵ or 65.8 Å at 70°C for DOPE³⁶, for example. This may be directly related to the ability of
152 squalane to release the chain frustration at H_{II} phases with high a_{hex} .

153 III. Higher negative curvature in presence of 2.5 mol% of squalane

154 By further adding squalane, the system presents a coexistence of a lamellar and HII phases at
155 low temperature and no longer a coexistence of lamellar and cubic phases, which is indicative
156 of a further increase of the membrane negative curvature. As for the 1 mol% squalane lipid
157 system, the lamellar phase is present up to 40°C, but in contrast, it seems to be far less sensitive
158 to pressure (Figure S3). At 85°C, the hexagonal phase coexists with two cubic phases, probably
159 Im3m. Remarkably, at 55°C and 70 °C, HHP induces a phase transition to a second hexagonal
160 phase coexisting with the one already presented at low pressures (Figure 4a). We propose that
161 this phase separation could result from the rearrangement of squalane between a squalane rich
162 and a squalane poor phase. As observed before, the hexagonal phase is much more sensitive to
163 temperature and HHP than the lamellar phase. The a_{hex} decreases from 94.6 ± 0.5 Å at 10°C to
164 77.4 ± 0.5 Å at 85°C at 1 bar and increases from 94.6 ± 0.5 Å to 103.1 ± 0.5 Å at 1 bar to 1000
165 bar at 10°C (Figure S3b). Last, at 85°C, two pressure-sensitive Im3m phases appear in
166 coexistence with the H_{II} phase (Figure 4b). The a_{cubic} varies from 248 ± 2 Å and 264 ± 2 Å at 1 bar,
167 to 264 ± 2 Å and 279 ± 2 Å, at 1000 bar for the two phases (Figure S3c). The presence of these
168 two Q_I phases may be due to different hydration levels as observed at 1 mol% squalane.

169

170

171 **IV. A competition between lamellar and inverted hexagonal phase appears by**
172 **adding 5 mol% squalane**

173 In presence of 5 mol% squalane at 1 bar, the system presents a mixture of one lamellar and one
174 inverted hexagonal phase at temperatures up to 55°C (Figure 5a). As expected, temperature
175 increases the membrane's negative curvature and at 70°C and above only the HII is present. In
176 contrast, pressure promotes the lamellar phase, which can be readily observed at 70°C, where
177 the lamellar phase appears above 350 bar. Higher HHP values further increase the structuration
178 of the lamellar phase (Figure 5b). At their highest temperature tested, e.i. 85°C, the lamellar
179 phase appears at 1000 bar. In presence of 5 mol% squalane, the lamellar-to-hexagonal transition
180 is a direct conversion as no cubic phase appears.

181 Interestingly, HHP swells much more the HII phase than the lamellar one. At 10°C, a_{lam} remains
182 almost constant at $57.8 \pm 0.4 \text{ \AA}$ – $58.0 \pm 0.4 \text{ \AA}$ from 1 bar to 1000 bar but, due to the capability
183 of squalane to reduce chain frustration, the hexagonal phase is able to swell from $111.8 \pm 0.5 \text{ \AA}$
184 at 1 bar to $121.2 \pm 0.5 \text{ \AA}$ at 1000 bar for the same temperature (Figure 5c and Figure S4).

185 The different pressure sensitivities of the L and HII phases are easily observed when plotting Δa ,
186 e.i. the difference between the lattice parameter at HHP and at ambient pressure (Figure 6a).

187 The Δa of the lamellar phase is ca. $0.2 \text{ \AA} \cdot \text{kbar}^{-1}$ and not significative. In contrast, the HII phases
188 swell up to $9.5 \text{ \AA} \cdot \text{kbar}^{-1}$ at 10°C and $1.5 \text{ \AA} \cdot \text{kbar}^{-1}$ at 85°C. Familiar $L\alpha$ phases usually swell up to
189 $2 \text{ \AA} \cdot \text{kbar}^{-1}$ while H_{II} may swell as much as $6 \text{ \AA} \cdot \text{kbar}^{-1}$ ³⁴. The swelling of HII decreases with
190 temperature. This unexpected behaviour can be explained by the coexistence of the HII and
191 lamellar phases in the system. Indeed, one can observe the change of slope of Δa at 70°C and
192 450 bar (Figure 6b), which coincides with the appearance of the lamellar phase in this lipid
193 system: in absence of the lamellar phase, Δa is $\sim 1 \text{ \AA} \cdot \text{kbar}^{-1}$, in presence of the lamellar phase it
194 increases to $5.2 \text{ \AA} \cdot \text{kbar}^{-1}$. At 85°C, at which no lamellar phase is observed below 1000 bar, the
195 slope of Δa remains close to $1 \text{ \AA} \cdot \text{kbar}^{-1}$. Such differences may be explained by the partition of

196 two polar headgroups and the apolar molecule squalane between the two phases in coexistence.
197 Indeed, in this lipid mixture we have two lipids with very different curvature. Phospholipids
198 containing PC headgroups present a zero-curvature monolayer and self-assemble in a lamellar
199 phase. DoPhPE easily bends under negative curvatures and therefore induce the formation of
200 inverted non-lamellar phases, such as the H_{II} . Accordingly, when L and H_{II} phases are present,
201 the system may present a “lipid-separation” and DoPhPE may have the tendency to adopt the
202 H_{II} phase with its preferred curvature. When the lamellar phase is present, as at 70°C and
203 pressures above 450 bar, DoPhPE may principally form the H_{II} phase (Figure 6c). This effect was
204 already observed in bacterial-like lipids, which better accommodate in L_{β} or L_{α} phases. For
205 example, a decrease in temperature induces domains enriched in saturated phospholipids with
206 small quantities of unsaturated lipids³⁷. As DoPhPE has a higher lateral compressibility, H_{II}
207 presents a higher Δa slope when most of DoPhPE is partitioned in the non-lamellar phase and
208 excluded from the L phase. However, the presence of methyls in the hydrocarbon chains could
209 reduce the lateral diffusion of these phospholipids and squalane may aggregate in the chain
210 frustration points and most especially at the interphase points, to fill the voids and release
211 packing tension. The chain-packing frustration increases as the diameter of the hexagonally
212 packed cylindrical inverse micelles increases. When the lamellar phase disappears, the apolar
213 molecule can better distribute along the H_{II} phase and squalane aggregates may disappear
214 (Figure 6c). Therefore, the lattice parameter of the H_{II} phase decreases and consequently its
215 pressure response.

216

217 **V. Only the inverted hexagonal phase is present when 10 mol% squalane is**
218 **added**

219 At 10 mol% squalane in the DoPhPC:DoPhPE (9:1) mixture only the H_{II} phase is observed under
220 all temperatures and pressures studied. At ambient pressure, we could detect a slow phase

221 transition between two HII phases. The axially integrated 1-D patterns at 55°C, 70°C and 85°C
222 (Figure 7a) shows the apparition of a HII phase at higher q and the gradual disappearance of the
223 peaks characteristics of the second hexagonal phase. The spectrum at 85°C stills shows
224 shoulders corresponding to the low temperature HII phase. The lattice parameters of HII range
225 from $104.8 \pm 0.5 \text{ \AA}$ at 10°C and 1 bar to $92.8 \pm 0.5 \text{ \AA}$ at 85°C and 1 bar (Figure 7c and Figure S5).
226 As thermal motions increase lipid dynamics, the HII phase is much more pressure sensitive at HT
227 (Figure 7b and 7c). Δa expansion increases from $0.1 \text{ \AA} \cdot \text{kbar}^{-1}$ at 10°C to $3.9 \text{ \AA} \cdot \text{kbar}^{-1}$ at 85°C
228 (Figure 7d). One can notice that the Δa curve as a function of pressure at 40°C presents 2 slopes,
229 consistent with a phase transition between two hexagonal phases. This phase transition also
230 possibly occurs at lower temperature, although very close to 1 bar. Such phase transition is not
231 visible at temperatures above 40°C. These low hexagonal phases differ principally by their ability
232 to swell with pressure, which is congruent with a higher pressure-dependent lateral
233 compressibility of the high temperature hexagonal phase.

234

235 **Conclusions**

236 X-ray diffraction experiments clearly show that the addition of squalane drastically changes
237 membrane curvature of a lipid DoPhPC:DoPhPE (9:1) mixture, an effect comparable to that of
238 cholesterol at biological relevant quantities on eukaryote-like lipids^{38,39}. SAXS data displays the
239 presence of highly stable non-lamellar lipid phases dependent on mol% of squalane,
240 temperature and pressure. The complete understanding of the observed structural transitions
241 will require a deeper study as it will be necessary to consider different kind of interactions, such
242 as lipid-water, lipid-lipid and lipid-squalane. However, the outstanding acubic values and
243 resistance revealed by the system may be useful for the stabilization or modification of
244 cubosomes and to adjust lipid membrane properties of archaeosomes^{40,41}.

245 The fact that small quantities of squalane (1 – 10 mol%) are capable to impose negative intrinsic
246 curvatures on the system has biological significance. Our results indicate that archaeal
247 membranes simply require to regulate squalane proportions in the cell membrane to tune its
248 curvature and flexibility. Therefore, due to the presence of small amounts of the apolar molecule,
249 archaeal cells would be capable to manage essential cell processes such as fusion and fission⁴².
250 Furthermore, recognizing that squalane is present in archaeal cell membranes and considering
251 the high metastability of all phase transitions in a large range of temperatures and pressures,
252 we can predict that non-lamellar phases and particularly, inverted cubic phases, may be locally
253 present in archaeal membranes.

254

255 **Conflicts of interest**

256 There are no conflicts to declare.

257

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262

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322 **Figures**

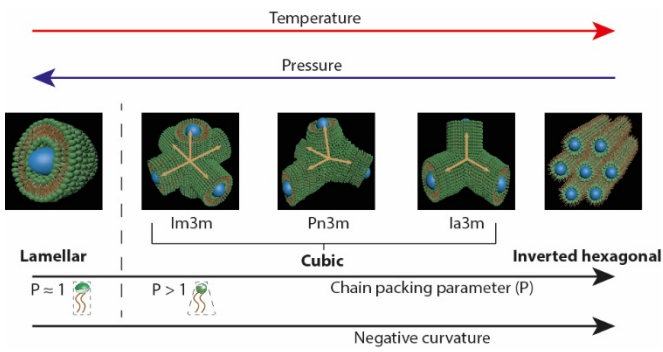


Figure 1. Schematic representation of different lipid phases as a function of lipid curvature. The lipid headgroups are represented in green, the hydrocarbon chains in brown and water location in blue. The arrows indicate the direction for the increase of the different parameters: chain packing parameter (P), negative intrinsic curvature of the membrane, pressure and temperature.

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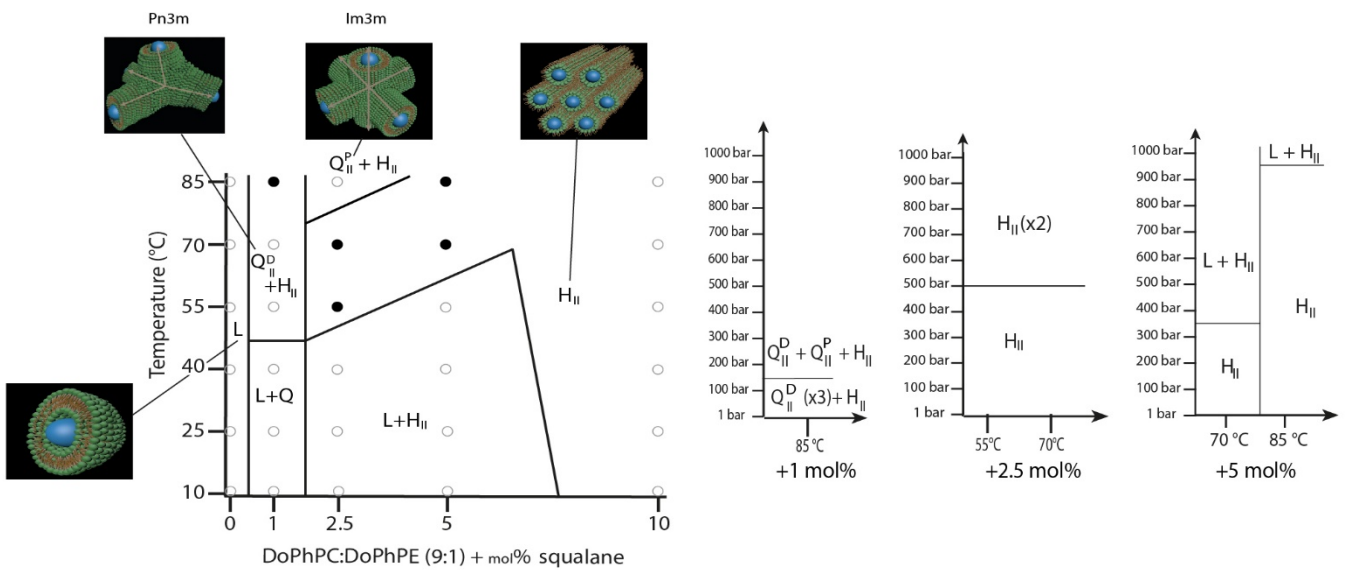


Figure 2. Phase diagrams of the DoPhPC:DoPhPE (9:1) in presence of excess water as a function of the proportion of squalane, temperature (Left panel) and pressure (Right panel) Left, sketches of each phase (same colour scheme as in figure 1). Full circles indicate conditions under which pressure-induced phase transitions are observed and detailed in the right panel, and open circles those under which no pressure-induced phase transition is observed between 0 and 1000 bar.

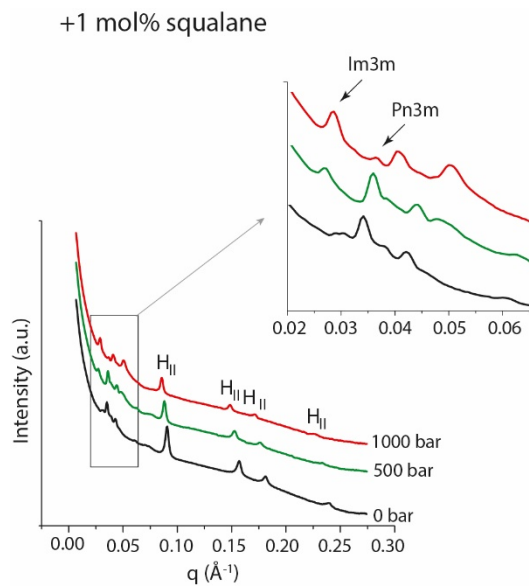


Figure 3. SAXS spectra of the mixture DoPhPC:DoPhPE +1 mol% squalane. At 85°C and ambient pressure (black), 500 bar (green) and 1000 bar (red). Top inset, zoom into the cubic phase region.

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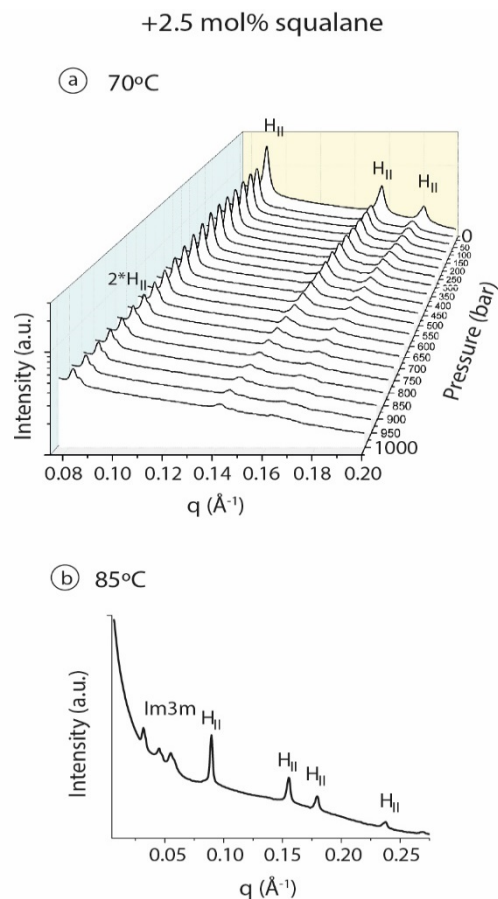


Figure 4. SAXS spectra of the mixture DoPhPC:DoPhPE + 2.5 mol% squalane. a) At 70°C as a function of applied pressure (from 0 to 1000 bar, 50 bar steps). Two H_{II} coexist above 600 bar. b) At 85°C at ambient pressure, Q_{II} Im3m and the H_{II} phases coexist.

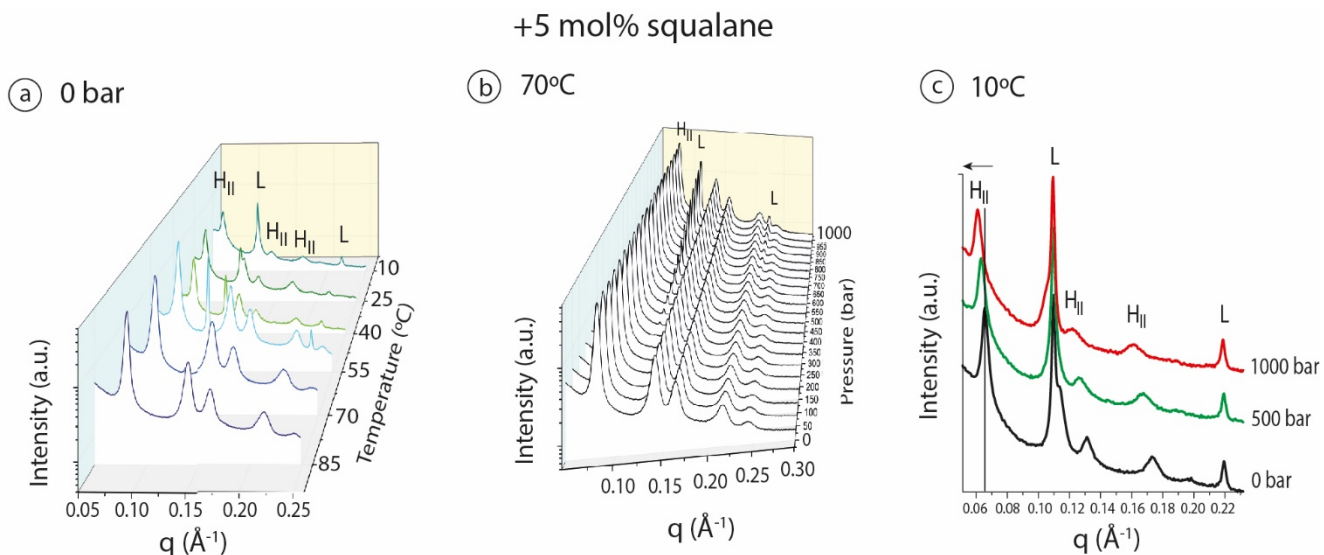


Figure 5. SAXS spectra of the mixture DoPhPC:DoPhPE + 5 mol% squalane. **a)** As a function of temperature at ambient pressure (0 bar applied). **b)** at 70°C as a function of pressure, from 0 to 1000 bar of pressure applied, 50 bar steps. A lamellar phase appears at 350 bar. **c)** At 10°C as a function of pressure. The L and H_{II} phases coexist at ambient pressure (black), 500 bar (green) and 1000 bar (red).

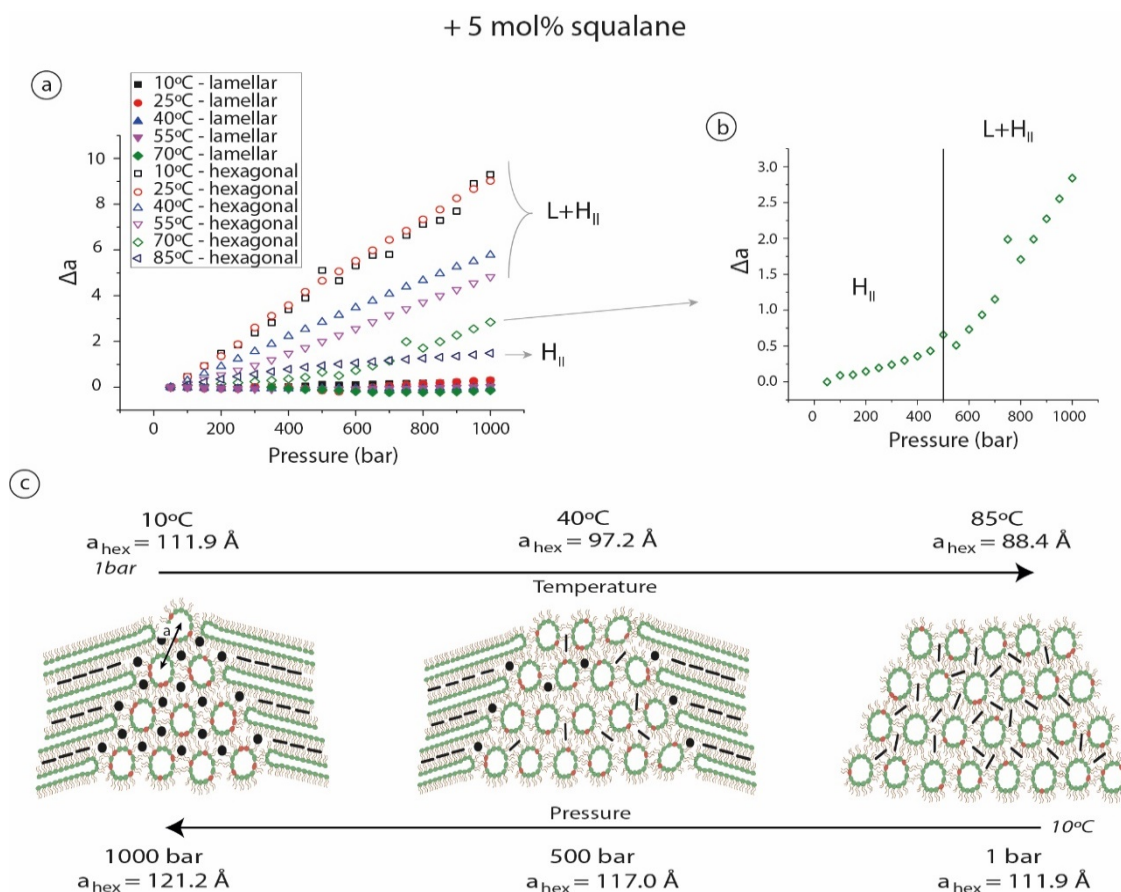


Figure 6. Squalane distribution between lamellar and hexagonal phases. **a)** Normalized lattice parameter (Δa) as a function of hydrostatic pressure. The lattice parameter has been normalized to that at ambient pressure for each temperature. L phase (full symbols); H_{II} (empty symbols). Up to 55°C, both, L and H_{II} coexist. **b)** Detail on Δa as a function of pressure at 70°C. **c)** Schemes of phase coexistence and lattice parameter evolution as a function of pressure and temperature in the DoPhPC:DoPhPE:5 mol% squalane system. Squalane is represented in black, black spheres represent squalane aggregates, lipid hydrocarbon chains are in brown, lipid headgroups are shown in green (PC) and in red (PE).

+ 10 mol% squalane

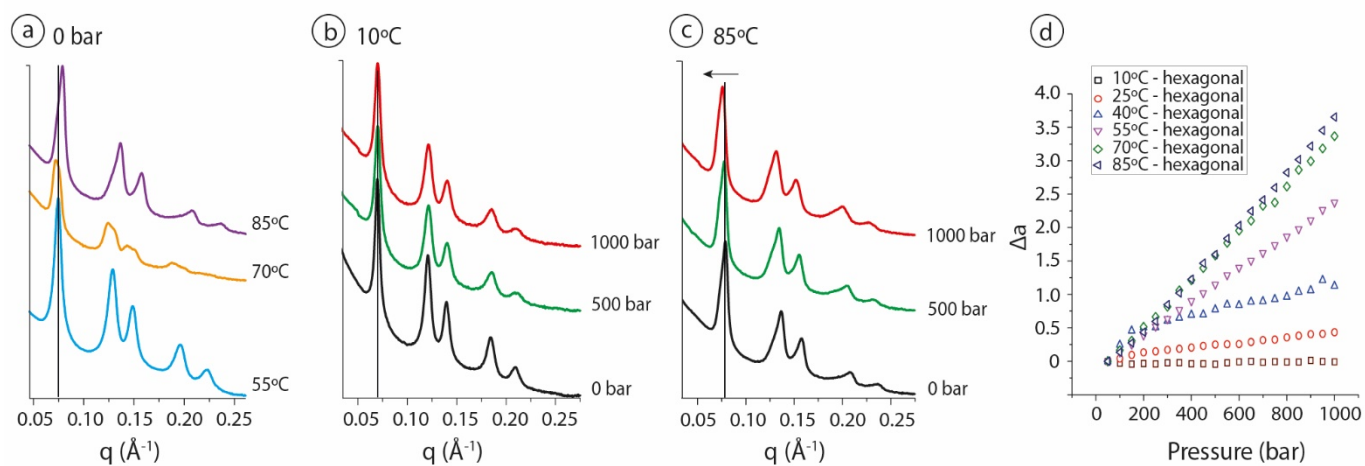


Figure 7. SAXS spectra of the mixture DoPhPC:DoPhPE + 10 mol% squalane. a) SAXS spectra at ambient pressure for 55°C (blue), 70°C (yellow) and 85°C (magenta). **b-c)** SAXS spectra at 10°C (b) and 85°C (c) of H_{II} phase as a function of pressure. **d)** Normalized lattice parameter of the H_{II} phase as a function of pressure and temperature. Lattice parameter has been normalized to ambient pressure conditions.

Electronic Supplementary Information

**Induction of non-lamellar phases in archaeal lipids at high
temperature and high hydrostatic pressure by apolar
polyisoprenoids**

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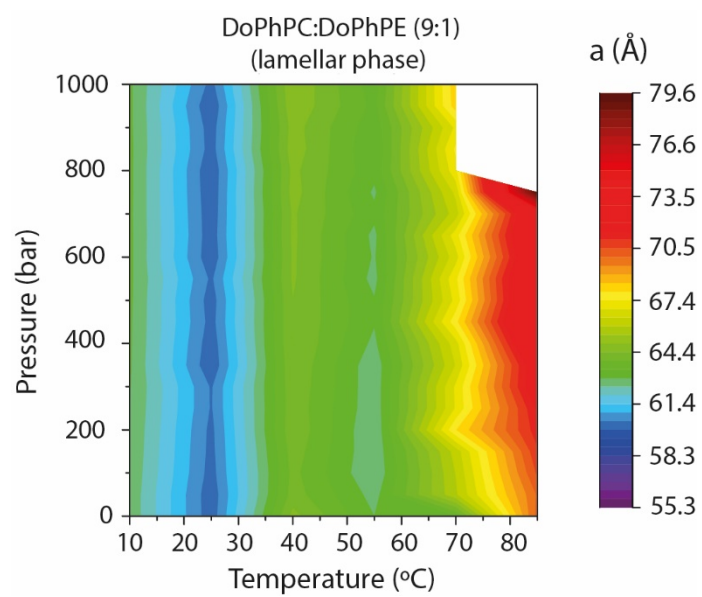


Figure S1. P,T color map of lattice parameters for the lamellar phase of DoPhPC:DoPhPE (9:1).

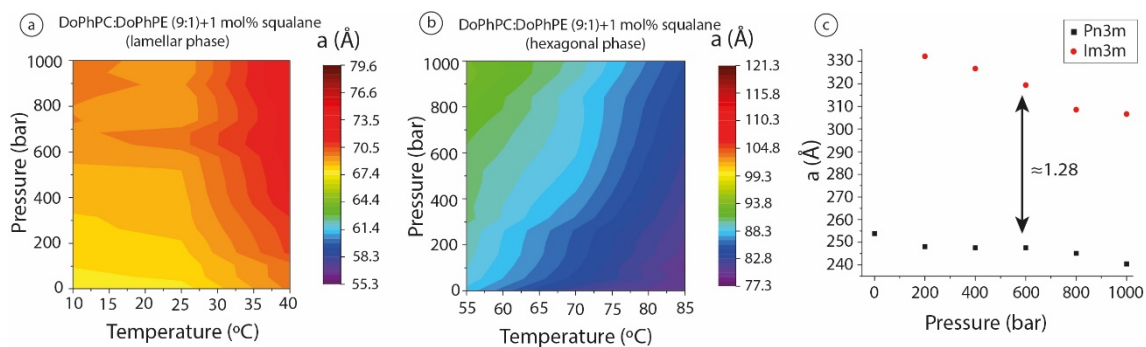


Figure S2. **a)** P,T color map of lattice parameters for the lamellar phase of DoPhPC:DoPhPE (9:1) + 1 mol% squalane. **b)** P,T color map of lattice parameters for the inverted hexagonal phase of DoPhPC:DoPhPE (9:1) + 1mol%. **c)** lattice parameters obtained for the cubic phases Pn3m (black) and Im3m (red) found at 85°C for the DoPhPC:DoPhPE (9:1) + 1mol% mixture.

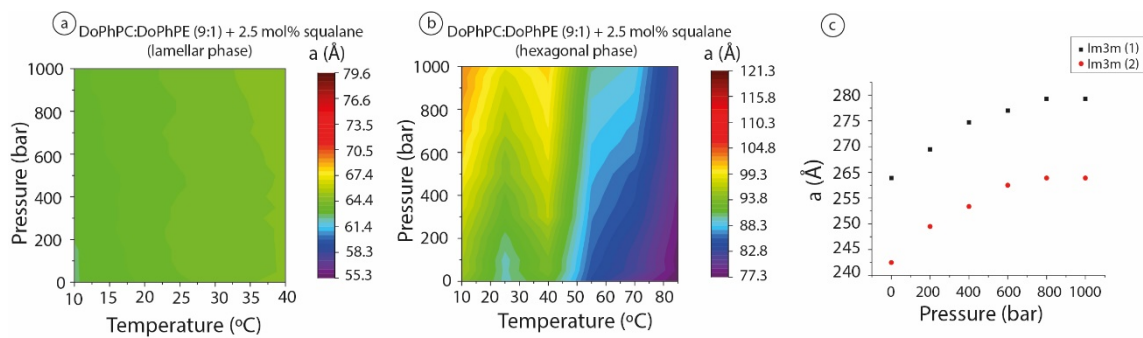


Figure S3. a) P,T color map of lattice parameters for the lamellar phase of DoPhPC:DoPhPE (9:1) + 2.5 mol% squalane. **b)** P,T color map of lattice parameters for the inverted hexagonal phase of DoPhPC:DoPhPE (9:1) + 2.5 mol%. **c)** lattice parameters obtained for the cubic phases $Im3m$ (red) found at 85°C for the DoPhPC:DoPhPE (9:1) + 2.5 mol% mixture.

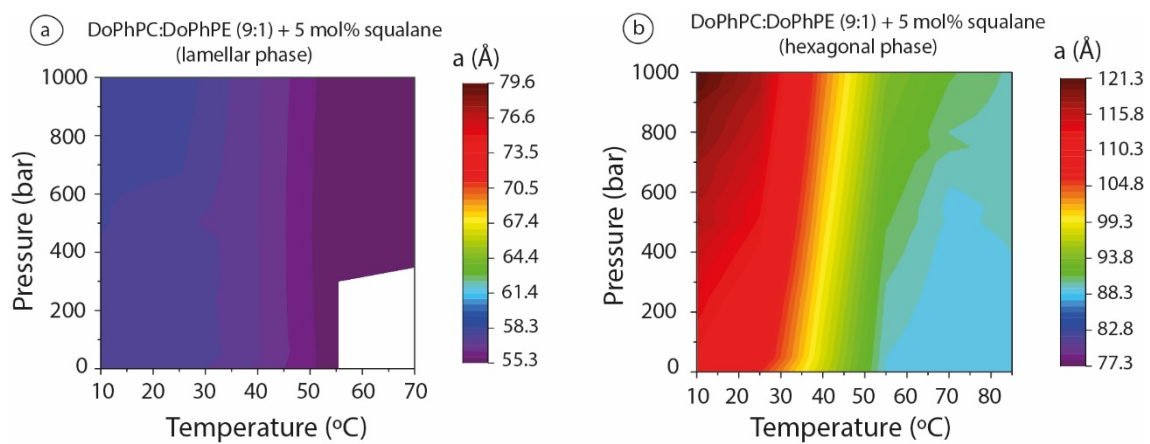


Figure S4. a) P,T color map of lattice parameters for the lamellar phase of DoPhPC:DoPhPE (9:1) + 5 mol% squalane. **b)** P,T color map of lattice parameters for the inverted hexagonal phase of DoPhPC:DoPhPE (9:1) +5 mol%.

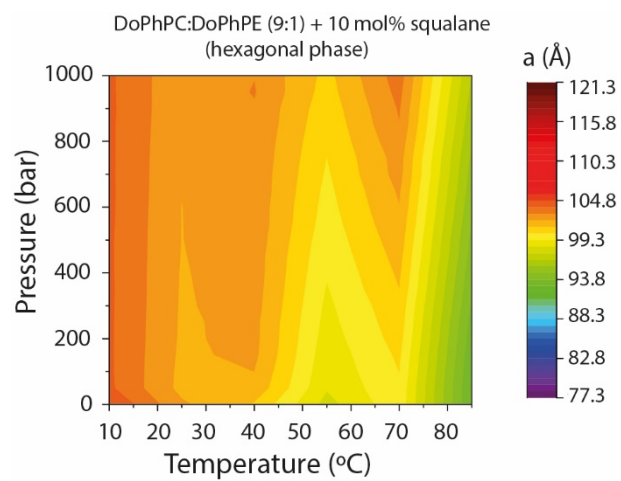


Figure S5. P,T color map of lattice parameters for the inverted hexagonal phase of DoPhPC:DoPhPE (9:1) +10 mol%.