Supporting Information

Black et al. 10.1073/pnas.1300963110

SI Materials and Methods

Flat-bottom, nonsterile, clear polystyrene 96-well plates were from Thermo Fisher Scientific. Decanoic acid was from Fluka/Sigma, glucose from Thermo Fisher Scientific, xylose from Calbiochem/EMD Millipore, phosphate-buffered saline (PBS) from Mediatech, Inc., and hypoxanthine from Acros Organics. All other chemicals were from Sigma. All solutions were prepared in 18 MΩ·cm water.

Decanoic Acid Solutions. Decanoic acid was dissolved, with heating, in 190 mM NaOH to yield a 180-mM solution. This stock then was 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) 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). Epifluorescence microscopy was performed with a 60× or 10× air objective on a Nikon Y-FL microscope with a CoolSNAP HQ CCD camera (Photometrics).

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. Next, 0.4 mL of each titrated solution was placed in a Slide-A-Lyzer with a cutoff of 3.5 kDa (Pierce/Thermo Fisher Scientific). Two solutions were dialyzed side by side in beakers containing 200 mL of a solution of 180 mM acetic acid/190 mM NaOH adjusted to pH 7.8. Samples of 200 μL were withdrawn from the dialysis buffer at intervals of 1 or 2.5 min, dried down with a centrifugal vacuum evaporator (SPD121P SpeedVac; Thermo Fisher Scientific), and resuspended in 30 μL water. The absorbance of each sample at 260 nm was measured with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific).

Absorbance data were fit to a linear function, with best-fit slopes and associated fit uncertainties determined using MATLAB software (MathWorks). Uncertainties stated for individual experiments (i.e., the two discrete experiments shown in Fig. 1*B*) 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 ± 5% lower from decanoic acid than from acetic acid, because 24/5 ~ 5, i.e., a 5-sigma change. Similarly, it is insignificant that the rate of release of uracil was 8 ± 7% greater from decanoic acid than from acetic acid, because 8/7 ~ 1. Uncertainties quoted in the main text for the mean difference between the rates of release from decanoic vs. acetic acids, over several experiments, account for both the uncertainty in fit and variation among replicates.

Langmuir Trough Studies. In Langmuir monolayer studies, the surface pressure is determined 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: the 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 ref. 1 using a NIMA trough with a subphase temperature of 22 °C. The subphase contained either 10 mM or no (control) adenine in PBS, which was prepared from a 10× stock solution; the inclusion of adenine did not change the pH detectably. Stearic acid in chloroform was deposited at the air–water interface using a Hamilton syringe. Ample time (10 min) 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 used, ≤0.3 mM, the bases did not alter pH detectably. Solutions of guanine and xanthine, which have low solubility, were centrifuged at 3,000 × *g* for 10 min in conical-bottomed tubes, and the supernates were used for the assay. All base solutions then were treated equally. Typically, 2 mL was placed in an Amicon Ultra-4 3K filter (Millipore) and centrifuged at 3,000 × *g* for 10 min in a Sorvall Legend RT swinging-bucket centrifuge (Thermo Fisher Scientific). Aliquots of the starting solution (taken before centrifugation), the retentate (after gentle agitation to dislodge aggregates on surfaces), and the filtrate then were 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 (2); 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 generally was 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 generally was only 0–2% of the starting concentration, as expected if retention of base in the experiments with 180 mM decanoic acid is primarily the result of 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 the solutions were vortexed briefly immediately after the addition. Including 30 mM adenine in the solutions altered the pH only slightly, 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 photographs of samples within test tubes in a rack on a black mat. In the case of samples above room temperature, photographs were taken promptly upon removal from the indicated temperature.

NaCl-Induced Flocculation in 96-Well Plates. Bases and sugars were dissolved in a solution of 80 mM decanoic acid/100 mM bicine/pH 7.9. The inclusion of bicine ensured that the effects of bases or sugars on flocculation were not the result of changes in pH. Typically, 19 μL of 4 M NaCl was added to 231 μL of the test

solutions, and each sample was vortexed immediately. After 5 min, the samples were vortexed again and 100-μL aliquots were placed in a 96-well plate. Bubbles introduced as the result of pipetting were eliminated by lancing with a hypodermic needle. The plate was wrapped in Saran Wrap and placed in a 60 °C incubator for 17 min. The plate then was read in a SpectraMax M5 plate reader (Molecular Devices) at 490 nm. On the initial reading, the 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 reflocculation had occurred. The plate then was reread 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 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. 2*E*: 81 ± 6% reduction in absorbance for ribose and 28 ± 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 to eliminate any reaction of the sugars with the buffer compound. Second, the pH was lowered to 7.5, because the stability of sugars decreases with increasing pH. Third, after heating the solution of 80 mM decanoic acid/300 mM NaCl 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.

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 then were titrated in a beaker dropwise with HCl at 0.0625–1 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-μL 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). 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. Then, 100 μL 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 then was wrapped in Saran Wrap, placed in a 60 °C incubator for 10 min, and read again. The % 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 as a result of 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)—first, 11 times through an 800-nm pore membrane and then 11 times through a 100-nm pore membrane. Then, 30 mM adenine or thiouracil (or an equivalent volume of buffer) was 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 so 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) operated at a wavelength of 659 nm and at 25 °C. A 300-μL sample was used for each measurement. The hydrodynamic radius at each reported time point was determined by averaging five 2-min runs. At the end of the experiment, the pH of all solutions was measured to ensure it had not changed.

1. Narayanan R, Stottrup BL, Wang P (2009) Surface packing characterization of Langmuir monolayer-anchored enzyme. *Langmuir* 25(18):10660–10665.

2. Namani T, Walde P (2005) From decanoate micelles to decanoic acid/dodecylbenzenesulfonate vesicles. *Langmuir* 21(14):6210–6219.

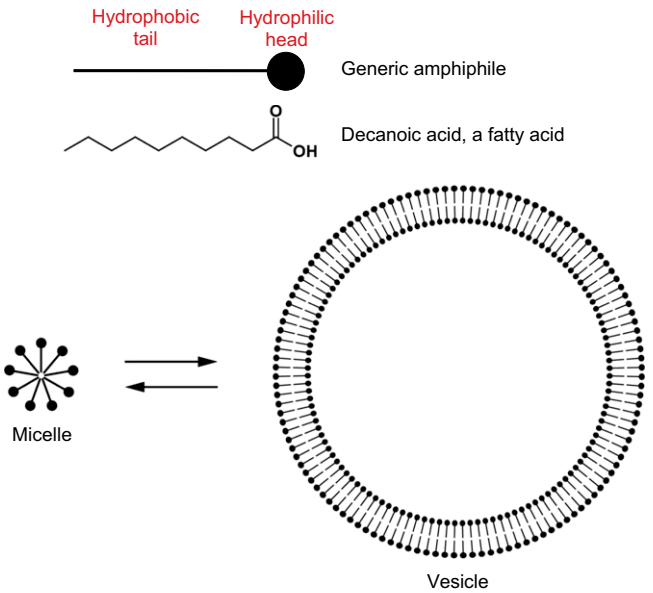

Fig. S1. Fatty acids, like other amphiphiles, can form both micelles and vesicles.

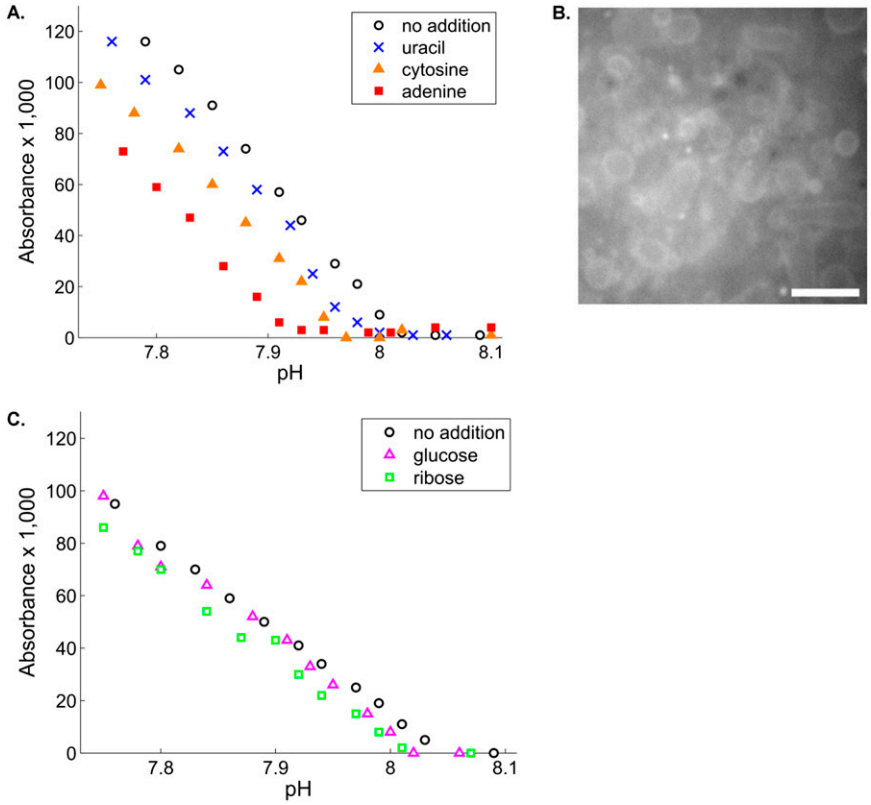

Fig. S2. Nucleobases and ribose lower the pH at which vesicles form in decanoic acid solutions when the pH is decreased by titrating with HCl. (*A*) Nucleobases at 30 mM lower the pH below which 80 mM decanoic acid/100 mM NaCl forms vesicles. The density of vesicles is related to the turbidity of the solution (the absorbance at 490 nm). Results shown are representative of 10 experiments with adenine and 4 with cytosine and uracil. (*B*) Epifluorescence microscopy shows that the turbidity corresponds with 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 three with glucose.

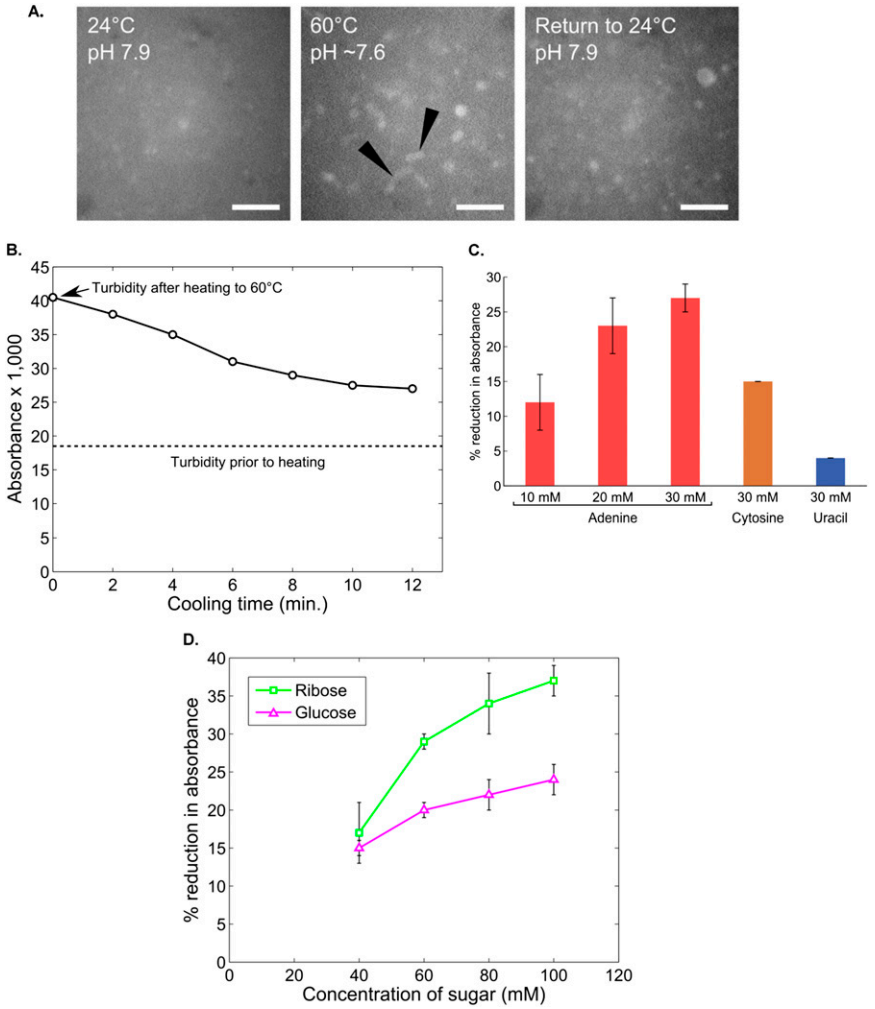

Fig. S3. Nucleobases and sugars decrease vesicle formation when pH is lowered by heating decanoic acid solutions containing bicine. To verify and quantify the results in Fig. S2, we established an alternate procedure for changing solution pH based on the temperature dependence of the pK_a of bicine. 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. (*A*) Vesicles form when the 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 μm. (*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 *A*, *Left*). The plate was reread after heating to 60 °C (0 time, corresponding to *A*, *Center*) 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. (*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. The percent 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.

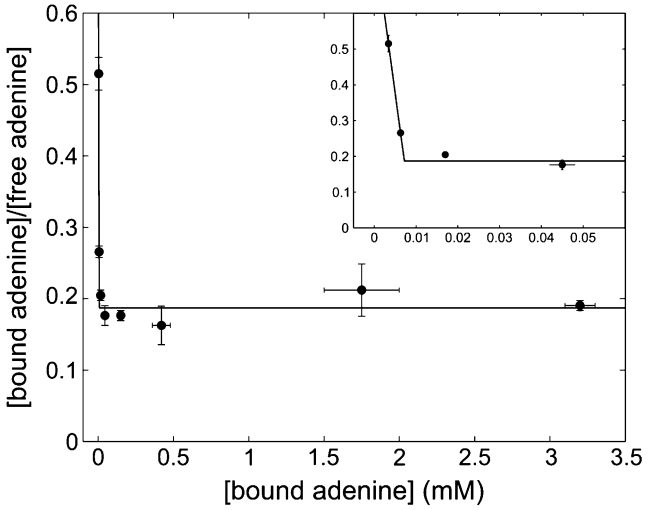

Fig. S4. Scatchard analysis of adenine binding to decanoic acid micelles. Binding was measured with the filtration assay described in *SI Materials and Methods*. We confirmed that the binding is dependent on the presence of micelles across the entire range of adenine concentrations tested: no significant retention was observed when we used decanoic acid at 20 mM, below the critical micelle concentration, instead of 180 mM decanoic acid.

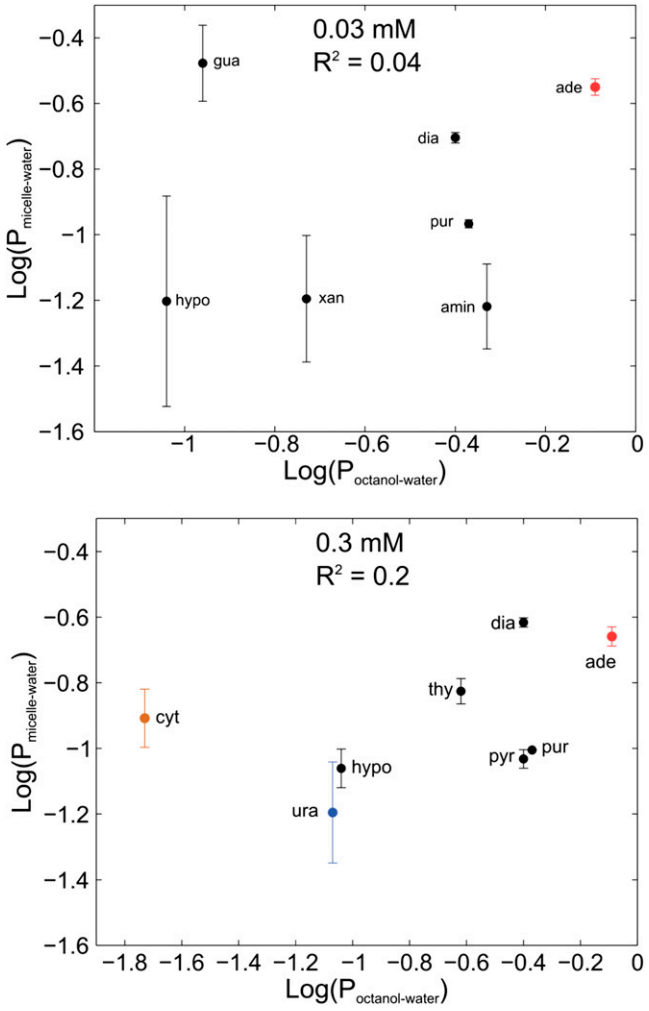

Fig. S5. Binding of bases to micelles does not correlate with their hydrophobicity. Binding is expressed as a partitioning between micelles and water; we define $P_{micelle\text{-}water}$ as the ratio of the base associated with micelles to the base that is not. Values are from Fig. 1*D*. Hydrophobicity is measured by the partitioning between octanol and water; $P_{octanol\text{-}water}$ 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, using Virtual Computational Chemistry Laboratory (www.vcclab.org, 2005).

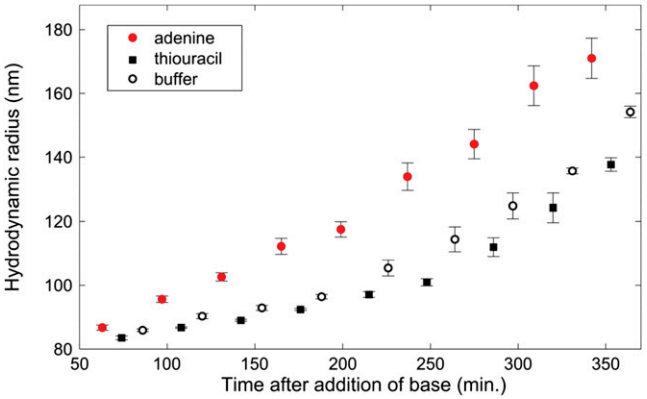

Fig. S6. Vesicles grow faster in the presence of adenine. First, vesicles were extruded through 100-nm filters; then buffer, adenine, or thiouracil was added (to 10 mM for the bases), and size was measured periodically by dynamic light scattering. Error bars represent the SE of the five runs at each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

Table S1. Inhibition of salt-induced flocculation: Arabinose is indistinguishable from ribose, and lyxose is indistinguishable from xylose

Sugar	% reduction in absorbance	No. of experiments
Ribose	70 ± 14	9
Arabinose	72 ± 21	9
Xylose	26 ± 6	5
Lyxose	32 ± 5	5

The flocculation assay was carried out as described in *SI Materials and Methods*, with all sugars at 90 mM. Ribose and arabinose were assayed together in the nine experiments reported, and xylose and lyxose were assayed together in the five experiments reported. The values are means ± SD.