Growth of Prebiotically Plausible Fatty Acid Vesicles Proceeds in the Presence of Prebiotic Amino Acids, Dipeptides, Sugars, and Nucleic Acid Components

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ABSTRACT: Fatty acid vesicles may have played a role in the origin of life as a major structural component of protocells, with the potential for encapsulation of genetic materials. Vesicles that grew and divided more rapidly than other vesicles could have had a selective advantage. Fatty acid vesicles grow by incorporating additional fatty acids from micelles, and certain prebiotic molecules (e.g., sugars, nucleobases, and amino acids) can bind to fatty acid vesicles and stabilize them. Here, we investigated whether the presence of a variety of biomolecules affects the overall growth of vesicles composed of decanoic acid, a prebiotically plausible fatty acid, upon micelle addition. We tested 31 molecules, including 15 dipeptides, 7 amino acids, 6 nucleobases or nucleosides, and 3 sugars. We find that the initial radius and final radius of vesicles are largely unaffected by the presence of the additional compounds. However, three dipeptides enhanced the initial rates of growth compared to control vesicles with no small molecules added; another three dipeptides decreased the initial rates of growth. We conclude that vesicles can indeed grow in the presence of a wide range of molecules likely to have been involved in the origin of life. These results imply that vesicles would have been able to grow in complex and heterogeneous chemical environments. We find that the molecules that enhance the initial growth rate tend to have hydrophobic groups (e.g., leucine), which may interact with the lipid membrane to affect growth rate; furthermore, the molecules that cause the largest decrease in initial growth rate are dipeptides containing a serine residue, which contains a hydroxyl group that could potentially hydrogen-bond with the fatty acid carboxylate groups.

INTRODUCTION

Fatty acids are thought to be important for the origin of life because they can spontaneously self-assemble in aqueous solutions to form vesicles that may encapsulate soluble components, including biopolymers (Figure 1).1−4 It has been postulated that the first protocells were bounded by fatty acids rather than the phospholipids in modern membranes because fatty acids were likely available on early Earth through synthesis on metal catalysts,5,6 electrochemistry,7,8 and/or photochemistry.9,10 In addition, fatty acids have been found in meteorites,11−14 indicating that these molecules were delivered to the early Earth. For further discussion on the sources of fatty acids on the early Earth, see Cohen et al.15

Although modern cells have proteins that are dedicated to the task of membrane growth and division, it was presumably crucial that fatty acid protocells grew and divided before proteins arose. Dynamic assemblies of fatty acids are well-suited to this task, since fatty acid membranes grow and divide much more readily than membranes of phospholipids. When the solution pH is above the fatty acid pK_a, fatty acids assemble into micelles. If the solution pH is near the fatty acid pK_a, assembly into vesicles is favored. Membranes can grow in a prebiotically plausible manner through the addition of fatty acid micelles (pH > pK_a) to a buffered solution of fatty acid vesicles (pH ∼ pK_a), see Figure 2).16−18 Vesicles that grow and divide more rapidly than others may have had a selective advantage in the competition for resources, perhaps leading to initiation of Darwinian evolution.19−21

Given the likely complexity of the prebiotic chemical environment, we sought to investigate how vesicle growth would occur in the presence of other prebiotic molecules. If the presence of a set of molecules results in advantages to a population of vesicles, chemical selection could have occurred prior to the arrival of biology. Prior work in the field has laid the foundation for addressing this question.

Previously, Adamala and Szostak22 found that oleic acid vesicles (18 carbons and 1 double bond) containing a dipeptide catalyst that synthesizes another dipeptide out-compete a population of vesicles without the dipeptides,
yielding a potential for selective evolution. The newly synthesized molecule in Adamala and Szostak’s system, N-acetyl-L-phenylalanine leucinamide, bound to the membrane, thereby influencing vesicle growth. While N-acetyl-L-phenylalanine leucinamide is not necessarily prebiotic, other prebiotically plausible small molecules have been shown to bind to fatty acid vesicles. For example, nucleobases and prebiotic amino acids can bind to vesicles and stabilize them against 10 mM magnesium salt, which would otherwise destabilize the vesicles. RNA catalysis and nonenzymatic RNA replication use moderately concentrated divalent cations, so vesicles stabilized in increased Mg concentrations may have helped to enable RNA activity within vesicles.

Given the suggestions from past work that small molecules can bind to vesicles and that vesicle growth can be affected by the binding of other molecules, we sought to examine vesicle growth in the presence of a wide range of prebiotically plausible small molecules. Here, we test 31 small molecules that are important within prebiotic contexts to discover whether they influence the growth of vesicles. We test amino acids, dipeptides, nucleobases, nucleosides, and sugars, all of which are building blocks of biological polymers. Furthermore, the vesicles and micelles in our experiments are composed of decanoic acid, a saturated fatty acid with only 10 carbons; it is widely considered to be among the more prebiotically plausible membrane formers. Our goal is to discover whether the small molecules influence the growth rate and final size of vesicles and to thereby determine whether interactions between small molecules and membranes could have driven chemical evolution toward biological polymers.

### EXPERIMENTAL SECTION

Chemicals were purchased from Sigma-Aldrich and BACHEM, in purities >95%. Lipid dyes N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (NBD-PE) and Lissamine rhodamine B 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Rh-DHPE) were purchased from Avanti Polar Lipids.

To measure the growth of vesicles, we employed a Förster resonance energy transfer (FRET) assay, in which the FRET pair Rh-DHPE and NBD-PE were incorporated (1:1000 FRET fluorophores/lipids) into 60 mM decanoic acid vesicle solutions, buffered to pH 7.6 in 200 mM 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES) buffer. Vesicles were made by diluting concentrated stock solutions of the components into water and then adjusting the pH to the desired value of 7.6. Vesicles were then extruded through 100 nm filters. To test the effect of various molecules, in most cases, we weighed out a solid compound corresponding to 10 mM solutions of the molecule and added the extruded vesicle solution to the solid. After 60 s, the samples were vortexed.

To solve the problem that acidic amino acids/dipeptides (asp, glu, Gly-Glu) could significantly change the pH of the solution when added as solids, 1 M concentrated solutions were prepared in 50 mM HEPES buffer, and the pH was adjusted to ~7.6. This solution was then diluted 1:100 into the vesicle prep. As a control, the same process was performed without the additional acidic amino acid or dipeptide, and the pH was verified to be unchanged from 7.6. As another control, we added glycine as a solution and found the results to be consistent with when glycine was added as a solid.

To initiate growth, a 5% volume of 1 M decanoic acid micelles (pH ~10) was added to the samples, and the fluorescence was measured over a timecourse of 90 min. The mixing of the sample and initiation of the measurements took on the order of 30 s, so the initial measurement was not precisely at time 0, which might introduce small errors into the quantitative values for initial growth rates. However, this error is present in all samples, so comparisons between vesicles with and without additional molecules should still be valid. The addition of micelles increases the pH by only a small amount (~0.1 pH units) consistently across all samples, allowing vesicles to still be the favored form of fatty acids in the sample and for accurate comparison of vesicle growth across all samples.

Fluorescent donor and acceptor molecules were excited at 485 nm, and emission was measured at 538 and 589 nm, respectively, with a Thermo Labsystems Fluoroskan Ascent FL microplate reader. The ratio of the two fluorescence signals decreases as vesicles grow because the fluorescent dyes disperse over a larger surface area. A standard curve (Figure S5) was used to convert the fluorescence ratio to an effective dye concentration and, therefore, vesicle size (see Supporting Information Section 2 for details).

Day-to-day variation in sample preparation can be significant, so all data for vesicles with amino acids, dipeptides, sugars, or nucleobases/sides were analyzed as ratiometric quantities, normalized to data for vesicles alone, without those additional molecules.

We determined the normalized radius ($R_{nom}$) for vesicles with an additional molecule (e.g., amino acid, dipeptide, sugar, or nucleobase/side) by dividing the initial radius of vesicles without an additional molecule, as follows

$$R_{nom} = \frac{r_{z,x}}{r_{0,x}}$$

(1)
where \( r_{t+X} \) is the radius at time \( t \) of vesicles with an additional molecule, \( X \), and \( r_{0-X} \) is the radius at time zero of vesicles without an additional molecule.

We quantified the initial rate of vesicle growth by fitting a linear curve to the normalized radii as a function of time over the first 10 min. The slopes (\( \frac{dr}{dt} \)) were determined for vesicles with and without an additional molecule (+\( X \) and −\( X \), respectively). The comparative growth rates are given by

\[
\frac{k_{\text{with}}}{k_{\text{without}}} = \frac{\frac{dr}{dt}_{\text{with}}(\text{normalized})}{\frac{dr}{dt}_{\text{without}}(\text{normalized})}
\]

(2)

Table 1. Comparison of Vesicle Growth with or without Each Additional Molecule

| DIPEPTIDES | AMINO ACIDS |
|------------|-------------|
| Molecule   | Normalized rate, \( k \) | \( \tau_t(\text{with}) \) | \( \tau_t(\text{without}) \) | Molecule   | Normalized rate, \( k \) | \( \tau_t(\text{with}) \) | \( \tau_t(\text{without}) \) |
| Ala-Ala     | 0.67 ± 0.30 | 1.01 ± 0.03 | 0.98 ± 0.04 | Alanine     | 0.39 ± 0.63 | 1.02 ± 0.04 | 1.01 ± 0.04 |
| Ala-Glu     | 0.78 ± 0.12 | 1.00 ± 0.02 | 0.99 ± 0.01 | Aspartic acid| 0.88 ± 0.78 | 1.02 ± 0.03 | 1.00 ± 0.02 |
| Ala-Leu     | 3.29 ± 1.98 | 1.00 ± 0.05 | 1.08 ± 0.09 | Glutamic Acid| 0.87 ± 1.18 | 1.03 ± 0.03 | 1.00 ± 0.02 |
| Gly-Glu     | 0.45 ± 0.03 | 1.03 ± 0.01 | 0.96 ± 0.01 | Glycine     | 0.53 ± 1.12 | 1.00 ± 0.02 | 0.98 ± 0.06 |
| Gly-Gli     | 0.68 ± 0.22 | 1.07 ± 0.08 | 1.02 ± 0.04 | Leucine     | 0.77 ± 0.70 | 1.01 ± 0.04 | 0.99 ± 0.04 |
| Gly-Leu     | 1.89 ± 2.15 | 1.03 ± 0.05 | 1.11 ± 0.12 | Proline     | 0.88 ± 0.59 | 1.02 ± 0.02 | 1.02 ± 0.005 |
| Gly-Pro     | 1.11 ± 0.35 | 1.03 ± 0.10 | 1.01 ± 0.08 | Serine      | 1.02 ± 0.33 | 1.04 ± 0.05 | 1.02 ± 0.04 |
| Gly-Ser     | 0.98 ± 0.36 | 1.01 ± 0.02 | 0.99 ± 0.02 | | | | |
| Leu-Ala     | 2.23 ± 0.65 | 1.02 ± 0.02 | 1.03 ± 0.02 | | | | |
| Leu-Glu     | 2.20 ± 0.10 | 1.00 ± 0.01 | 1.02 ± 0.01 | Adenine     | 0.81 ± 0.30 | 1.09 ± 0.18 | 1.06 ± 0.15 |
| Leu-Leu     | 2.41 ± 1.39 | 1.02 ± 0.03 | 1.06 ± 0.07 | Adenosine   | 1.00 ± 0.34 | 1.02 ± 0.02 | 1.01 ± 0.01 |
| Pro-Glu     | 0.97 ± 0.32 | 1.22 ± 0.31 | 1.21 ± 0.27 | Cytosine    | 1.17 ± 0.31 | 1.04 ± 0.01 | 1.05 ± 0.05 |
| Ser-Leu     | 0.38 ± 0.34 | 1.00 ± 0.05 | 0.96 ± 0.04 | Guanosine   | 1.24 ± 0.30 | 1.05 ± 0.01 | 1.05 ± 0.02 |
| Ser-Ser     | 0.51 ± 0.36 | 1.01 ± 0.05 | 0.96 ± 0.02 | Uracil      | 0.91 ± 0.63 | 1.02 ± 0.04 | 1.04 ± 0.03 |
| Uridine     | 1.30 ± 0.28 | 1.02 ± 0.03 | 1.01 ± 0.03 | | | | |

| SUGARS |
|--------|
| Molecule | Normalized rate, \( k \) | \( \tau_t(\text{with}) \) | \( \tau_t(\text{without}) \) |
| Arabinose | 1.21 ± 0.30 | 1.05 ± 0.02 | 1.04 ± 0.02 |
| Lyxose     | 1.09 ± 0.09 | 1.04 ± 0.01 | 1.02 ± 0.04 |
| Ribose     | 1.28 ± 0.42 | 1.07 ± 0.04 | 1.06 ± 0.07 |

Values of 1.0 indicate that there is no difference between the two samples. Uncertainties correspond to the standard deviation (1σ) from ≥2 independent experiments. Statistically significant differences of the normalized growth rate, \( k \), from 1.0, based on a \( p \)-value of <0.05, are highlighted in green (positive effects) or red (negative effects), even in cases where the difference is marginal (See Supporting Information, Section 4 for calculation details and \( p \)-values). The growth curves for all molecules are shown in Supporting Information, Section 5. \( \tau_t \) is the initial radius and \( r_f \) is the final radius of vesicles.

RESULTS AND DISCUSSION

We measured the growth of decanoic acid vesicles upon the addition of ~1 equiv of decanoic acid micelles in the presence and absence of different molecules. We tested the effects of four broad classes of molecules: dipeptides, amino acids, nucleobases or nucleosides, and sugars (see Table 1 for the list of molecules and Figures S1–S4 for chemical structures). We chose molecules that would be present in prebiotic chemistry, with varying charge, hydrophobicity, and structure. All small molecules were used at concentrations of 10 mM aside from the sugars, which were used at 100 mM (the concentration required for their inhibition of salt-induced flocculation).

To measure the growth of vesicles, we employed a Förster resonance energy transfer (FRET) assay. The FRET signal
decreases as vesicles grow because the dyes disperse over a larger surface area.

Using the FRET assay, we monitored the growth of vesicles upon micelle addition for a total of 90 min. A control experiment without a small molecule was performed alongside all of the growth experiments in the presence of test molecules. Figure 3 shows example vesicle growth curves, where a nucleoside (adenosine, panels A and B) and a dipeptide (Ser-Ser, panels C and D) were used. Panels B and D show the normalized radius vs time for the first 10 min of panels A and C, respectively, where the radius is normalized to the initial radius in the control experiment. The first 10 min (shaded in yellow in panels A and C) are used to calculate comparative initial growth rates (panels B and D). In panel B, the slopes of the lines do not differ significantly, so there is no significant effect of adenosine on the initial growth rate. In contrast, in panel D, the initial growth rate for vesicles with Ser-Ser is significantly smaller than for control vesicles without Ser-Ser. Error bars are standard deviations of the fluorescence measurements from three aliquots of the same sample. Corresponding graphs for replicates (≥2) of all 31 small molecules are shown in Figures S6–S36 in the Supporting Information.

Figure 4. Comparison of normalized initial vesicle growth rate, $k$, in the presence or absence of other molecules. Values of 1.0 (dashed line) indicate that there is no difference between vesicles with or without the molecules listed on the x-axis. Molecules are grouped by category: dipeptides are in the red-shaded region, sugars in green, amino acids in yellow, and nucleobases/sides in blue. Uncertainties correspond to the 1σ standard deviation from ≥2 independent experiments. The molecules with statistically significant deviations from 1.0 ($p < 0.05$) are shown in open circles; those consistent with no effect within 1σ uncertainty are shown in filled circles.
Ser, panels C and D) were added. To determine the initial rate of growth, we calculated the rate of change of radius with time over the first 10 min of growth (panels B and D). In panel B, the slopes of the lines with and without adenosine are not significantly different, indicating no effect in the initial growth rate due to adenosine. However, in panel D, the slope of the rate of change of radius with time with Ser-Ser present is significantly smaller than that without Ser-Ser, showing that Ser-Ser slows the initial growth of vesicles. For details of the analysis procedures and calculations, see Experimental Section and Supporting Information Section 3.

Experiments as shown in Figure 3, were repeated for 31 different potentially prebiotic molecules in at least two replicates to assess the effect of small molecules on vesicle growth. From the resulting summary data (Figure 4), it is clear that some of the small molecules result in large increases (twofold or higher) in the initial rate of vesicle growth. Overall, we find that growth rates; Ala-Leu, Gly-Leu, and Ser-Leu show growth rates consistent with or slower than control vesicles. Hydrophobic peptides may increase the growth rate of fatty acid vesicles by forming nanoscale clusters that connect the membrane with a nearby micelle.23 While the precise mechanism by which these dipeptides enhance growth rates is not the focus of this paper, it will be interesting to explore the properties of various leucine-containing dipeptides and other similar molecules that may allow vesicles to have a selective advantage in terms of growth rate.

All molecules causing the pronounced decreases in initial growth rate are dipeptides (Gly-Glu, Ser-Leu, and Ser-Ser). Two of these dipeptides contain serine residues. Serine contains a hydroxyl group, which may be able to hydrogen-bond with the carboxylate group in the fatty acid, again possibly influencing vesicle growth. Interestingly, a third dipeptide containing serine (Gly-Ser) does not show significant effects on the rate of vesicle growth. Sample growth curves for serine-containing dipeptides are shown in Figure 5. The Ser-Leu dipeptide is intriguing because both of its amino acid residues seem to affect the rate of vesicle growth. Any positive influence of the leucine residue is less important than the apparent negative effect of the serine residue, yielding a net decrease in the initial rate of growth in the presence of Ser-Leu. Determining the precise mechanisms for enhanced or decreased growth rates would require additional studies, outside the scope of the current report.

In addition to examining the initial rates of growth, we also compared the initial radii ($R_{\text{initial}}$) and final radii of vesicles ($R_{\text{final}}$) with and without small molecules added. The radii for vesicles with a small molecule were normalized by the corresponding control vesicle radius, without a small molecule added (Table 1). Molecules with statistically significant differences in the comparative growth rate, $\kappa$ (p-value <0.05, see Supporting Information Section 3) are highlighted (green for positive effect, red for negative effect). Overall, we find no differences in the initial and final radii of vesicles with and without an additional small molecule, indicating that despite any potential differences in initial growth rate, vesicles grow to essentially the same size in the presence of an additional small molecule as without a small molecule present (~60–70 nm, assuming an initial radius of 50 nm) after 90 min. This
observation could be due to an exhaustion of the added micelles.

During the origin of life, prebiotic molecules, including nucleobases, nucleosides, nucleotides, amino acids, dipeptides, sugars, and potentially their polymers, could have been present together with fatty acid vesicles. The interactions of these types of molecules and polymers could have had a large influence on the eventual emergence of life. Our results indicate that vesicles can grow after micelle addition in the presence of a wide range of polymer building blocks. Certain small molecules increase the rate of vesicle growth relative to control vesicles (most significantly, dipeptides containing the hydrophobic leucine residues), while others result in a decreased rate of growth. In our 90-min experiments, all vesicles grew to approximately the same size as control vesicles without an additional molecule present. In a natural environment, repeated cycles of growth may have amplified any small differences in rates of growth.

CONCLUSIONS

We have measured the growth of prebiotically plausible fatty acid vesicles upon the addition of fatty acid micelles, with and without a range of small molecules that could have been present during the origin and evolution of early life. We found that many molecules tested (a range of dipeptides, sugars, amino acids, nucleobases, and nucleotides) have little, if any, effect on the growth of vesicles in terms of initial growth rate, initial radius, and final radius. Moreover, there are some interesting results for dipeptides, especially those containing leucine and serine residues. We found three molecules with reduced initial growth rates: Gly-Glu, Ser-Leu, and Ser-Ser. We also found three molecules with faster initial growth rates: Leu-Ala, Leu-Gly, and Leu-Leu. Interestingly, all molecules found to increase the initial growth rate were leucine-containing dipeptides; however, not all dipeptides containing a leucine residue have a positive effect on the growth rate. Independent of initial growth rates, all vesicles generally reached a consistent residue have a positive effect on the growth rate. Independent of initial growth rates, all vesicles generally reached a consistent size by the end of the experiment, regardless of the presence of an additional molecule. Our results suggest that fatty acid vesicles would have grown in complex chemical environments on the early Earth and that a few molecules tested may have accelerating or slowing effects on vesicle growth in complex populations.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.langmuir.2c02118.

Chemical structures; experimental details; analysis procedures; all growth curves, and additional control experiments (PDF)

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Z.R.T., Z.R.C., and R.A.B. carried out experiments. Z.R.T., Z.R.C., and R.A.B. analyzed the data. All authors contributed to the writing of the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Deamer, D.; Dworkin, J. P.; Sandford, M. P.; Bernstein, L. J.; Allamandola, L. J. The first cell membranes. *Astrobiology* 2002, 2, 371–381.

(2) Deamer, D. W.; Pashley, R. M. Amphiphilic components of the Murchison carbonaceous chondrite: surface properties and membrane formation. *Origins Life Evol. Biophores* 1989, 19, 21–38.

(3) Walde, P.; Wick, R.; Fresta, M.; Mangone, A.; Luisi, P. L. Autoctonous self-reproduction of fatty acid vesicles. *J. Am. Chem. Soc.* 1994, 116, 11649–11654.

(4) Apel, C.; Deamer, D. W.; Mautner, M. N. Self-assembled vesicles of monocarboxylic acids and alcohols: conditions for stability and for the encapsulation of biopolymers. *Biochim. Biophys. Acta, Biomembr.* 2002, 1559, 1–9.

(5) de Klerk, A. Fischer–Tropsch Synthesis. In *Fischer–Tropsch Refining*; John Wiley & Sons, Ltd., pp 73–103.

(6) Schulz, H.; Beck, K.; Erieh, Mechanism of the Fischer Tropsch Process. *In Studies Surface Science Catalysis*; Bibby, D. M.; Chang, C. D.; Howe, R. F.; Yurchak, S., Eds.; Elsevier, 1988; Vol. 36, pp 457–471.

(7) Criado-Reyes, J.; Bizzarri, B. M.; Garcia-Ruiz, J. M.; Saladino, R.; Di Mauro, E. The Role of Borosilicate Glass in Miller–Urey Experiment. *Sci. Rep.* 2021, 11, no. 21009.

(8) Allen, W. V.; Ponnamperuma, C. A Possible Prebiotic Synthesis of Monocarboxylic Acids. *Biosystems* 1967, 1, 24–28.

(9) Groth, W. E.; Weyssenhoff, H. V. Photochemical Formation of Organic Compounds from Mixtures of Simple Gases. *Planet. Space Sci.* 1960, 2, 79–85.

(10) Bonfio, C.; Caumes, C.; Duffy, C. D.; Patel, B. H.; Percivalle, C.; Tsanakopoulou, M.; Sutherland, J. D. Length-Selective Synthesis of Acylglycerol-Phosphates through Energy-Dissipative Cycling. *J. Am. Chem. Soc.* 2019, 141, 3934–3939.
(11) Yuen, G. U.; Kvenvolden, K. A. Monocarboxylic acids in Murray and Murchison carbonaceous meteorites. *Nature* 1973, 246, 301–303.

(12) Lawless, J. G.; Yuen, G. U. Quantification of mono carboxylic acids in the Murchison carbonaceous meteorite. *Nature* 1979, 282, 396–398.

(13) Yuen, G.; Blair, N.; Des Marais, D. J.; Chang, S. (1984) Carbon isotope composition of low molecular weight hydrocarbons and mono carboxylic acids from Murchison meteorite. *Nature* 1984, 307, 252–254.

(14) Naraoka, H.; Shimoyama, A.; Harada, K. Molecular distribution of mono carboxylic acids in Asuka carbonaceous chondrites from Antarctica. *Origins Life Evol. Biospheres* 1999, 29, 187–201.

(15) Cohen, Z. R.; Todd, Z. R.; Wogan, N.; Black, R. A.; Keller, S. L.; Catling, D. C. Plausible sources of membrane-forming fatty acids on the early Earth: a review of the literature and an estimation of amounts. *ACS Earth Space Chem.*, in press; DOI: 10.1021/acsearthspacechem.2c00168.

(16) Hanczyc, M. M.; Fujikawa, S. M.; Szostak, J. W. Experimental models of primitive cellular components: encapsulation, growth, and division. *Science* 2003, 302, 618–622.

(17) Chen, I. A.; Szostak, J. W. A kinetic study of the growth of fatty acid vesicles. *Biophys. J.* 2004, 87, 988–998.

(18) Berclaz, N.; Muller, M.; Walde, P.; Luisi, P. L. Growth and transformation of vesicles studied by ferritin labeling and cryo-transmission electron microscopy. *J. Phys. Chem. B* 2001, 105, 1056–1064.

(19) Szathmáry, E.; Demeter, L. Group selection of early replicators and the origin of life. *J. Theor. Biol.* 1987, 128, 463–486.

(20) Szostak, J. W.; Bartel, D. P.; Luisi, P. L. Synthesizing life. *Nature* 2001, 409, 387–390.

(21) Cavalier-Smith, T. Obcells as proto-organisms: membrane heredity, lithophosphorylation, and the origins of the genetic code, the first cells, and photosynthesis. *J. Mol. Evol.* 2001, 53, 555–559.

(22) Adamala, K.; Szostak, J. W. Competition between model protocells driven by an encapsulated catalyst. *Nat. Chem.* 2013, 5, 495–501.

(23) Black, R. A.; Blosser, M. C.; Stottrup, B. L.; Tavakley, R.; Deamer, D. W.; Keller, S. L. Nucleobases bind to and stabilize aggregates of a prebiotic amphiphile, providing a viable mechanism for the emergence of protocells. *Proc. Natl. Acad. Sci. U.S.A.* 2013, 110, 13272–13276.

(24) Cornell, C. E.; Black, R. A.; Xue, M.; Litz, H. E.; Ramsay, A.; Gordon, M.; Mileant, A.; Cohen, Z. R.; Williams, J. A.; Lee, K. K.; Drobny, G. P.; Keller, S. L. Prebiotic amino acids bind to and stabilize prebiotic fatty acid membranes. *Proc. Natl. Acad. Sci. U.S.A.* 2019, 116, 17239–17244.

(25) Monnard, P.-A.; Apel, C. L.; Kanavaroti, A.; Deamer, D. W. Influence of ionic inorganic solutes on self-assembly and polymerization processes related to early forms of life: Implications for a prebiotic aqueous medium. *Astrobiology* 2002, 2, 139–152.

(26) Walton, T.; Szostak, J. W. (2017) A kinetic model of nonenzymatic RNA polymerization by cytidine-5′-phosphoro-2′-aminoimidazolide. *Biochemistry* 2017, 56, 5739–5747.

(27) Izgu, E. C.; Bjorkbom, A.; Kamat, N. P.; Lelyveld, V. S.; Zhang, W.; Jia, T. Z.; Szostak, J. W. N-Carboxyamide-mediated fatty acylation of amino acids and peptides for functionalization of protocell membranes. *J. Am. Chem. Soc.* 2016, 138, 16669–16676.

(28) Moore, D. S.; McCabe, G. P.; Craig, B. A. *Introduction to the Practice of Statistics*; W.H. Freeman: New York, 2009.

(29) Wei, C.; Pohorille, A. Fast bilayer-micelle fusion mediated by hydrophobic dipptides. *Biophys. J.* 2021, 120, 2330–2342.