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Detection of Sunlight Exposure with Solar-Sensitive Liposomes that Capture and Release Food Dyes

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Supporting Information

ABSTRACT: Direct assessment of sunlight exposure can be challenging over extended periods or in multiple remote locations. We optimized liposome formulations that included egg and hydrogenated soy lipids as well as small amounts of a chlorophyll-derived phospholipid (PoP) to confer sunlight reactivity and triggered-release of entrapped dyes. Dye release occurred with PoP-mediated photooxidation of unsaturated phospholipids and could be tuned by varying the unsaturated (egg) to saturated (soy) lipid ratio or by varying the amount of PoP included. Numerous food coloring dyes were entrapped within the liposomes and, when applied onto a polyacrylamide gel, remained intact and immobilized for at least a month in the dark. When exposed to sunlight, the dyes were released and rapidly diffused through the gel, creating a detection system for assessing sunlight exposure by the naked eye based on dye diffusion. Acid Blue 9 and Acid Yellow 23 were particularly effective reporter food dyes in this system. By using liposomes with varying PoP amounts, light-induced dye release could be tuned to coincide with varying amounts of solar exposure. Additionally, exposure-dependent, color-coded release patterns were generated using colloidal mixtures of separately formed liposomes loaded with different dyes. Using customized 3D printed enclosures, a small and readily deployable device was created for visualizing solar-sensitive liposome sunlight reporting. These studies show proof-of-principle that PoP liposomes can serve as simple nonelectric reporters for visually assessing sunlight exposure based on diffusion of entrapped food coloring.

KEYWORDS: *liposomes, sunlight, dyes, triggered release, sensors, porphyrin, chlorophyll*

INTRODUCTION

The sun is the source of all energy and life on our planet, so measuring sunlight at the Earth's surface is of importance. Numerous digital devices exist to do so, including pyranometers and other light meters for spot measurements. Most information on sunlight exposure is inferred indirectly from algorithms that incorporate data from satellite imagery of atmospheric conditions. Such approaches are not reliable in conditions where variable shade is prominent, such as in canopy growth or urban areas. Sun tracking systems exist that can directly measure extended periods of sunlight exposure, but are expensive devices and thus not highly accessible and cannot be used easily at multiple sites.[1] Of frequent interest for sunlight measurement is photosynthetically active radiation (PAR), which can be obtained following instrument calibration based on the instrumentation spectral response.

A variety of light-sensitive nanoparticles and materials has been developed in recent years.[2−8] Liposomes have been the focus of much research activity for light-triggered release[9] in conjunction with polymers, which share similar release mechanisms[10−12] and improved biocompatibility.[13] Numerous methods for light-triggered release from liposomes include photo-cross-linking,[14−16] photoisomerization,[17−24] photocleavage,[25−29] photothermal release,[30−38] and light-induced lipid oxidation.[39] We previously found that a chlorophyll derivative, pyropheophorbide-a, can be conjugated to a lipid to generate porphyrin-phospholipid (PoP), which in turn can be incorporated into standard liposomes to induce light-triggered cargo release.[40−42] The mechanism of PoP-mediated light-induced membrane permeabilization appears related to PoP-mediated photooxidation of unsaturated lipids within the bilayer, as evidenced by multiple studies involving liquid chromatography and mass spectrometry (LC/MS) lipidomics analysis of liposomes before and after irradiation.[43−46] Other studies also demonstrate the importance of dissolved oxygen for the photooxidation to occur.[47]

Some studies have examined ultraviolet and sunlight peroxidation of lipids, although not in the context of

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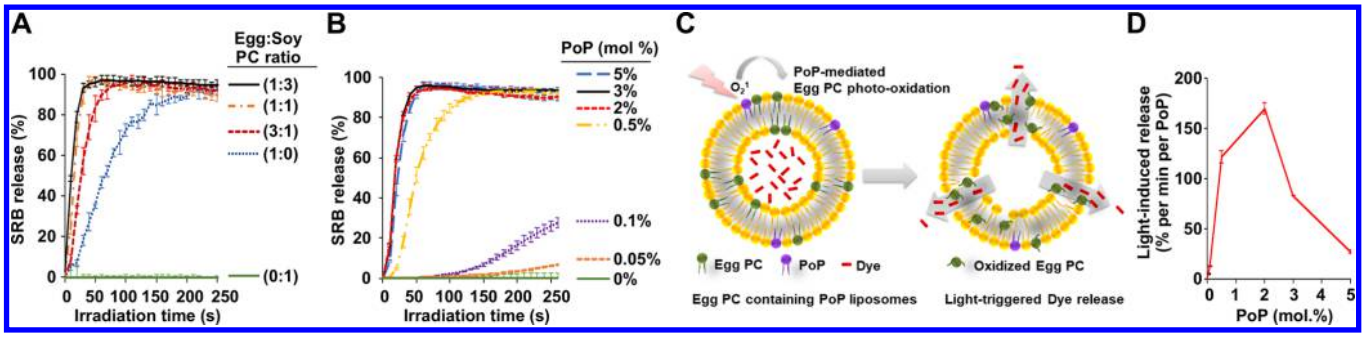

Figure 1. Light-triggered release in PoP liposomes comprising soy and hydrogenated soy lipids. (A) SRB dye release from PoP liposomes (0.5 mol % PoP) containing varying molar ratios of egg PC and hydrogenated soy PC. (B) SRB release from PoP liposomes containing varying amounts of PoP. (C) Putative mechanism of light-triggered release of dyes from PoP liposomes. (D) Laser-induced SRB release rate in liposomes containing 1:3 ratio of egg PC:HSPC at the indicated mol % PoP. A 665 nm laser diode at a fluence rate of 300 mW/cm^2 was used for light-triggered cargo release.

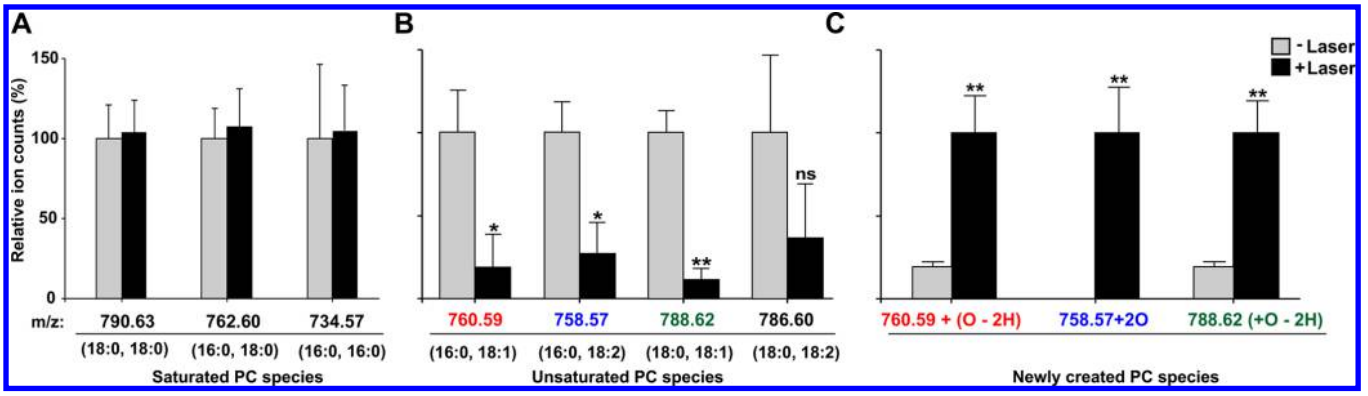

Figure 2. Light-induced PoP-mediated unsaturated lipid oxidation. (A) HSPC species show no significant changes after laser irradiation. (B) Decreased ion counts of four major lipids present in egg PC after laser irradiation. (C) Appearance of three new oxidized species after laser irradiation (color-matched to unsaturated species as indicated).

controllable release of cargo.[48,49] PoP has not yet been used in applications involving sunlight activation. To the best of our knowledge, nanoscale reporters of sunlight exposure have not been reported. In this work, we develop a nanoparticulate reporter system derived from chlorophyll (which naturally forms the basis for PAR) as a small, diffusion-based, nonelectric, and tunable sensor for sunlight exposure that can be detected by the naked eye.

RESULTS AND DISCUSSION

Liposome Formulation and Light-Triggered Release. Light-triggered cargo release in liposomes containing small amounts of PoP was shown to be impacted by the presence of an unsaturated lipid, dioleoylphosphatidylcholine (DOPC), which was prone to PoP-mediated photooxidation.[44] A similar liposome formulation was developed here, but the naturally derived lipids of hydrogenated soy PC (HSPC) and egg PC were used. Liposomes were formed with a small amount of PoP and loaded with the hydrophilic fluorophore sulforhodamine B (SRB) at self-quenching concentrations. Initial experiments for formulation optimization and light-triggered release characterization were performed using a 665 nm laser diode directly connected to a fluorometer which enabled the simultaneous excitation of the sample and measurement of cargo release in real time. The ratio of HSPC and egg PC strongly influenced cargo release (Figure 1A). A mixture of both unsaturated and saturated lipids was found to release faster than when one of them was used alone as the main lipid. In the absence of egg PC, light-triggered release in PoP liposomes was negligible. The fastest light-induced release was found at molar ratios of 1:1 and 1:3 of egg PC to HSPC. The amount of PoP included in the liposome formulation (using a 1:3 ratio of egg PC to HSPC) also influenced the rate of light-triggered release (Figure 1B). With inclusion of PoP over 0.5 mol %, complete release of cargo was observed within a few minutes, while liposomes containing lower amounts of PoP had slower release. Thus, both PoP and egg PC were required for light-triggered release in these conditions. As shown in Figure 1C, the mechanism is believed to relate to PoP-mediated photo-oxidation of unsaturated lipids in egg PC. This leads to destabilization of the bilayer and release of entrapped cargo. When the rate of release is normalized by the amount of PoP present in the liposomes, 2 mol % provided the optimal amount of PoP for rapid release (Figure 1D).

As a chlorophyll derivative, PoP has two absorption peaks with a Soret band in the blue and Q-bands in the red. PoP liposomes were irradiated with 50 mW/cm^2 illumination from laser diodes of either color; blue (405 nm) or red (665 nm). As shown in Figure S1, the blue laser induced more rapid release, which is expected given the greater absorption intensity of the Soret band over the Q-bands. To further characterize release properties, the liposomes were assessed for thermal stability in the absence of light during brief incubation periods (15 min). Figure S2 shows that PoP liposomes have high thermal stability with no substantial release observed at temperatures up to 75 °C and only ~15% cargo release even at 95 °C. To assess the impact of temperature on light triggered release, 665 nm irradiation was applied to PoP liposomes in a stirring cuvette at

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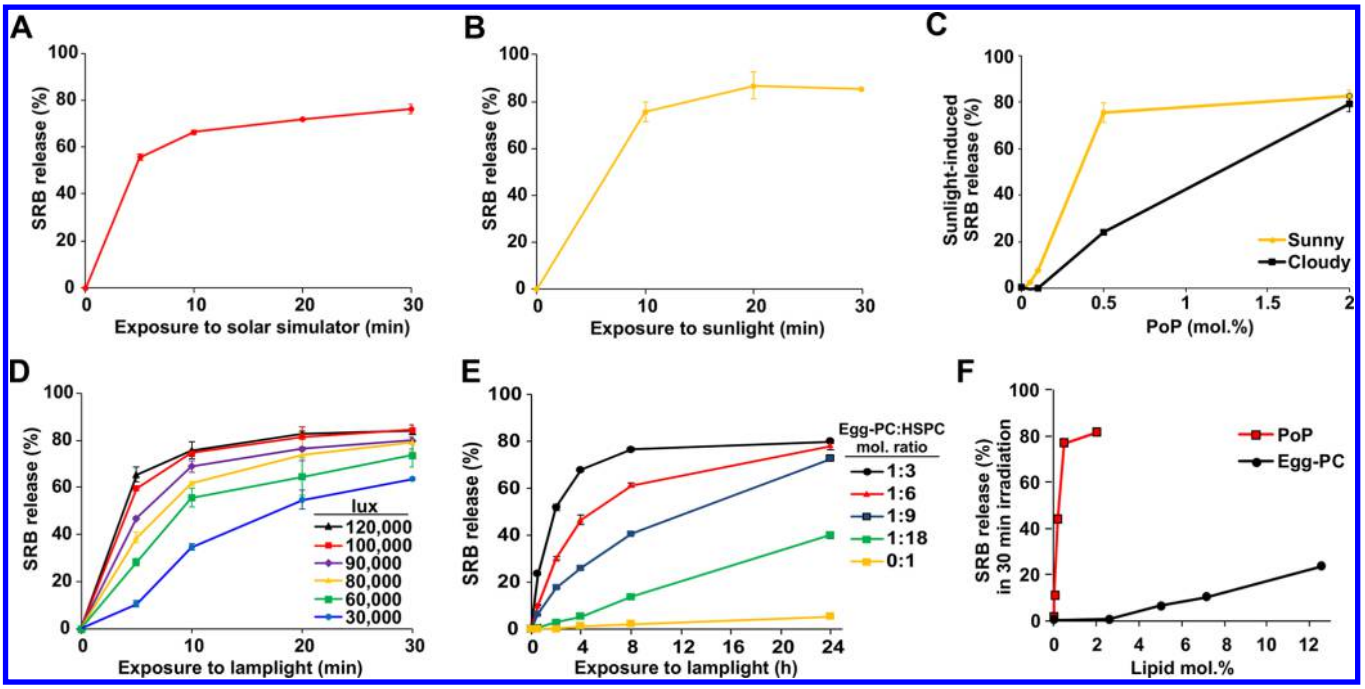

Figure 3. Light-induced SRB release from PoP liposomes using different light sources and conditions. (A) Solar simulator-induced SRB release at 0.5 PoP mol % during 30 min irradiation (lux ≈ 125 100). (B) Sunlight-induced SRB release at 0.5 PoP mol % during 30 min irradiation (lux ≈ 98 370). (C) Sunlight-induced SRB release on a sunny day vs cloudy day (lux ≈ 98 370 and 11 350, respectively) as a function of PoP mol % at 10 min irradiation time-point. (D) Cool white LED-induced SRB release at 0.5 PoP mol % during 30 min irradiation at variable illuminance (lux). (E) Twenty-four hours of cool white LED-induced SRB release at variable egg PC:HSPC mol % ratio at 0.05% PoP mol % (lux ≈ 90 000). (F) SRB release as a function of variable egg PC mol % (0.05 mol % PoP) and variable PoP mol % using 1:3 egg PC:HSPC molar ratio with 30 min irradiation with cool white LED (lux ≈ 90 000).

temperatures of 20, 25, 30, and 35 °C. As shown in Figure S3, there was no substantial difference in the rate of light-triggered release within this temperature range.

We further investigated the mechanism of PoP-mediated phototriggered cargo release. Egg PC and hydrogenated soy comprise a mixture of different phospholipids, ranging in alkyl side chain length, and for egg PC, the number and position of double bonds. Based on LC-MS, 3 saturated lipids were clearly detected in the PoP liposomes that would be expected from hydrogenated soy PC, formed from combinations 18:0 and 16:0 alkyl side chains (Figure 2A). Notably, following laser treatment, the abundance of these soy lipids was not impacted. Unsaturated egg PC lipids were also identified based on their molecular weights, including mixed side chains such as monounsaturated (16:0, 18:1) and diunsaturated (16:0, 18:2) species (Figure 2B). Unlike the saturated soy PC, laser treatment drastically decreased the abundance of unsaturated egg PC lipids (Figure 2B). At least three unsaturated egg PC lipids significantly were depleted from the PoP liposomes. When liposomes lacking PoP were irradiated, no reduction in unsaturated egg PC lipids was observed, demonstrating the photooxidation process is PoP-mediated (Figure S4). As the likely mechanism of the treatment is photooxidation of unsaturated lipids, corresponding peroxidized products were identified in significantly increased abundance (Figure 2C). These LC-MS conditions are limited in providing definitive structural information on the newly emerging species. Full details on observed species are listed in Table S1. On the basis of the m/z we observed, we propose a demonstrative peroxidized structure for m/z = 790.5593 in Figure S5. SRB-loaded liposomes did not show any significant change in size (Figure S6A), polydispersity (Figure S6B), or spherical and unilamellar morphology (Figure S6C) before and after laser irradiation, consistent with our prior observations of light-induced membrane permeabilization, which was found to be a transient phenomenon.[40,43]

Cargo Release in Response to Solar Simulation, Sunlight, and a Cool White LED Lamp. Solar simulators simulate the solar spectrum at the Earth's surface.[50] When PoP liposomes (containing 0.5 mol % and a 3:1 ratio of soy:egg PC) were placed in a solar simulator, nearly full cargo release was observed during 30 min of irradiation (Figure 3A). Despite the low amount of PoP in the formulation, approximately 60% of entrapped SRB was released in the first 10 min of exposure. Liposomes were next exposed to actual outdoor sunlight. Figure 3B shows that when exposed to sunlight on a sunny day, light triggered release was induced. Cargo release was somewhat faster than that observed for samples irradiated with the solar simulator with nearly 80% cargo release in the first 10 min of exposure.

Solar irradiance on a sunny day typically varies between 80 000 to 98 000 lx,[51] which matches with the average lux measurements observed in our experiments (lux ≈ 98 370). Solar irradiance on Earth is not constant throughout time due to several factors which influence the amount of solar energy passing through the atmosphere and finally arriving at the ground level.[52,53] Different solar irradiance means different light levels being delivered to the liposomes. Because chlorophyll naturally detects PAR, we anticipate these liposomes are detectors for irradiance directly related to plant energetics. On a cloudy day, cargo release from PoP liposomes was significantly slower compare to that on a sunny day (Figure 3C). We further assessed whether the amount of PoP included in the formulation could impact sunlight triggered release. Indeed,

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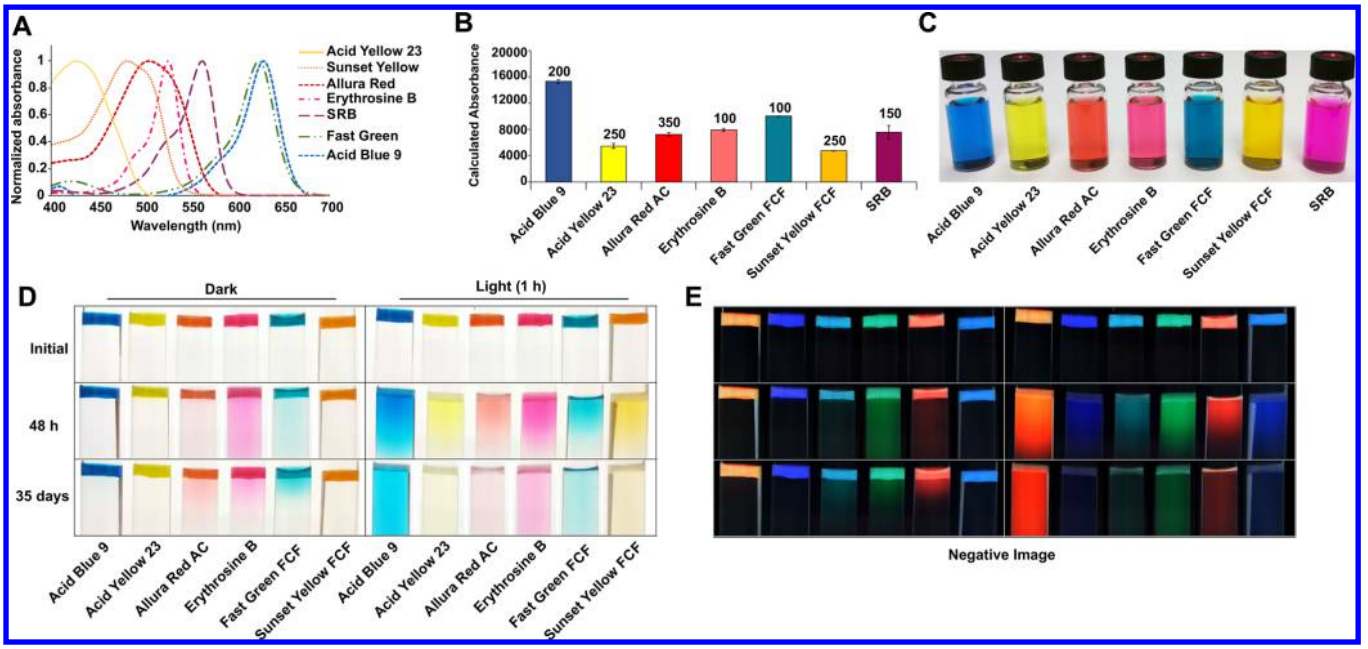

Figure 4. Food dyes as visual reporters of light-triggered liposome permeabilization. (A) Normalized absorbance spectra of food dyes used for encapsulation. (B) Maximum calculated absorption of each food dye. The numbers on top of each dye indicate the maximum water solubility (in mM) that was tested. (C) Food dyes in aqueous solutions at 10 μM. (D) Food dyes were entrapped in PoP liposomes and placed at the top of a 5% acrylamide gel in tubes. One set of tubes was exposed to light for 1 h, and then both sets of tubes were placed in the dark and photographed after 48 h and 35 days. (E) Negative image of panel D.

with 2 molar % PoP, full release was observed with 30 min of sunlight exposure on either a cloudy or a sunny day. Liposomes with 0.5 mol % PoP produced a greater differential of dye release between sunny and cloudy conditions. Liposomes containing less PoP did not release substantial amounts of cargo with just 30 min of exposure.

For improved irradiation reproducibility within our own lab, we assessed a cool white LED lamp as the irradiation source. By varying the distance from the lamp to the irradiated samples, different power densities were achieved (Figure 3D). Increasing cargo release occurred with increasing lux. Irradiance rates of >60 000 lx produced reasonably similar release kinetics as the solar simulator or sunlight, even though the LED lamp spectrum (Figure S7) represents only a fraction of the mix of all wavelengths observed in the sunlight.[53] The LED light source could be used to achieve similar rates of release as natural sunlight using 90 000 lx irradiation and was used for further experiments unless indicated otherwise.

Because spot measurements of sunlight can adequately be measured with hand-held monitors, we aimed to generate liposomes that could be activated with substantially longer periods of sunlight exposure. We decreased PoP mol % to 0.05 and then compared different formulations with decreasing amounts of unsaturated lipids in relation to the saturated ones (Figure 3E). PoP liposomes with a 3:1 ratio of hydrogenated soy:egg PC liposomes still produced fast release, although full release decreased to a period of 8 h of simulated sunlight exposure. Decreasing the amount of egg PC resulted in decreased light-triggered release due to decreasing amounts of unsaturated lipids. In the absence of egg PC, very little cargo release was observed. Figure 3F shows the relationship between the amount of PoP and egg PC in the liposomes with respect to light-triggered release. These two components can be adjusted to robustly fine-tune liposome solar sensitivity, with PoP having a relatively profound impact between 0 and 2 mol % inclusion.

Encapsulation and Release of Food Dyes. SRB and other commonly used hydrophilic markers of liposome permeability are useful with fluorometric analysis. However, the necessity of a fluorescence detector or imaging system would limit the versatility and ease of use of a small liposome-based sunlight exposure sensing device. Commonly used food dyes were considered as potential cargos for reporting membrane permeabilization. The advantages of using food dyes are that they are inexpensive and available in large quantity, are generally consumed by humans so are expected to be safe, tend to be extremely water-soluble, and are readily detected by eye. Despite these advantages, there have been few, if any, reports on entrapping food dyes in liposomes to the best of our knowledge. Acid Blue 9, Acid Yellow 23, Allura Red AC, Erythrosine B, and Fast Green FCF (as well as the previously used SRB fluorescent dye) were used, and their absorption spectra in water are shown in Figure 4A. Absorption peaks ranged from about 420 nm (Acid Yellow 23) to 620 nm (Acid Blue 9). We dissolved the food dyes at concentrations increasing in 50 mM increments from 100 to 350 mM until the maximum solubility of each dye was found. These ranged from about 100 mM (Fast Green) to 350 mM (Allura Red), with a median dye solubility of 200 mM (Figure 4B). When dissolved at the maximum solubility, the absorption of each dye was measured and determined to be extremely high. With the dilution factor taken into account, calculated absorption values at the peak food dye wavelength ranged from about 4000 to 16 000 (for Acid Blue 9). A photograph of a 10 μM aqueous solution of each dye is shown in Figure 4C. It should be noted that this concentration is generally more 10 000-fold diluted compared to the maximum solubility.

Food dyes were encapsulated into PoP liposomes, and free dye was removed. Liposomal dyes were then loaded on top of 5% polyacrylamide gel within glass tube. The tubes were exposed to light and photographed after 2 and 35 days (Figure

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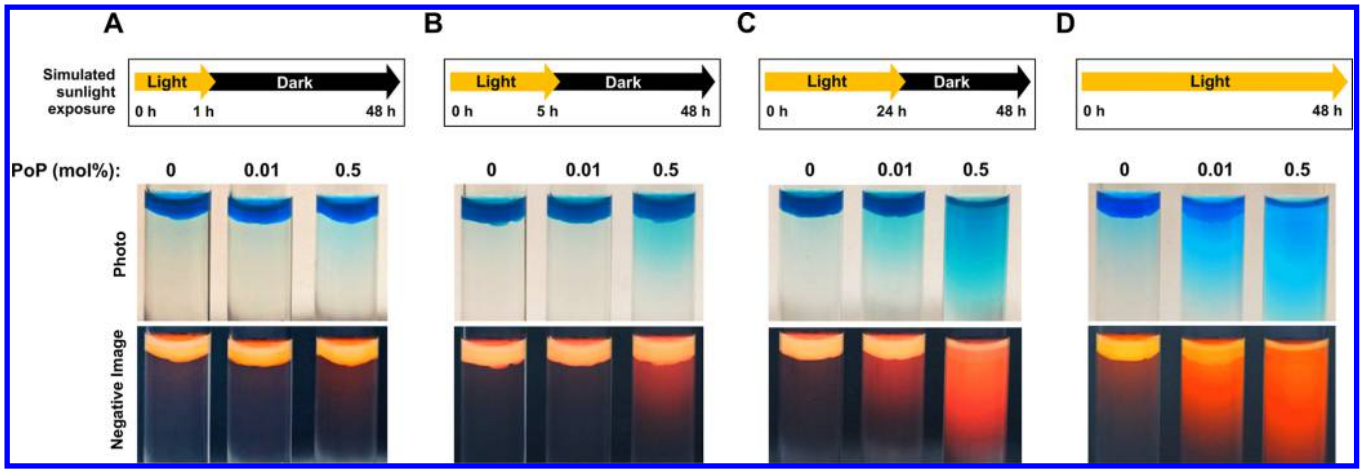

Figure 5. Tuning PoP liposomes for visual detection of variable light exposure. Liposomes containing indicated amounts of PoP were loaded with Acid Blue 9, placed on acrylamide gels, and exposed to 1, 5, 24, or 48 h of simulated sunlight. (A–D). A photograph was taken at the 48 h time point. The bottom row is the negative image of the white light photograph.

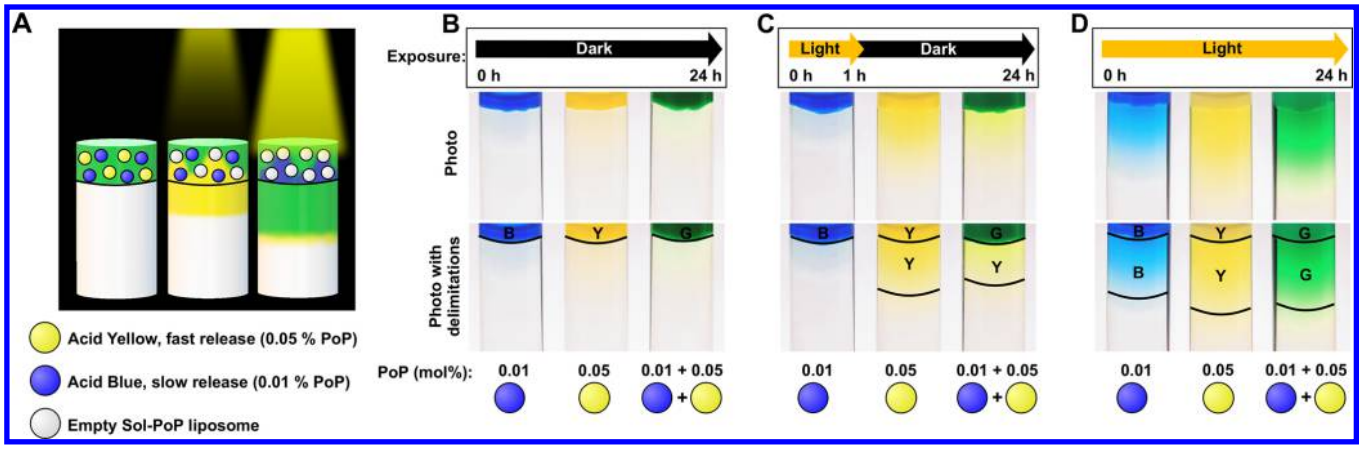

Figure 6. PoP liposome mixtures for color-coded reporting of light exposure. (A) Schematic representation for the use of solar-sensitive PoP liposome mixtures to produce color-coded gel permeation patterns based on light exposure. PoP liposomes loaded with Acid Blue 9 and Acid Yellow 23 were loaded separately or mixed on acrylamide gels and exposed to simulated sunlight for 0, 1, or 24 h (B–D). Green permeation into the gel indicates release of both Acid Yellow 23 and Acid Blue 9. The bottom row annotates the observed release patterns (B = blue; Y = yellow; G = green).

4D). The liposomes themselves were too large to permeate the gel and so remained on the top of the tube in all cases. After light exposure, all of the dyes were able to escape the PoP liposomes and permeate the gel. This diffusion-based system provides for a simple visual test of sunlight exposure. In the absence of laser light, liposomal Allura red AC, Erythrosine B, and Fast Green FCF had some degree of leakage after 48 h, indicating these dyes were not stable in the liposomes. Acid Blue 9, Acid Yellow 23, and Sunset Yellow FCF showed great stability, and the dye remained completely entrapped even after 35 days but was completely released when exposed to laser irradiation. These results reveal that the stability of the cargo is not only dependent on the formulation being used in PoP liposomes, but it is also intrinsic to the nature of the dye. Figure 4E shows a negative image of 4D, which may be helpful to show the release patterns more clearly, especially to readers with impaired color vision.

Tunable Visual Reporting of Light Exposure. Given the extreme aqueous visible absorption of Acid Blue 9 (>16 000) and favorable dark vs light release characteristics following PoP liposome encapsulation, Acid Blue 9 was chosen for additional optimization studies. Acid Blue 9 was encapsulated in liposomes containing reduced amounts of PoP to further increase the amount of sunlight exposure required for dye release. Zero, 0.01, and 0.5 mol % PoP was used. As shown in Figure 5, samples were placed on an acrylamide gel and exposed to varying amounts of simulated sunlight ranging 1–48 h, and all samples were photographed at the 48 h time point. For samples exposed to 1 h of simulated sunlight, minimum release was observed with a very faint amount of dye release detectable with the liposomes containing 0.5% PoP. Increasing irradiation time to 5 h was insufficient to trigger Acid Blue 9 release for liposomes containing 0 or 0.01% PoP, but increased dye release was apparent for 0.5% PoP liposomes. Twenty-four hours of constant irradiation was sufficient to trigger release of most of the dye from 0.5% PoP liposomes as well as a small amount of dye release from 0.01% PoP liposomes. Liposomes lacking PoP did not release any cargo with 24 h of simulated sunlight exposure. After 48 h of continuous irradiation, a very small amount of dye release was observed in liposomes lacking PoP, which may have been due to photooxidation of unsaturated phospholipids. However, the liposomes containing 0.01% PoP had easily detectable dye release, as did the liposomes with 0.5% PoP. Thus, these data imply that different liposomes can be formed that clearly release their cargo in

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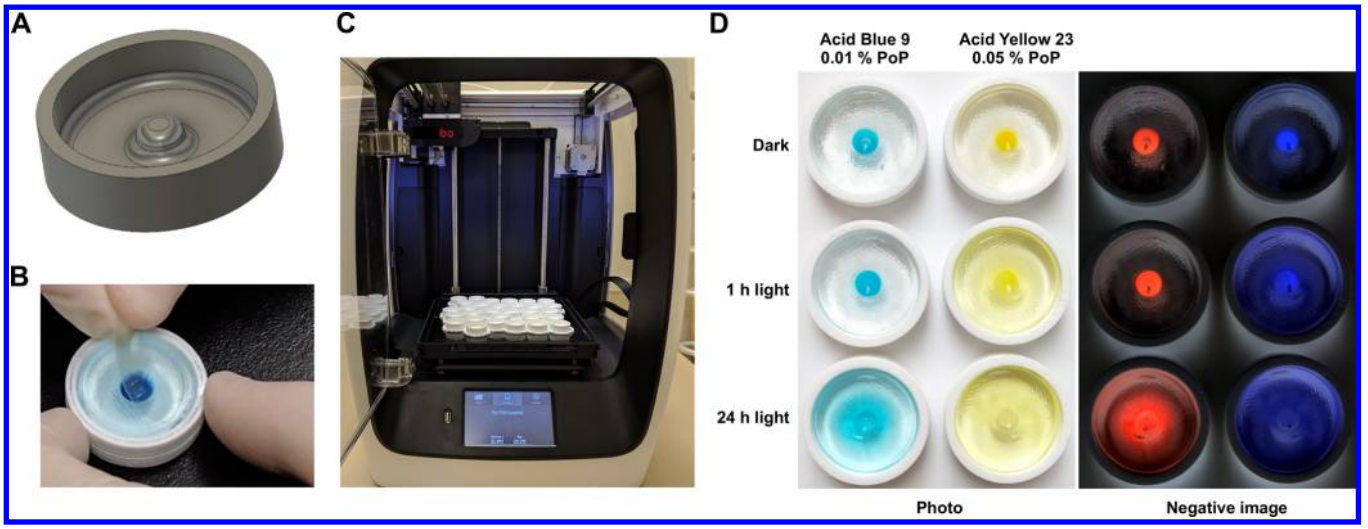

Figure 7. 3D printed deployable devices for PoP liposome reporting of light exposure. (A) 3D rendering of the selected mold dimensions for polyacrylamide gels and PoP liposome loading designed in Autodesk Fusion 360. (B) Placement of a clear adhesive circular seal to prevent gel dehydration. (C) A photo of the 3D printer following the build of 25 molds (white) simultaneously. (D) Photo of Acid Blue 9 and Acid Yellow 23 gel permeation in the printed device from PoP liposomes containing 0.01 and 0.05 PoP mol %, respectively, following simulated sunlight exposure. The negative image is shown on the right.

response to certain threshold values of sunlight exposure and that this can be detected by the naked eye.

We next developed a system for color-coded detection of solar exposure. This was achieved by mixing two different variants of PoP liposomes with different entrapped dyes and different solar sensitivity, as shown in Figure 6A. Acid Blue 9 and Acid Yellow 23 liposomes (effective diameter: 251 ± 11 and 301 ± 22 nm, respectively) containing 0.01 or 0.05 PoP mol %, respectively, were combined into a single colloidal mixture. Liposomes were then loaded alone or together on top of polyacrylamide gel. When combined, the yellow and blue liposomes created a dark green color. Without irradiation, the liposomes remained stable and at the top of the acrylamide gel (Figure 6B). With 1 h of irradiation, Acid Yellow 23 was released from the PoP liposomes with greater solar sensitivity (i.e., 0.05 mol % PoP). The amount of yellow permeation into the gel was similar whether or not they were combined or separate (Figure 6C). With exposure to 24 h of simulated sunlight, both 0.01 and 0.05 mol % PoP liposomes released their respective blue and yellow food coloring dyes, creating green permeation through the gel (Figure 6D). For Acid Blue 9 PoP liposomes with 0.01 mol % PoP, the dye releases with around 6 h of continuous light irradiation at 90 000 lx (Figure S8), but the time gap between the first and second released could be easily tuned by adjusting the amount of PoP or unsaturated lipid in the lipid formulation or simply by assessing the release at longer time points. The delayed corelease can be useful as a means of determining how much sunlight PoP liposomes received, just by knowing how long it would take for the two distinct colors (or maybe even more than two) to appear.

While glass test tubes are convenient to use for acrylamide gel permeation studies, glass is prone to breaking, and it would be difficult to deploy many of such devices at multiple locations in remote locations without special holders. Using a Robo3D R2 printer, we designed and tested several small acrylamide molds and holders for field deployment to accommodate polyacrylamide gels with variable diameter and sample volume (Figure S9A and B). The mold was printed using polylactic acid (PLA) filament and a layer thickness of 0.1 mm. We assessed the dimensions that would provide good detectability of dye release and studied the spread of free dye on these flat surfaces. A design with a 24 mm outer diameter and 7 mm inner diameter was found to work well (Figure 7A). Dye diffusion into the gel could clearly be visualized (Figure S9C). One caveat to using the 3D printed molds and holders was that the exposed acrylamide gel would evaporate and dehydrate. Further, in an outdoor environment, rain can enter the gel and possibly interfere with liposome behavior. These issues were addressed by sealing the printed holder with a clear circular adhesive that is transparent in the visible range (Figure 7B). Future versions of the mold could be modified to better improve functional design (e.g., protection from evaporation and rain; enabling maximum sunlight exposure; small size; optimal visual detection). A mold with these specifications uses a total of 3.96 g of PLA when printed, which represents a cost of 12 cents (USD). One advantage of 3D printing for this application is the accessibility and capacity for printing high volumes of molds on demand (Figure 7C).

Acid Blue 9 and Acid Yellow 23 PoP liposomes were loaded into polyacrylamide gels formed in the 3D molds and were exposed to simulated sunlight (Figure 7D). A similar dye distribution pattern as shown in Figure 6 was observed in the 3D printed devices, demonstrating that release can be tuned by adjusting the amount of PoP in the liposomes for varying degrees of sunlight sensitivity.

■ CONCLUSION

In this work, we developed liposome formulations formed from naturally derived egg and soy lipids that can be tuned to release cargo in response to varying amounts of sunlight exposure. Unsaturated lipids were depleted during irradiation, strongly suggesting a mechanism related to PoP-mediated photo-oxidation leading to membrane permeabilization. Release could be tuned by varying the unsaturated lipid content (i.e., egg PC content) or by varying the amount of PoP. Food dyes were encapsulated within PoP liposomes with the stability varying depending on the dye being encapsulated. Acid Blue 9

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and Acid Yellow 23 showed retention in PoP liposomes in the dark and release from PoP liposomes exposed to sunlight. These dyes are also highly absorbing in the visible range, so that dye permeation into acrylamide gels could be used for convenient monitoring of sunlight exposure by the naked eye. By using colloidal liposome mixtures, color-coded permeation colors could be used to report on sunlight exposure. Small, deployable 3D printed devices were prototyped and found to be useful for monitoring dye release.

EXPERIMENTAL SECTION

Materials. Non-hydrogenated egg phosphatidylcholine (egg PC) and hydrogenated soybean phosphatidylcholine (HSPC) were purchased from NOF America (COATSOME NC-50 and COATSOME NC-21, respectively). Cholesterol was purchased from Nu-Chek Prep, Inc. (#CH-800). SN-1-palmitoyl SN-2-pyropheophorbide phosphatidylcholine (Pyro-lipid (PoP)) was synthesized as previously described.[54] Acid Blue 9, Acid Yellow 23, Allura Red AC, Erythrosine B, and Sunset Yellow FCF were purchased from TCI (#3844-45-9, #1934-21-0, #25956-17-6, #16423-68-0, and #2783-94-0, respectively). Fast Green FCF was purchased from Acros Organics (#2353-45-9). Sulforhodamine B (SRB) was purchased from Biotium (#80100). Acrylamide: Bis Solution 19:1 OmniPur 40% solution (w/v) was purchased from EMD Millipore (#1290-OP). Ammonium persulfate (APS) was purchased from Amresco (#0486-25G). TEMED (*N*,*N*,*N*′,*N*′-tetramethylethylenadiamine, 99%, extra pure) was purchased from Acros Organics (#110-18-9). Glass tubes were purchased from VWR (#47729-568). All the other reagents used in the experiments were prepared in our laboratory. All water used was Type I reagent grade water purified with a Barnstead Nanopure water purification system.

Preparation and Characterization of PoP Liposomes. Unless otherwise stated, most experiments were performed with liposomes constituted of egg PC:HSPC:Chol:PoP at a molar ratio of 12.3:37.1:50:0.5 mol %, although in several experiments, the unsaturated to saturated lipid ratio and the amount of PoP were varied as indicated. Liposomes were prepared by the ethanol injection method. Briefly, 20 mg/mL lipids were dissolved in 200 μL of ethanol and mixed in glass tubes at 60 °C. Lipids were injected with 800 μL of different aqueous solutions containing varying food dyes at their maximum soluble concentration in water or 50 mM SRB in water at 60 °C. To remove the nonencapsulated components, size-exclusion chromatography was used. Columns containing Sephadex G-75 were loaded with 1 mL of liposome samples, and select liposome-containing fractions were collected. Free dye was further removed by dialyzing the samples against water several times until no color was detected in the external medium. Samples were protected from light during most parts of the experiment to avoid unwanted leakage of the encapsulated contents. The size of PoP liposomes was determined by dynamic light scattering in a NanoBrook 90Plus PALS.

Maximum Solubility Tests of Dyes. The maximum solubility concentration in water was determined by centrifugation at 20 000*g* of varied concentrations of dye solutions until no pellet was detected in the tubes. Absorbance measurements of the supernatants were obtained with a PerkinElmer Lambda 35 UV/vis Spectrometer.

Laser and Heat Induced Release of Fluorescent Cargo. Cargo release from PoP liposomes loaded with the self-quenched fluorophore SRB was assessed using a power-tunable 665 nm laser diode (RPMC laser, LDX-3115-665). The fluence rate was 300 mW/cm^2. SRB fluorescence was constantly monitored using a fluorometer (PTI) at 585 nm emission wavelength. The influence of the temperature in the laser-induced SRB release was performed as mentioned above but with the adjustment of the temperature by the PTI fluorometer. For comparison of SRB light-induced release by a blue laser (420 nm) or a red laser (665 nm), the fluence rate was set to 50 mW/cm^2 for both lasers. Heat-induced release experiments were performed using a PCR Sprint thermocycler by incubating 200 μL of SRB-loaded PoP liposomes (49.5 mol % [1:3 egg PC:HSPC mol. ratio], 50 mol % cholesterol, and 0.5 mol % PoP) in water for 15 min each at indicated temperature. Differences in fluorescence upon incubation at different temperatures was accessed by using a TECAN Safire fluorescence microplate reader. Full release was determined following liposome lysis with 0.1% Triton X-100.

Transmission Electron Microscopy (TEM). The morphology and size of liposomes were measured by TEM with a JEOL JEM-2010 microscope at a working voltage of 200 kV. TEM samples were prepared for imaging by dropping dilute liposomes onto a continuous carbon film coated copper TEM grid and staining with 2% uranyl acetate successively.

Solar Simulator, Cool White LED, and Sunlight-Induced Release Experiments. Solar irradiation simulation was performed using a Newport Oriel 91191-1000 Solar Simulator with an AM1.5G filter. The resulting illuminance from the solar simulator was measured by using a Leaton Digital Luxmeter placed under the lamp at the same level as the samples. Cool white LED-induced release was performed using a QUANS 5W 5 × 1W Cool White 19.68′′ High Power LED Lamp. Samples were positioned at variable distances from the light source. The light dose per second can be translated into shorter or longer exposure times depending on the experiment objective. The illuminance was measured by using a Leaton Digital Luxmeter. For the sunlight experiments, samples were placed outside our laboratory area in an open space without shadows in either a sunny or a cloudy day. The illuminance was measured by using a Leaton Digital Luxmeter.

LC-QTOF Characterization of Egg PC and Potential Oxidation Products. Lipid extraction from liposomes was performed as we described previously.[43,44] The detailed chromatographic condition and MS parameters can be found in our previous studies[43,44] with slight modifications on the LC gradient as follows: the gradient started after 5 min at 0% B and then increased to 100% B over 40 min followed by 100% B for 7 min before equilibration for 8 min at 0% B. Targeted analysis of lipid species was performed using MassHunter Qualitative Analysis (version B.06.00, Agilent Technologies).

PC Species. Egg PC is a mixture of PCs with major component of PC (16:0, 18:1), PC (16:0, 18:2), PC (18:0, 18:1), and PC (18:0, 18:2).[55] For targeted analysis of PCs, the corresponding m/z of each species [PC (16:0, 18:1) m/z = 760.5851; PC (16:0, 18:2) m/z = 758.5694; PC (18:0, 18:1) m/z = 788.6164; PC (18:0, 18:2) m/z = 786.6007] was extracted, and peak areas were manually integrated. These areas were presented as ion counts. m/z values corresponded to $[M + H]^+$ adduct of each lipid.

The identity of these lipids was confirmed by MS/MS (experimental details on data acquisition can be found in Luo et al.[43]). Based on m/z = 184.0773, 522.3499, and 577.5274 fragments, m/z = 760.5847 was confirmed as PC (16:0, 18:1). Based on m/z = 184.0718, 520.3415, and 575.5048 fragments, m/z = 758.5667 was confirmed as PC (16:0, 18:2). Based on m/z = 184.0761, 524.3744, and 605.5446 fragments, m/z = 788.6157 was confirmed as PC (18:0, 18:1). Based on m/z = 184.0768, 524.3737, and 603.5276 fragments, m/z = 786.5983 was confirmed as PC (18:0, 18:2).

Oxidized PC Species. Oxidized forms of PC (16:0, 18:1), PC (16:0, 18:2), and PC (18:0, 18:1) were observed as m/z = 774.5643, m/z = 790.5593, m/z = 802.5956, respectively (correspond to $[M + H]^+$ adducts). These m/z values were assigned as oxidized PCs based on the choline headgroup (m/z = 184.0773) and oxidized forms of the intact lipid or the lysolipid chains fragment. These fragments are reported below: for m/z = 774.5652: m/z = 184.0728, 496.3373, 591.4974, and 758.5560 fragments were observed. For m/z = 790.5549: m/z = 184.0711, 478.3225, 754.5299, and 772.5398 fragments were observed. For m/z = 802.5976: m/z = 184.0763 and 786.6073 fragments were observed. Statistical analysis for the comparison between lipid species from liposomes treated or not with light was performed using Student's t test.

Dye Release in Gels in Glass Tubes and 3D Printed Molds. PoP liposomes or free dye were loaded on top of 5% polyacrylamide gels. The gels were prepared combining an acrylamide solution (40% solution, w/v) with water at a proportion of 1:6.9 mL, with later addition of 10 μL of 10% ammonium persulfate per mL of gel and 1 μL of TEMED per mL of gel. The gels were prepared in both glass

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tubes or in 3D printed molds designed specifically to hold the gels. The molds contained variable outer diameter and inner post sizes (which would form small wells on the gel, serving as a reservoir for the samples). The molds were printed using a Robo3D R2 printer and polylactic acid plastic filament. The molds were developed in Autodesk Fusion 360, and gcode for the printer was sliced in Cura software. For the release experiments, 50 μL of PoP liposomes containing acid blue or acid yellow were first loaded onto the wells formed in the gel. The gels were placed under a cool white LED lamp (QUANS 5W 5 × 1W Cool White 19.68" High Power LED Lamp) and irradiated for 1 or 24 h and photographed immediately. A control sample was kept in the dark for 24 h.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsanm.8b00435.

Supporting Table S1: LC-MS analysis of lipid composition of irradiation liposomes. Figures S1−S: Influence of irradiation wavelength on light-triggered SRB release from PoP liposomes; light-induced change in unsaturated lipid content of liposomes lacking PoP; possible structure of peroxidated unsaturated lipid; PoP liposome morphology before and after light-treatment; spectra of irradiation lamp; release kinetics of Acid Blue 9 and Acid Yellow 23; dye release in 3D printed molds with varying dimensions (PDF)

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Notes

The authors declare no competing financial interest.

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