Prebiotic vesicles retain solutes and grow by micelle addition after brief cooling below the membrane melting temperature

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ABSTRACT:
Replication of RNA genomes within membrane vesicles may have been a critical step in the development of protocells on the early Earth. Cold temperatures near 0°C improve the stability of RNA and allow efficient copying, while climate models suggest a cold early Earth, so the first protocells may have arisen in cold-temperature environments. However, at cold temperatures, saturated fatty acids, which would have been available on the early Earth, form gel phase membranes that are rigid and restrict mobility within the bilayer. Two primary roles of protocell membranes are to encapsulate solutes and to grow by incorporating additional fatty acids from the environment. We test here whether fatty acid membranes in the gel phase accomplish these roles. We find that gel-phase membranes of 10-carbon amphiphiles near 0°C encapsulate aqueous dye molecules as efficiently as fluid-phase membranes do, but the contents are released if the aqueous solution is frozen at -20°C. Gel-phase membranes do not grow measurably by micelle addition, but growth resumes when membranes are warmed above the gel-liquid transition temperature. We find that longer, 12-carbon amphiphiles do not retain encapsulated contents near 0°C. Together, our results suggest that protocells could have developed within environments that experience temporary cooling below the membrane melting temperature, and that membranes composed of relatively short-chain fatty acids would encapsulate solutes more efficiently as temperatures approached 0°C.

INTRODUCTION:

Many models for the formation of protocells on the early Earth involve encapsulation of RNA by a membrane[1]. However, it remains unclear whether environmental conditions that enhance the stability and function of RNA were beneficial, neutral, or detrimental to the stability and function of membranes. We have previously explored aspects of this question in the context of evaporating lakes that concentrate salts[2]. Here, we explore the effect of temperature.

Cold temperatures could have enabled development of RNA genomes[3]. In particular, nucleobases[4] and RNA[5,6] are more stable at temperatures near 0°C. Partially frozen water-ice eutectic mixtures and freeze-thaw cycles allow synthesis of nucleobases[7,8], oligomerization of nucleotides[9–13], and catalysis by ribozymes[14–17]. Coupled global carbon cycle and climate simulations show that the average surface temperature of the Earth may have been near 0°C from about 4.4 to 4.0 billion years ago when life likely originated[18].

How would membranes have fared in low temperature environments? Although the permeability and gel-liquid transitions of modern, phospholipid membranes are well-studied[19,20], corresponding data for prebiotic, fatty acid membranes are sparser. Fatty acids assemble into membranes when the solution pH is within about half a unit of the fatty acid pKa in the bilayer[21]. Fatty acids were delivered to the early Earth via meteorites[22], but they also could have been synthesized by the Fischer-Tropsch mechanism[23] or sparking reactions[24].

Historically, experiments with fatty acids have focused on membranes that are above their melting temperature[25], where the fatty acids are in a fluid phase characterized by high lateral mobility and mobile acyl chains (Fig. 1). A fluid, fatty acid membrane would have conferred many advantages to a protocell. Fluid vesicles have been shown to grow by incorporating additional fatty acids from micelles[26–28] or other vesicles[29]. Because these vesicles accumulate excess surface area as they grow, gentle shear forces can induce vesicle division[27], providing a simple, environmentally-driven mechanism for reproduction. Fluid membranes are permeable to small molecules while retaining larger ones, enabling synthesis of genetic polymers inside vesicles[30]. In addition, the building blocks of RNA and protein polymers bind to fluid-phase fatty acid vesicles, which could concentrate these molecules from dilute solutions in the early Earth environment[31–34].

Below their melting temperatures, fatty acid membranes are in a gel phase[35–37], characterized by amphiphiles with low lateral mobility and rigid acyl chains. It was previously unknown whether rigid, gel-phase fatty acid vesicles can retain encapsulated contents and whether they can grow via micelle addition. Here, we test permeability and growth in membranes of prebiotic single-chain amphiphiles with intermediate (10-carbon and 12-carbon) chain lengths.

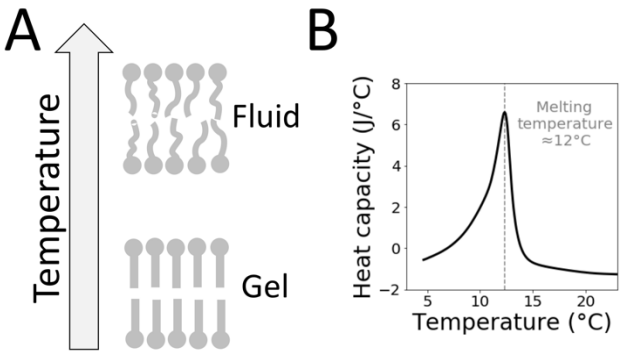

Figure 1: (A) The melting temperature of a fatty acid membrane is the transition from the gel to fluid phase. (B) For membranes composed of 1:1 decanoic acid:decanol at pH 8.7 (± 0.05), we measured a melting temperature of ~12°C using differential scanning calorimetry (see methods and results), which is consistent with previous results[36].

METHODS:

Materials:

Decanol, dodecanoic acid, dodecanol, and carboxyfluorescein were purchased from Sigma-Aldrich (St. Louis, MO). Decanoic acid was from Nu-Chek Prep (Elysian, MN) and $NaHCO_3$ was from EMD Chemicals (Darmstadt, Germany). Sepharose 4B was used for size-exclusion chromatography (Sigma-Aldrich, St. Louis, MO). Triton X-100 was from Supleco (Bellefonte, PA). Rhodamine B dihexadecanoyl-phosphoethanolamine (rhodamine-DHPE), N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dihexadecanoyl-phosphoethanolamine (NBD-PE), and 96-well polystyrene plates were from ThermoFisher (Waltham, MA). A 300-µL quartz cuvette #23-3.45-Q-3 was from Starna Cells (Atascadero, CA).

Preparation of vesicle solutions:

1.0 M stock solutions of decanoate and dodecanoate were made by dissolving solid decanoic or dodecanoic acid in an equimolar NaOH solution, followed by gentle heating to 60°C and rocking for 1.25 hr. Vesicle solutions were prepared in the following order: (1) Stock solutions of 1.0 M $NaHCO_3$ and 0.10 M carboxyfluorescein were diluted into ~18 mΩ-cm water. (2) 1.0 M decanoate (or 1.0 M dodecanoate) stock solution was added. (3) Liquid decanol (or dodecanol) was added, which induced vesicles to form. At this step, the solution contained 0.10 M $NaHCO_3$ and 60 mM carboxyfluorescein, both inside and outside the vesicles. The pH was adjusted to 8.70 ± 0.05 by adding small volumes of concentrated HCl or NaOH solutions**.** To avoid insolubility of dodecanoate and dodecanol at low temperatures, their stock solutions were heated at 60°C for 1 hr before mixing. The resulting solution was maintained at 60°C, and the solution was housed in a test tube held in an aluminum block at 60°C during pH adjustment.

Determining membrane melting temperature using calorimetry:

Heat capacities of vesicle solutions were measured using a Malvern Panalytical (Malvern, UK) MicroCal VP-capillary differential scanning calorimeter. 0.10 M $NaHCO_3$ at pH 8.7 (± 0.05) was used as a reference solution. Temperature sweeps were conducted at 60°C/hr.

Size-exclusion chromatography (SEC):

To encapsulate concentrated dye in vesicles, we allowed vesicles to self-assemble in a self-quenched (60 mM) carboxyfluorescein solution as described above, eluted the resulting solution through a SEC column, and collected aliquots enriched in vesicles and depleted of free dye. SEC columns were prepared with 6.9 mL Sepharose 4B resin. Before each SEC run, the column was flushed with at least two volume equivalents of running buffer. 500 µL vesicle solutions in the concentrations listed in Table 1 were added to the SEC column, and 400 µL fractions were collected by hand. After all fractions were collected, the column was flushed with at least 2 volume equivalents of 0.2 M bicine (pH 9) for storage. The SEC running buffer contained 0.10 M $NaHCO_3$ to match ionic conditions in the vesicle sample and 20 mM fatty acid (either decanoic acid or dodecanoic acid, corresponding to the vesicles) so that the total amphiphile concentration remained above the critical vesicle concentration[2]. The pH of the running buffer was adjusted to 8.70 (± 0.05) by adding small volumes of concentrated HCl or NaOH solutions. The fractions containing vesicles were combined and further diluted in SEC running buffer for analysis.

Table 1:

Expt.	Vesicles	Carbons	Ratio	Conc. before SEC	Conc. after SEC + dilution	Melting Temp	T_{melt} Data
Fig. 1	decanoic acid / decanol	10	1:1	37.5 mM / 37.5 mM	–	~12°C	Fig. S1
Fig. 2A, Fig. 5	decanoic acid / decanol	10	2:1	250 mM / 125 mM	~50 mM / ~25 mM	~9°C	Fig. S2
Fig. 2B	decanoic acid / decanol	10	1:1	112.5 mM / 112.5 mM	~37.5 mM / ~37.5 mM	~12°C	Fig. S1
Fig. 3	decanoic acid	10	–	60 mM	–	~17°C	Fig. S3
Fig. 4	dodecanoic acid / dodecanol	12	1:1	112.5 mM / 112.5 mM	~37.5 mM / ~37.5 mM	~31°C	Fig. S4
Fig. S13, S14	tetradecanoic acid / tetradecanol	14	1:1	112.5 mM / 112.5 mM	~37.5 mM / ~37.5 mM	~51°C	Fig. S5
Fig. S15, S16	nonanoic acid / nonanol	9	1:1	112.5 mM / 112.5 mM	~37.5 mM / ~37.5 mM	< 4°C	Fig. S6

Release of encapsulated dye at constant temperature:

After SEC, vesicle solutions contained ~50 mM decanoic acid and ~25 mM decanol, and the vesicles encapsulated self-quenched carboxyfluorescein at ~60 mM. Vesicle solutions were held in a glass cuvette at 23.8-25.6°C (for fluid-phase membranes) and 4.5-5.9°C (for gel-phase membranes), including during measurement. Carboxyfluorescein fluorescence (ex475/em520) was measured every hour for 5 hours using a Horiba Fluorolog (Kyoto, Japan). After 5 hours, Triton X-100 was added (final concentration 0.3% by weight) to disrupt vesicles, leading to complete release of encapsulated carboxyfluorescein and corresponding increase in the fluorescence signal.

Release of encapsulated dye during temperature cycling:

After SEC, vesicle solutions contained ~37.5 mM decanoic acid and ~37.5 mM decanol, and the vesicles encapsulated self-quenched carboxyfluorescein at ~60 mM. 350-µL aliquots of vesicle solution were distributed to 1.5 mL Eppendorf tubes, and three types of experiments were

performed. In Experiment 1, samples were maintained in an oven at 25°C (fluid phase) for 120 min. In Experiment 2, samples were maintained at –2.7 to 1.8°C (gel-phase fatty acid membranes in a fluid-phase solution) for 120 min using either a refrigerator or an ice bath. In Experiment 3, temperature was cycled three times, starting with 20 min at the cold temperature (–2.7 to 1.8°C, either in a refrigerator or ice bath), then 20 min in an oven at 25°C, for a total of 120 min. The 20-min equilibration times were verified to be long enough for completion of the gel-liquid transition of all vesicles.

Triplicate 100 μL aliquots from each vesicle sample were then transferred into a 96-well plate. Carboxyfluorescein fluoresence was measured at room temperature using a Thermo Labsystems (Gulph Mills, PA) Fluoroskan Ascent FL Fluorescence Microplate Reader with ex485/em520. At the end of the experiment, Triton X-100 (final concentration 0.3% by weight) was added to disrupt vesicles and release all encapsulated carboxyfluorescein.

Vesicle surface area assay:

Hydrophobic dyes were added to solutions of nominally 100-nm vesicles as follows. First, vesicles self-assembled in solutions of 60 mM decanoic acid and 200 mM HEPES, with pH adjusted to 7.60 (+/- 0.05) by the addition of small volumes of concentrated NaOH solution. Next, stock solutions of dye in chloroform (10 mM NBD-PE and 5 mM rhodamine-DHPE) were dried in glass vials under N_2 followed by 30 min under vacuum. The vesicle solution was added to the vials corresponding to a final concentration of dye of 60 μM. The vials were rocked for 2 days to incorporate dye into the vesicles. The vesicles were extruded 9 times through 100-nm filters. 90 μL aliquots of vesicle solution were distributed to wells in a 96-well plate, which was then placed on a metal heating block (Fig. S7). Temperature was maintained at 24.7-26.4°C for fluid-phase vesicles and at 0.9-1.5°C for gel-phase vesicles.

Vesicles grew in the presence of micelles for 0-40 min. The protocol for adding micelles to vesicle solutions (Fig. 3B) was designed so that all Förster resonance energy transfer (FRET) data were collected simultaneously at room temperature (~20°C) because membrane FRET is inefficient at ~0°C. 1) We prepared parallel samples of vesicles in the fluid or the gel phase in a 96-well plate. 2) The 96-well plate was equilibrated to the temperature of fluid or gel-phase vesicles, along with a solution of decanoic acid micelles (1.0 M decanoic acid in 1.0 M NaOH). 3) In 5 min intervals, 10 μL of micelle solution (~2 equivalents of decanoic acid) was added to a different vesicle sample in the 96-well plate to achieve growth times of 0-40 min (the time prior to addition of the micelle solution is referred to as the "wait time" in Figure 3). The total time elapsed from the start of the experiment was the same for each vesicle sample, 40 min. One 100-μL aliquot of vesicles without micelles was reserved as a control. 4) Over an additional 2 min, the plate was brought to 25°C, then room temperature (~20°C), and 5) FRET was measured in all wells simultaneously (ex485/em538 and ex485/em589). Thus, micelle addition and vesicle growth occurred in either gel or fluid phase, and surface area was always measured on fluid-phase vesicles. 6) Vesicles that had been in the gel phase were then held at room temperature for an additional 10 min, and FRET was measured again.

Using a phenomenological relationship between the FRET intensity (I, taken as em538/em589) and the concentration of dye molecules in the membrane (Fig. S8), the vesicle surface area (A) was calculated as:

$$A = 4\pi r^2 \frac{C_0}{C_t} \quad (1)$$

In the equation, r is the initial radius of the vesicles (taken as 50 nm), C_0 is the initial concentration of FRET-pairs in the membrane (0.1%), and C_t is the concentration of those FRET-pairs at time t (determined from Fig. S8). Surface area was normalized by the value from a control without added micelles.

Release of dye from vesicles of 12-carbon amphiphiles:

After SEC, vesicle solutions contained ~37.5 mM dodecanoic acid and ~37.5 mM dodecanol, and the vesicles encapsulated self-quenched carboxyfluorescein at ~60 mM. Two 350-µL volumes of vesicle solution were distributed to 1.5-mL Eppendorf tubes. One was maintained at 25°C for 120 min; the other was maintained on ice for 120 min. The membranes were in the gel phase at both temperatures. Triplicate 100-µL aliquots from each volume were transferred into a 96-well plate. Carboxyfluorescein fluorescence was measured at room temperature using a Thermo Labsystems (Gulph Mills, PA) Fluoroskan Ascent FL Fluorescence Microplate Reader with ex485/em520. At the end of the experiment, Triton X-100 (final concentration 0.3% by weight) was added to disrupt vesicles, which released all encapsulated carboxyfluorescein.

Release of dye from vesicles during complete freezing:

After SEC, vesicle solutions contained ~50 mM decanoic acid and ~25 mM dodecanol, and the vesicles encapsulated self-quenched carboxyfluorescein at ~60 mM. Two 500-µL samples of vesicle solution were distributed to glass tubes. One sample was maintained at 25°C for 180 min; the other sample was frozen at -20°C for 120 min, and then maintained at 25°C for 60 min to thaw. Both samples were incubated for a total time of 180 min. Triplicate 100-µL aliquots from each vesicle sample were transferred to a 96-well plate. Carboxyfluorescein fluorescence was measured at room temperature using a Thermo Labsystems (Gulph Mills, PA) Fluoroskan Ascent FL Fluorescence Microplate Reader with ex485/em520. At the end of the experiment, Triton X-100 (final concentration 0.3% by weight) was added to disrupt vesicles, which released all encapsulated carboxyfluorescein.

RESULTS AND DISCUSSION:

Our goal is to determine whether cold conditions, which enhance the stability of RNA, would have been detrimental to membranes of protocells, either by causing leakage of aqueous contents or by preventing growth. Specifically, we investigated conditions in which fatty acid membranes were in a gel phase rather than a fluid phase.

Our focus is on prebiotic fatty acids, which include decanoic acid (10-carbons), and, to a lesser extent, dodecanoic acid (12-carbons) [22–24]. We added a prebiotic long-chain alcohol (decanol or dodecanol) to the vesicles in order to improve retention of encapsulated dye after size-exclusion chromatography (SEC). Unless otherwise specified, vesicles were multilamellar with a heterogenous distribution of sizes up to ~10 µm. Because the melting temperature of fatty

acid membranes is not necessarily the same as for pure fatty acids [35–37], we measured membrane transitions, which vary from < 4°C to 51°C (Table 1, Fig. S1-S6).

To quantify leakage across membranes, we filled fatty acid vesicles with high (60 mM) concentrations of self-quenched carboxyfluorescein dye. Subsequent leakage of the dye from the self-quenched interior of the vesicle to the dilute exterior produced a robust fluorescence signal. We held vesicles at temperatures above or below their membrane melting transition and found that dye leaks from the interior of gel-phase vesicles at a similar, slow rate as from fluid-phase vesicles (Fig. 2A). Moreover, gel-phase vesicles maintain encapsulation of dye over long periods (24 hours) near 0°C (Fig. S12). Dye retention is similarly high when fatty acid vesicles are quickly cycled across the gel-liquid transition (Fig. 2B-C). For example, when vesicles of 1:1 decanoic acid:decanol (with a melting temperature of ~12°C as in Fig. S1) were cycled three times between ~25°C and 0.0°C ± 2.7°C, the leakage of encapsulated carboxyfluorescein dye was indistinguishable from leakage from vesicles that were maintained in only fluid or gel phases (Fig. 2B). The ability of gel-phase membranes to retain encapsulated solutes suggests that protocells composed of fatty acid membranes could have survived within cold, unfrozen environments.

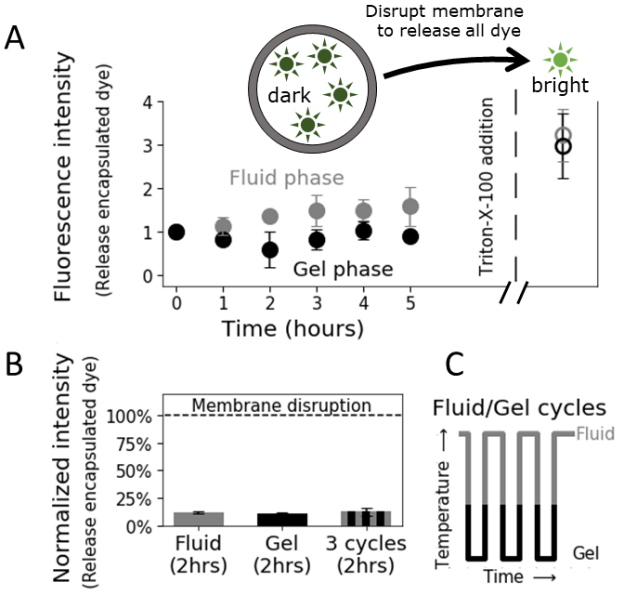

Figure 2: Gel phase fatty acid vesicles encapsulate dye as efficiently as fluid phase vesicles do. (A) Complete disruption of vesicle membranes with Triton X-100 releases all encapsulated dye and increases fluorescence. In contrast, leakage of dye is slow across fluid- and gel-phase membranes. In Panel A, vesicles of 2:1 decanoic acid:decanol (melting temperature ~9°C, Fig. S2) were held in the gel (5.0 ± 0.9°C) or fluid phase (25.0°C ± 1.2°C) up to 5 hours, and fluorescence was measured in a fluorimeter. (B-C) Cycling temperature three times between the fluid (25.0°C) and gel phases (0.0 ± 2.7°C) does not increase leakage relative to holding vesicles at a constant temperature. Vesicles of 1:1 decanoic acid:decanol (melting temperature ~12°C, Fig. S1) were held at constant temperature or cycled for 2 hours. Fluorescence was measured in a plate-reader before and after disruption of membranes with Triton X-100. Fluorescence intensity data are normalized as a percent after disruption. Normalized intensities are related to (but not necessarily proportional to) the number of dye molecules that leak from vesicles. Error bars are standard deviations from 3-4 independent experiments.

Our results in Fig. 2B are surprising in the context of phospholipid membranes. Although the permeability of phospholipid membranes is similar in the fluid and gel phases, it spikes near the phospholipid's melting temperature [19,38]. In contrast, fatty acid membranes do not appear to experience higher permeability as they repeatedly pass through their melting temperature. Overall, vesicles of fatty acids are regarded as more permeable than vesicles of modern phospholipids [39]. In some scenarios, permeability is an advantage: replication of RNA in protocells may have been enabled by the movement of small molecules across the membrane while larger molecules were retained in the protocell's lumen[30]. It is difficult to place our results on gel-phase vesicles in the context of other fatty acid membranes because we are unaware of any other tests of membrane permeability (or growth) for gel-phase fatty acid membranes.

Having established that gel-phase vesicles can retain their contents, we next examined if they grow upon addition of micelles. In short, we did not observe convincing signs of growth after the addition of micelles to gel phase vesicles. However, subsequent heating of vesicles above their melting temperature allows them to grow again (Fig. 3). We conducted our experiments by adding decanoic acid micelles to fluid (25.0 ± 1.4°C) or gel (1.0 ± 0.5°C) vesicles with melting temperatures of ~17°C (Fig. S3), then monitoring vesicle growth over times ranging from 0-40 min. Specifically, we measured Förster resonance energy transfer (FRET) between two dyes in the membrane, and we converted fluorescence intensities to membrane surface areas, as described in the Methods.

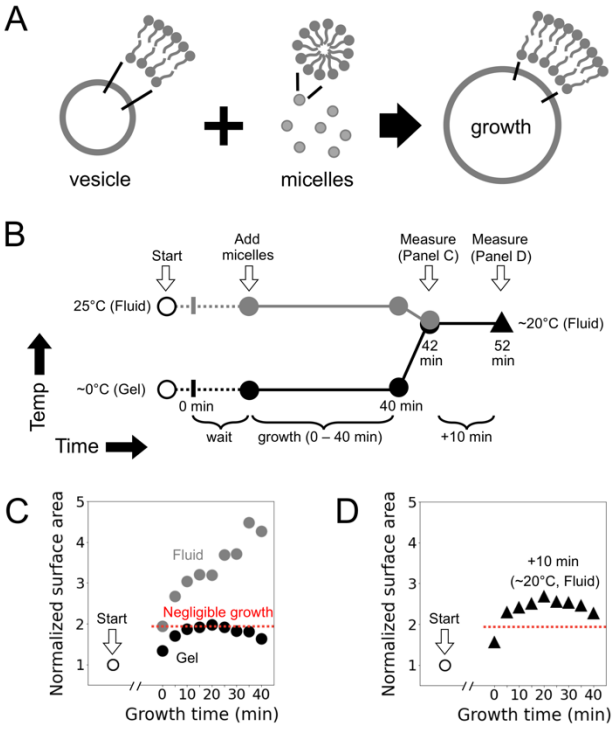

Figure 3: In the presence of micelles, the surface area of fluid-phase (but not gel-phase) vesicles increases with time. (A) Vesicles grow by incorporating fatty acids from micelles. (B) Protocol for introducing micelles to 100-nm diameter decanoic acid vesicles (melting

temperature ~17°C) in the fluid (25.0 ± 1.4°C) or gel (1.0 ± 0.5°C) phase. Growth times ranged from 0-40 min, and the sum of the growth time and wait time was always 40 min. Over the next 2 min, all samples were brought into the fluid phase (25.0°C), and FRET was measured at ~20°C (data in Panel C). After an additional 10 min at ~20°C, FRET was measured again for the samples that had been in the gel phase (data in Panel D). Gel-phase vesicles showed negligible growth during the timecourse, relative to fluid-phase vesicles at zero growth time. However, the vesicles in these samples did grow during an additional 10 min in the fluid phase (~20°C). Data in panels C and D are from a single experiment, and are consistent with other independent trials (Fig. S9-S10).

In detail, our vesicle growth results were as follows. First, we generated unilamellar, 100 nm vesicles by extrusion. When micelles were added to fluid-phase vesicles, the membrane area increased with time (Fig. 3C), consistent with previous observations[26–28]. In controls without micelles, membrane area remained constant, as expected (Fig. S11). When micelles were added to gel-phase vesicles, membrane area showed negligible growth – it did not exceed the area of fluid-phase vesicles at zero time (Fig. 3C and Fig. S9). When micelles were added to gel-phase vesicles (at each point in the timecourse) and subsequently the set of samples was held in the fluid phase (~20°C for 10 min), vesicle surface area increased (Fig. 3D and S10). These results were reproducible over multiple independent experiments (Fig. S9-S10).

In addition to decanoic acid, which was used in the experiments above, saturated fatty acids with longer carbon chains were likely present on early Earth as well[22–24]. Could these longer-chain fatty acids assemble into the membranes of protocells if the Earth was cold? Longer-chain fatty acids are often used in model protocells because they have lower critical vesicle concentrations[35], and because they encapsulate aqueous solutes at higher temperatures[40]. We next asked: if temperature is much lower (near 0°C), do longer-chain fatty acids encapsulate solutes more (or less) effectively than decanoic acid?

We produced vesicles of longer chain 12-carbon amphiphiles (1:1 dodecanoic acid:dodecanol) with a gel-liquid transition at ~31°C (Fig. S4). This transition temperature is higher than for membranes composed of 1:1 decanoic acid:decanol (~12°C), and the solubility of 12-carbon amphiphiles is lower than for 10-carbon amphiphiles. When the vesicles of 12-carbon amphiphiles are held in the gel phase close to the gel-liquid transition (25°C), they retain encapsulated dye (Fig. 4) like the vesicles of 10-carbon amphiphiles. However, at lower temperatures (~0°C, on ice), they completely release the dye, unlike the 10-carbon vesicles at ~0°C (Fig. 2). It is unclear if this difference is due to the larger, 30°C offset between the membrane's melting temperature and the sample temperature or due to the low solubility of 12-carbon chains at 0°C. We observe similar behavior with vesicles of 14-carbon amphiphiles (1:1 tetradecanoic acid: tetradecanol, Fig. S13-14), whereas short-chain 9-carbon amphiphiles (1:1 nonanoic acid: nonanol) retain encapsulated dye at 0°C (Fig. S15). Our results imply that membranes composed of relatively short-chain fatty acids would have had an advantage as temperatures approached 0°C.

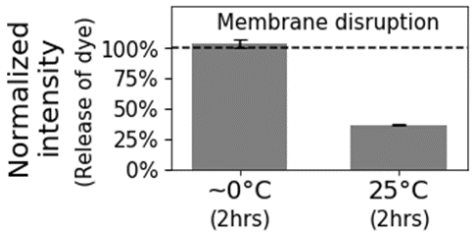

Figure 4: Vesicles of 12-carbon amphiphiles (1:1 dodecanoic acid:dodecanol) release encapsulated dye after 2 hrs at ~0°C, but retain dye after 2 hrs at 25°C. Fluorescence intensity is normalized by the fluorescence intensity after membrane disruption by Triton X-100, and expressed as a percent. Error bars are standard deviations from three independent experiments.

Our results above report on the behavior of model protocell membranes in the gel phase, when the vesicles are in unfrozen solution near 0°C. But what if the early Earth experienced even colder temperatures such that the bulk solution froze completely? To investigate this, we examined if fatty acid vesicles retain encapsulated solutes after freezing and thawing of the surrounding solution.

We froze solutions of vesicles composed of 2:1 decanoic acid: decanol at -20°C, and we observed that all encapsulated dye leaked from the vesicles upon thawing (Fig. 5). We observed similar behavior for vesicles of 1:1 nonanoic acid: nonanol (Fig. S16). This is in contrast to modern, phospholipid membranes, which retain some of their encapsulated contents after complete freezing[41]. Our vesicles of 2:1 decanoic acid:decanol have a membrane transition temperature of ~9°C, so there is ~30°C offset between the membrane transition temperature and the sample temperature. This may be analogous to our experiments with 12-carbon amphiphiles because we create a similar ~30°C offset, and we observe release of encapsulated dye in both experiments.

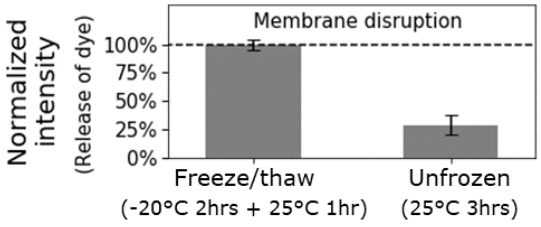

Figure 5: Vesicles of 10-carbon amphiphiles (2:1 decanoic acid:decanol) release encapsulated dye after 2 hrs of freezing at -20°C and 1 hr of thawing at 25°C. A unfrozen sample of the vesicles retains dye after 3 hrs at 25°C. Fluorescence intensity is normalized by the fluorescence intensity after membrane disruption by Triton X-100, and expressed as a percent. Error bars are standard deviations from three independent experiments.

CONCLUSIONS:

Temperatures near 0°C enable synthesis of RNA genomes[3–12,14–17], making cold brines an attractive environment for the development of protocells on the early Earth. Here, we have

shown that vesicles composed of 10-carbon amphiphiles (decanoic acid and decanol) retain encapsulated solutes when they are transiently cooled to temperatures near 0°C, even though the vesicle membranes are in a gel phase at these cold temperatures. Gel-phase membranes of decanoic acid do not grow by micelle addition, but membrane growth ensues when the temperature is increased above the melting transition. Suppressing the melting temperature could enable the growth of fatty acid membranes near 0°C. Sugars, nucleobases, amino acids, and peptides in the prebiotic environment could bind to fatty acid vesicles[31,32,34] and decrease the membrane melting temperature, as membrane proteins do in cell-derived phospholipid membranes[42]. Similarly, polyaromatic hydrocarbons incorporate into fatty acid membranes[43] and could behave analogously to sterols by fluidizing the membrane.

We have shown that vesicles of 12-carbon amphiphiles (dodecanoic acid and dodecanol) do not encapsulate solutes after cooling near 0°C, which suggests that protocells composed of relatively short-chain amphiphiles may have had an advantage in cold conditions on the early Earth. Additionally, complete freezing disrupts encapsulation by fatty acid vesicles, which may have constrained protocell development to unfrozen solutions. Our results imply that fatty acid vesicles could have contributed to the formation of the earliest protocells within cold, unfrozen environments on the early Earth

ASSOCIATED CONTENT:

The following files are available free of charge:

Supplementary data and descriptions of methods (PDF)

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AUTHOR CONTRIBUTIONS:

Z.R.C and Z.R.T. collected data. Z.R.C., Z.R.T., R.A.B, S.L.K, and D.C.C. analyzed data and wrote the paper.

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ABBREVIATIONS:

$NaHCO_3$, sodium bicarbonate; NaOH, sodium hydroxide; SEC, size-exclusion chromatography; FRET, Förster resonance energy transfer.

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