

Hidden Beauty, Assembling Lipids: Modular Hands-On Outreach Experiments Showcasing Lipid Assembly Chemistry

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ABSTRACT: Successful demonstration of lipid assembly chemistry to a general audience can be challenging on both physical (nanoscopic dimensions of lipid assemblies) and intellectual (complex, interconnected concepts) accounts. Quite often, an instructor has to leave key concepts out of what is being offered due to time constraints, which unfortunately hampers students' developing a coherent understanding. In this demonstration, we present an approach to address this conundrum through modular and progressive learning. Consisting mainly of three straightforward hands-on experiments, this demonstration enables most of the key concepts in lipid assembly chemistry to be conveyed to interested students. Lipid phase separation, an important area of current membrane biophysics research, is also covered.



KEYWORDS: High School/Introductory Chemistry, First-Year Undergraduate/General, Fatty Acids, Membranes, Micelles, Lipids, Phases/Phase Transitions/Diagrams

INTRODUCTION

As nature's material choice for cell packaging and compartmentalization, lipids are fascinating to study and their assemblies elegant to behold. With their biocompatibility virtually a given, lipid-assembled materials have become an indispensable ingredient in numerous industrial applications and products, highlighted perhaps most resoundingly by the recent advancement in COVID-19 mRNA vaccines.^{1–3} The underlying assembly chemistry of lipids is quite complex, however, characteristic of intertwined geometric, thermodynamic, and mechanical concepts and factors spanning specific locations and dimensions. Conveying such complex chemistry effectively in an outreach setting is challenging, because one has to balance carefully the detail/depth to be covered with the "big picture" outline. Given the short time and fast pace typical of such outreach offerings, it is hardly avoidable for an instructor to leave certain key concepts out, which, unfortunately, hampers students' developing a coherent understanding. In this demonstration, we wish to present an approach to address this conundrum through modular and progressive learning.

A substantial body of pedagogical work has been dedicated to lipids and their assemblies, covering their biochemistry,^{4–9} biophysics,^{10–14} assembly chemistry,^{15,16} food chemistry,^{17–19} and analysis and characterization.^{20–22} The prevalence of these species in our everyday life is thoroughly demonstrated, for example, by various types of food^{18–22} employed in these studies. As an important class of closed lipid architecture, liposomes of various sizes have been covered by several demonstrations. Among these, nanosized liposomes have been

shown to be an excellent membrane model illustrating the stability^{8,9} and permeability¹⁴ of lipid bilayers, whereas their larger, micro-sized cousins enable direct visualization¹⁶ of individual formations with optical and fluorescence microscopy. From more recent work, we also start to see the integration of computer simulations²³ and portable electronic devices²⁴ in lipid-themed outreach efforts.

RATIONALE AND METHODOLOGY

While the familiar cartoon depiction of lipid bilayers generally serves as a good starting point for the students to comprehend amphiphilicity of lipids, its *static-and-final* undertone should be recognized and dispelled. It is essential to make students understand, from the very start, that as amphiphiles, lipids *dynamically* respond to the surroundings to minimize their energy, of which a bilayer is only one of the many possible forms of aggregation (i.e., polymorphism²⁵). Upon taking water away or subjecting them to a different environment, therefore, lipids may not assemble into a bilayer. Moreover, as recent research^{26–28} has amply revealed, biomembranes often display asymmetry across and lateral heterogeneity along their

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bilayer(s). Since such asymmetry and heterogeneity are critically tied with biological functions of lipids, it seems a good educational opportunity lost if we do not attempt to make such knowledge updates and connections.

Working with lipid assemblies for the past decade and half, our laboratory has participated in multiple outreach activities targeting K–12 students with group size ranging from half a dozen to a few hundred. Quite often, we had students with additional questions during the event, which, in order to be answered reasonably satisfactorily, call for additional concepts and detailed delineation. Moments like this have motivated us to make improvements in our delivery, so that the students can come out of the event feeling better informed and more fulfilled. The hands-on experiments described below resulted from our recent outreach effort, titled “*Hidden Beauty, Assembling Lipids*”, toward this goal. These experiments were offered to groups of 12–16 high school students (11th–12th grade) selected into the Summer Science Institute program at our institution in 2019 and 2021.

As depicted in Figure 1, this demonstration comprises primarily three experimental modules, which can be offered to

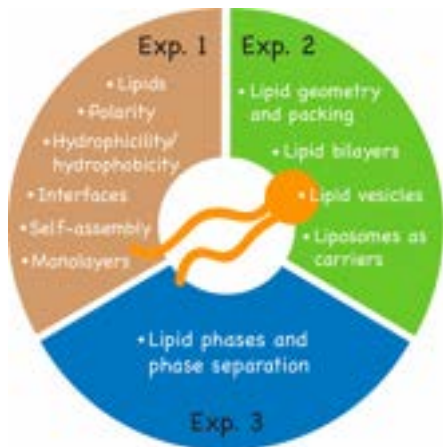


Figure 1. Activity design, with three main experiments illustrating a dozen core concepts of lipid assembly chemistry. Experiments are (1) lipid spreading at a water/oil interface, (2) liposomes as carriers of hydrophobic species in water, and (3) lipid phase separation.

interested audience either separately or progressively. The first experiment centers on lipid spreading, in which lipids subjected to a water/oil interface quickly spread and cover the interface to minimize the tension therein. As a result, lipids self-assemble into a stable monolayer in this case. In the second, the focus is shifted to the hydrocarbon region of the bilayer, which presents a *de facto* 2D organic solvent environment for hydrophobic species. This very property underlies much of the industrial use of small liposomes as a carrier in food,²⁹ cosmetics,³⁰ and drug delivery.^{31–33} The last experiment deals mainly with lipid phases and their separation. Together, these three experiments allow us to discuss most of the core concepts in lipid assembly chemistry with the students. These concepts are disassembled and described in detail in Tables 1–3. Depending on the nature of the outreach event (e.g., audience and time available), these tables may be used either by the instructor as a guide to develop lectures or as handouts to the students.

MATERIALS AND EQUIPMENT

Experiment 1

The dye-labeled lipid used in this experiment is 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (ammonium salt) (rho-DOPE) obtained from Avanti Polar Lipids (Alabaster, AL) in chloroform. Chloroform (Sigma-Aldrich) and deionized (DI) water of 18.2 MΩ cm (Millipore) were used to prepare the water/oil interface.

Experiment 2

The lipid used for liposome preparation is 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) from Avanti Polar Lipids (Alabaster, AL). Sudan III as the hydrophobic dye in this experiment was obtained from Sigma-Aldrich. DI water of 18.2 MΩ cm (Millipore) was used throughout this experiment. A general-purpose bath sonicator is needed for dispersing Sudan III dye particles in aqueous solutions.

Experiment 3

All lipids, including 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (ammonium salt) (rho-DOPE), and 23-(dipyrrometheneboron difluoride)-24-norcholesterol (Bodipy-cholesterol), were products of Avanti Polar Lipids (Alabaster, AL). Other chemicals, including poly(vinyl alcohol) (PVA, MW: ~145,000), cholesterol, and chloroform, were obtained from Sigma-Aldrich. Poly(dimethylsiloxane) (PDMS) elastic films were prepared from a thermally curable kit (Sylgard 184, Dow Corning). A standard compound epifluorescence microscope (OMAX 40–1600× trinocular, in our case) is needed to observe the liposome formation and lipid phase separation.

SAFETY REMARKS

Chloroform is carcinogenic and acutely toxic and can cause eye/skin irritation. While the chance for direct chloroform exposure in experiment 1 is very low, safety protocols and disposal kits should be in place in case of its accidental spill. Samples containing chloroform should be collected and properly disposed of after the experiments. Sudan III (experiment 2) has been classified as a category 3 carcinogen by the International Agency for Research on Cancer; care needs to be taken in handling microscope glass slides (experiment 3). Students should wear lab gloves throughout this event.

DESCRIPTION OF HANDS-ON EXPERIMENTS

Experiment 1. Lipid Spreading at Water/Oil Interface

Water/oil interfaces were formed by layering 1.5 mL of DI water on chloroform of the same volume in 1 dram glass vials. To minimize chloroform exposure, these samples were prepared in ventilation hoods in our own lab and presented to the participants in cap-sealed vials, one for each student. As a colored lipid sample, 2 μL of 0.1 mM Rho-DOPE in chloroform were gently injected into the top aqueous phase using a 10 μL microsyringe (Hamilton, Reno, NV). To ensure safety and consistency, the injection was performed by the instructor, leaving the students to focus on the experimental observation alone. By gravity, the injected lipid droplet would fall until it reaches the interface. Thereafter, lipid spreading normally follows in a few seconds, which can be easily observed by the lateral propagation of pink color along the interface.

Table 1. Concepts Covered in Experiment 1

Core Concepts	Conceptual Breakdown	Descriptive/Instructional Remarks
1. Lipids	Tail vs headgroup	While the circle-and-wiggly lines cartoon comes in handy, it is worthwhile pointing out the structural diversity associated with the two moieties.
	Phospholipid	The most abundant type of lipids in mammalian cells; ³⁷ Containing two fatty acid chains and a phosphate (which is further attached with a headgroup) linked to a glycerol via ester bonds;
	Surfactant	A general term covering all species with surface activities, including, e.g., detergents and emulsifiers; Lipids, with their intrinsic amphiphilicity, can be viewed as a special class of surfactants when their surface-related properties are emphasized, e.g., in pulmonary surfactant membranes. ³⁸ Lipids act as the main emulsifier in many food products, for example, in milk and mayonnaise. See also entry 5 below.
2. Polarity	Solubility	Polarity refers to the level of partial charge separation within individual molecules; at the extreme of the scale lies total charge separation, which yields ions. Polar molecules interact with each other primarily via dipole–dipole interactions. Often, such interactions can be substituted by a second polar species without significantly raising the total energy in the system. This gives rise to a typical “like dissolves like” scenario. Water is a very special polar molecule in its ability to hydrogen-bond with neighbors, making their association not only stronger (than common dipole–dipole interactions) but also more ordered. Conversely, water does not accommodate species whose addition causes disruption of its H-bonding network, which corresponds to a thermodynamic penalty in form of entropy. ³⁹
	Immiscibility	As such, polar and nonpolar species, such as water and oil, do not mix, causing their mutual immiscibility. Immiscibility between lipids is a subtle phenomenon, see Table 3.
3. Hydrophilicity/hydrophobicity	Amphiphilicity	Two opposite terms coined with regard to the solvent of life: water. Generally, species containing mainly charged or polar groups are hydrophilic, and those with mostly nonpolar groups are hydrophobic. Amphiphiles contain both identities arranged in a directional manner. Lipids are amphiphiles owing to their nonpolar acyl chains and polar (or charged) head groups.
		Whenever two substances of dissimilar state or phase are put into direct contact, an interface is created. The water/chloroform interface is clearly visible due to (1) contrast in their refractive index (1.33 for water vs 1.44 for chloroform) and (2) curvature caused by differential adhesion of the two liquids on glass (see also quiz 1 in SI). Since the chemical composition and physical characteristics change sharply across the interface, this region differs greatly from the bulk and abounds with interesting (interfacial) phenomena.
5. Interfacial tension	Surface energy	Facing the oil phase (chloroform) directly, those “front-line” water molecules experience a net cohesive pull from adjacent water molecules in the bulk, collectively producing a tension at the interface. ^{39,40} By contrast, water molecules in the bulk do not experience this interfacial force, because, being surrounded by other water molecules all around, no net force would emerge.
6. Self-Assembly		Amphiphiles tend to occupy a water/oil interface, with their hydrophilic/hydrophobic segments aligned with the water/oil phase, respectively. This spontaneous process (hence <i>self-assembly</i>) can be understood in the context of energy lowering that benefits all parties in the system: for the water/oil interface, lipid insertion therein lowers the interfacial tension, and for the assembling lipids, there is a harmonious dispersion of their amphiphilic moieties.
7. Monolayers		As such, an oil/water interface typically only affords a single layer of amphiphiles, i.e., a monolayer. Knowing the average area taken by a single lipid in a monolayer formation, the students can estimate roughly the total number of lipids needed to occupy the available interfacial area in this experiment (see quiz 2 in SI).

Experiment 2. Liposomes as Carriers of Hydrophobic Species in Water

The DPPC liposomes employed in this experiment were prepared by sonication in two simple steps. To start, desired quantities of DPPC stock solution in chloroform were added in 1 dram vials, and the solvent was removed thoroughly under vacuum. In place of vacuum, solvent removal may be achieved by setting the vials on a 60 °C hot plate for 30 min. In the second step, DI water was added into the same vial with dried lipid films, sealed, and sonicated at room temperature for 30 min to yield liposomes. In our offering of summer 2021, each student received 2 mL of such sample containing 0.3 mM DPPC.

Sudan III was chosen as the indicator dye of this demonstration for its intense red color and low water solubility (<0.1 mg/mL), qualifying its use as a lipophilic model drug.³⁴ Prior to meeting with the students, an aqueous suspension of this dye was prepared by adding 0.5 g of solid to 20 mL of DI water. During the hands-on session, 0.2 mL portions of such suspensions were pipetted into the DPPC liposome solution by

the instructor; the same operation was repeated with DI water in a separate vial as control. To avoid particle settlement and ensure equal sampling, the suspension was briefly shaken before each sample injection.

Experiment 3. Phase Separation in Lipid Systems

The procedure described here follows generally our earlier report³⁵ on Janus liposomes prepared via gel-assisted hydration. To achieve liquid/liquid phase separation at room temperature, three lipids, DPPC, DOPC, and cholesterol, are mixed at a 35:35:30 mol ratio. While the phospholipids are the main component of the liquid-ordered (l_o , DPPC) and liquid-disordered (l_d , DOPC) phases, cholesterol is more broadly distributed and helps maintain the lipid fluidity and packing order in the system. Two fluorescent lipids, Bodipy-cholesterol (l_o phase indicator) and Rho-DOPE (l_d phase indicator), are also included in the lipid precursor at 0.2 mol % each, so that the lipid phase separation and liposome formation can be followed by fluorescence microscopy. The total lipid concentration in the final precursor (mixed in chloroform) was 2 mM.

Table 2. Concepts Covered in Experiment 2

Core Concepts	Conceptual Breakdown	Descriptive/Instructional Remarks
8. Lipid geometry and packing ^a	Lipid polymorphism	Dispersed in water, amphiphilic molecules tend to pack together to shield their hydrophobic segment(s) from water. Packed too close, however, repulsion forces rise quickly among their headgroups and hydrophobic tails. There exists an optimal packing scenario, therefore, which depends on the structure and geometry of amphiphiles. ³⁹ Three geometric factors together decide how amphiphiles pack in water, ³⁹ length (l), volume (v) of their hydrophobic segment(s), and polar headgroup area (a), yielding the packing parameter (P), v/al . $P > 1$, if the hydrophobic portion geometrically dominates, and $P < 1$, if the headgroup is relatively large. In both cases, the resultant assemblies tend to be self-limiting in terms of number of molecules accommodated per formation. $P \approx 1$ for many phospholipids, which affords them the important freedom to grow relatively unlimited along the lateral direction. As a result, stable lipid assemblies can exist from nm to μm and beyond, contributing to their polymorphism. ^{25,39}
	Cell membranes	In particular, lipids of $P \approx 1$ self-assemble into bilayers, in which two monolayers of lipids come together in an opposing formation to produce a largely 2-dimensional hydrophobic interior. Facing away from each other, the headgroups make up the exterior of the bilayer. With good stability (with free lipid concentrations normally in nM) and polymorphism, lipid bilayers are nature's material choice for cellular packaging and compartmentalization. Finger rolls (bread) can be used as an effective everyday subject to illustrate lipid bilayer formation, in place of the oft-used cigar example.
9. Lipid bilayers	Liposomes	As a dynamic, assembled architecture, lipid bilayers are soft, which allows them to wrap into spherical, seamlessly sealed sacs in three dimensions, lipid vesicles. ⁴¹ Typically, lipid vesicles contain a single aqueous core separated by the bilayer(s) from the bulk aqueous phase. The term "vesicle" is more general and often used in describing naturally occurring lipid sacs or vacuoles. Liposomes specifically refer to vesicles that are artificially made with known composition and controlled size.
	Lamellarity	Vesicles/liposomes can contain more than one bilayer, which is described by their lamellarity, e.g., monolamellar, bilamellar, etc. Onions serve well as an everyday example for its similarity (concentric multilayers typically) and dissimilarity (filled throughout vs water core) vs lipid vesicles.
	Micelles	Amphiphiles with $P < 1$ tend to aggregate into micelles, another spherical formation featuring instead a filled hydrophobic core and a polar exterior. Compared to liposomes, micelles are more dynamic (less stable) and often monodisperse in size (lack of polymorphism). Their monodispersity arises from the geometric requirement for optimal packing, a point that can be very well illustrated in 2D using pizza slices as an example.
10. Vesicles	Liposomes	The hydrocarbon region of liposomes serves a quasi-2D organic solvent environment to host hydrophobic species, whereas their aqueous interior can be used to encapsulate water-soluble species. ^{42,43} As a biocompatible carrier, liposomes have found many applications, ^{29–31} ranging from drug delivery to food/cosmetic formulations.
	Liposomes as carriers	

^aNumbering continues from Table 1.

Table 3. Concepts Covered in Experiment 3

Core Concepts	Conceptual Breakdown	Descriptive/Instructional Remarks
12. Lipid phases	Lipid chain melting temperature (T_m)	Analogous to melting point for pure substances (e.g., water), single-component lipid aggregates are often characterized by a specific temperature, T_m , at which phase transition occurs. Typically, lipids condense into a solid-like gel phase below T_m and stay melted in liquid-like phase(s) above T_m .
	Gel vs liquid phases	The two main lipids employed in this experiment, DPPC (T_m : 41 °C) and DOPC (T_m : -17 °C), form gel and liquid phases at room temperature, respectively.
	Phase separation (in mixed lipid systems)	Lipid bilayers consisting of lipids with dissimilar T_m values can segregate laterally into different phases (domains), each displaying distinct chemical (lipid composition) and physical (lipid order, fluidity, and thickness) characteristics. ⁴⁴ Driving such phase separation is the structural dissimilarity in participant lipids, in particular, length and saturation level of lipid acyl chains; it is the "like associates with like" principle again, only this time in 2D.
	Liquid-ordered vs liquid-disordered phases	Analogous to the case of water/chloroform (experiment 1), two immiscible lipid liquid phases can coexist. The two liquid phases differ from each other in the order of acyl chain packing, which is more ordered in the phase enriched with the high-melting lipids. The liquid-disordered phase is enriched instead with the low-melting lipids. ^{44,45}
	Cholesterol, the lipid phase modulator	Such coexistence formation is facilitated primarily by cholesterol, which lubricates and softens the high-melting lipids to transition from the gel to the liquid phase. ⁴⁶ A ubiquitous component in cell membranes, cholesterol is a small sterol-type lipid species featuring a single hydroxyl group as the head and a rigid fused-ring body (see quiz 5).
	Line tension	An analogy can be drawn once again with water/chloroform, whose 3D bodies produce a 2D interface along which the interfacial tension develops. Likewise, two immiscible lipid liquid phases in a 2D bilayer produce a quasi-1D interface, from which line tension emanates. The analogy goes on. Water tends to bead up on hydrophobic surfaces to minimize direct contact and hence surface tension. Similarly, the line tension between dissimilar lipid domains contributes to lipid budding and fission, which are essential in numerous cellular processes, such as cell division.
	Lipid rafts	An emerging biomembrane hypothesis/model ^{26,28,44} stressing the importance of heterogeneous lipid organization in cellular functions; This model postulates nanoscopic lipid/protein patches (domains) as a functional platform/unit in cell membranes.

Phase-separated liposomes are produced from the above lipid precursor in three steps, with the first two prepared in our lab

and the last carried out by the students. (1) PVA gel preparation, which is done by spreading a small amount of PVA solution on a

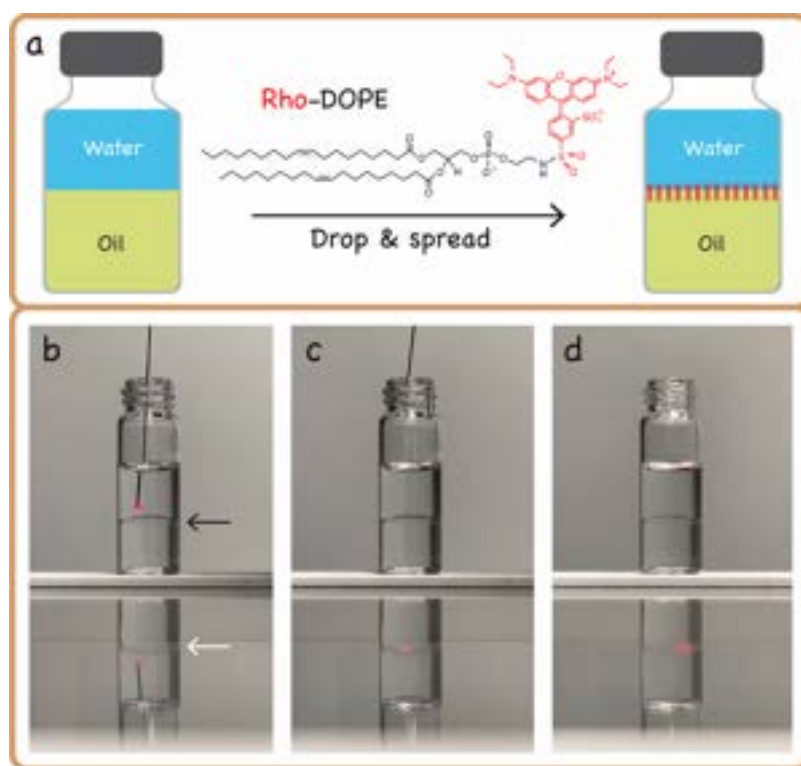


Figure 2. Lipid spreading at water/oil interfaces. (a) Schematic of the hands-on experiment, in which a rhodamine-labeled lipid (rho-DOPE) is employed as a visual indicator of the process. (b–d) Snapshot images taken from a video recording (Movie S1) of the lipid spreading experiment at 8, 9, and 11 s, respectively. Arrows in image b mark the actual water/oil interface (in black) and its reflection (in white) off glass, respectively.

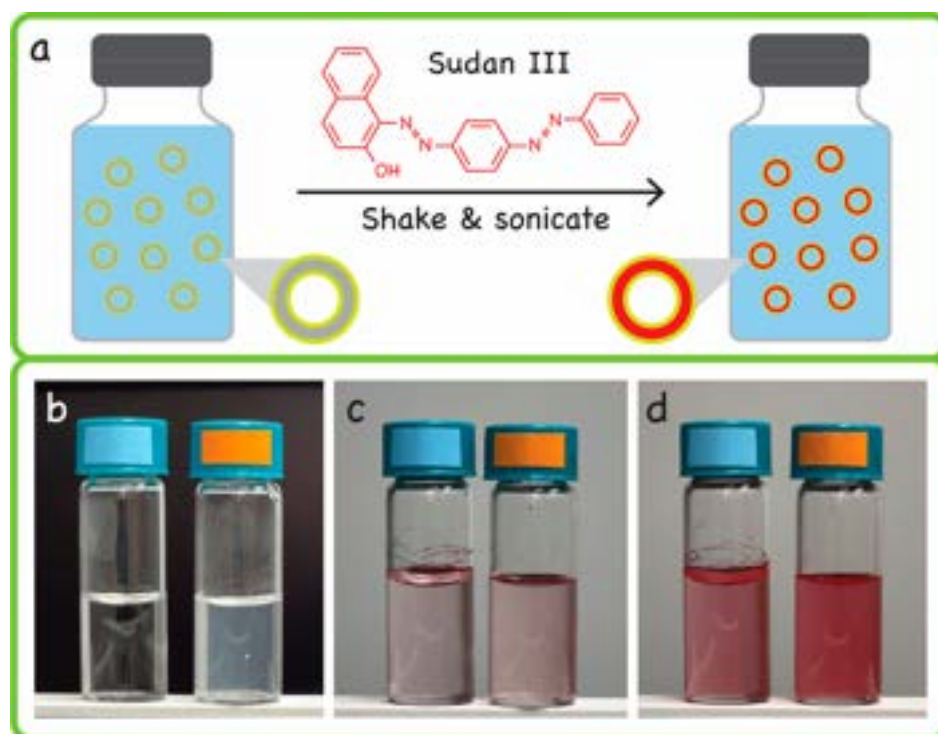


Figure 3. Liposomes as carriers of hydrophobic species in water. (a) Schematic of the hands-on experiment, in which a red hydrophobic dye (Sudan III) is employed as a visual indicator of the process. Photo b shows a liposome sample (right vial) and its water control (left) taken before the dye addition. Photos c and d are taken after the dye addition upon brief shaking (c) and sonicating (d) the vials.

microscope glass cover slide and then drying it on a hot plate at 50 °C for 0.5 h. This produces a clear and smooth gel layer on the glass slide. The PVA aqueous solution used in this procedure

contains 5 wt % polymer, which is dissolved by continuously stirring the polymer in DI water maintained at 80 °C. An optional PDMS patch, on which a ~5 mm diameter hole is

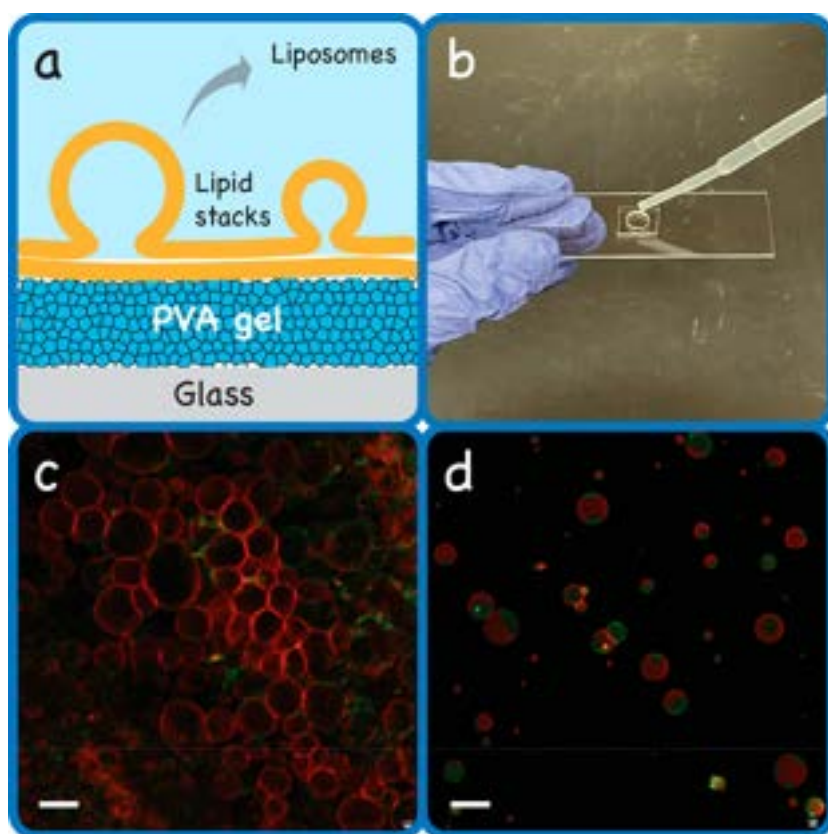


Figure 4. Phase separation in lipid systems. (a) Schematic illustration of the liposome formation process via gel-assisted lipid hydration, adapted from our previous work (ref 35) with permission. Copyright 2018, American Chemistry Society. (b) Photo of the experimental setup. (c) Confocal fluorescence image of liposomes taken during hydration at room temperature. (d) Confocal fluorescence image of phase-separated liposomes harvested after 20 min hydration at room temperature. Scale bar: 25 μm .

punctured, may be further affixed to the PVA gel layer as a fluid reservoir (see below). (2) Lipid deposition on PVA gel. To achieve this, a few microliters of lipid precursor is first cast on the PVA gel film using a microsyringe. This quickly produces a lipid thin film upon solvent evaporation, which is further dried under vacuum overnight in the dark at room temperature. Lipid samples thus prepared can be stored under vacuum for weeks with satisfactory results. (3) Liposome production, which is initiated by hydrating the lipid films deposited on PVA gel with a few drops of DI water. Formation of liposomes becomes evident under a microscope normally after a few minutes.

RESULTS AND DISCUSSION

Several details are worth mentioning to ensure success in experiment 1. To start, the lipid drop should be injected close to the center of the upper (water) phase (Figure 2b). This way, it lands naturally at the center of the water/oil interface upon falling, where the subsequent breakup of the droplet and lipid spreading generally take place in a few seconds (Movie S1). When the same microsyringe is used for multiple injections, the operator should wipe dry the surface of the needle prior to next injection. This simple step prevents the lipid drop from “floating” to the top of the water phase via the residual solvent on the needle. Also key to the success is the location for making observations: Due to the extremely low (monolayer) quantity of lipids involved in the spreading, it can be difficult for the students to see directly at the curved interface.³⁶ This potential issue has an easy fix, however. As shown in Figure 2c,d, the lipid spreading becomes quite evident if the glass vial is placed on

glass (e.g., microscope glass slides) and the observation is focused on the reflected interface instead.

A movie captured in slow motion in addition reveals that the lipid drop bounces and rolls at the water/oil interface before spreading (Movie S2). This interesting result could be used to illustrate the concept of interfacial tension to the students among other possibilities.

An ideal entry point into experiment 2 is the appearance of DPPC liposome solution. Despite being largely nanoscopic and transparent in the visible region, the colloidal suspension appears slightly milky as a result of light scattering, which is easily distinguishable from water against a dark background (Figure 3b). Stored at 4 $^{\circ}\text{C}$, this sample remains usable for at least a few weeks. By contrast, Sudan III samples should be prepared and used freshly. In our hands, we found that fine Sudan III particles tend to stay suspended in water in old samples (i.e., a few days to a few weeks), producing a red suspension that can sometimes be hard to discern from liposome samples.

Pronounced differences can be identified when equal amounts of freshly prepared Sudan III suspensions are added into water vs DPPC liposome solution. (1) Upon brief shaking, the dye particles can be seen to suspend throughout the liposome solution, whereas fine dye residues are clearly visible from the wall of the vial containing water only (Figure 3c). Here, similarity may be drawn between the liposome solution and common detergents used for grease removal. (2) Upon brief sonication, a more intense and evenly distributed red color can be seen from the liposome sample (Figure 3d). In this case,

ultrasound sonication not only helps break the dye solid down into still finer particles but also helps speed up the latter's mass transfer and interaction with liposomes.

Experiment 3 is quite fun in that students get to see directly phase-separated liposomes as they are forming. Since the setup is complex, involving multiple prep steps and 5 lipid species, we normally assembled it in our own lab the day before the actual event. This leaves the students only one operation to perform, adding water into the preassembled reservoir (Figure 4b), so they can focus on the final microscopic observation. In terms of phase separation in the DPPC/DOPC/cholesterol tertiary system, ideally, the lipid hydration (upon water addition) should be carried out above 45 °C, so that both DPPC and DOPC exist in their liquid states to begin the process.³⁵ For the purpose of this demonstration, however, we find that formation of phase-separated liposomes can proceed quite satisfactorily at room temperature (Figure 4c,d). This thus removes the need for temperature control in experiment 3.

■ QUIZZES

If time permits, the instructor can offer a section of quizzes to the students toward the end of this outreach event, so that they have a chance to practice and reinforce the chemistry they have just learned. In our 2021 summer outreach event which ran 3 h, this was given as a team competition, where students in two groups were tasked with 8 multiple-choice problems. The team that answers more of the first 7 quizzes correctly wins, and if tied, the last quiz can be used as a tiebreaker. After both teams turned in their answers, we revealed and explained the correct answers to the students. These quiz problems are given in the [Supporting Information](#).

■ STUDENT RESPONSE/FEEDBACK

The uniformly positive response and feedback received from past participants of this outreach activity point to its effectiveness in engaging students and facilitating their learning lipid assembly chemistry. This can be clearly seen from, for example, many spot-on questions the students were able to ask during the event as well as their high success rate in answering quizzes correctly. In return, the instructor had received numerous thank-you and other complementary remarks from the students.

■ CONCLUDING REMARKS

In summary, we have presented new outreach experiments designed to convey the beauty as well as the complexity of lipid assembly chemistry to the general audience. Consisting mainly of three straightforward hands-on experiments involving relatively simple setups, this demonstration enables an instructor to disseminate most of the key concepts in lipid assembly chemistry among interested students. Lipid phase separation, an important area of current membrane biophysics research, is also covered. As the future-generation scientists and engineers continue to embrace the beauty in lipid assemblies, they need to be prepared to face the underlying complexity at the same time. In the regard, we sincerely hope that the work described here could contribute in a small way to such efforts.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available at <https://pubs.acs.org/doi/10.1021/acs.jchemed.1c00827>.

Movie S1, video recording of lipid spreading (experiment 1) in real time ([MOV](#))

Movie S2, video recording of lipid spreading (experiment 1) in slow motion (1/4 normal speed) ([MOV](#))

Quizzes for instructors ([PDF](#))

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Notes

The authors declare no competing financial interest.

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