ABSTRACT: We report on photolipid doping of giant unilamellar vesicles (GUVs) via vesicle fusion with small unilamellar photolipid vesicles (pSUVs), which enables retroactive optical control of the membrane properties. We observe that vesicle fusion is light-dependent, if the phospholipids are neutral. Charge-mediated fusion involving anionic and cationic lipid molecules augments the overall fusion performance and doping efficiency, even in the absence of light exposure. Using phosphatidylcholine analogs with one or two azobenzene photoswitches (azo-PC) and dazo-PC affects domain formation, bending stiffness, and shape of the resulting vesicles in response to irradiation. Moreover, we show that optical membrane control can be extended to longer wavelengths using red-absorbing photolipids (red-azo-PC). Combined, our findings present an attractive and practical method for the precise delivery of photolipids, which offers new prospects for the optical control of membrane function.

INTRODUCTION

Photoswitchable lipids ("photolipids") are versatile molecular nanagoeds for controlling lipid-mediated processes and membrane properties with light.1 Phosphatidylcholine derivatives bearing a photoswitchable azobenzene group in one of their hydrocarbon tails2−5 have been used to control lipid diffusion,6 domain formation,7 bilayer rigidity,2 permeability,7 and protein molecular diffusion8 in synthetic bilayer membranes. However, in all of these examples, the photolipids were already added during the preparation of the vesicles and lipid bilayer assemblies. For many applications in synthetic biology or pharmacology, the postdoping of photolipids into an already formed bilayer membrane would be highly desirable to take advantage of the full potential offered by photolipids to control membrane properties. So far, amphiphilic or membrane-targeted photoswitchable molecules and fatty acids9 have been used in the context of the cell membrane and protein modification, allowing one to modulate neuronal firing,10 ion channel excitability,11 and cell signaling.12−14

Phospholipid doping and vesicle fusion can in principle be achieved by modulating the lipid synthesis pathway or by introducing specific phospholipid molecules via vesicle fusion or lipid uptake from solution. While the incorporation of photolipids via synthesis was recently achieved,15 this is not applicable to artificial systems lacking the required enzymatic pathways. Light-dependent and retroactive doping could present a highly attractive alternative and modular strategy to render membranes photoswitchable.

A number of chemical and physical strategies for introducing synthetic phospholipids into lipid membranes have been demonstrated in recent years, including the use of fusogenic proteins16 and ligands,17 osmotic gradients,18 electrofusion,19 or charge-mediated fusion.20,21 In the case of photolipids, another possibility is added since the photoswitching process itself can aid the fusion process. Suzuki et al.22 reported that azobenzene surfactants could increase the tension of bilayer membranes, which facilitates photocontrolled fusion of cell-sized vesicles. For photolipid membranes, Scheidt et al.23 reported that light-triggered lipid splay, attributed to the conformational change between the trans and cis forms, plays a causal role in vesicle coalescence. Successful membrane mixing was observed during UV exposure and trans-to-cis isomerization using vesicles that contained up to 20 mol % of photolipids. Similar observations were reported by Morgan et al.20 In this case, the authors showed that vesicles of binary dipalmitoylphosphatidylcholine (DPPC)/photolipid mixtures can interact with dye-loaded gel-phase DPPC vesicles leading to enhanced permeability and dye release only after the photolipids were subject to UV photoysis. However, postsynthetic photolipid doping into fluid cell-sized vesicle membranes and subsequent reversible photocontrol of the membrane mechanics and bilayer order has not been reported to date. To achieve this aim, two factors have to be considered. On the one hand, a suitable strategy to introduce enough photolipids into bilayer membranes is required to obtain an...
appreciable photoswitching effect on membrane properties. On the other hand, the photolipids have to display a significant conformation change to achieve a strong impact on the bilayer properties.

In this work, we investigate the possibility for retroactive photolipid doping in synthetic bilayer membranes by comparing two fusion strategies with three different types of photolipids. For this, we compare photoinduced and charge-mediated fusion with photolipids that have an azobenzene group in either one (azo-PC) or both (dazo-PC) hydrocarbon tails. We observe that photolipid uptake in 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) vesicles by photoisomerization is possible in both cases, although charge-mediated fusion via cationic and anionic lipids further enhances the overall fusion process to obtain photocontrol of membrane properties. Importantly, we demonstrate that the properties of photolipid-doped giant unilamellar vesicles (GUVs) can be controlled with light after the fusion. Photoisomerization of azo-PC allows one to reversibly affect the membrane order and phase separation, while dazo-PC doping enables optical control of membrane fluctuations and vesicle shape. In addition, we show that charge-mediated fusion is also adequate to postsynthetically dope the red-shifted photolipid red-azo-PC into regular GUVs and obtain subsequent photocontrol of the vesicle stiffness. These red-azo-PC lipids can be switched with tissue-penetrating red light, which increases the biocompatibility for in vivo studies. Overall, our findings pave the way for applications in cell membrane systems as the doping of lipid molecules via vesicle fusion is a viable and compatible strategy in a cellular context.

### EXPERIMENTAL SECTION

**Preparation of Small Unilamellar Photolipid Vesicles (pSUVs).** We prepared pSUVs by tip sonication as previously reported. Briefly, 100 μL of photolipids (azo-PC or dazo-PC) dissolved in chloroform (amylene stabilized, Merck) at 6.36 mM were mixed with 1 mol % TexasRed-DHPE (TexasRed 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, Thermo Fisher) and dried by air to deposit a lipid film on the bottom of a glass flask. The lipid film was then rehydrated with 1.5 mL of deionized water. Next, the solution was tip sonicated on ice twice for 30 s (Bandelin, Sonopuls) and centrifuged for 10 min with a relative centrifugal force (RCF) of 35.8 rpm$^2$ m before being stored at 4 °C until further use. Fluorescent red-azo-PC pSUVs were prepared from 99 mol % red-azo-PC and 1 mol % Atto633-DPPE (Atto633 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, Atto-Tec). Cationic pSUVs contained 3 mol % of the positively charged lipid DOTAP (1,2-dioleoyl-3-trimethylammonium-propane, Avanti Polar Lipids). The overall molar contents of these pSUVs were 94 mol % photolipids, 3 mol % DOTAP, and 1 mol % dye-labeled lipids. We again used TR-DHPE or Atto633-DPPE to label cationic azo-PC and dazo-PC pSUVs or red-azo-PC pSUVs, respectively.

**Preparation of Giant Unilamellar Vesicles.** We prepared GUVs as previously reported in a home-built device based on the electroformation method. To synthesize label-free GUVs, DOPC
(1,2-dioleoyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids) lipids were first dissolved in chloroform at a concentration of 6.36 mM. For experiments on charge-mediated fusion, 5 mol% of PA (1,2-distearyl-sn-glycero-3-phosphate, Avanti Polar Lipids) were added. An amount of 5 µL of the solution was spread with a syringe on two platinum wires which are 3 mm apart and span a Teflon chamber. Then, 1.5 mL of a sucrose solution (0.3 M), enough to cover the wires, was added. The chamber was heated to 70 °C, and an actuated electric field (10 Hz, 3 V) was applied for 120 min to the wires. The 0.3 M solution containing the GUVs was stored at room temperature for further use. No further buffering components or salts were added. To facilitate microscopy measurements, the GUV solution was diluted with a 0.3 M glucose solution (1:1 ratio GUV solution/glucose solution) to induce sedimentation of the GUVs at the sample bottom.

**Microscopy.** Sample imaging was done on an inverted microscope (IX81, Olympus) with a 100x objective (NA = 1.35, UPlanSapo, Olympus) in epifluorescence configuration. For epifluorescence microscopy, an HBO lamp was used, and imaging as well as excitation of the respective photolipid isomers were realized with suitable filter cubes (blue filter set (λ_{exc}: 470–490 nm, λ_{em} > 520 nm), green filter set (λ_{exc}: 510–550 nm, λ_{em} > 590 nm), a UV filter set (λ_{exc}: 330–385 nm, λ_{em} > 420 nm), and a red filter set (λ_{exc}: 623/32 nm, λ_{em} > 680/42 nm). To prevent unwanted switching and photobleaching, suitable optical filters were utilized. In addition, reflectance interference contrast microscopy (RICM) was used to visualize label-free GUVs. RICM was achieved using a filter cube containing a 50:50 beamsplitter (PBSW-532R, Thorlabs) in the dichroic position and a narrow bandpass filter (FL532-10, Thorlabs) to select the excitation wavelength, λ_{exc} = 532 nm. The vesicle samples were imaged with a Canon EOS 6D or a CCD camera (iXon Ultra, Andor). The photoluminescence (PL) intensities of dye-containing vesicle contours were determined by summing the intensities I of all pixels p_{ij} within a circle of radius R_{circle} = 4R_{vesicle}/3 around the center of the vesicle of each image frame according to

\[
I = \sum_{p_{ij} \in A_{vesicle} = R_{vesicle}/3} I(p_{ij}) - \sum_{p_{ij} \in A_{background} = R_{vesicle}/3} I(p_{ij})
\]

The subtrahend serves for background correction.

**RESULTS AND DISCUSSION**

**Photolipid Molecules and Vesicles.** The photolipid azo-PC, which contains an azobenzene unit in the sn2 tail, was synthesized according to a previous protocol.\(^3\) Photoswitching between the isomeric forms, trans- and cis-azo-PC, is achieved with blue and UV-A light, respectively (Figure 1A). The dazo-PC lipids were synthesized over two steps following a new synthetic method for the preparation of sufficiently large amounts of photolipids required for elaborate biophysical studies (Figure 1B, Supporting Information, S1). In the first step, AzoLPC bearing a single azobenzene group was prepared by selective monoacetylation of 1-α-glycerylphosphorylcholine (α-GPC) in 30% yield. In the second step, the photoswitchable fatty acid FAAz-o-4 was installed at the sn2 position via a Yamaguchi esterification, and dazo-PC was obtained with an overall yield of 27%. In the dark-adapted state (prior to any photoswitching), dazo-PC assumes the thermally favored trans conformation. NMR measurements revealed a trans/cis ratio of 99:1 (Supporting Information, S2). For azo-PC lipotails, a trans/cis ratio of 100:0 was reported.\(^2\) As shown for regular azo-PC, the newly synthesized dazo-PC lipids can also be switched between the trans and cis form using UV-A (trans/cis = 2.98) and blue light (trans/cis = 79:21) over many switching cycles without a sign of sample decomposition (NMR data and absorbance spectra are included in the Supporting Information, Figure S2A,B). Moreover, the switching kinetics of monomeric azo-PC and dazo-PC (free lipids dissolved in CHCl_3) are similar, while those of azo-PC and dazo-PC pSUVs differ by a factor of ~2 (Supporting Information, Figure S2C), which is similar to previous studies of azobenzene-containing surfactants and micelles.\(^2\)

We performed DLS measurements of dazo-PC pSUVs to study liposome stability (Supporting Information, Figure S3). The average diameters of dazo-PC pSUVs increase from (264 ± 6) to (313 ± 11) nm upon photoswitching from trans to cis, which corresponds to an average size change of (15.6 ± 1.1)%.

In comparison, a reversible size change of only ~3% was reported for azo-PC pSUVs.\(^2\) The vesicles could be switched...
Fluorescence quenching by the
that saturates after
images.

7.3) s and (4.2
decrease of the fluorescence intensities by 5
with DOPC vesicles, the sample was first illuminated with
thermodynamically stable
formed using a green (510
dye
via
are (18.0
intensities increase exponentially. The average PL rise times
During green-light exposure, the photoluminescence (PL)
acquisition settings. This results in prefusion of a tiny fraction
of pSUVs, which is sufficient to locate individual GUVs. We
then defined t = 0 as the starting point for the switching cycles
(Figure 2).

The sequential pSUV uptake was quantified by determining
the fluorescence intensity increase of the vesicle contours.
During green-light exposure, the photoluminescence (PL)
intensities increase exponentially. The average PL rise times
are (18.0 ± 3.7) s and (14.7 ± 4.8) s for azo-PC and dazo-PC,
respectively. For UV-A illumination, an immediate exponential
decrease of the fluorescence intensities by 5–30 % is observed
that saturates after ∼20 s. The mean PL decay times are (14.4
± 7.3) s and (4.2 ± 0.6) s for azo-PC and dazo-PC,
respectively.

This PL decay can be attributed to the stronger TR
fluorescence quenching by the cis compared to the trans
isomer. In general, azobenzenes are well-known dark
quenchers for certain fluorophores, and such effects must
be considered for the analysis of the fluorescence microscope
images.27 In azobenzene-containing aggregates, isomer-specific
photomodulation is observed, where cis azobenzenes generally
induce a larger decrease in the fluorophore emission.28,29 To
account for photomodulation by the two isomers, we
quantified the PL intensities of azo-PC and dazo-PC pSUVs,
which contained 1 mol % of TR-DHPE (Supporting Information, S6).
In both samples, the TR emission intensities are lower in the presence of cis isomers compared to trans
photolipids (Supporting Information, Figure S6). The photo-
modulation efficiencies are 79% and 72% for azo-PC and dazo-
PC, respectively.

Taking a closer look at the PL curves of the fusion experiment shown in Figure 2, one finds that the intensity
increases measured for green-light exposure are almost
continuous, only interrupted by the UV illumination steps.
The average TR emission intensity differences prior (PL\text{ prior})
and after (PL\text{ after}) UV-A light exposure, i.e., ⟨ΔI⟩ = (PL\text{ after} −
PL\text{ prior}) with I = 3 representing the number of switching cycles,
are only (7 ± 3)% and (2 ± 3)% for azo-PC and dazo-PC,
respectively. This suggests that fusion with pSUVs is enhanced
during cis-to-trans isomerization, while the reverse direction
plays a minor role. Furthermore, vesicle fusion occurs only
during lipid isomerization and slows down or stops when a cis/
trans equilibrium is reached. Notably, the positive average
difference (⟨ΔI⟩ > 0) further indicates that photobleaching is
negligible. This is also supported by the saturation of the PL
decrease during UV-A exposure at a constant intensity level.
This observation was further confirmed by control
experiments, where green-light illumination was extended over a
longer time period (Supporting Information, Figure S7). No
further increase in the fluorescence intensity was observed in
this case, indicating that membrane reorganization due to
changes in lipid conformation is important for the fusion
process. For cis-to-trans isomerization, bilayer defects are
introduced due to the rapid reduction of the cross-sectional
area of azo-PC.7 Such defects or membrane voids have been
reported as a driving force for membrane fusion.30 Transient
pore formation during cis-to-trans switching was observed in
pure photolipid membranes, while permeability after trans-to-
cis isomerization was found for lipid mixtures and binary
membrane compositions with only a 2–12 mol % amount of
photolipids.31–34 This indicates that the formation of
membrane voids is strongly dependent on the amount of
photolipids, which is also in agreement with the recent work by
Scheidt et al.35 demonstrating UV-A-triggered fusion between
liposomes made of POPC or DOPC and up to 20 mol % azo-
PC.

However, the efficiency of light-triggered vesicle fusion was
not sufficient to obtain visible photocontrol over membrane
properties of photolipid-doped GUVs, even after extending the
fusion time to 30 min. The vesicles showed no sign of
membrane fluctuations, domain formation, shape transforma-
tions, or any other form of photoinduced effects that
were observed for pure photolipid vesicles, suggesting that not
enough photolipids were fused into the GUV bilayer by light-
mediated fusion alone.

Charge-Mediated Vesicle Fusion. To enhance the
photolipid uptake, we followed a second strategy, where we
added anionic and cationic lipids to enable charge-mediated fusion
via attractive electrostatic interactions and subsequent
membrane adhesion and coalescence.31 First, the viability of
this approach was tested with regular nonswitchable vesicles.
We prepared anionic DOPC GUVs with 5 mol % of the
negatively charged phospholipid PA (1,2-diaryl-sn-glycero-
3-phosphate) and cationic, fluorescent DOPC SUVs with 3
mol % of the positively charged lipid DOTAP. While PA itself
favors fusion due to its cone shape and subsequent negative
curvature,36 DOTAP, on the other hand, becomes fusogenic in
the presence of neutral colipids like e.g., DOPE and DOPC.36,37
Mixing the oppositely charged SUVs and GUVs
resulted in vesicle fusion (Supporting Information, Figure S8).
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Figure 3. Optical control of photolipid-doped GUVs. (A) Fluorescence images of azo-PC-doped GUV. The box colors represent the illumination wavelengths, i.e., green light of 510−550 nm (green boxes) and UV-A light of 330−385 nm (pink boxes). For both illumination conditions, dark and bright areas are visible on the vesicle contour, indicating phase separation in the GUV bilayer. (B) Fluorescence images of dazo-PC-doped GUV at different time steps. The vesicle shape changes from sphere-like to elliptical upon isomerization with UV-A (pink) and green (green) light. The vesicle contour plots illustrate the change of the membrane fluctuations and vesicle shape. (C) UV-A exposure for 32 s results in a budding transition. Green-light illumination restores the initial spherical shape, which is highlighted in the kymograph. Scale bars: 10 μm. The GUVs were composed of 95 mol % DOPC and 5 mol % PA. The SUVs contained 96 mol % photolipids (azo-PC or dazo-PC), 1 mol % TR-DHPE, and 3 mol % DOTAP.

We then prepared cationic fluorescent pSUVs with 3 mol % DOTAP and added them to a solution of anionic DOPC GUVs (5 mol % PA). After mixing, the GUVs were imaged under the microscope using green light (Figure 3). The uptake of azo-PC vesicles immediately led to dark and bright areas of the GUV membrane, suggesting phase separation of the DOPC and azo-PC lipids (Figure 3A). Vesicles of binary compositions containing azo-PC/DPHPC or azo-PC/DOPC have been suggested to feature domains in the presence of trans photolipids due to dipolar interactions and H-aggregate formation, while no phase separation was observed for cis photolipids. Domain formation can be further enhanced by the presence of the two oppositely charged lipids DOTAP and PA. Vequi-Suplicy et al., for example, have reported that the fusion of DOTAP-labeled liposomes with anionic ones results in domain formation of the oppositely charged lipid species.

We changed the illumination conditions to UV-A exposure to convert the photolipids to the cis form and found that the domains started to merge, while upon back switching to trans, they disassembled again into smaller domains. The average number (n) and area (DA) of the GUV domains (Figure 3A) changed between ntrans = (8 ± 2) and n cis = (3 ± 1), and DAtrans = (55.6 ± 13.3)% and DA cis = (72.6 ± 16.9)% in more detail by determining the vesicle contours as a function of the polar angle. An increase of the mean (μ) and the standard deviation of two switching cycles, respectively. The number of domains and the domain sizes, i.e., their surface areas, were determined from a three-dimensional (3D) image that was reconstructed from multiple two-dimensional (2D) epifluorescence images (Supporting Information, Movie S1). All six vesicles studied displayed phase separation and domain formation upon azo-PC pSUV uptake. These findings hint at a fractional lipid reorganization during photoswitching, which could be a consequence of charged lipid domains preventing complete mixing of the cis photolipids and DOPC. Binary trans-azo-PC/ DOPC vesicles have been suggested to display nanodomains, while lipid mixing occurs in cis-azo-PC-containing vesicles. The charged lipid species can aid domain formation in the presence of trans-azo-PC leading to the observed micrometer-sized domains.

For charge-mediated dazo-PC doping, we found that vesicle fusion even allows to gain control over membrane fluctuations and vesicle shape transformations, owing to the presence of photoswitches in both lipid tails (Figure 3B,C). The dazo-PC-doped GUV shown in Figure 3B already exhibits a slightly elliptical shape during green-light exposure. Switching to UV-A light results in the appearance of membrane fluctuations within milliseconds and further elongation (Supporting Information, Movie S2). Furthermore, budding events were observed for 48% of the GUVs after prolonged UV-A exposure (Figure 3C; Supporting Information, Movie S3). Back-switching to trans with green light allowed for reversing the shape transition and recouping a spherical contour (Supporting Information, Movie S4). Although the process was repeatable, a clear correlation between UV-A exposure time and budding events was not observed. We analyzed the membrane undulations of this GUV in more detail by determining the vesicle contours as a function of the polar angle. An increase of the mean (μ) and the standard deviation of two switching cycles, respectively. The number of domains and the domain sizes, i.e., their surface areas, were determined from a three-dimensional (3D) image that was reconstructed from multiple two-dimensional (2D) epifluorescence images (Supporting Information, Movie S1). All six vesicles studied displayed phase separation and domain formation upon azo-PC pSUV uptake. These findings hint at a fractional lipid reorganization during photoswitching, which could be a consequence of charged lipid domains preventing complete mixing of the cis photolipids and DOPC. Binary trans-azo-PC/ DOPC vesicles have been suggested to display nanodomains, while lipid mixing occurs in cis-azo-PC-containing vesicles. The charged lipid species can aid domain formation in the presence of trans-azo-PC leading to the observed micrometer-sized domains.

We confirmed this by control measurements where we analyzed the membrane fluctuations of pure dazo-PC GUVs using a previously reported protocol (Supporting Information, Movie S9). We found an average bending stiffness increase from...
Figure 4. Doping with red-azo-PC. (A) Red-azo-PC can be switched between the trans and cis isomer using green/red and purple/blue light, respectively. (B) Vesicle images after the uptake of red-azo-PC upon blue- and red-light exposure. The contour plots represent the membrane fluctuations in the photostationary state (PSS) reached with blue (left) and red (right) light, respectively. Scale bars: 10 μm.

\[ \kappa_{\text{trans}} \approx 10^{-20} \text{ J} \quad \text{to} \quad \kappa_{\text{cis}} \approx 10^{-17} \text{ J} \], which was previously reported to be in the range of gel-to-fluid-like membrane phase transitions.\(^4\)\(^3\) Notably, long integration times (200 ms) are required for vesicle contour analysis in our experiment. The membrane bending rigidity could therefore not be analyzed with the same level of accuracy typically obtained in membrane fluctuation spectroscopy.\(^4\)\(^2\) The photosensitive nature of the photolipids demands careful control of the illumination conditions to avoid any unintentional switching. Label-free techniques such as e.g., phase-contrast or darkfield microscopy are therefore not suitable due to their extended UV/vis imaging range. Instead, we relied on fluorescence microscopy with dyes that are excitable at the same wavelengths as the photoswitch itself. While TR-DHPE is excitable with UV-A light, it only shows a low absorbance and therefore low emission, which requires rather long integration times for fluorescence imaging (Supporting Information, Figure S4). For comparison, short integration times of <10 ms are typically applied in fluctuation spectroscopy to account for thermal undulations and to assess membrane bending rigidity with high accuracy.\(^4\)\(^2\) However, even with these experimental constraints, our measurements clearly show that the bending rigidity of pure dazo-PC GUVs and subsequently also of photolipid-doped GUVs can change significantly. To further support this conclusion, we performed fluorescence recovery after photo-bleaching (FRAP) experiments and determined the diffusion coefficients of supported dazo-PC bilayers in the trans and cis states that were labeled with 1 mol % TR-DHPE (Supporting Information, S10). The bilayers displayed average diffusion coefficients of \(D_{\text{trans}} = (0.11 \pm 0.01) \mu m^2 s^{-1}\) and \(D_{\text{cis}} = (1.3 \pm 0.1) \mu m^2 s^{-1}\), which, again, agrees well with a typical increase of membrane fluidity from gel-like to fluid.\(^4\)\(^3\)\(^4\)

In total, 48% of 25 studied GUVs exhibited budding events, while 16% showed at least enhanced membrane fluctuations and 8% displayed further vesicle shape deformations such as vesicle splitting and pearling transitions upon trans-to-cis isomerization (Supporting Information, S11). The bright spot at the neck region of the vesicle budding event shown in Figure 3C indicates a local accumulation of TR at the GUV surface. Since the GUVs themselves were not labeled, these dyes stem from the pSUVs that were added to instigate vesicle fusion. Steinkühler et al.\(^4\)\(^5\)\(^6\) have reported that protein binding to GUVs can control the spontaneous membrane curvature, leading to budding and eventually even splitting of single vesicles into daughter cells. Potentially, the observed accumulation of lipid dyes or adsorbed pSUVs on the outer GUV surface could therefore facilitate vesicle budding as well, in particular in combination with the membrane stiffness modulation obtained by photoswitching (Supporting Information, S9).

Photolipid-Dependent Fusion Efficiency. For azo-PC-containing GUVs, we previously suggested that the addition of DOPC can lead to phase separation and domain formation in the binary trans-azo-PC/DOPC vesicles.\(^2\) Dazo-PC, however, can exhibit phase separation also in the absence of a second lipid species, which we observed for GUVs only containing dazo-PC and 1 mol % TR-DHPE (Supporting Information, Figure S12). This raises the question of why the GUVs do not display domains after vesicle fusion and dazo-PC accumulation, as is the case for azo-PC (Figure 2). A possible explanation could be a lower fusion efficiency of dazo-PC than of azo-PC. To support this hypothesis, we applied a fluorescence-based fusion assay and mixed the cationic red-fluorescent pSUVs with DOPC GUVs that not only contained PA but also green-fluorescent Atto465-DOPE (Supporting Information, S13). After fusion, the doped GUVs displayed both colors, red and green (Supporting Information, Figure S13). The fluorescence intensities of the two dyes changed in response to the lipid uptake. We compared the ratios of these emission intensities with calibrated PL ratios, which we derived from fluorescence measurements of vesicle samples where the lipids were already added during the liposome preparation. These samples contained defined amounts of TR-DHPE, Atto465-DOPE, PA, DOTAP, DOPC, and azo-PC or dazo-PC to resemble the fusion process and reflect various fusion efficiencies and photolipid doping levels. The measurements indicate that the average photolipid uptake are \((40 \pm 30)\%\) for azo-PC and \((20 \pm 15)\%\) for dazo-PC, which shows that azo-PC uptake is more effective.

Red-azo-PC Doping. As a perspective for future applications in biological systems, we also tested the fusion assay with the red-shifted photolipid red-azo-PC that contains a tetra-ortho-chlorinated azobenzene unit in the sn2 tail (Figure 4A).

The ortho-substitution induces a spectral shift of the isomerization wavelengths rendering photoswitching with blue and red light possible.\(^4\)\(^0\) This is of particular advantage for biological applications since red light is less absorbed by...
tissue than UV-A light allowing for deeper penetration depths. The pSUVs were prepared from 96 mol % red-azo-PC, 1 mol % Atto633-DPPE, and 3 mol % DOTAP. Prior to use, they were again stored in the dark to convert them to a photostationary state (PSS) with mostly trans lipids. After mixing the pSUVs with the nonfluorescent anionic DOPC GUVs, we initially imaged the doped vesicles with blue light using a blue filter cube (470–490 nm) to maintain the trans-rich state (Figure 4B). We then changed to red-light (600–645 nm) illumination to induce the trans-to-cis isomerization. The vesicle contour started to immediately fluctuate (Supporting information, Movie S5). These fluctuations were again studied in more detail by determining the vesicle contour profiles as a function of the polar angle. The membrane fluctuations displayed in Figure 4B increase by a mean factor of ~2.7 from (0.7 ± 2.1) to (1.7 ± 3.5) μm, which corresponds to the mean value and standard deviation of an averaged spectrum derived from the five single spectra, when switching the red-shifted lipids from trans to cis. This result agrees well with photo-triggered membrane undulations in pure red-azo-PC GUVs and shows that the reached red-azo-PC doping level is sufficient to gain optical control of the membrane mechanics retroactively via fusion of charged vesicles. Overall, 83% of the GUVs showed enhanced membrane fluctuations upon red-azo-PC uptake and trans-to-cis isomerization. This might seem surprising, considering that the doping of azo-PC, which also contain a single azobenzene unit in the sn2 lipid tail, results in the formation of μm-sized domains. However, trans-azo-PC and -dazo-PC photolipids display H-aggregate formation when assembled to bilayer membranes (Supporting Information, S2). This is not the case for red-azo-PC. Hence, a more homogeneous distribution of the red-azo-PC photolipids after their uptake can be expected, which is in agreement with the homogeneous coloring of the vesicle membrane contours shown in Figure 4B.

■ CONCLUSIONS

In conclusion, we have shown that regular GUVs can be efficiently doped with photolipid molecules via photo-controlled and charge-mediated vesicle fusion with 3–5 mol % of the charged lipid species DOTAP and PA. Azo-PC doping leads to a change of membrane organization and domain formation in the GUV bilayer, while dazo-PC and red-azo-PC doping render membrane fluctuations, shape transformation, and vesicle budding events photoswitchable. Altogether, our findings demonstrate that the photocontrol of membrane properties via isomerization of azobenzene-based photolipids can be transferred to regular, nonswitchable lipid membranes, which emphasizes the potential of photolipid molecules as optical nanoagents for applications in life science.

■ ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.langmuir.2c01685.

Additional information on the synthetic procedures, materials, methods, and analytical/supporting data (PDF)

Domains in azo-PC-doped GUV (Movie S1) (AVI)

Photo-triggered membrane fluctuations and elongation of dazo-PC-doped GUV (Movie S2) (AVI)

UV-A-triggered budding event of dazo-PC-doped GUV (Movie S3) (AVI)

Back-switching (cis-to-trans) of dazo-PC-doped GUV/ reverse of the shape transition (budding event) (Movie S4) (AVI)

Photo-triggered membrane fluctuations of red-azo-PC-doped GUV (Movie S5) (AVI)

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Notes

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