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Supplementary Information for

Prebiotic amino acids bind to and stabilize prebiotic fatty acid membranes

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MATERIALS AND METHODS

Materials. Sodium chloride was purchased from Thermo Fisher Scientific (Waltham, MA), centrifugal filters from MilliporeSigma (Burlington, MA), and decanoic acid from Nu-Chek Prep (Elysian, MN). L-leucine ($^{13}C_6$), decanoic-10,10,10-$^{2}H_3$ acid and D_2O were from Cambridge Isotope Laboratories (Andover, MA). Decanoic-2,2-$^{2}H_2$ acid was from C/D/N Isotopes (Quebec, CA). All other chemicals were from Sigma. Except for NMR experiments, all solutions were prepared in 18 MΩ-cm water.

Decanoic acid solution. Decanoic acid was dissolved, with heating, in 190 mM NaOH to yield a 180 mM stock solution. This stock, 0.5 M monosodium phosphate, and 4 M NaCl were diluted into water to yield a solution of 50 mM decanoic acid, 30 mM sodium phosphate, and 100 mM NaCl. The pH was then adjusted to 6.83 by adding a small volume of 1 M HCl. This resulting solution is referred to as “the decanoic acid solution” and was used in all experiments except where indicated otherwise. Generally, "decanoic acid" in the text refers to mixtures of the protonated and unprotonated forms.

Diffusion NMR. Pulsed field gradient nuclear magnetic resonance (PFG-NMR) experiments were performed on a Bruker Avance III 700 MHz NMR instrument with a 5 mm Broadband Observe (BBO) probe at 25 °C. The “stebpgp1s” pulse sequence was applied to probe each amino acid's translational diffusivity (D), which was found via $\ln(I/I_0) = -\gamma^2G^2\delta^2D(\Delta-\delta/3)$. In this equation, G is the gradient strength, which varied from 10% to 95% of the maximum strength (53.5 Gauss/cm) for samples in which amino acids were in the presence of decanoic acid, and from 10% to 75% of the maximum strength for samples in the absence of decanoic acid. The variable I is the observed ^{1}H NMR peak intensity corresponding to each G, where I_0 is the intensity at the initial G value. γ is the gyromagnetic ratio of ^{1}H (4257.64 Hz/G), δ is the length of the gradient pulse (set to 0.002 s), and Δ is the diffusion time (set to 0.3 s). PFG-NMR experiments detected a series of 1D ^{1}H spectra and the diffusion coefficients were extracted by fitting the signal intensity decay at increasing gradient strengths to the equation above. Data were analyzed by extracting NMR peak intensities using the TopSpin 3.5 software package (Bruker) and exporting peak intensities to OriginPro (OriginLab, Northampton, MA), which fit the equation above and generated uncertainties of the fit. For PFG-NMR experiments, samples contained 50 mM of the amino acids leucine, glycine and serine or 10 mM lysine in the presence or absence of 50 mM deuterated decanoic acid, and were prepared in a solution of 50 mM sodium phosphate and 50 mM NaCl in D_2O at pH7 and room temperature.

REDOR. 10 mM leucine uniformly labeled with ^{13}C was mixed with 72 mM decanoic acid labeled with ^{2}H at either the 2- or 10-carbon, and ^{2}H-free water, at pH 7.6. Samples were then lyophilized and analyzed in a 11.74 T Bruker Avance III 500 MHz, narrow-bore spectrometer using a Bruker TriGamma probe tuned for ^{1}H-^{13}C-^{2}H triple resonance. The REDOR (xy-8) pulse sequence was used (1). Samples were spun at 10 kHz magic-angle spinning frequency. S0 and S1 were collected for dephasing times of 0.8, 3.2, 5.6, 8.0 and 10.4 ms and required 1024, 2048, 2048, 2048 and 4096 scans, respectively. The CP time was 1.5 ms. During this time the ^{1}H power was ramped from 70% to 100%. Field strengths were: 83 kHz ^{1}H π/2 pulse, 56 kHz ^{13}C π pulses, and 66 kHz ^{2}H π pulses. During dephasing and acquisition, 100 kHz SPINAL-64 decoupling was applied on the ^{1}H channel. Data were processed with 20 Hz Gaussian line broadening and

baseline correction. Chemical shift referencing was achieved externally using adamantane. The methylene ^{13}C shift was set to 38.5 ppm.

Filtration assay. The decanoic acid solution was added to solid base or amino acid such that the final concentration of the compound was 10 mM. The compound was ground with the end of a stirring rod to facilitate dissolution and vortexed for ~6 sec. After 30 min, 1.5 ml of solution was transferred to an Amicon Ultra-4 3K filter and centrifuged in a Beckman Allegra X-30R swinging bucket centrifuge at 3,000 *g* for 10 min at 21 °C. To assay bases (adenine and thiouracil) in the starting material and filtrate, absorbance at 260 nm was measured using an Agilent 8453 spectrophotometer, with uncertainties reported as standard error of the mean of 3 trials. To assay amino acids, (a) samples were diluted 225-fold in 0.2 M bicine at pH 9.1, (b) 750 µL of 1.5 mg/ml fluorescamine dissolved in dimethylsulfoxide was added to 315 µL of each diluted sample, and (c) after ≥ 20 min, fluorescence was measured with excitation at 400 nm and emission at 460 nm using a Perkin Elmer LS-50B fluorimeter. The assay had a standard error of ~1%.

NaCl and $MgCl_2$ effects. Methods for evaluating the flocculation of decanoic acid by NaCl are described in (2). $MgCl_2$ was added to the decanoic acid solution from a 50 mM stock solution, prior to adjusting the pH to 6.83. The resulting solution was then added to a test tube containing a solid amino acid (or to an empty test tube for the control) and vortexed briefly.

Fluorescence microscopy. This procedure was conducted as in (2); decanoic acid vesicles were labeled with the dye rhodamine 6G.

Cryo transmission electron microscopy. The decanoic acid solution was added to enough solid amino acid (or nothing for a control) to yield a 10 mM solution. After 1 min, samples were vortexed for ~6 sec. Samples were then applied to glow-discharged c-flat Holey Carbon grids (Electron Microscopy Science, Hatfield, PA) and plunge-frozen into liquid ethane using a Vitrobot Mark IV (FEI, Hillsboro, OR) at 4 °C and 100% humidity. The images were collected on a FEI T12 Spirit TEM (FEI, Hillsboro, OR) under-focused by 1.9 µm at a nominal magnification of 52,000x. Linear contrast enhancement was applied in ImageJ, a public domain program available for free download at https://imagej.nih.gov/ij/.

Turbidity. For most experiments, the decanoic acid solution was added to a test tube containing solid amino acid (or to an empty tube as control); for Fig. S7 and parts of Fig. 4B, 10 µL of a 1 M solution of amino acid in 50 mM sodium phosphate, pH 6.83 (or the buffer alone) was layered on 990 µL of the decanoic acid solution. Samples were mixed with a vortexer about one min after the addition of the solution to solid amino acid for the experiment reported in Fig. 3C. The timing of mixing in the other turbidity experiments is described in the figure legends. Turbidity values for individual samples were found as follows: each sample was plated in triplicate, and absorbance at 490 nm was measured three times with a Multiskan Spectrum microplate reader. The turbidity of that sample was taken to be the average of the nine readings, less the value of a water blank. The figures report the average of the sample values from multiple independent experiments, or, for Fig. 4B, from multiple replicate samples in one experiment. The reported standard deviations are based on these sets of values.

Critical vesicle concentration. This method is described in the legend of Fig. S10.

Critical aggregate concentration. Solutions of various decanoic acid concentrations, containing 30 mM sodium phosphate and 100 mM NaCl at pH 6.83, were added to solid amino acid such that the final amino acid concentration was 10 mM. Controls contained no amino acid. After ~30 sec, the solution was vortexed for ~6 sec. 30 min later, 8 µL of a 1 mg/mL solution of merocyanine 540 in 1:1 ethanol:water was added to 2.5 mL of the sample. Absorbance was measured with an Agilent 8453 Diode Array UV-Vis spectrophotometer from 400 nm to 700 nm, and the background at 650 nm was subtracted. The normalized intensity is reported as the intensity at 564 nm divided by the intensity at 530 nm, as detailed in (3).

Rehydration. Samples were dried in a 60 °C oven. Water was added to restore the original volume. After 30 min, samples were agitated gently by hand. After another 30 min, samples were heated for ~3 sec in an 80 °C water bath and then vortexed for ~2 sec. Absorbance at 490 nm was measured one day later with a Multiskan Spectrum microplate reader.

Size exclusion chromatography (SEC). Vesicles were prepared as described in the "Decanoic acid solutions" section above, with or without amino acids, except that 5 mM calcein was added prior to decanoic acid. Free calcein was separated from vesicles by size exclusion chromatography over a column of Sepharose 4B equilibrated with a solution of 20 mM decanoic acid, 30 mM sodium phosphate, and 100 mM NaCl, at pH 6.83. Specifically, 0.5 ml of each sample was applied to a column containing 5 ml of Sepharose 4B, and 0.5 ml fractions were collected. Vesicles elute from the columns in the first few fractions. In each experiment, one of the first four fractions was imaged by fluorescence microscopy.

Dynamic light scattering. Decanoic acid solutions were prepared with 25 mM decanoic acid (rather than 50 mM) in order to avoid multiple scattering effects. Solutions were analyzed at 25 °C on a Zetasizer Nano ZS (Malvern, Worcestershire, United Kingdom) with a 633 nm helium-neon laser using back-scattering detection (at an angle of 173° to the incident light). The average diameter and polydispersity index from the cumulant method were determined using Zetasizer software (Dispersion Technology Software version 5.00). The reported data are the averages of three runs with a single sample for each condition; variation among runs was negligible. A strong indication that the comparison is valid is that both samples had a polydispersity index of 0.3, which meets Malvern's criteria for acceptable data (4). Dynamic light scattering has been previously used as an indicator of fatty acid vesicle size (5, 6); here we used it to compare two samples rather than to determine absolute values.

Availability. All protocols are described above. All materials are commercially available, as is NMR code. Fluorescence images are original files to which only a linear contrast has been applied such that the brightest and darkest pixels span the contrast range. Other data analysis calculated only averages and associated uncertainties. Unprocessed NMR spectra and other data are available upon request to Roy Black.

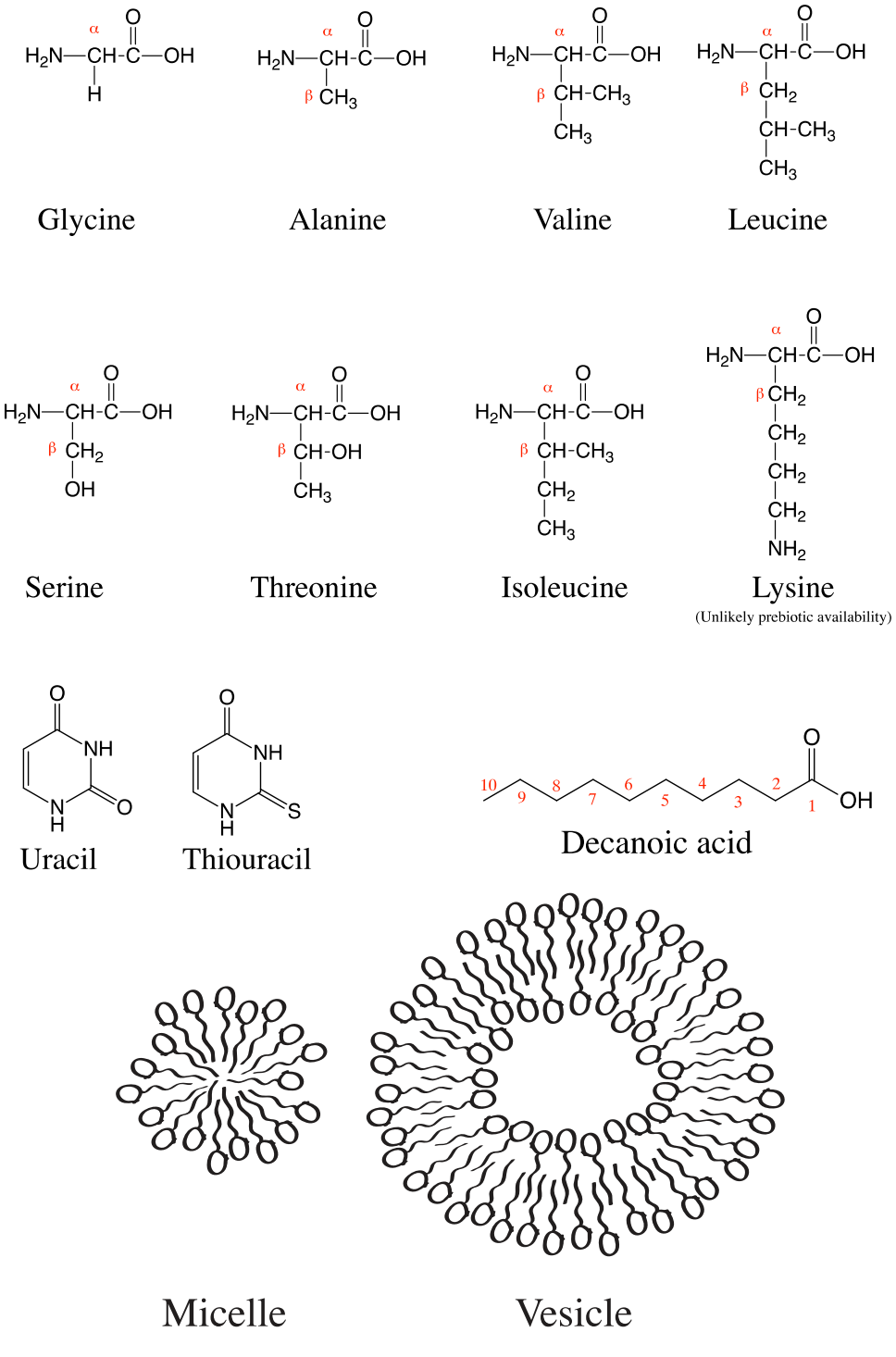

Fig. S1. Structures of amino acids, the nucleobase uracil and its related base thiouracil, decanoic acid, a micelle, and a vesicle.

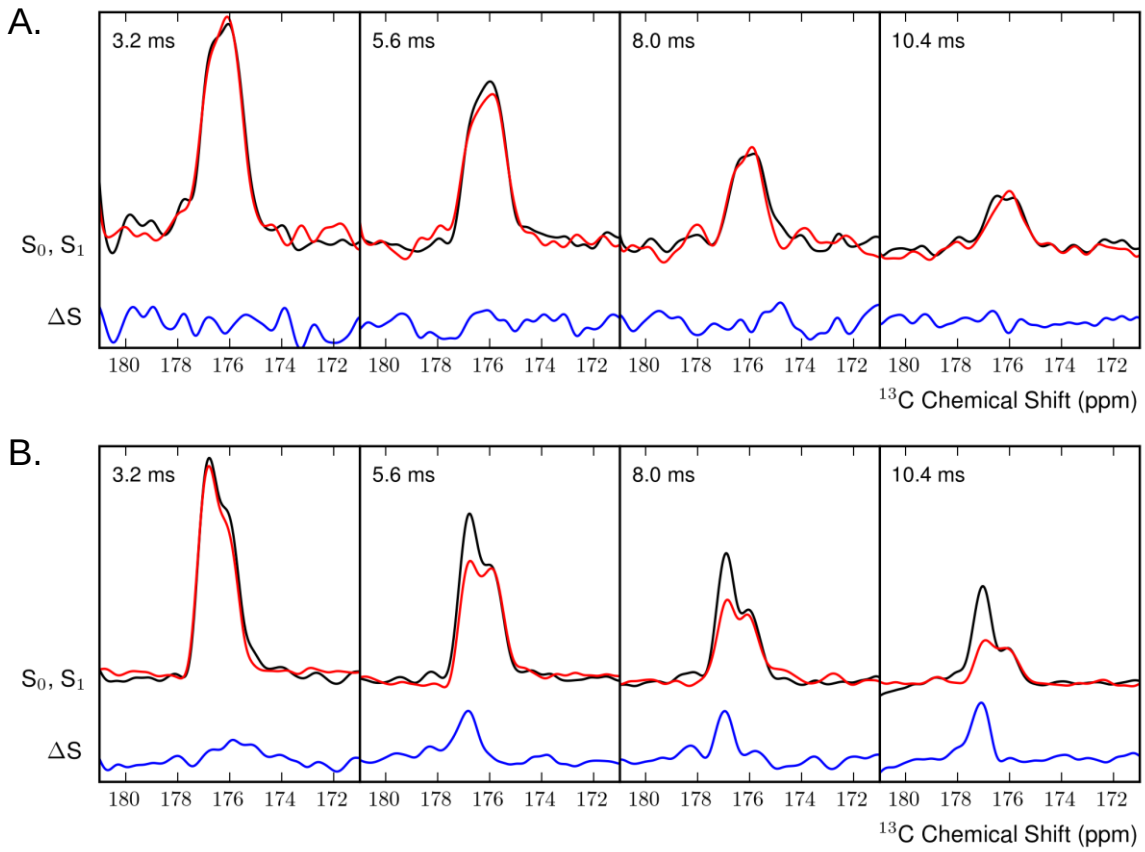

Fig. S2. Dephasing as a function of time of leucine uniformly labeled with ^{13}C. Dephasing is due to (A) decanoic acid ^{2}H-labeled at the terminal methyl group of the carbon chain (decanoic-10,10,10-$^{2}H_3$ acid) and (B) decanoic acid ^{2}H-labeled near the carbonyl group (decanoic-2,2-$^{2}H_2$ acid). The spectrum without dephasing (S0) is shown in black and the spectrum with dephasing (S1) is shown in red. ΔS is shown in blue, with a vertical offset for clarity. Note that the broad peak does not uniformly diphase. The two distinct peaks likely represent leucine molecules in two different environments: one at 177 ppm in which leucine interacts with the decanoic acid and one at 176 ppm in which it does not.

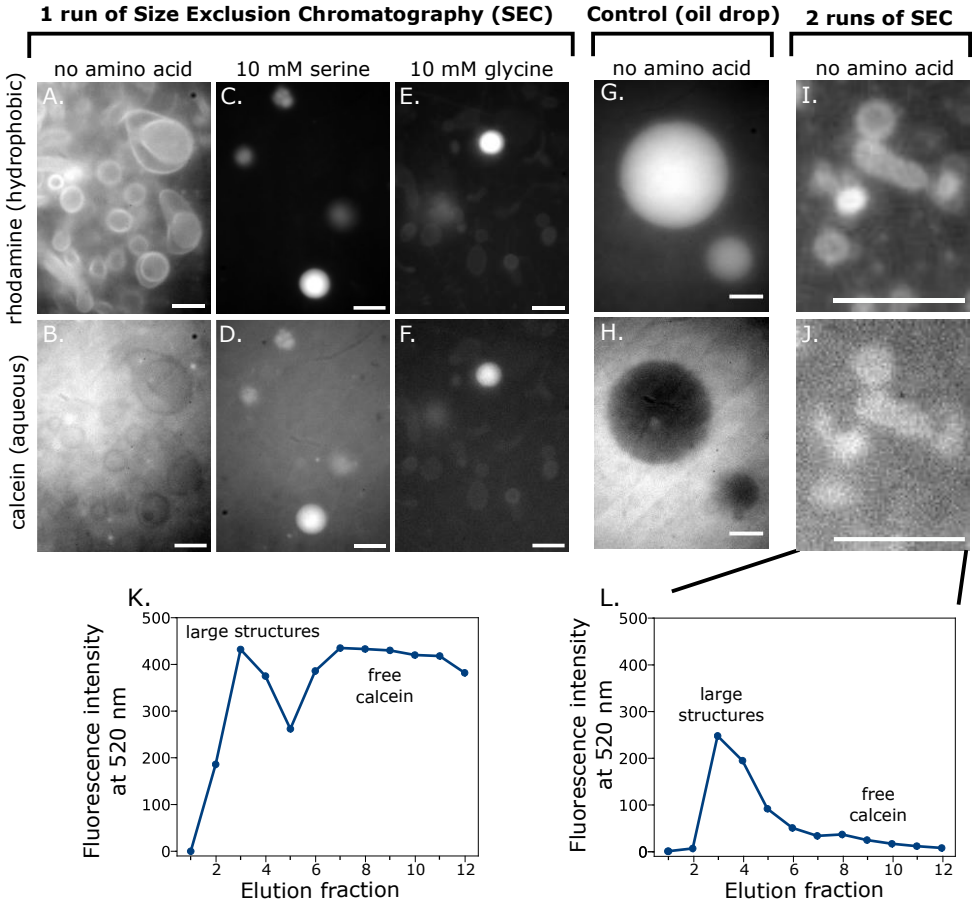

Fig. S3. Solutions of decanoic acid (with or without serine or glycine) contain individual, ~10 µm vesicles with aqueous interiors that can be labeled with calcein. Top panels show structures labeled by rhodamine 6G, a probe that labels fatty acid membranes. Bottom panels show fluorescence from calcein, a negatively-charged hydrophilic probe that does not label membranes. Calcein that was not encapsulated in vesicles was removed by running solutions through size exclusion chromatography (SEC) column(s), as described in the Methods. All scale bars are 10 µm. **A-B.** In the absence of amino acid, the decanoic acid solution contains individual, ~10 µm vesicles with membranes labeled by rhodamine 6G. After only one purification step by SEC, calcein is seen both inside and outside the vesicles. **C-F.** When 10 mM serine or glycine is included in the decanoic acid solution, bright structures are observed in both the rhodamine 6G and calcein channels, with little calcein fluorescence outside the vesicles, consistent with multilamellar vesicles that retain calcein in their interiors.

(Figure caption is continued on the next page.)

We carried out three controls to validate our SEC procedure:

1: We verified that the structures in Panels A-F are inconsistent with oil droplets.
2: We confirmed that the interiors of the vesicles in Panels A-B are aqueous.
3: We verified that our size-exclusion chromatography separates vesicles from calcein.

G-H. Control 1: We lowered the pH of the calcein-containing decanoic acid solution to 6.3 to induce the formation of oil droplets. Panel G shows that oil droplets are labeled by rhodamine 6G, and Panel H shows that calcein is excluded from oil droplets.
I-J. Control 2: We ran a calcein-containing decanoic acid solution over a SEC column, merged fractions 3 and 4, ran 0.5 mL of this pool over a second SEC column to further remove excess free calcein, and then analyzed early-eluting fractions from the second run by fluorescence microscopy. As observed after only one run (Panels A-B), vesicle membranes were labeled by rhodamine 6G and the lumen was labeled by calcein. As expected, the second SEC run removed more unencapsulated calcein such that the calcein fluorescence inside the vesicles became clearly greater than outside the vesicles (unlike in panel B). The vesicles that emerged from the second SEC run were consistently smaller than after the first run.
K-L. Control 3: We measured the fluorescence intensity due to calcein (at 520 nm) for each fraction eluted from the first (Panel K) and second (Panel L) SEC runs described within Control 2. To eliminate any vesicles that might affect calcein fluorescence, the data in Panels K-L were collected after adding NaOH to each sample (to a final concentration of 1 M), which converts decanoic acid vesicles to decanoate micelles. After the first SEC run, total fluorescence from free calcein in solution appeared higher than the fluorescence associated with vesicles. After the second SEC run, the level of free calcein in solution was much lower than the level in vesicles.

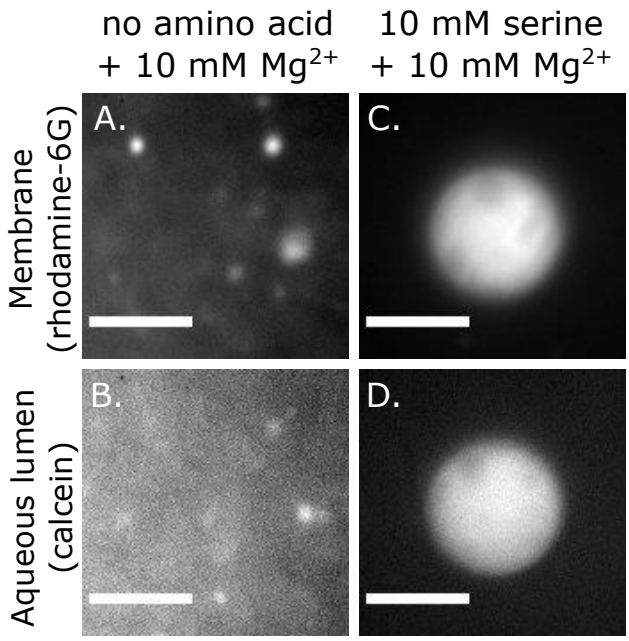

Fig. S4. Individual, ~10 μm decanoic acid vesicles with serine are not disrupted by 10 mM Mg^{2+}; they retain calcein in their lumens. Vesicle solutions were prepared as described in the Methods, except that 5 mM calcein was added before the decanoic acid. The pH of the solutions was then adjusted to 6.83 and serine was either added (to a final concentration of 10 mM) or not. Next, $MgCl_2$ was added to a concentration of 10 mM, the solutions were briefly vortexed, and the pH was re-adjusted to 6.83.

Free calcein was separated from vesicles by size exclusion chromatography as described in the Methods, and early-eluting fractions were used for fluorescence microscopy. Top panels show structures labeled by rhodamine 6G, a probe that labels decanoic acid membranes, and bottom panels show fluorescence due to calcein, a negatively-charged, hydrophilic probe. **A-B.** In the absence of serine, only small structures are observed. In some cases, these structures are labeled by both rhodamine 6G and calcein, which suggests that at least some of the structures are vesicles. However, few of the vesicles approach 1 μm in size, and the poor correspondence between structures labeled with rhodamine 6G and calcein implies that the membranes allow significant leakage of vesicle contents. **C-D.** In the presence of both serine and Mg^{2+}, ~10 μm structures are observed that are labeled by both rhodamine 6G and calcein. These structures are indistinguishable from those prepared in the absence of Mg^{2+} (compare to SI Appendix, Fig. S3 C-D). Scale bars are 10 μm.

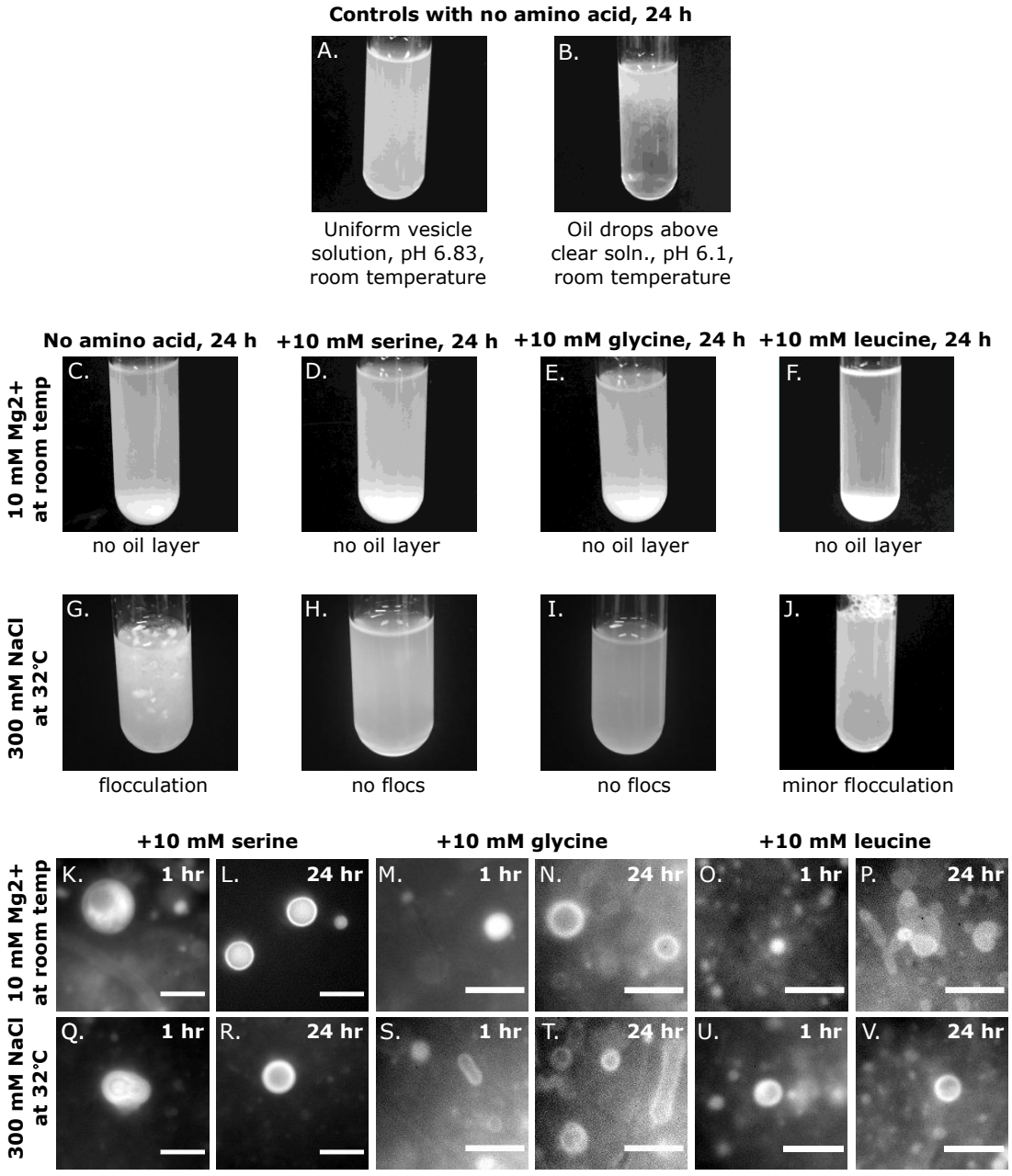

Fig. S5. No millimeter-scale or micron-scale evidence of oil droplets appears in solutions of decanoic acid at pH 6.83, whether or not the solutions contain amino acids (serine, glycine, or leucine). All samples containing NaCl were prepared with 80 mM decanoic acid at pH 7.65, as in reference 2. NaCl was added at room temperature, and then the samples were brought to 32 ˚C, where they remained for the duration of the experiment. In the images, all test tubes are 13 mm wide and were photographed with an iPhone camera under ambient light.

(Figure caption is continued on the next page.)

Millimeter-scale Behavior: A. Decanoic acid solutions at pH 6.83 appear uniformly cloudy because they contain paucilamellar micron-scale vesicles. **B.** Shifting the pH to 6.1 causes oil droplets of decanoic acid to form. When solutions with oil droplets are allowed to sit for 24 h, the bottom half of the solutions becomes relatively clear because oil droplets have floated to the top of the test tube. **C-F.** The appearance of solutions containing 10 mM Mg^{2+} is inconsistent with the formation of oil droplets: the solution at the bottom of the test tubes is not more clear than at the top. Mg^{2+} destabilizes individual, ~10 μm vesicles (Fig. 2). Mg^{2+} also appears to cause some precipitate to form at the bottom of the test tubes. **G-J.** The appearance of solutions containing 300 mM NaCl is also inconsistent with the formation of oil droplets: the solution at the bottom of the test tubes is not more clear than at the top. In Panel G, millimeter-scale flocs are observed, and reduced flocculation is seen in Panel J.

Micrometer-scale Behavior: All samples contain rhodamine 6G, a fluorophore that labels decanoic acid membranes and oil droplets. All scale bars on fluorescence micrographs are 10 μm. **K-N, Q-V.** In solutions containing serine, glycine, or leucine, with Mg^{2+} or NaCl as indicated, micron-scale structures persist after 24 h without significantly increasing in size. The lumens of at least some of the structures are not labeled by rhodamine 6G, in contrast to oil droplets, which have interiors labeled by this dye (SI Appendix, Fig. S3, Panel G) and should coalesce over time. **O-P.** In solutions containing Mg^{2+} and leucine, vesicles with distinct lumens appear after 24 h.

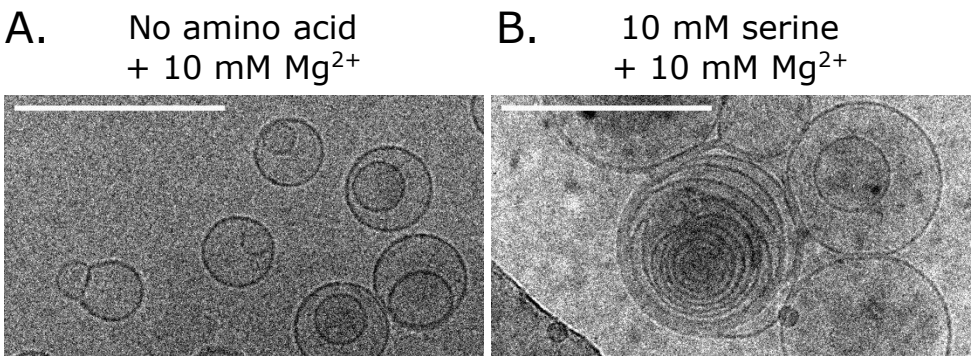

Fig. S6. Cryo TEM shows that with serine present, multilamellar vesicles persist in the presence of 10 mM Mg^{2+}. A. No amino acid added. B. 10 mM serine added. Cryo TEM records images of vesicles that are smaller than vesicles seen in fluorescence micrographs because vesicles larger than 300 nm are not retained on TEM grids. Vesicles less than 50 nm in diameter persist in the presence of 10 mM Mg^{2+} even without addition of any amino acid; these vesicles would be unresolvable by fluorescence microscopy. Scale bars are 100 nm.

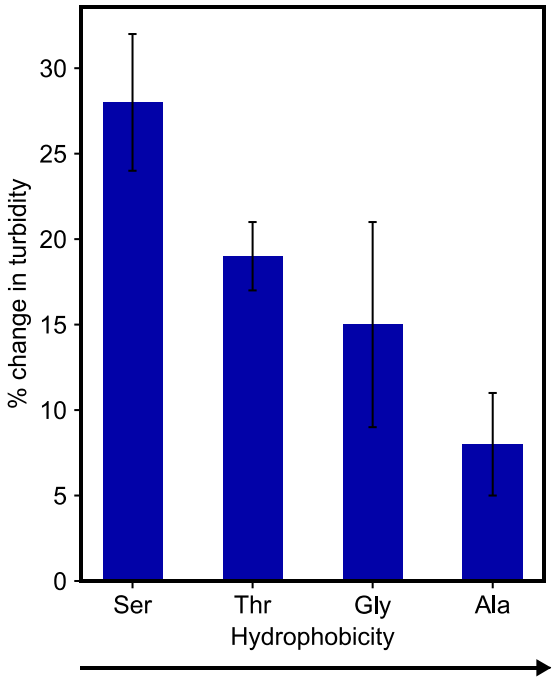

Fig. S7. Amino acids increase the turbidity (the absorbance at 490 nm) of the decanoic acid solution when added as concentrated solutions. 10 µL of a 1 M solution of each amino acid in 50 mM sodium phosphate at pH 6.83 ± 0.03 was overlaid on 990 µL of a solution that contained 50 mM decanoic acid, 30 mM sodium phosphate, and 100 mM NaCl at pH 6.83. Twenty seconds later, the sample was vortexed for ~6 seconds. For a control, 10 µl of the same phosphate buffer (with no amino acid) was added. The relative effectiveness of the amino acids, with diminished turbidity for more hydrophobic amino acids, is similar to that seen when the amino acids are added as solids (c.f. Fig 3C in the main text).

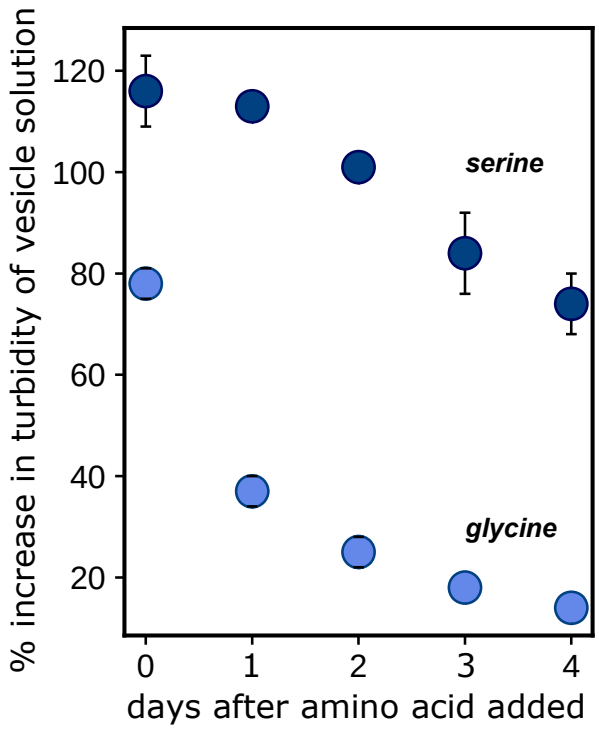

Fig. S8. The increase in turbidity in decanoic acid solutions caused by the addition of serine and glycine decays over a period of days. The decanoic acid solution was added to solid amino acid such that the resulting solution contained 10 mM amino acid, or to an empty tube as a control. One minute later, samples were vortexed for ~6 seconds. Turbidity (absorbance at 490 nm) of the samples was determined 30 minutes later and then daily. The figure records the percentage change of the test with respect to the control. Three experiments were conducted: one extended through Day 2 and two extended through Day 4. Standard errors of the mean are shown for Days 0, 1, and 2, and average errors are shown for Days 3 and 4, unless the errors are smaller than the symbols.

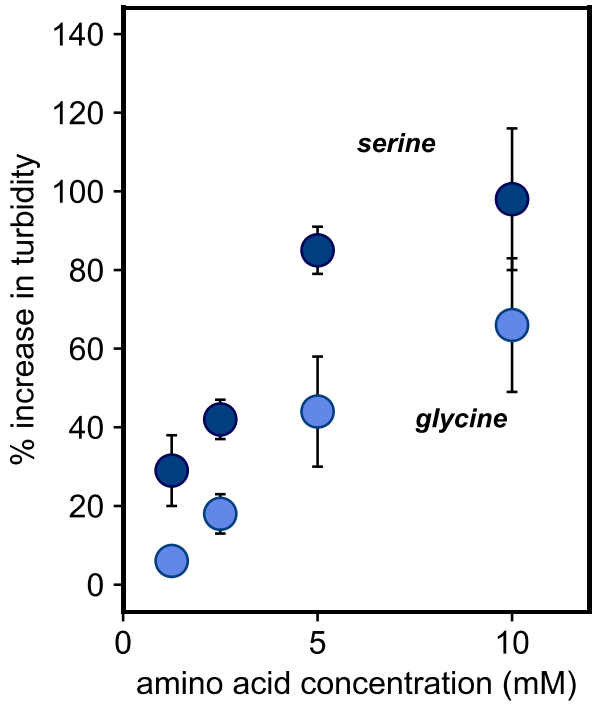

Fig. S9. At concentrations of 1.25 mM and higher, amino acids serine and glycine significantly increase turbidity of solutions containing decanoic acid vesicles, and the effect increases with concentration of the amino acid. The decanoic acid solution was added to a test tube containing solid amino acid such that the final amino acid concentration was 0, 1.25, 2.5, 5 or 10 mM. One minute after each addition, samples were vortexed for ~6 seconds. Turbidity, measured by absorbance at 490 nm, was determined 30 minutes later. The graph shows the percent increase in turbidity relative to the control with no amino acid. Each point is the average of at least three experiments. Error bars denote standard deviations, including when the error is smaller than the symbol size.

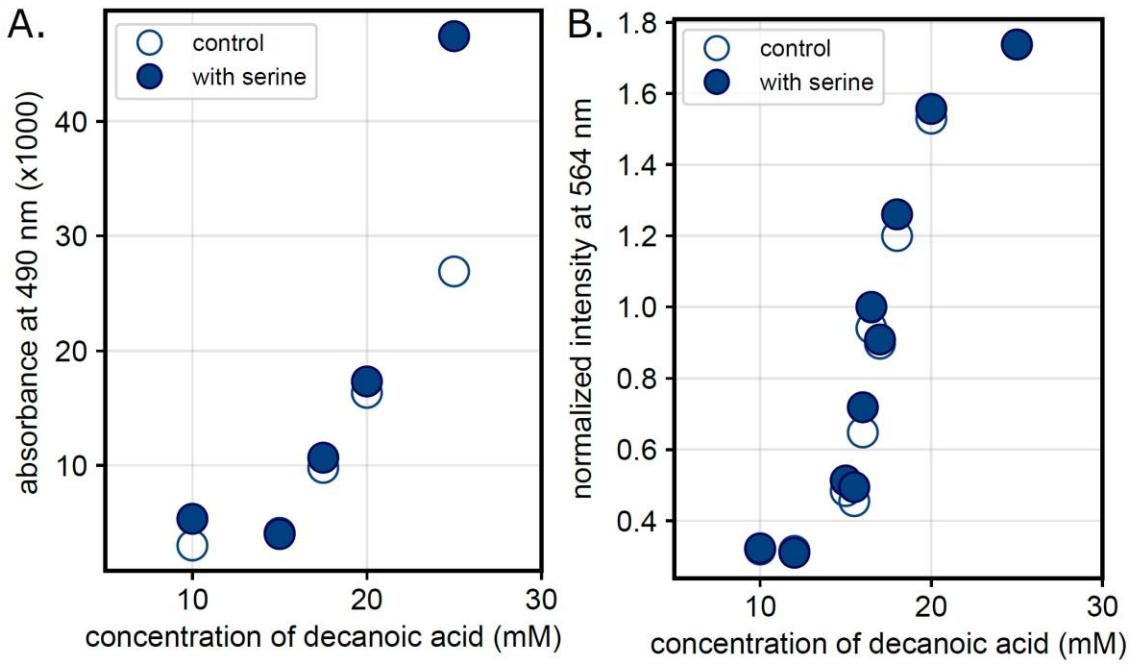

Fig. S10. Serine does not affect the critical vesicle concentration of decanoic acid solutions, as determined by two methods. **A.** Solutions containing 25 mM decanoic acid, 30 mM sodium phosphate, and 100 mM NaCl at pH 6.83, with and without 10 mM serine, were diluted until the concentration of decanoic acid was as low as 10 mM. The diluting solution contained all of the same compounds except decanoic acid. The critical vesicle concentration was identified as a sharp change in the absorbance of the sample at 490 nm. **B.** The critical vesicle concentration was measured via an assay that employs merocyanine 540, a dye. The absorption spectrum of this dye shifts in hydrophobic environments, including surfactant aggregates (7). Solutions containing 10-25 mM decanoic acid with or without 10 mM serine were prepared as in the Methods. At least 30 min later, 8 µL of 1 mg/mL merocyanine 540 that had been dissolved in 1:1 water:ethanol was added to 2.5 mL of each decanoic acid solution. The critical aggregate concentration was measured as in (3). Briefly, each sample's absorbance was measured at 530, 564, and 630 nm (denoted A_{530}, A_{564}, and A_{630}). The critical aggregate concentration was identified by a sharp change in the quantity: $(A_{564} - A_{630})/(A_{530} - A_{630})$.

Table. S1. Summary of Results.

	Lys	Ser	Thr	Gly	Ala	Val	Leu	Ile		Fig
		(These seven amino acids are considered prebiotic.)								
BIND MEMBRANE	**Diffusion NMR**	**Yes**	**Yes**	**–**	**Yes**	**–**	**–**	**Yes**	**–**		**1B**
	Strength of evidence	Strong	Strong	–	Strong	–	–	Strong	–		
	Retention w/ vesicles	**–**	**Maybe**	**Maybe**	**Maybe**	**Yes**	**Yes**	**Yes**	**Yes**		**1C**
	Strength of evidence	–	Error bars overlap control	Error bars overlap control	Error bars overlap control	Clearly exceeds control	Clearly exceeds control	Clearly exceeds control	Clearly exceeds control		
PROTECT ~10 µm VESICLE OR INCREASE # LAMELLAE	**Cryo TEM w/ more layers**	**–**	**Yes**	**–**	**–**	**–**	**–**	**–**	**–**		**3**
	Strength of evidence	–	Strong	–	–	–	–	–	–		
	Increase in brightness	**–**	**Yes**	**–**	**Yes**	**–**	**–**	**No**	**–**		**2 & 3**
	Strength of evidence	–	Strong	–	Strong	–	–	Strong	–		
	Protects vs. 10mM Mg2+	**–**	**Yes**	**–**	**Yes**	**–**	**–**	**No**	**–**		**2, S4 & S5**
	Strength of evidence	–	Strong	–	Strong	–	–	Strong	–		
	Protects vs. 300 mM NaCl	**–**	**Yes**	**–**	**Yes**	**–**	**–**	**Some**	**–**		**2 & S5**
	Strength of evidence	–	Strong	–	Strong	–	–	Strong	–		
	Increase in turbidity	**–**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**No**	**No**		**3**
	Strength of evidence	–	Strong data. Indirect result likely due to more lamellae	Strong data. Indirect result likely due to more lamellae	Strong data. Indirect result likely due to more lamellae	Strong data. Indirect result likely due to more lamellae	Strong data. Indirect result likely due to more lamellae	Strong data. Indirect result likely due to more lamellae	Strong data. Indirect result likely due to more lamellae		

Table. S2. Diffusion coefficients of amino acids and water in solutions without and with decanoic acid.

		Diffusion Coefficient (m^2/s)		
		fast	**slow**	**water**
Samples *without* decanoic acid vesicles	Glycine	$7.6445\times10^{-10} \pm 1.6\times10^{-13}$	none	$1.69715\times10^{-9} \pm 3.7\times10^{-13}$
	Leucine Trial 1	$5.20932\times10^{-10} \pm 8.4\times10^{-14}$	none	$1.67917\times10^{-9} \pm 2.8\times10^{-13}$
	Leucine Trial 2	$5.21152\times10^{-10} \pm 7.4\times10^{-14}$	none	$1.68409\times10^{-9} \pm 3.1\times10^{-13}$
	Serine	$6.3733\times10^{-10} \pm 3.86\times10^{-13}$	none	$1.69553\times10^{-9} \pm 5.7\times10^{-13}$
	Lysine	$4.8876\times10^{-10} \pm 5.8\times10^{-13}$	none	$1.70501\times10^{-9} \pm 2.0\times10^{-13}$
Samples *with* decanoic acid vesicles	Glycine	$7.3974\times10^{-10} \pm 2.4\times10^{-13}$	$1.04\times10^{-12} \pm 3.1\times10^{-13}$	$1.59499\times10^{-9} \pm 3.3\times10^{-13}$
	Leucine Trial 1	$4.9420\times10^{-10} \pm 1.5\times10^{-13}$	$2.98\times10^{-12} \pm 1.9\times10^{-13}$	$1.52258\times10^{-9} \pm 2.8\times10^{-13}$
	Leucine Trial 2	$4.9601\times10^{-10} \pm 1.33\times10^{-13}$	$2.98\times10^{-12} \pm 1.8\times10^{-13}$	$1.53912\times10^{-9} \pm 1.9\times10^{-13}$
	Serine	$6.1322\times10^{-10} \pm 6.3\times10^{-13}$	$1.76\times10^{-12} \pm 1.07\times10^{-12}$	$1.5932\times10^{-9} \pm 5\times10^{-13}$
	Lysine	$4.580\times10^{-10} \pm 1.7\times10^{-12}$	$1.03\times10^{-11} \pm 2.1\times10^{-12}$	$1.56629\times10^{-9} \pm 2.0\times10^{-13}$

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