Title

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Nucleobases bind to and stabilize aggregates of a prebiotic amphiphile: a viable mechanism for the emergence of protocells

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Abstract:

Primordial cells presumably combined RNAs, which functioned as catalysts and carriers of genetic information, with an encapsulating membrane of aggregated amphiphilic molecules. Major questions regarding this hypothesis include how the four bases and the sugar in RNA were selected from a mixture of prebiotic compounds and co-localized with such membranes, and how the membranes were stabilized against flocculation in salt water. To address these questions, we explored the possibility that aggregates of decanoic acid, a prebiotic amphiphile, interact with the bases and sugar found in RNA. We found that these bases, as well as some but not all related bases, bind to decanoic acid aggregates. Moreover, both the bases and ribose inhibit flocculation of decanoic acid by salt. The extent of inhibition by the bases correlates with the extent of their binding, and ribose inhibits to a greater extent than three similar sugars. Finally, the stabilizing effects of a base and ribose are additive. Thus, aggregates of a prebiotic amphiphile bind certain heterocyclic bases and sugars, including those found in RNA, and this binding stabilizes the aggregates against salt. These mutually reinforcing mechanisms could have driven the emergence of protocells.

Introduction:

The origin of RNA (1) and how it became associated with amphiphilic membranes in primordial cells is unclear. RNA is a polymer of units containing the sugar ribose covalently bound to one of four nucleobases; amphiphiles are molecules that possess both a hydrophobic and a hydrophilic moiety and can therefore aggregate into membranes in water. We know that two of the four units of RNA can be synthesized under simulated prebiotic conditions (2), that simple amphiphiles such as fatty acids spontaneously aggregate into vesicles in an aqueous environment (3), and that such vesicles can encapsulate nucleic acid and its building blocks (4, 5). Fundamental questions remain, however, regarding how the bases and sugar in RNA were selected from a heterogeneous mixture of prebiotic organic compounds, concentrated sufficiently to react, and co-localized with vesicles. It is also unclear how the first membranes were stabilized in sea water given that fatty acids precipitate at high salt concentrations (6).

Previous lines of research suggest possible answers to these questions. Prebiotic chemical processes could have preferentially generated at least two of the four nucleotides (consisting of a base bound to ribose and phosphate) from simple organic precursors (2). These building blocks could then have polymerized on mineral surfaces (7), which also stimulate fatty acid vesicle formation (8). Finally, the incorporation of alcohols and glycerol monoesters in fatty acid membranes could have increased their stability in sea water (4, 9, 10, 11).

We hypothesize a simpler, more integrated scenario that complements these mechanisms. In this scenario, aggregates of amphiphiles preceded RNA and facilitated its synthesis by binding and concentrating the bases and sugar of which it is composed. The observation that the assembly of

amphiphilic aggregates proceeds spontaneously, whereas the synthesis of RNA requires energy, supports this scenario. Moreover, the planar structure of the bases and the hydrogen-bonding potential of sugars suggest mechanisms by which these compounds could interact with fatty acid aggregates. We further hypothesize a functional consequence of the binding: stabilization of the amphiphilic aggregates in the presence of salt. The mechanisms we hypothesize are mutually reinforcing and under prebiotic conditions could drive the emergence of vesicles enriched in components of RNA.

The array of bases that we investigated is shown in Fig. 1A, including the nucleobases found in RNA: adenine, guanine, cytosine and uracil. We primarily employed decanoic acid (a carboxyl group attached to a chain of nine additional carbons) as our amphiphile because it is synthesized under prebiotic conditions (12) and is long enough to self-assemble into vesicles (13). (We use the term "decanoic acid" to refer to both the protonated and unprotonated forms of the molecule.) Vesicles enclose an aqueous volume, as a cell does, in contrast to smaller aggregates like micelles that have no aqueous core (Fig. S1). Above pH 8, decanoic acid forms only micelles. Vesicles typically start to form as the proton concentration becomes sufficient, below pH 8, to bridge carboxyl groups by hydrogen bonding, thereby reducing surface charge (13).
Due to the sensitivity of decanoic acid aggregates to pH, this parameter must be tightly controlled, and our procedures for doing so are described in the Supporting Information.

Results:

In a series of preliminary experiments (Figs. S2-S3), we found that nucleobases and ribose interact with decanoic acid strongly enough to alter the pH at which vesicles form within a solution of micelles (results summarized in Table 1). Among the nucleobases tested, the

magnitude of the pH shift was in the order of adenine > cytosine > uracil. (Guanine was not sufficiently soluble to test.) Between the sugars, ribose had a greater effect than glucose. The differences in the magnitudes of these effects suggest that they are due to direct interaction of the compounds with the decanoic acid aggregates, rather than to a change in nonspecific parameters of the solution such as ionic strength or viscosity.

Binding of nucleobases to aggregates of fatty acids. To confirm direct interaction between the bases and the aggregates, as well as to better quantify the strength of interaction, we employed three independent assays for binding. In these experiments, we focused on fatty acid micelles and monolayers rather than vesicles in order to differentiate between adsorption and encapsulation.

First, we determined that adenine dialyzes more slowly from decanoic than from acetic acid (21 ± 7% slower averaged over 6 experiments, p ~ 0.003; Fig. 1B). This result suggests that adenine binds to micelles, because acetic acid has the same hydrophilic moiety as decanoic acid but a hydrophobic tail too short (one carbon) to support micelle formation. As controls, we tested two compounds, uracil and thiouracil, that show weak or no interaction with decanoic acid aggregates by other measures (Figs. S2-S3, Fig. 1D). We found that the rates of uracil dialysis from decanoic and acetic acids are indistinguishable within experimental uncertainty (3 ± 10% faster, not slower, from decanoic acid, n=2, p > 0.05; Fig. 1B), and the difference in rates of thiouracil dialysis is also insignificant (6 ± 4% faster, not slower, from decanoic acid, n=2, p > 0.05). These results suggest that the slower dialysis of adenine from decanoic versus acetic acid

is due to its binding to micelles rather than to a nonspecific property of the solution, such as viscosity.

In a second test for interaction between adenine and long-chain fatty acids, we found that the base interacts with a fatty-acid monolayer in a Langmuir trough. In these experiments, a fatty acid is dispersed over the surface of an aqueous solution, altering the surface tension at the air-solution interface. The change in surface tension is expressed as surface pressure, defined for Langmuir monolayers as the surface tension of pure water minus the surface tension of the system under study. Decreasing the surface area, by moving a barrier, concentrates the fatty acid molecules and increases the surface pressure. We found that the presence of adenine in solution below a stearic acid monolayer increases the surface pressure observed at a given surface area (Fig. 1C). This result suggests that adenine adsorbs to or inserts in the monolayer of fatty acid molecules.   In the absence of a stearic acid monolayer, surface pressures of an adenine solution and of a buffer-only solution are indistinguishable, indicating that adenine alone does not partition to the air-solution interface enough to measurably affect surface pressure.

We employed ultrafiltration as our third binding assay. Samples were centrifuged through a 3 kD-cut-off filter, which retains decanoic acid micelles and, presumably, any bases associated with them. We found that RNA bases are retained with decanoic acid micelles, and the extent of their retention differs, with adenine ≈ guanine > cytosine > uracil (Fig. 1D, Table 1).   Moreover, adenine and guanine are retained to a greater extent than all five other purines tested, and the three pyrimidines in RNA or DNA are retained to a greater extent than thiouracil (Fig. 1D).

We conclude from these three diverse binding assays that (a) nucleobases bind to fatty acid aggregates, (b) the strength of nucleobase binding to fatty acid aggregates correlates well with the magnitude of the pH shifts that they induce in micelle-vesicle transitions (Table 1), and (c) structurally related bases exhibit substantial variation in binding.

We quantitatively assessed the affinity of adenine binding to decanoic acid micelles by repeating the filtration assay over a range of adenine concentrations, 0.01-3 mM. Scatchard analysis of the results suggests two modes of binding, one with a K_d of about 11 μM and one, with much lower affinity, that is not saturated at the highest adenine concentration tested (Fig. S4). In contrast, 2-aminopurine appears to lack a high affinity binding mode; whereas the percentage of adenine retained with micelles increases from 18 ± 1% at 0.3 mM to 22 ± 1% at 0.03 mM (Fig. 1D), retention of 2-aminopurine declines over this concentration range from 9.3 ± 0.8% (n=3) to 5.7 ± 1.6% (n=3). The relatively low absorbance of 2-aminopurine and the other purines besides adenine precluded testing them at the low concentrations required to further evaluate for high affinity binding.

We found that the mechanism by which bases bind to decanoic acid micelles is not simply related to hydrophobicity. Including 0.4 M NaCl in the filtration assay with 0.03 mM adenine increased the amount of the base retained with micelles, by 68 ± 2% (average of duplicates), suggesting that a hydrophobic interaction is involved. However, we found no strong correlation between extent of binding and the hydrophobicity of the bases, as measured by their partitioning into octanol versus water (R^2 = 0.2 and 0.04 for binding measured at 0.3 and 0.03 mM respectively) (Fig. S5).

Inhibition of decanoic acid flocculation by nucleobases and ribose. Having established the plausibility of a scenario in which aggregates of amphiphiles could have facilitated RNA synthesis by binding its components, we next tested the functional element of our hypothesis, that these components could have stabilized the aggregates against precipitation by salt. Salt concentrations in ancient oceans were likely at least as high as in modern oceans (14), and decanoic acid flocculates in the presence of even modest concentrations of NaCl (Fig. 2A) (a phenomenon previously reported as precipitation (6)). We began our investigation with adenine because it exhibits strong interaction with fatty acid aggregates in all our assays (Table 1).

We found that adenine inhibits salt-induced decanoic acid flocculation, thereby preserving vesicles. Salt-induced flocs in a decanoic acid solution dissolve upon heating, and in the absence of adenine they begin to re-form as the temperature falls to about 32 ˚C (Fig. 2A). With the inclusion of adenine, however, the solution remains relatively clear at this temperature, and epifluorescence microscopy shows that instead of flocs, vesicles as large as ~10 μm form (Fig. 2A). Moreover, we found that in addition to inhibiting re-flocculation upon cooling, adenine at 32 ˚C substantially eliminates pre-existing flocs (Fig. 2B). Adenine's inhibition of flocculation persists to temperatures as low as 30 ˚C; at room temperature the base has no apparent effect (Fig. 2A). Stabilization of vesicles could account for this shift in equilibrium between decanoic acid vesicles and flocs; this explanation is consistent with our finding (in the absence of salt) that vesicles extruded to about 100 nm in diameter grow faster in the presence of adenine than in the presence of the nonbinding base thiouracil (Fig. S6).

To determine the concentration dependence and specificity of adenine's effect on flocculation temperature, we established the following high-throughput assay using a 96-well plate: Decanoic acid solutions are flocculated by the addition of salt and then heated to 60 °C, which dissolves the flocs and renders the solutions virtually clear. Solution turbidity is then measured as the solutions cool and flocs re-form. At 32 °C and below, the turbidity of decanoic acid solutions containing 300 mM NaCl is due primarily to flocs (Fig. 2A), so turbidity can be used as a measure of flocculation.

We found that as little as 2.5 mM adenine inhibits NaCl-induced flocculation (Fig. 2C). The other nucleobases tested also inhibit flocculation, in the order adenine>cytosine>uracil (Fig. 2C, inset). This is the same order seen in the extent of their binding to fatty acid aggregates (Fig. 1D, Table 1), suggesting that the inhibition of flocculation is related to binding as we hypothesized. Moreover, the correlation between inhibition of flocculation and binding is generalizable to a large group of bases (Fig. 2D).

Several sugars, too, inhibit flocculation of decanoic acid due to NaCl, and ribose does so more effectively than glucose or xylose (Fig. 2E). This order is noteworthy for three reasons. (a) Ribose is the sugar found in RNA and DNA. (b) For sugars, as with bases, the extent of inhibition of flocculation correlates with the shift they cause in pH dependence of vesicle formation (Table 1). (c) Diastereomers are not equally effective, since xylose is less inhibitory than ribose. Ribose is indistinguishable from arabinose in the flocculation assay, and xylose is indistinguishable from lyxose (Table S1).   The downward orientation of the C3 hydroxyl group (in standard projections) common to ring structures of ribose and arabinose but not present in

xylose or lyxose could cause the difference in efficacy, if hydroxyl groups of sugars are involved in the binding to fatty acid aggregates.

Finally, we found that the inhibitory effects of adenine and ribose on salt-induced flocculation are approximately additive, at least when adenine alone inhibits by less than 50% and ribose alone inhibits by over 50% (n=5). In one such experiment, for example, 3 mM adenine alone inhibited by 26 ± 11%, 90 mM ribose alone inhibited by 64 ± 5%, and the combination inhibited by 86 ± 2% (uncertainties expressed as average deviation of duplicate samples). The additivity of the adenine and ribose effects suggests that the two compounds can bind to decanoic acid aggregates simultaneously.

Discussion:

Taken together, our observations support a scenario in which the bases and sugar required for RNA were selected and concentrated by binding to aggregates of prebiotic amphiphiles. Further, the resulting stabilization of the aggregates against salt could have created a positive feedback loop in which vesicles that bound bases and sugar resisted flocculation, thereby preserving more surface area to bind additional bases and sugar, further enhancing stability.

The prebiotic presence of these components at significant concentrations is plausible. Long-chain fatty acids are found in meteorites (15) and can be formed by natural processes on earth (16, 17). Nucleobases have also been found in meteorites (18) and are produced by plausible earth-based prebiotic reactions (19). Recent work describes how ribose could have been generated prebiotically (20, 21). Under prebiotic conditions, organic matter could have been relatively long-lived, and processes such as adsorption and encapsulation could have

concentrated these components (22). We suggest that the binding of bases and sugars to amphiphilic aggregates was one of these processes. Since we have shown that multiple compounds bind to these aggregates and that their stabilizing effects are additive, bases and sugars need not have reached concentrations at which they alone would have stabilized vesicles.

Mechanisms by which bases and sugars bind to fatty acid aggregates could involve several variables including planarity, hydrophobicity, and hydrogen bonding. All the bases are planar, and planarity may facilitate insertion in a lipid membrane. The increase in adenine binding in the presence of 0.4 M salt suggests that charge is not a major factor and implicates hydrophobic interactions, despite the lack of a simple correlation between binding and hydrophobicity (Fig. S5). Amines on the bases could hydrogen bond with the carboxyl groups of fatty acids, and most of the bases that are retained at higher fractions with micelles have amine groups. Sugars may interact with a fatty acid aggregate through hydrogen bonding between the carboxyl groups and hydroxyl groups of the sugar, as has been suggested for the hydroxyl groups in glycerol monoesters (11). The unique configuration of hydroxyl groups in ribose has been noted previously to explain its exceptionally rapid permeation of protocells (23,24).

Following the co-localization of the nucleobases and ribose, the next logical step in the emergence of life is the formation of the glycosidic bond, which may be facilitated by orientation of the base and sugar on amphiphilic aggregates.

Materials and Methods:

A detailed description of materials and methods is provided in the Supporting Information.

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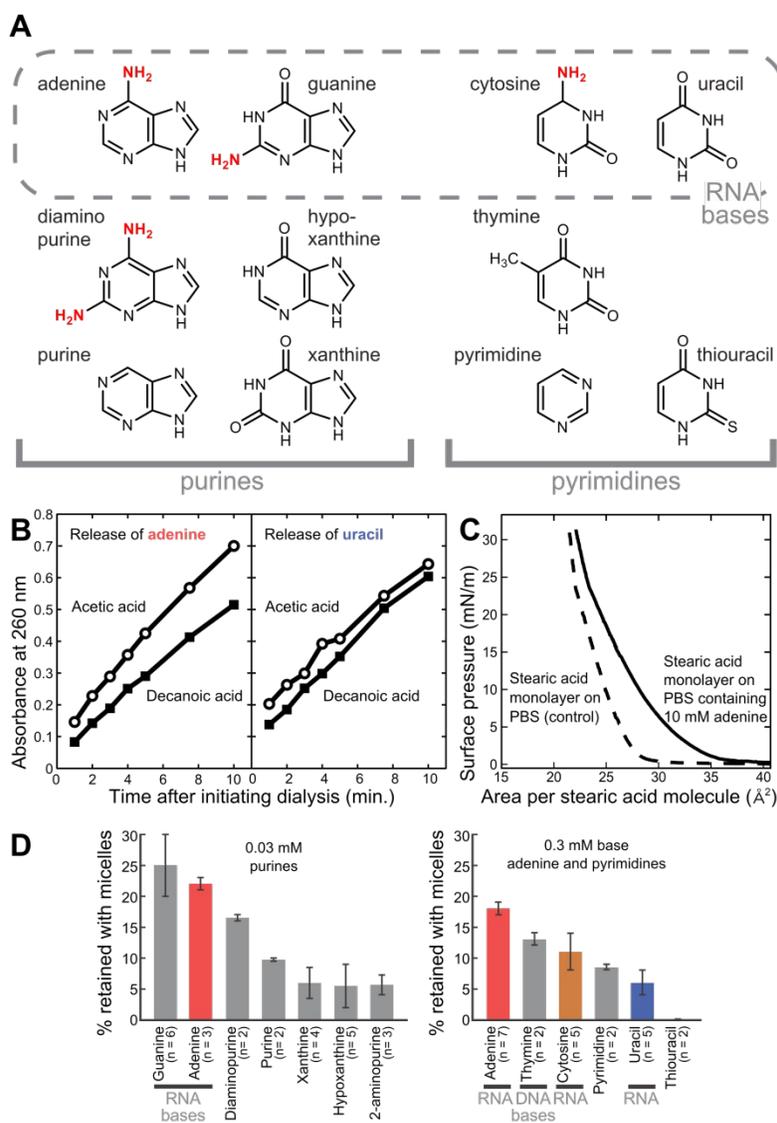
Fig. 1

Fig. 1. Decanoic acid aggregates selectively bind heterocyclic nitrogenous bases.

(*A*) Structures of purines and pyrimidines tested for interactions with decanoic acid aggregates. Diaminopurine contains an amine at the 2-position in addition to the 6-position as in adenine; 2-aminopurine, not shown, has an amine only at the 2-position. Amine substituents are indicated in red.

(*B*) Adenine dialyzes more slowly from a decanoic acid solution than from an acetic acid solution. The left panel shows results of a representative experiment in which adenine, at 15 mM, diffused from either 180 mM decanoic acid or from 180 mM acetic acid. Aliquots of dialysis buffer were collected at indicated times and assayed for adenine by measuring absorbance at 260 nm. The rate of release was $24 \pm 5\%$ lower from decanoic acid ($p < 0.05$). The right panel shows results of a corresponding representative control experiment with uracil in place of adenine. The rate of release was $8 \pm 7\%$ greater, not lower, from decanoic acid ($p > 0.05$).

(*C*) The presence of 10 mM adenine in a subphase of phosphate-buffered saline (PBS) increases the surface pressure of a Langmuir monolayer of stearic acid. Measurement uncertainty is ± 1 mN/m. Stearic acid (18 carbons) was used instead of decanoic acid because the latter does not form a stable Langmuir monolayer.

(*D*) Nucleobases are retained with decanoic acid micelles during ultrafiltration. A solution of 180 mM decanoic acid and each base at 0.03 mM (for purines) or 0. 3 mM (for pyrimidines) was partially centrifuged through a 3 kD-cut-off filter. These concentrations optimize both the percentage of base retained by micelles and the detection of base by absorbance; adenine was evaluated at both 0.3 and 0.03 mM to enable comparison of all the bases. Values are

averages, and error bars represent average deviations. (The difference between the means for cytosine and uracil is significant based on Student's t-test: p=0.028 by a one-tailed test and 0.056 by a two-tailed test.)
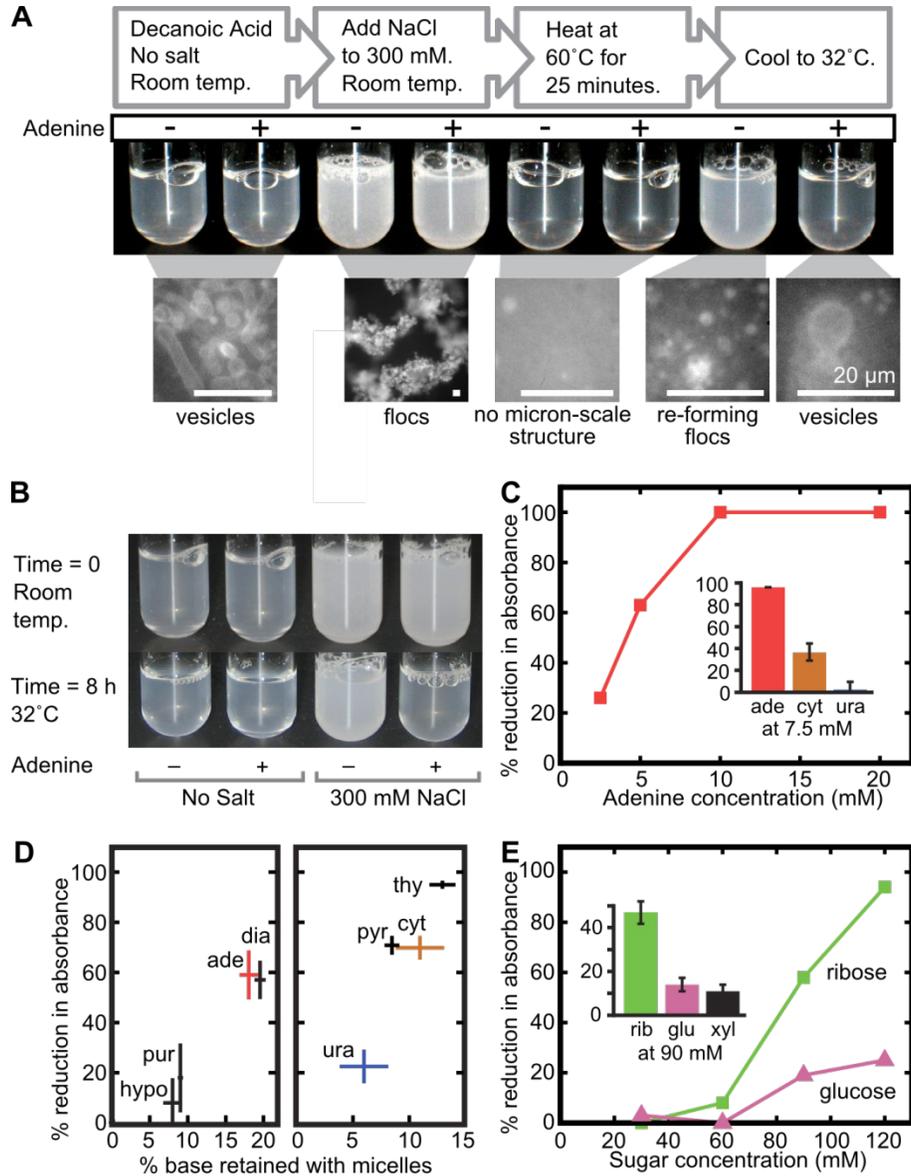
Fig. 2. Nucleobases and sugars inhibit flocculation of decanoic acid induced by salt.

(*A*) Adenine reduces re-flocculation of decanoic acid, and enables vesicle formation, after

dissolution of flocs by heat. Test-tube solutions of 80 mM decanoic acid/pH 7.65, without

and with 30 mM adenine, were treated as indicated. Corresponding samples for microscopy

contained 10 μM rhodamine 6G as a dye and were heated and cooled to the indicated

temperatures on the microscope stage. Scale bars are 20 μm.

(*B*) Incubation with adenine at 32 ˚C reduces pre-existing flocs. 80 mM decanoic acid/pH

7.6, with and without 25 mM adenine and 300 mM NaCl as indicated, is shown before and

after incubation at 32 ˚C for 8 h. (The larger volume used for the pre-incubation set was

arbitrarily chosen.) To quantitate the effect, aliquots were incubated in a 96-well plate in

parallel, and turbidity was measured after 8 h; the presence of adenine reduced absorbance at

490 nm by $74 \pm 1\%$ (average of duplicates).

(*C*) Nucleobases inhibit salt-induced flocculation of decanoic acid. The main panel shows

the percent reduction in absorbance of a solution of 80 mM decanoic acid/300 mM NaCl

(compared to controls with no base added) vs. concentration of adenine, in the plate-based

assay for flocculation described in the text; results are representative of three experiments.

Inset shows percent reduction in absorbance of a solution of 80 mM decanoic acid/300 mM

NaCl containing 7.5 mM adenine, cytosine or uracil (compared to controls with no base

added); error bars represent average deviations of duplicate samples.

(*D*) The inhibition of salt-induced flocculation of decanoic acid by nitrogenous bases

correlates with their binding to decanoic acid micelles. The purines (left panel) were tested

in the plate-based assay for flocculation at 2.5 mM, and the pyrimidines (right panel) were

tested at 10 mM; samples were run in duplicate, and error bars represent average deviations.

Values for percent retained with micelles are from the filtration assay run with bases at 0.3 mM.

(*E*) Sugars inhibit salt-induced flocculation of decanoic acid. Main panel shows percent reduction in absorbance of a solution of 80 mM decanoic acid/300 mM NaCl (compared to controls with no sugar added) vs. concentration of sugar, in the plate-based assay for flocculation; the results are representative of three experiments. Inset shows percent reduction with 90 mM sugar; error bars represent average deviations of duplicate samples.

Table 1

Test for interaction	Bases	Sugars
Altered pH of vesicle transition	A > C > U (Figs. S2, S3)	rib > glu (Figs. S2, S3)
Retention during dialysis	A > U (Fig. 1B)	
Retention during ultrafiltration	A ~ G > C > U (Fig. 1D)	
Reduction in flocculation	A > C > U (Fig. 2C)	rib > glu (Fig. 2E)

Table 1. The rank order of effects of nucleobases and sugars is consistent across several tests for interaction with decanoic acid aggregates. The tests are described in the text and figure legends.

Supporting Information for

Nucleobases bind to and stabilize aggregates of a prebiotic amphiphile: a viable mechanism for the emergence of protocells

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Materials

Flat bottom, nonsterile, clear polystyrene 96-well plates were from Thermo Fisher Scientific (Waltham, MA). Decanoic acid was from Fluka/Sigma (St. Louis, MO), glucose from Thermo Fisher Scientific (Waltham, MA), xylose from Calbiochem/EMD Millipore (Billerica, MA), PBS from Mediatech, Inc. (Manassas, VA), and hypoxanthine from Acros Organics (Geel, Belgium). All other chemicals were from Sigma. All solutions were prepared in 18 MΩ-cm water.

Methods

Decanoic acid solutions

Decanoic acid was dissolved, with heating, in 190 mM NaOH, to yield a 180 mM solution. This stock was then diluted to obtain 80 mM decanoic acid with or without 100 mM bicine (diluted from a 1 M stock solution) or 100-300 mM NaCl (diluted from a 4 M stock solution). The pH was adjusted by adding HCl, typically from 0.5 or 1 M solutions.

Imaging vesicles

All samples contained 10 µM Rhodamine 6G and were placed between two coverslips sealed with vacuum grease. In experiments involving temperature changes, the bottom coverslip was coupled with thermal paste (Omega Engineering, Stamford, CT) to the microscope stage. Temperature control of the stage was achieved with a Wavelength controller connected to a Peltier device and a thermistor temperature probe with a manufacturer-quoted accuracy of 0.02°C (Wavelength Electronics, Bozeman, MT). Epifluorescence microscopy was performed with a 60x or 10x air objective on a Nikon Y-FL microscope (Nikon, Melville, NY) with a Coolsnap HQ charge-coupled device camera (Photometrics, Tucson, AZ).

Dialysis

Adenine, uracil or thiouracil was dissolved to 15 mM in solutions of either 180 mM decanoic acid/190 mM NaOH or 180 mM acetic acid/190 mM NaOH. Solutions were brought to the same temperature and then titrated with HCl to the same pH, typically about 7.8. 0.4 ml of each titrated solution was placed in a Slide-A-Lyzer with a cut-off of 3.5 kD (Pierce/Thermo Fisher Scientific, Rockford, IL). Two solutions were dialyzed side-by-side in beakers containing 200 ml of a 180 mM acetic acid/190 mM NaOH solution adjusted to pH 7.8. Samples of 200 µl were withdrawn from the dialysis buffer at intervals of 1 or 2.5 minutes, dried down with a centrifugal vacuum evaporator (SPD121P Speedvac, Thermo Fisher Scientific, Pittsburg, PA), and resuspended in 30 µl water. The absorbance of each sample at 260 nm was measured with a NanoDrop1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

Absorbance data were fit to a linear function, with best fit slopes and associated fit uncertainties determined using MATLAB software (Mathworks, Natick, MA). Uncertainties stated for individual experiments (*i.e.,* the two discrete experiments shown in Fig. 1B) reflect only the uncertainty in the fit. We set the criterion that differences in slopes are significant only if they are greater than zero with a probability of $p < 0.05$ (equivalent to a difference in slope at least twice as large as the experimental uncertainty). For example, it is significant that the rate of release of adenine was $24 \pm 5\%$ lower from decanoic acid than from acetic acid since 24/5 ~ 5, *i.e.* a 5-sigma change. Similarly, it is insignificant that the rate of release of uracil was $8 \pm 7\%$

greater from decanoic acid than from acetic acid since 8/7 ~ 1. Uncertainties quoted in the main text for the mean difference between the rates of release from decanoic versus acetic acids, over several experiments, account for both uncertainty in fit and variation among replicates.

Langmuir trough studies

In Langmuir monolayer studies, the surface pressure is determined by using a balance to measure forces on a plate of filter paper or platinum partially immersed in the subphase. The force on the plate results from three components: buoyant force of water, force of gravity, and surface tension of water. The only one of these terms that changes during our experiments is the surface tension. Our   studies were performed as in reference 25 using a Nima trough (Coventry, England) with a subphase temperature of 22 ˚C. The subphase contained either 10 mM or no (control) adenine in phosphate-buffered saline, which was prepared from a 10x stock solution; the inclusion of adenine did not detectably change the pH. Stearic acid in chloroform was deposited at the air-water interface using a Hamilton syringe. Ample time (10 minutes) was allowed for chloroform to evaporate before data were taken.

Filtration assay

Bases were dissolved in 180 mM decanoic acid/pH 8.25; at the concentrations employed, $\leq$ 0.3 mM, the bases did not detectably alter pH. Solutions of guanine and xanthine, which have low solubility, were centrifuged at 3000 x g for 10 minutes in conical-bottomed tubes and the supernates were used for the assay. All base solutions were then treated equally. Typically, 2 ml were placed in an Amicon Ultra-4 3 K filter (Millipore, Billerica, MA) and centrifuged at 3000 x g for 10 minutes in a Sorvall Legend RT swinging-bucket centrifuge (Thermo Fisher Scientific, Waltham, MA). Aliquots of the starting solution (taken prior to centrifugation), the retentate (after gentle agitation to dislodge aggregates on surfaces), and the filtrate were then measured for absorbance at 280 nm for 2,6-diaminopurine, 300 nm for 2-aminopurine, 250 nm for hypoxanthine, 242 nm for pyrimidine, or 260 nm in all other cases. To confirm that decanoic acid micelles are retained in the retentate, we used a pinacyanol chloride assay for aggregated lipids (13) ; we found that the retentate contained over 10-fold more aggregated decanoic acid than the filtrate.

The decrease in concentration of base in the filtrate relative to the starting material was used as the measure of base retained with the micelles. This decrease was generally of the same magnitude as the increase in base concentration in the retentate, and was more reproducible. This agreement provides evidence against nonspecific loss of base on the surfaces of the centrifuge tube and filter. Further evidence against nonspecific loss came from control experiments in which bases were dissolved in a 20 mM decanoic acid solution, which is below the critical micelle concentration. The amount of base retained in these experiments was generally only 0-2% of the starting concentration, as expected if retention of base in the experiments with 180 mM decanoic acid is primarily due to binding to micelles.

NaCl-induced flocculation in test tubes

NaCl was added to 80 mM decanoic acid/pH 7.60-7.65 solutions to a final concentration of 300 mM by diluting from a 4 M stock solution, and solutions were briefly vortexed immediately after the addition. Including 30 mM adenine in the solutions only slightly altered pH, lowering it about 0.03 units, and adenine inhibited flocculation equally well when bicine was included to eliminate pH changes. A Canon PowerShot SD600 camera was used to take photos of samples

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within test tubes in a rack on a black mat. In the case of samples above room temperature, photos were taken promptly upon removal from the indicated temperature.

NaCl-induced flocculation in 96-well plates

Bases and sugars were dissolved in an 80 mM decanoic acid/100 mM bicine/pH 7.9 solution. Inclusion of bicine ensured that effects of bases or sugars on flocculation were not due to changes in pH. Typically, 19 ⬜l of 4 M NaCl was added to 231 ⬜l of the test solutions, and each sample was immediately vortexed. After 5 minutes, samples were vortexed again and 100 ⬜l aliquots were placed in a 96-well plate. Bubbles introduced due to pipetting were eliminated by lancing with a hypodermic needle. The plate was wrapped in Saranwrap and placed in a 60 °C incubator for 17 min. The plate was then read in a SpectraMax M5 plate-reader (Molecular Devices, Sunnyvale, CA) at 490 nm. On the initial reading, samples showed virtually no absorbance above background (the absorbance of a solution of 80 mM decanoic acid/pH 8.2 with no salt added), because no significant re-flocculation had occurred. The plate was then re-read roughly every minute until control samples with no base or sugar showed substantial absorbance (about 0.5), typically after about 6 min. The reported percent reduction in absorbance (relative to the control value with no base or sugar added) is based on this time point. Values for percent reduction in absorbance vary from experiment to experiment because the cooling time at which the measurement was made varied.

To verify that ribose and glucose, rather than derivatives that formed during heating to 60 ˚C, inhibit flocculation, we conducted a control experiment in which the sugars were not added until the solutions had cooled to 40 ˚C; the results were identical within experimental uncertainty to those in Fig. 2E: $81 \pm 6\%$ reduction in absorbance for ribose and $28 \pm 7\%$ for glucose, in duplicate trials, with the sugars at 120 mM. For this control experiment, we made three changes to the general procedure. First, bicine was omitted in order to eliminate any reaction of the sugars with the buffer compound. Second, pH was lowered to 7.5, because the stability of sugars decreases with increasing pH. Third, after heating the 80 mM decanoic acid/300 mM NaCl solution to 60 °C to dissolve the flocs, we cooled it to 40 °C before adding 120 mM sugar; we then transferred the samples to a 96-well plate, and measured absorbance as the solutions cooled further and flocculation occurred.

Methods for Supporting Information

Titration of decanoic acid solutions

Bases or sugars were dissolved in 80 mM decanoic acid/100 mM NaCl that had been adjusted to pH 8.25 with HCl. Solutions were then titrated in a beaker dropwise with HCl at 0.0625 to 1 M (depending on the volume of the solution and the point in the titration, to yield small, even decreases in pH). After each new pH was established, a 100 ⬜l aliquot was withdrawn to a 96-well plate for subsequent measuring of the absorbance at 490 nm with a SpectraMax M5 plate-reader (Molecular Devices, Sunnyvale, CA). Ribose and glucose solutions showed some absorbance (< 0.01) even at pH values above the point at which turbidity increased, and baselines were normalized accordingly.

Measuring turbidity induced by heating decanoic acid solutions containing bicine

Bases or sugars were dissolved in 80 mM decanoic acid/100 mM bicine/pH 7.9. 100 ⬜l of

each solution was placed in a well of a 96-well plate, in duplicate, and the absorbance at 490 nm was measured. The plate was then wrapped in Saranwrap, placed in a 60 °C incubator for 10 minutes, and read again. % reduction in absorbance is the percentage of the control value (with no base or sugar added) by which a base or sugar reduced the increase in absorbance of a sample due to the heat-induced drop in pH.

Dynamic light scattering

   A solution of 90 mM decanoic acid/100 mM bicine/pH 7.66 was extruded through polycarbonate membranes (Avanti Polar Lipids, Alabaster, AL), first 11 times through an 800 nm pore membrane and then 11 times through a 100 nm pore membrane. 30 mM adenine or thiouracil (or an equivalent volume of buffer) was then diluted into the extruded preparation to yield a final concentration of 10 mM base. Because pH falls when decanoic acid vesicle preparations are diluted, the additions were in 100 mM bicine at higher pH, such that the pH of the final solution was maintained at 7.66. Dynamic light scattering measurements were carried out on a ZetaPlus analyzer (Brookhaven Instruments, Holtsville, NY) operated at a wavelength of 659 nm and at 25 ºC. A 300 ⎕l sample was used for each measurement. The hydrodynamic radius at each reported time point was determined by averaging 5 two-minute runs. At the end of the experiment, the pH of all solutions was measured to ensure that it had not changed.
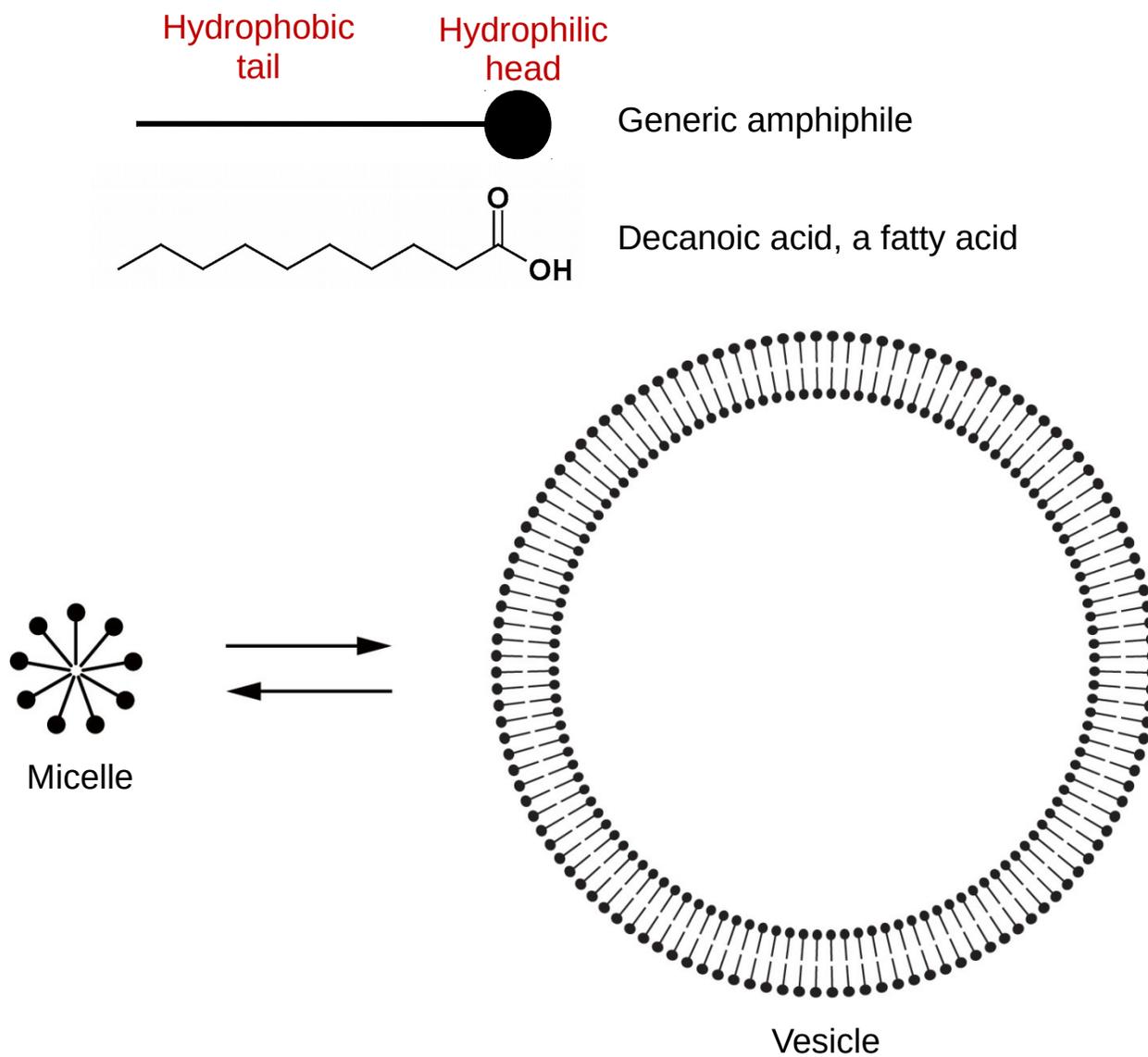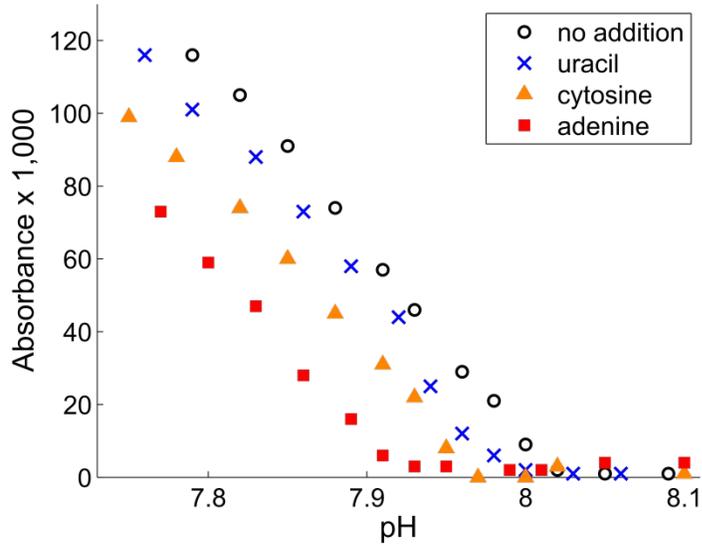
Fig. S1

Fatty acids, like other amphiphiles, can form both micelles and vesicles.

A. Nucleobases at 30 mM lower the pH below which 80 mM decanoic acid/100 mM NaCl forms vesicles. The density of vesicles is related to the turbidity of the solution (the absorbance at 490 nm). Results shown are

representative of ten experiments with adenine and four

with cytosine and uracil.
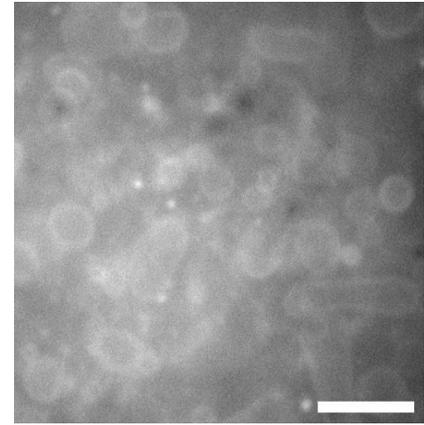
B. Epifluorescence microscopy confirmed a correspondence between turbidity and the presence of vesicles. Here, a solution of 80 mM decanoic acid/100 mM NaCl was titrated with HCl to pH 7.8, and 10 μM rhodamine 6G was added for

imaging. Scale bar, 10 μm.
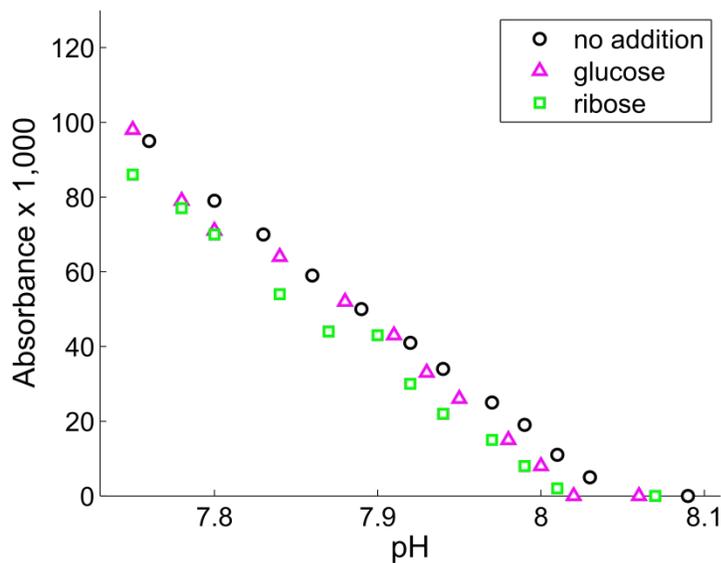
C. Ribose at 120 mM lowers the pH required for 80 mM decanoic acid/100 mM NaCl to form vesicles, whereas glucose has minimal effect. The density

of vesicles is related to the turbidity of the solution (the absorbance at 490 nm). Results shown are representative of four experiments with ribose and

Fig. S2

Nucleobases and ribose lower the pH at which vesicles form in decanoic acid solutions when the pH is decreased by titrating with HCl.
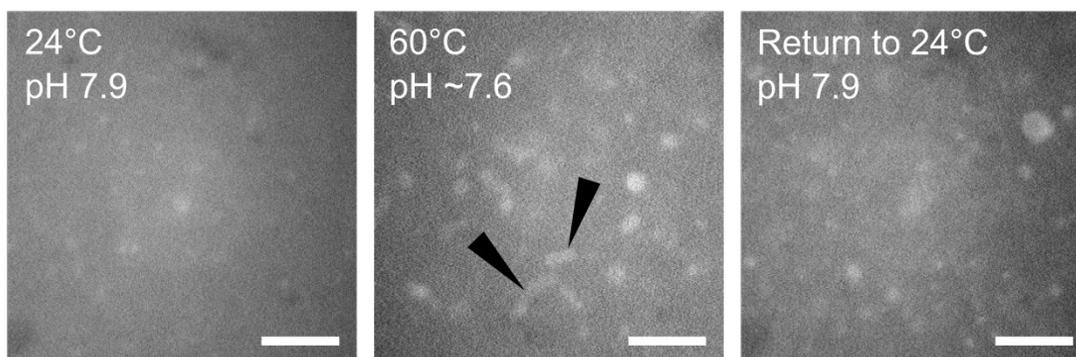
A. Vesicles form when pH is decreased by heating a decanoic acid solution containing bicine to 60 °C. A solution of 80 mM decanoic acid/100 mM bicine/10 μM rhodamine 6G was imaged by epifluorescence microscopy as it was cycled from room temperature (24 ˚C) at pH 7.9, to 60 ˚C at pH ~7.6, and back again. Arrows indicate the appearance of tubular vesicles, which disappeared rapidly upon the return to 24 °C. Scale bar, 10 ⎕m.
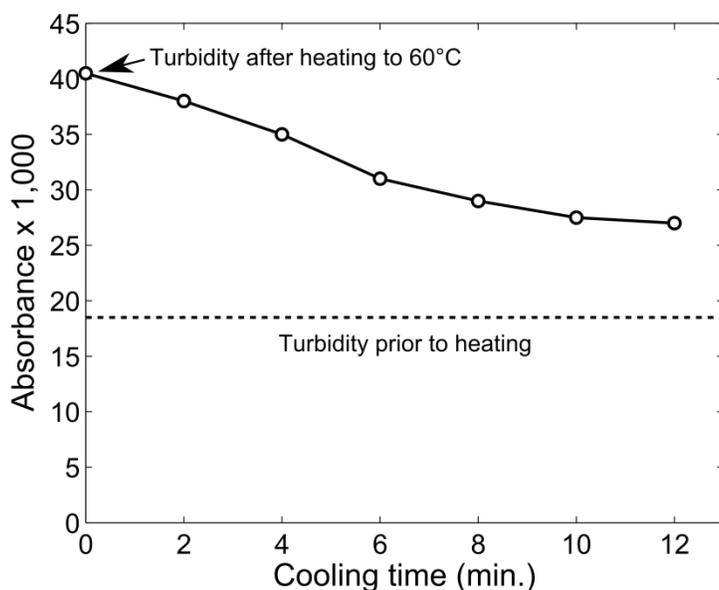

B. Quantification of the heating-induced increase in vesicle density and subsequent decrease upon cooling. The dotted line shows the absorbance at 490 nm of 80 mM decanoic acid/100 mM bicine/pH 7.9 in a 96-well plate at room temperature (corresponding to first panel in S3A). The plate was re-read after heating to 60 °C (0 time, corresponding to the middle panel) and at various times thereafter as the plate cooled (and pH rose). Values are the average of duplicate wells, and average deviations are smaller than the symbols. No increase in absorbance occurs upon heating if bicine is omitted from the solution.

Fig. S3

Nucleobases and sugars decrease vesicle formation when pH is lowered by heating decanoic acid solutions containing bicine. To verify and quantify the results in Fig. S2, we established an alternate procedure for changing solution pH based on the temperature-dependence of the pK_a of bicine.   Heating a solution of 80 mM decanoic acid/100 mM bicine/pH 7.9 from room temperature (24 ˚C) to 60 °C causes a drop in pH to ~7.6.
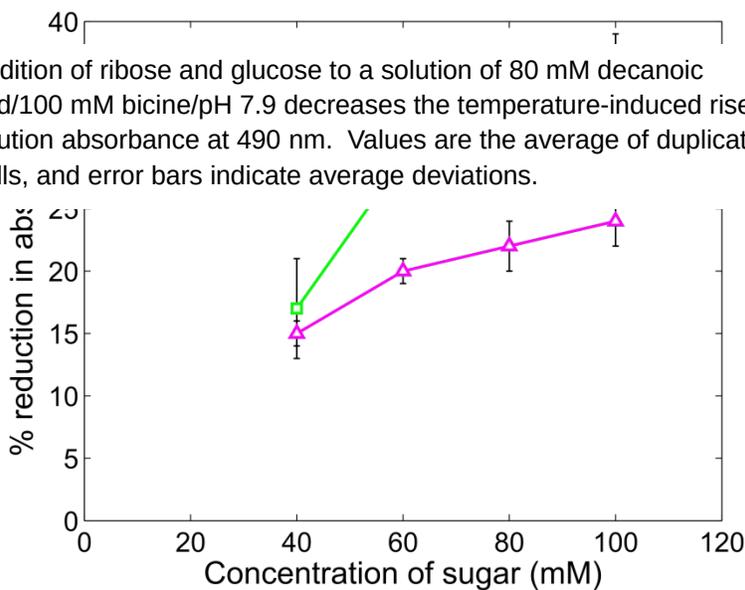
c. Addition of nucleobases to a solution of 80 mM decanoic acid/100 mM bicine/pH 7.9 decreases the temperature-induced rise in solution absorbance at 490 nm. Values are the average of duplicate wells, and error bars indicate average deviations. Percent reduction is the fraction of the control value (with no base added) by which the base decreased the temperature-induced rise in absorbance.

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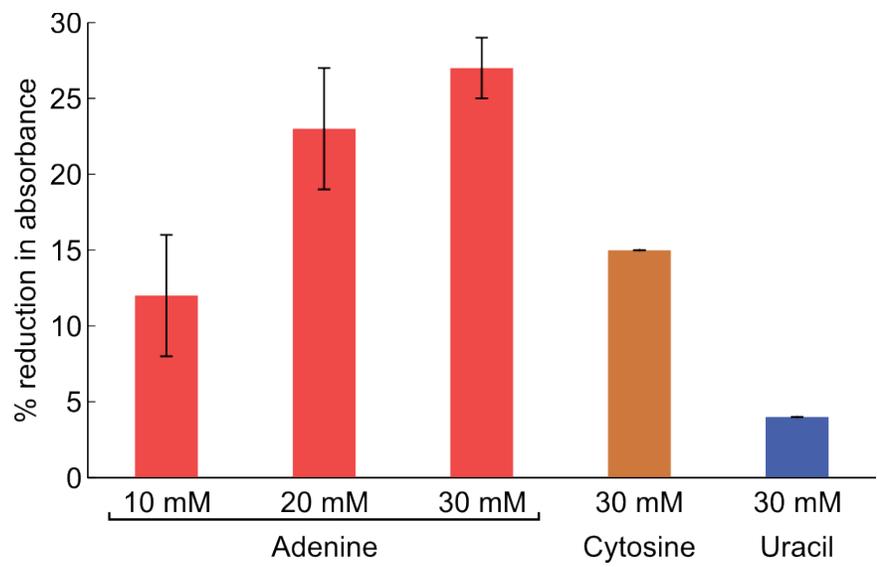
C. Addition of nucleobases to a solution of 80 mM decanoic acid/100 mM bicine/pH 7.9 decreases the temperature-induced rise in solution absorbance at 490 nm. Values are the average of duplicate wells, and error bars indicate average deviations. % reduction in absorbance is the percentage of the control value (with no base added) by which the base decreased the temperature-induced rise in absorbance.

D. Addition of ribose and glucose to a solution of 80 mM decanoic acid/100 mM bicine/pH 7.9 decreases the temperature-induced rise in solution absorbance at 490 nm. Values are the average of duplicate wells, and error bars indicate average deviations.

Fig. S3

Continued.

d. Addition of ribose and glucose to a solution of 80 mM decanoic acid/100 mM bicine/pH 7.9 decreases the temperature-induced rise in solution absorbance at 490 nm. Values are the average of duplicate wells, and error bars indicate average deviations.
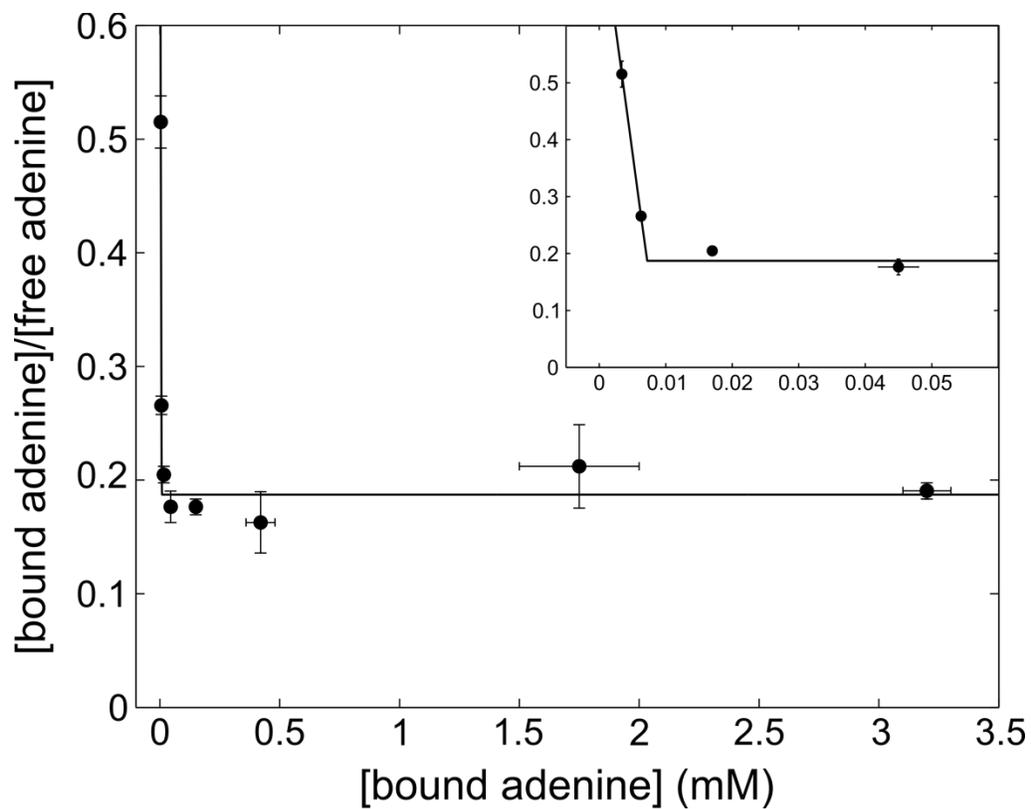
Fig. S4
Scatchard analysis of adenine binding to decanoic acid micelles. Binding was measured with the filtration assay described in Methods. We confirmed that the binding is dependent on the presence of micelles across the entire range of adenine concentrations tested: no significant binding was observed when we used decanoic acid at 20 mM, below the critical micelle concentration, instead of 180 mM decanoic acid.
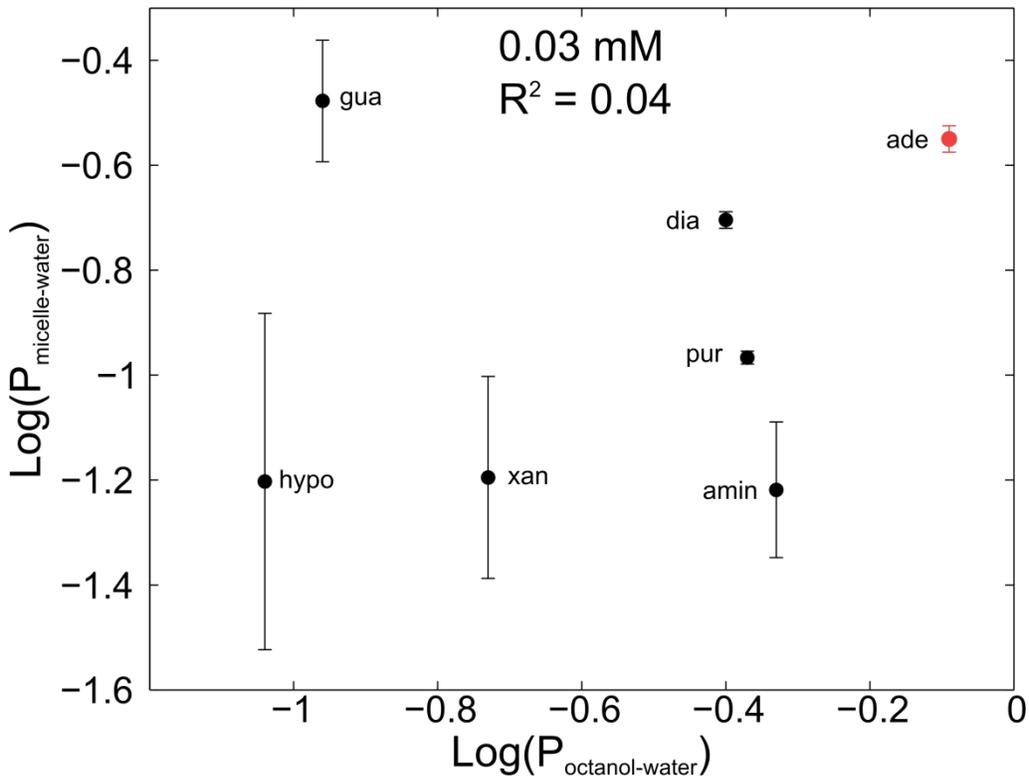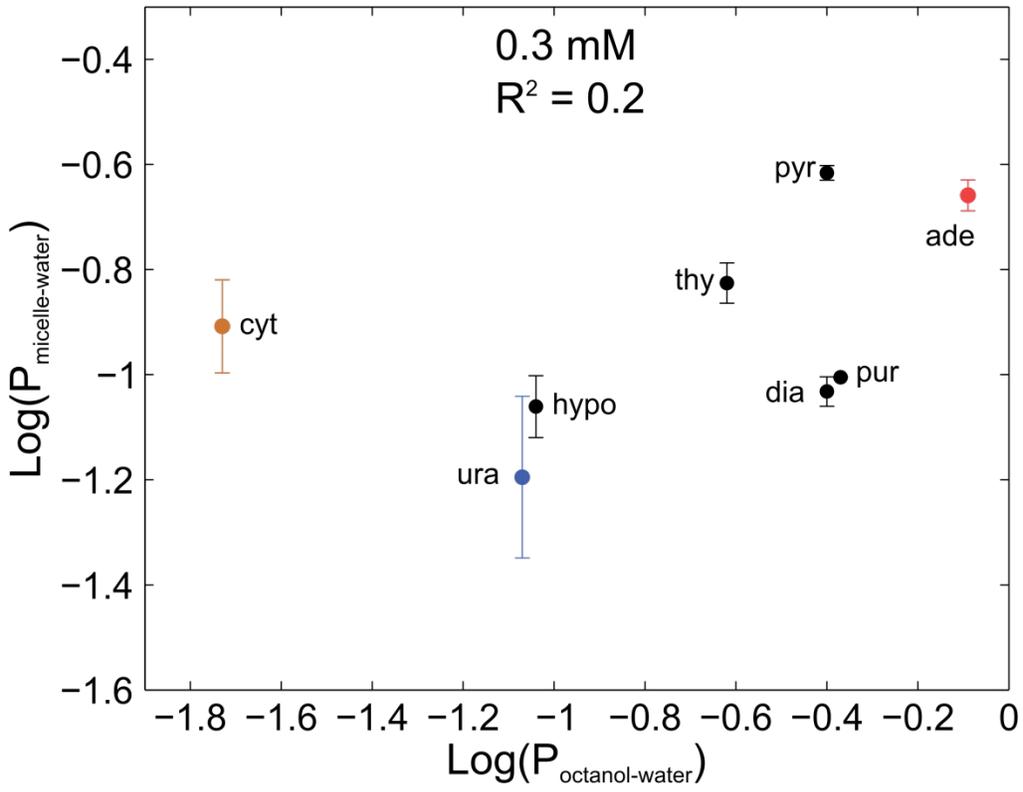
Fig. S5
Binding of bases to micelles does not correlate with their hydrophobicity. Binding is expressed as a partioning between micelles and water; we define $P_{micelle-water}$ as the ratio of the base associated with micelles to the base that is not. Values are from Fig. 1D. Hydrophobicity is measured by the partitioning between octanol and water; $P_{octanol-water}$ is defined as [base in octanol]/[base in water]. Most of the values for octanol-water partitioning are the recommended values from Sangster (http://logkow.cisti.nrc.ca/logkow/index.jsp). For diaminopurine and aminopurine, no literature values exist to the best of our knowledge, and we predicted those in the graph, employing Virtual Computational Chemistry Laboratory, http://www.vcclab.org, 2005.
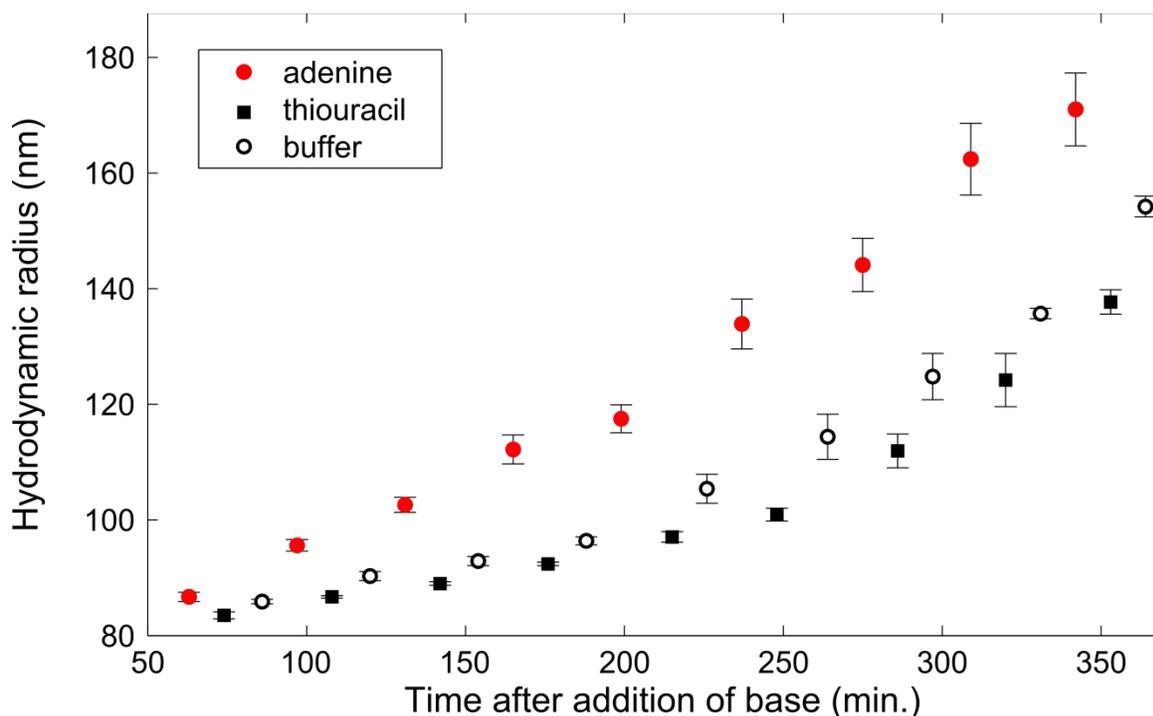

Fig. S6
Vesicles grow faster in the presence of adenine. First, vesicles were extruded through 100 nm filters; then buffer, adenine or thiouracil was added (to 10 mM for the bases), and size was measured periodically by dynamic light scattering. Error bars represent the standard error of the 5 runs at each time point. (The plot is representative of three experiments). See Methods for details.

Table S1

Sugar	% reduction in absorbance	Number of experiments
Ribose	70 ± 14	9
Arabinose	72 ± 21	9
Xylose	26 ± 6	5
Lyxose	32 ± 5	5

Table S1. Inhibition of salt-induced flocculation: arabinose is indistinguishable from ribose, and lyxose is indistinguishable from xylose. The flocculation assay was carried out as described in Methods, with all sugars at 90 mM. Ribose and arabinose were assayed side-by-side in the 9 experiments reported, and xylose and lyxose were assayed side-by-side in the 5 experiments reported. The values are means ± standard deviation.