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Light-Controlled Lipid Interaction and Membrane Organization in Photolipid Bilayer Vesicles

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Abstract: Controlling lateral interactions between lipid molecules in a bilayer membrane to guide membrane organization and domain formation is a key factor for studying and emulating membrane functionality in synthetic biological systems. Here, we demonstrate an approach to reversibly control lipid organization, domain formation, and membrane stiffness of phospholipid bilayer membranes using the photoswitchable phospholipid *azo*-**PC**. *azo*-**PC** contains an azobenzene group in the *sn*2 acyl chain that undergoes reversible photoisomerization on illumination with UV-A and visible light. We demonstrate that the concentration of the photolipid molecules, and also the assembly and disassembly of photolipids into lipid-domains, can be monitored by UV-Vis spectroscopy due to a blue-shift induced by photolipid aggregation.

Introduction.

The plasma membrane of living cells serves as a scaffold for enzymes and proteins whose proper function can be strongly influenced by the membrane composition[1-2] and its' physical properties[3-6]. It is important to note that lipid molecules are not merely passive elements of the bilayer matrix. On the contrary, the localization of specific lipids in the plasma membrane is crucial for the proper regulation of protein function[7], membrane stiffness[8], and signaling[9]. Furthermore, self-organization of lipids in lipid domains is a well-known intrinsic property of native cell membranes[10], and is important for the proper execution of certain cellular functions[11-13].

Lipid bilayers and vesicles made from synthetic phospholipids have been successfully used to emulate the fundamental properties of cell membranes.[14-17] A general shortcoming of most synthetic membrane models, however, is the inability to achieve fast and reversible control over the membrane properties. Phase transitions, for example, can be triggered by changing experimental parameters such as temperature or ion concentrations, but these effects are only slowly reversible, and require drastic changes to the physiological conditions around the membrane.[18-19] Nanoparticles or high-intensity laser pulses have been used to locally control the formation of transient membrane pores for ion exchange over short time scales.[20-23] These examples also require a strong external trigger, which may be disruptive or damaging when applied in a biological context. Molecules or surfactants can also be embedded in a bilayer membrane to influence lipid organization and membrane properties. However, changing the membrane composition is typically a non-reversible process.

Light-responsive molecules that change their structural conformation - and thus their lateral interaction - on illumination bear an exciting alternative for manipulating phospholipid bilayer

membranes *in situ*. Photoswitchable amphiphiles containing an azobenzene moiety, also coined "photolipids", have been used as powerful reagents to manipulate membrane permeability[24-27], to control the association and distribution of domains in lipid membranes[28-29], to induce liposome fusion[30], or to selectively induce vesicle bursting[31] with light. In most cases, the photolipids themselves could not sustain a bilayer membrane, and had to be incorporated into canonical bilayer systems.[26, 29] Recently, we devised a strategy to synthesize giant unilamellar vesicles (GUVs) entirely from a photoswitchable phosphatidylcholine derivative, *azo*-**PC**,[32] which contains an azobenzene group in the *sn*2 acyl chain that undergoes a reversible *trans*-to-*cis* isomerization upon illumination with UV-A and visible light. Switching the lipid conformation allows reversible manipulation of the mechanical properties of GUVs, such as the surface area and bending stiffness, which could induce shape transformations and even controlled fission of the photolipid vesicles. Importantly, we showed that the illumination power could regulate the temporal dynamics of these shape transformations, indicating that not all photoswitchable molecules changed their conformation simultaneously. However, monitoring the distribution of photolipids within a lipid bilayer membrane at any given point in time, which is a prerequisite for quantitative studies of photoswitchable membrane properties, has remained a challenge.

Here, we report a strategy for measuring the concentration of *azo*-**PC** lipids in a bilayer membrane. This is achieved by analyzing the concentration-dependent blue-shift of the absorption spectrum of *azo*-**PC** when assembled in a vesicle. This hypsochromic effect is a result of the dipole-dipole interaction between the *trans*-azobenzene groups of the lipid tails, and is therefore sensitive to the photolipid density in the bilayer. We demonstrate that this approach allows us to directly monitor phase separation and membrane domain formation in GUVs made from ternary lipid mixtures. Furthermore, we demonstrate that optical switching of photolipids allows to

reversibly control the assembly and disassembly of lipid domains in GUVs within milliseconds and under physiological conditions. We analyze the effect of photoswitching and domain formation on the mechanical properties of the photolipid bilayer membranes.

Experimental.

Synthesis of *azo*-PC and *iso-azo*-PC. Photolipids were prepared by a new synthetic route, which improved our previously reported procedure[29] by allowing for a faster access to *azo*-**PC** (supporting information). Our approach relied on selective mono-acylations of L-α-glycerylphosphorylcholine (α-GPC) which enabled the preparation of our target molecules in two steps. By modifying the literature procedure for the dibutyltin oxide-mediated selective primary alcohol acylation of 1,2-diols, we afforded 1-steaoryl-sn-glycero-3-phosphocholine (lysoPC) in good yields with steraroyl chloride as acylating agent.[33] In the following step, we found that esterification reactions between lysoPC and FAAzo-4[34] were accompanied with acyl chain migrations. This circumstance enabled the formation of the two products *azo*-**PC** and *iso-azo*-**PC** in a single procedure.

Preparation of Small Unilamellar Vesicles. SUVs were prepared as previously described[32]. Briefly, 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC, Avanti Polar Lipids) and *azo*-**PC** were dissolved in chloroform at a concentration of 6.36 mM and stored at -20°C. Immediately before preparation, lipids were mixed and 200 µl of the resulting solution was given into a round glass bottom flask and diluted with chloroform to a final volume of 2 ml. The solvent was removed with a rotary evaporator. The residue was then dissolved in 2 ml of ddH_2O. The solution was transferred to a centrifugation tube and tip sonicated (Bandelin Sonopuls) two times for 30s with

high intensity until the solution was clear. Finally, the vesicle solution was centrifuged for 10min at 10000rpm.

Preparation of Giant Unilamellar Vesicles. GUVs were prepared by electroformation as reported previously[32], using a home-built reaction chamber. Two platinum wires, 3 mm apart, span a chamber with a volume of 1.5 ml. Lipids and cholesterol were dissolved in chloroform at a concentration of 6.36 mM and mixed to the desired composition. Additionally, Texas Red labelled 1,2-dihexadecanoyl-*sn*-glycero-3-phospho-ethanolamine (TR-DHPE, Thermo Fisher Scientific) was added to a final lipid concentration of 1 mol%. The reaction chamber and a 300 mM sucrose solution were heated to 60°C. Then, 5 µl of the lipid solution were spread dropwise on the platinum wires. After evaporation of the chloroform, the reaction chamber was filled with 1.5 ml sucrose solution. The chamber was sealed and kept at 60°C. An electric field (10Hz, 3V) was applied for 120 min. Finally, the solution containing GUVs was stored at room temperature until further use.

Optical Spectroscopy. Absorption spectra of lipids in chloroform, SUVs and GUVs were obtained with a Cary 60 UV/Vis Spectrophotometer (Agilent Technologies). The solutions containing the sample were measured in quartz cuvettes. Spectra were taken either without or after illumination with a 365 nm high power LED light source (Prizmatix). Illumination periods were >1 min, to guarantee that a photostationary state was reached. For an unfocused LED (365 nm), the time constant for the isomerization process from *trans* to *cis* was typically 34 s (supporting information, Figure S3).

Fluorescence Microscopy. GUVs were imaged with a 20x oil immersion objective (Olympus UPlanSApo, NA 0.85) on an inverted microscope (IX81, Olympus). The solution containing GUVs was mixed with a 300 mM glucose solution. Due to the higher density of sucrose compared

to glucose, the GUVs are immobilized on the glass coverslide. For image acquisition, we used either a color CMOS camera (Canon, EOS 550D or EOS 5D Mark IV) or a monochrome CCD camera (Andor iXon Ultra 897). Green (U-MWG2, Olympus) and UV (U-MWU2, Olympus) filter sets were used to prevent unwanted switching.

Bending Rigidity Measurements. The mechanical properties of the photoswitchable GUVs were determined with micropipette aspiration[35]. The bending rigidity was calculated from the elongation of a membrane tube in a micropipette (Hilgenberg GmbH). The pressure was controlled with two liquid filled reservoirs and measured with a pressure transducer (Valydine Inc., DP15). The micropipette and the GUVs were imaged on an upright microscope (Zeiss, AXIO Scope.A1) in phase contrast configuration with a water immersion objective (Zeiss, Achroplan 100x, NA 1.0, Ph3). Image acquisition was done with a CMOS camera (Canon, EOS 550D). Experimental details and the analysis are discussed in the Supporting Information.

Results and Discussion.

Our previous synthesis of *azo*-**PC** suffered from an air/water-sensitive multi-step procedure, which prevented us from preparing larger amounts of material for more elaborate biophysical studies. To circumvent this limitation, we designed a new synthesis that enabled us to quickly prepare large amounts of *azo*-**PC**, and its' regioisomer *iso-azo*-**PC**, which contains the azobenzene on the *sn*1 acyl chain. Starting from α-GPC, *azo*-**PC** was prepared over two steps consisting of a selective acylation of the primary alcohol followed by a Yamaguchi esterification (Figure 1a).

In the dark, the azobenzene persists mostly in the thermally stable *trans* configuration (Figure 1b). The absorption spectrum of *trans-azo*-**PC** dissolved in $CHCl_3$ showed a strong absorbance in the UV-A region with a maximum at 335 nm (Figure 2a). This corresponds to the S0 → S2

transition[36] and is characteristic for a standard azobenzene. Furthermore, a weaker absorption band was observed at 440 nm, corresponding to the S0 → S1 transition, which is symmetry forbidden for *trans*-azobenzene.[36] On irradiation with UV-A light (365 nm), the absorbance spectrum of the photolipids changed until the photostationary state is reached, indicative for the isomerization of *azo*-**PC** from the *trans* to the *cis* conformation (Figure 2a). For *cis*-azobenzene, the S0 → S2 transition weakened and the maximum absorption shifted to 295 nm. At the same time, the intensity of the S0 → S1 transition increased as the symmetry of the azobenzene group changes on isomerization due to the structural change. The spectra of the photolipids in a photostationary state did not change for several minutes and even hours. Since all measurements presented in this study were conducted within seconds, the possibility of thermal isomerization of photolipids from *cis* to *trans* could be neglected.

Notably, the absorbance spectrum of *trans*-*azo*-**PC** changed when the lipids were assembled in a bilayer membrane. Compared to the measurement in $CHCl_3$, the absorption maximum shifted from 335 nm to 315 nm, and therefore towards higher energies. This hypsochromic- or blue-shift of the absorption peak has been observed previously in mono- or bilayers containing azobenzene groups and is attributed to the formation of H-aggregates.[26-27, 37] In addition to a hypsochromic shift, the appearance of a vibrational pattern was observed around the absorption band of the S0 → S2 transition (between 320 nm – 380 nm, supporting information, Figure S2). This vibronic progression is the result of the coupling between delocalized excitonic states and vibrational modes, and a characteristic feature of H-aggregation[38]. The transition dipole moments of the azobenzene groups can couple via dipole-dipole interactions. The observed hypsochromic shift of the absorbance maximum is a direct indicator for the coupling strength, and thus carries information about the density and intermolecular distance between the *azo*-**PC** lipids in the bilayer

membrane[27]. The prerequisite for H-aggregation is that the azobenzenes in the lipid tails are aligned parallel to each other, which is the case in a bilayer membrane.[39-42]

The absorption spectrum of the *cis*-isomer did not display a shift in the absorbance maximum (Figure 2a), indicating that the *cis*-photolipids were less aligned and oriented in the bilayer. This is in accordance with previous results[32], where the overall size of photolipid vesicles made from only *azo*-**PC** increased by 3% on photoswitching, and that photolipid membranes displayed strong fluctuations and decreased in bending rigidity after photoswitching (Figure 2a, inset).

Since the dipole-dipole coupling strength is distance dependent, the amount of photolipids in the bilayer membrane must have an influence on the position of the absorption maximum. In a dilute sample, fewer interactions between the photolipids should result in a less pronounced hypsochromic shift. Different mixtures of *azo*-**PC** in the dark-adapted state with DPhPC were tested to quantify this effect. For bilayer membranes composed of 100% to only 10% *azo*-**PC**, the maximum of the absorbance peak in the UV spectrum shifted from 315 nm to 335 nm, close to the absorption peak of the free photolipids in $CHCl_3$ (Figure 2b, supporting information, Figure S4). The strongest hypsochromic shift was observed for membrane mixtures with an *azo*-**PC** content above 75%, indicating that the photolipids are packed more closely together and aligned at higher concentrations. These results demonstrate that measuring the absorption spectra of the vesicle solution provides a straightforward way to determine the concentration of *trans*-*azo*-**PC** for a simple two-component system, provided that the molecules are evenly distributed within the bilayer and that no clustering or phase separation of the lipids can take place. However, this is not always the case for more complex membrane compositions, where phase separation and membrane domain formation can become a factor.

We then studied lipid domain formation of photolipids embedded in GUVs by preparing ternary mixtures of *azo*-**PC**, DPhPC and cholesterol (Figure 3). To observe the domain structure using epi-fluorescence microscopy, 1 mol% TR-DHPE was added to the lipid mixture. The excitation wavelength range between 510 nm and 550 nm keeps the *azo*-**PC** molecules in a photostationary state with a majority of photolipids being in the *trans* conformation. In this case, addition of cholesterol to the GUVs aids in the formation of large lipid domains. Different lipid mixtures were tested systematically in order to investigate the boundary conditions for phase separation and domain formation. First, a series of measurements was performed with a constant concentration of cholesterol (20 mol%) and a varying ratio of *azo*-**PC** and DPhPC (Figure 3a). Domain formation was observed between a 1:3 and a 3:1 ratio of *azo*-**PC**:DPhPC (Figure 3a). In the fluorescence images, we observed that the area covered by the dark domains increased with an increasing amount of *azo*-**PC**. The dark areas are therefore attributed to be *azo*-**PC** rich domains. Since TR-DHPE partitions preferentially into the liquid disordered phase[43], the dark domains are presumably in the liquid ordered phase and therefore rich in cholesterol as well[44]. Furthermore, a stronger hypsochromic shift of the S0 → S2 band was observed for increasing amounts of *azo*-**PC** in the bilayer (Figure 3b), which is well in accordance with the previous measurements of the binary lipid mixture (see Figure 2b).

Next, the ratio between *azo*-**PC** and DPhPC was kept constant, while the cholesterol concentration was increased. Domain formation was only observed for a cholesterol content between 10-35 mol% (Figure 3c). However, the S0 → S2 peak measured at different points of the phase diagram was blue-shifted at larger concentrations of cholesterol (Figure 3d). Above 40 mol% cholesterol, the formation of membrane domains was no longer observed (Figure 3c). Now,

the absorption peak of *azo*-**PC** also shifted towards longer wavelengths, as one would expect due to a decreasing concentration of *azo*-**PC** in the membrane.

The blue-shift of the spectrum observed for 10-35 mol% cholesterol was directly correlated to the presence of membrane domains. Clustering of *azo*-**PC** in large domains led to H-aggregation. As a consequence, a stronger hypsochromic shift is observed compared to the same concentration of lipids when they are homogeneously distributed within the membrane. The absorbance spectra of the phase separated GUVs in solution (Figure 3b, d) are a superposition of the spectra of all different conditions and domain sizes in the sample.

We then investigated the possibility to control the formation of membrane domains by switching *azo*-**PC** on irradiation. This was tested using GUVs composed of a 4:4:2 ratio of *azo*-**PC**, DPhPC and cholesterol. Again, the vesicles were labelled with 1 mol% TR-DHPE to observe the presence of large membrane domains using fluorescence microscopy (Figure 4a). On illumination with UV-A light, the membrane domains disappeared. However, this effect was fully reversible. After illumination of the vesicles with visible light (510 nm – 550 nm), the membrane domains formed again within seconds. Importantly, this process could be repeated over several illumination cycles until photobleaching of the dye prevented further observation.

Photoswitching of lipid domains can be viewed as a change of the molecular ratio of the photolipids that are in a *trans* or a *cis* state. Controlling the illumination conditions (e.g. intensity and duration) thus renders it possible to change the lipid composition between photostationary states, as depicted in the phase diagram (Figure 4b). On illumination with UV-A or visible light, the photolipids change their conformation until a photostationary state between the *cis* and *trans* configuration is reached. After visible light illumination of GUVs with *azo*-**PC** in a *cis* state, for

example, most molecules were switched to a *trans* state within ~300 ms, which was in accordance with the appearance of small membrane domains on the GUV surface. These small domains then fused to form large domains over the course of seconds (supporting information, Figure S5, video V1).

Changes in membrane composition and organization go hand in hand with a modulation of the mechanical properties such as membrane stiffness and bending rigidity.[45-46] For *azo*-**PC**, it was shown that membrane stiffness of GUVs decreased in the *cis* state.[32] In order to quantify this effect, the bending moduli of vesicles with different *azo*-**PC**:DPhPC compositions were measured by micropipette aspiration (Figure 5a). The experiment was first performed with pure *azo*-**PC**-GUVs. GUVs made from non-photoswitchable lipids were used as a control. The bending modulus of membranes made from 100% *azo*-**PC** ($k_c = 3.1x10^{-20}$ J) under white-light illumination was found to be one order of magnitude lower than the value for bilayers consisting purely of DPhPC ($k_c=1.2x10^{-19}$J) (Figure 5b, see also Supporting Information Figure S6 for mixtures of *azo*-**PC** with DOPC). After UV-A illumination, the bending modulus for *azo*-**PC** membranes decreased by a factor of 5 to $k_c = 6.4x10^{-21}$ J as the molecules are switched to the *cis* state. The measurement was repeated for different mixtures of *azo*-**PC** and DPhPC (Figure 5c). The largest difference between the two photostationary states was observed for mixtures containing 35% *azo*-**PC**.

To interpret these results, one should consider that the bending rigidity also carries information about the distribution of the lipid molecules within the bilayer. For two-component membranes, the function describing the bending rigidity in terms of the fraction of one lipid type has a positive curvature for systems with ideal mixing, and a negative curvature for systems displaying domains.[47] The curves for DPhPC and *azo*-**PC** mixtures were consistent with this observation (Figure 5c). The measured curve for the UV-A adapted state suggests a homogeneous lipid

distribution, indicating that the majority of lipids are indeed in a *cis* state. After illumination with blue light (465 nm) to switch the lipids back to *trans*, the bending rigidity displayed a negative curvature, indicative for de-mixing and the presence of small domains. Importantly, these small membrane domains could not be seen by fluorescence microscopy of DPhPC and *azo*-**PC** vesicles. Yet, the combination of micro-aspiration measurements and absorption measurements (cp. Figure 2b) provided clear evidence for the occurrence of photolipid aggregation and clustering, particularly at high *azo*-**PC** concentrations.

The local variation of the membrane stiffness due to membrane domains does have an effect on the function of proteins or enzymes within a bilayer membrane. Photolipids that self-organize into small, rigid domains can therefore be used to control the properties and function of synthetic biological systems. By devising strategies for embedding photolipids in the plasma membrane of living cells, the controlled switching of the lipid conformations and thus shift of lipid composition and mechanical properties with light could provide a powerful approach to control cellular function *in situ* and potentially also *in vivo.*

Conclusion.

In summary, we have shown that optical switching of *azo*-**PC** assembled in a bilayer membrane can be used to influence lipid organization and the mechanical properties in phospholipid bilayers with high temporal control and accuracy. H-aggregate formation of azobenzene molecules in the lipid tails renders it possible to measure the concentration of azobenzene molecules in a bilayer membrane by a hypsochromic shift of the absorbance maximum corresponding to the S0 → S2 transition. Controlling the isomerization process for ternary mixtures of *azo*-**PC** with cholesterol allows us to reversibly control membrane domains with light. The dynamic control of lipid

membranes and membrane self-organization with light paves the way towards new applications in synthetic biology, or as light sensitive reagents in native cell membranes to control cellular functions in space and time.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Experimental details for bending rigidity measurements, for the chemical synthesis, and additional data (PDF).

Video V1 (ZIP).

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REFERENCES

1. Bienvenüe, A.; Marie, J. S., Modulation of Protein Function by Lipids. In *Cell Lipids*, Hoekstra, D., Ed. Academic Press: 1994; Vol. 40, pp 319-354.
2. Spector, A. A.; Yorek, M. A., Membrane lipid composition and cellular function. *J. Lipid Res.* **1985,** *26* (9), 1015-35.
3. Andersen, O. S.; Koeppe, R. E., 2nd, Bilayer thickness and membrane protein function: an energetic perspective. *Annu. Rev. Biophys. Biomol. Struct.* **2007,** *36*, 107-30.
4. Anishkin, A.; Loukin, S. H.; Teng, J.; Kung, C., Feeling the hidden mechanical forces in lipid bilayer is an original sense. *Proc. Natl. Acad. Sci. U.S.A.* **2014,** *111* (22), 7898-905.
5. Ingolfsson, H. I.; Thakur, P.; Herold, K. F.; Hobart, E. A.; Ramsey, N. B.; Periole, X.; de Jong, D. H.; Zwama, M.; Yilmaz, D.; Hall, K.; Maretzky, T.; Hemmings, H. C., Jr.; Blobel, C.; Marrink, S. J.; Kocer, A.; Sack, J. T.; Andersen, O. S., Phytochemicals perturb membranes and promiscuously alter protein function. *ACS Chem. Biol.* **2014,** *9* (8), 1788-98.
6. Lundbaek, J. A.; Collingwood, S. A.; Ingolfsson, H. I.; Kapoor, R.; Andersen, O. S., Lipid bilayer regulation of membrane protein function: gramicidin channels as molecular force probes. *J. R. Soc., Interface* **2010,** *7* (44), 373-95.
7. Di Paolo, G.; De Camilli, P., Phosphoinositides in cell regulation and membrane dynamics. *Nature* **2006,** *443* (7112), 651-7.
8. Stoiber, K.; Naglo, O.; Pernpeintner, C.; Zhang, S.; Koeberle, A.; Ulrich, M.; Werz, O.; Muller, R.; Zahler, S.; Lohmuller, T.; Feldmann, J.; Braig, S., Targeting de novo lipogenesis as a novel approach in anti-cancer therapy. *Br. J. Cancer* **2018,** *118* (1), 43-51.
9. Simons, K.; Toomre, D., Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **2000,** *1* (1), 31-9.
10. Schmid, F., Physical mechanisms of micro- and nanodomain formation in multicomponent lipid membranes. *Biochim. Biophys. Acta, Biomembr.* **2017,** *1859* (4), 509-528.
11. Simons, K.; Ikonen, E., Functional rafts in cell membranes. *Nature* **1997,** *387* (6633), 569-72.
12. Simons, K.; Sampaio, J. L., Membrane organization and lipid rafts. *Cold Spring Harbor Perspect. Biol.* **2011,** *3* (10), a004697.
13. Laganowsky, A.; Reading, E.; Allison, T. M.; Ulmschneider, M. B.; Degiacomi, M. T.; Baldwin, A. J.; Robinson, C. V., Membrane proteins bind lipids selectively to modulate their structure and function. *Nature* **2014,** *510* (7503), 172-175.
14. Baumgart, T.; Hess, S. T.; Webb, W. W., Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension. *Nature* **2003,** *425* (6960), 821-4.
15. Veatch, S. L.; Gawrisch, K.; Keller, S. L., Closed-loop miscibility gap and quantitative tie-lines in ternary membranes containing diphytanoyl PC. *Biophys. J.* **2006,** *90* (12), 4428-36.
16. Veatch, S. L.; Keller, S. L., Organization in lipid membranes containing cholesterol. *Phys. Rev. Lett.* **2002,** *89* (26), 268101.
17. Veatch, S. L.; Keller, S. L., Separation of liquid phases in giant vesicles of ternary mixtures of phospholipids and cholesterol. *Biophys. J.* **2003,** *85* (5), 3074-83.
18. McNeil, P. L.; Steinhardt, R. A., Plasma membrane disruption: repair, prevention, adaptation. *Annu. Rev. Cell Dev. Biol.* **2003,** *19*, 697-731.
19. Endo, Y.; Torii, R.; Yamazaki, F.; Sagawa, S.; Yamauchi, K.; Tsutsui, Y.; Morikawa, T.; Shiraki, K., Water drinking causes a biphasic change in blood composition in humans. *Pfluegers Arch.* **2001,** *442* (3), 362-8.

20. Liu, Q.; Frerck, M. J.; Holman, H. A.; Jorgensen, E. M.; Rabbitt, R. D., Exciting cell membranes with a blustering heat shock. *Biophys. J.* **2014,** *106* (8), 1570-7.
21. Palankar, R.; Pinchasik, B. E.; Khlebtsov, B. N.; Kolesnikova, T. A.; Mohwald, H.; Winterhalter, M.; Skirtach, A. G., Nanoplasmonically-induced defects in lipid membrane monitored by ion current: transient nanopores versus membrane rupture. *Nano Lett.* **2014,** *14* (8), 4273-9.
22. Rettenmaier, A.; Lenarz, T.; Reuter, G., Nanosecond laser pulse stimulation of spiral ganglion neurons and model cells. *Biomed. Opt. Express* **2014,** *5* (4), 1014-25.
23. Urban, P.; Kirchner, S. R.; Mühlbauer, C.; Lohmüller, T.; Feldmann, J., Reversible control of current across lipid membranes by local heating. *Sci. Rep.* **2016,** *6*, 22686.
24. Cui, Z. K.; Phoeung, T.; Rousseau, P. A.; Rydzek, G.; Zhang, Q.; Bazuin, C. G.; Lafleur, M., Nonphospholipid fluid liposomes with switchable photocontrolled release. *Langmuir* **2014,** *30* (36), 10818-25.
25. Fujiwara, H.; Yonezawa, Y., Photoelectric Response of a Black Lipid-Membrane Containing an Amphiphilic Azobenzene Derivative. *Nature* **1991,** *351* (6329), 724-726.
26. Kuiper, J. M.; Engberts, J. B. F. N., H-Aggregation of Azobenzene-Substituted Amphiphiles in Vesicular Membranes. *Langmuir* **2004,** *20* (4), 1152-1160.
27. Song, X.; Perlstein, J.; Whitten, D. G., Supramolecular Aggregates of Azobenzene Phospholipids and Related Compounds in Bilayer Assemblies and Other Microheterogeneous Media: Structure, Properties, and Photoreactivity. *J. Am. Chem. Soc.* **1997,** *119* (39), 9144-9159.
28. Frank, J. A.; Franquelim, H. G.; Schwille, P.; Trauner, D., Optical Control of Lipid Rafts with Photoswitchable Ceramides. *J. Am. Chem. Soc.* **2016,** *138* (39), 12981-12986.
29. Hamada, T.; Sugimoto, R.; Nagasaki, T.; Takagi, M., Photochemical control of membrane raft organization. *Soft Matter* **2011,** *7* (1), 220-224.
30. Morgan, C. G.; Yianni, Y. P.; Sandhu, S. S.; Mitchell, A. C., Liposome Fusion and Lipid Exchange on Ultraviolet Irradiation of Liposomes Containing a Photochromic Phospholipid. *Photochem. Photobiol.* **1995,** *62* (1), 24-29.
31. Diguet, A.; Yanagisawa, M.; Liu, Y. J.; Brun, E.; Abadie, S.; Rudiuk, S.; Baigl, D., UV-induced bursting of cell-sized multicomponent lipid vesicles in a photosensitive surfactant solution. *J. Am. Chem. Soc.* **2012,** *134* (10), 4898-904.
32. Pernpeintner, C.; Frank, J. A.; Urban, P.; Roeske, C. R.; Pritzl, S. D.; Trauner, D.; Lohmüller, T., Light-Controlled Membrane Mechanics and Shape Transitions of Photoswitchable Lipid Vesicles. *Langmuir* **2017,** *33* (16), 4083-4089.
33. Fasoli, E.; Arnone, A.; Caligiuri, A.; D'Arrigo, P.; de Ferra, L.; Servi, S., Tin-mediated synthesis of lyso-phospholipids. *Org. Biomol. Chem.* **2006,** *4* (15), 2974-8.
34. Frank, J. A.; Moroni, M.; Moshourab, R.; Sumser, M.; Lewin, G. R.; Trauner, D., Photoswitchable fatty acids enable optical control of TRPV1. *Nat. Commun.* **2015,** *6*, 7118.
35. Kwok, R.; Evans, E., Thermoelasticity of large lecithin bilayer vesicles. *Biophys. J.* **1981,** *35* (3), 637-652.
36. Bandara, H. M.; Burdette, S. C., Photoisomerization in different classes of azobenzene. *Chem. Soc. Rev.* **2012,** *41* (5), 1809-25.
37. Kawai, T.; Umemura, J.; Takenaka, T., UV absorption spectra of azobenzene-containing long-chain fatty acids and their barium salts in spread monolayers and Langmuir-Blodgett films. *Langmuir* **1989,** *5* (6), 1378-1383.
38. Eisfeld, A.; Briggs, J. S., The J- and H-bands of organic dye aggregates. *Chem. Phys.* **2006,** *324* (2-3), 376-384.

39. Czikkely, V.; Försterling, H. D.; Kuhn, H., Light absorption and structure of aggregates of dye molecules. *Chem. Phys. Lett.* **1970,** *6* (1), 11-14.
40. Czikkely, V.; Försterling, H. D.; Kuhn, H., Extended dipole model for aggregates of dye molecules. *Chem. Phys. Lett.* **1970,** *6* (3), 207-210.
41. Kasha, M., Energy Transfer Mechanisms and the Molecular Exciton Model for Molecular Aggregates. *Radiat. Res.* **1963,** *20* (1), 55-70.
42. Kasha, M.; Rawls, H. R.; Ashraf El-Bayoumi, M., The exciton model in molecular spectroscopy. *Pure Appl. Chem.* **1965,** *11* (3-4).
43. Baumgart, T.; Hunt, G.; Farkas, E. R.; Webb, W. W.; Feigenson, G. W., Fluorescence probe partitioning between Lo/Ld phases in lipid membranes. *Biochim. Biophys. Acta, Biomembr.* **2007,** *1768* (9), 2182-94.
44. Heberle, F. A.; Feigenson, G. W., Phase Separation in Lipid Membranes. *Cold Spring Harbor Perspect. Biol.* **2011,** *3* (4), a004630-a004630.
45. Nagle, J. F., Experimentally determined tilt and bending moduli of single-component lipid bilayers. *Chem. Phys. Lipids* **2017,** *205*, 18-24.
46. Rawicz, W.; Olbrich, K. C.; McIntosh, T.; Needham, D.; Evans, E., Effect of Chain Length and Unsaturation on Elasticity of Lipid Bilayers. *Biophys. J.* **2000,** *79* (1), 328-339.
47. Illya, G.; Lipowsky, R.; Shillcock, J. C., Two-component membrane material properties and domain formation from dissipative particle dynamics. *J. Chem. Phys.* **2006,** *125* (11), 114710.

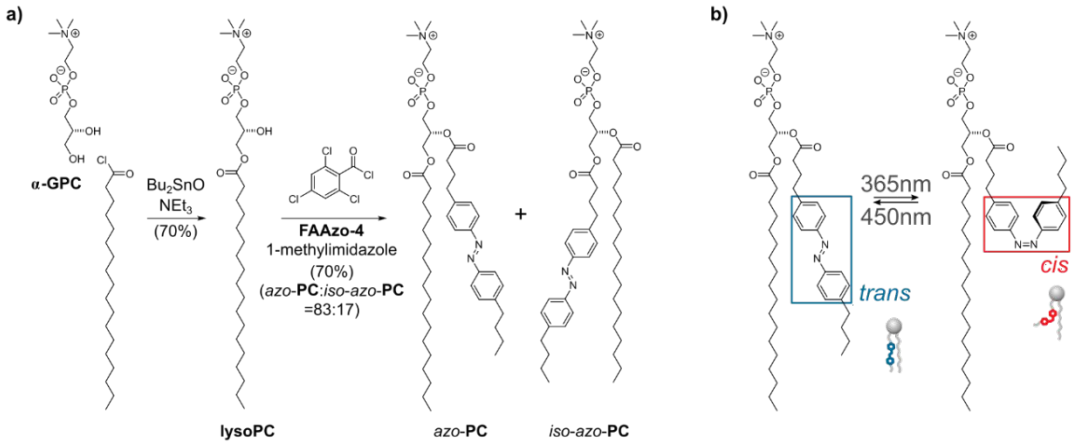

Figure 1. *azo*-**PC** photolipids (a) *azo*-**PC** and *iso*-*azo*-**PC**, which contain the azobenzene in the sn1 acyl chain, were prepared by a new synthetic route. Employing an optimized Yamaguchi esterification protocol afforded good yields and an 83:17 ratio in favor of *azo*-**PC**. (b) The molecule configuration can be switched reversibly from *trans* to *cis* by UV-A and visible light.

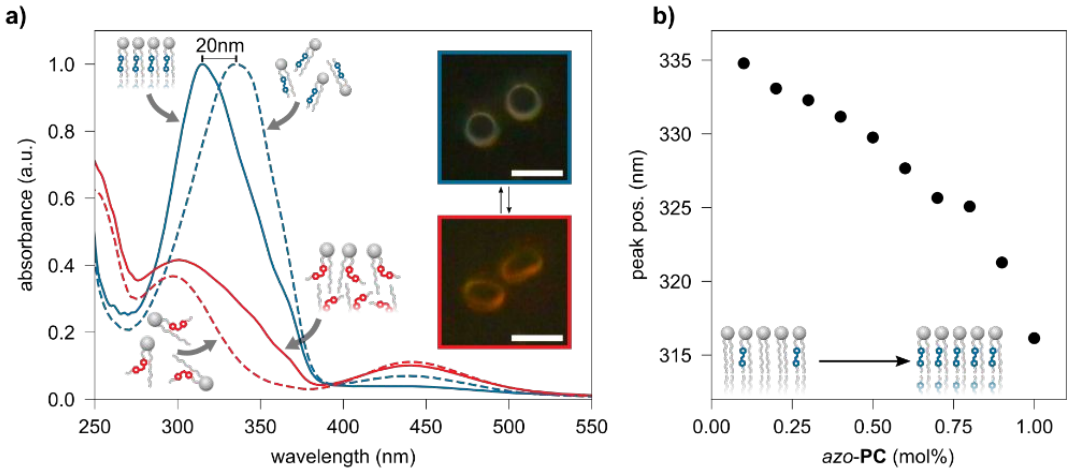

Figure 2. Aggregation of *azo*-**PC** in a phospholipid bilayer membrane. (a) Absorbance spectra of *azo*-**PC** in $CHCl_3$ (dashed line) and in a lipid bilayer (solid line) in the dark-adapted state (blue) and after illumination with UV light (red). A shift of 20 nm of the absorbance maximum is observed for photolipids in the *trans* state when they are assembled in a membrane. In dark-field microscopy, membrane fluctuations and shape changes of the *azo*-**PC** GUVs can be observed upon photoswitching the lipids from *trans* to *cis* (inset, scale bar = 5 µm). (b) The peak position of the S0 → S2 transition shifts for mixtures of *azo*-**PC** with DPhPC. The magnitude of the shift is depending on the concentration of *azo*-**PC** in the bilayer.

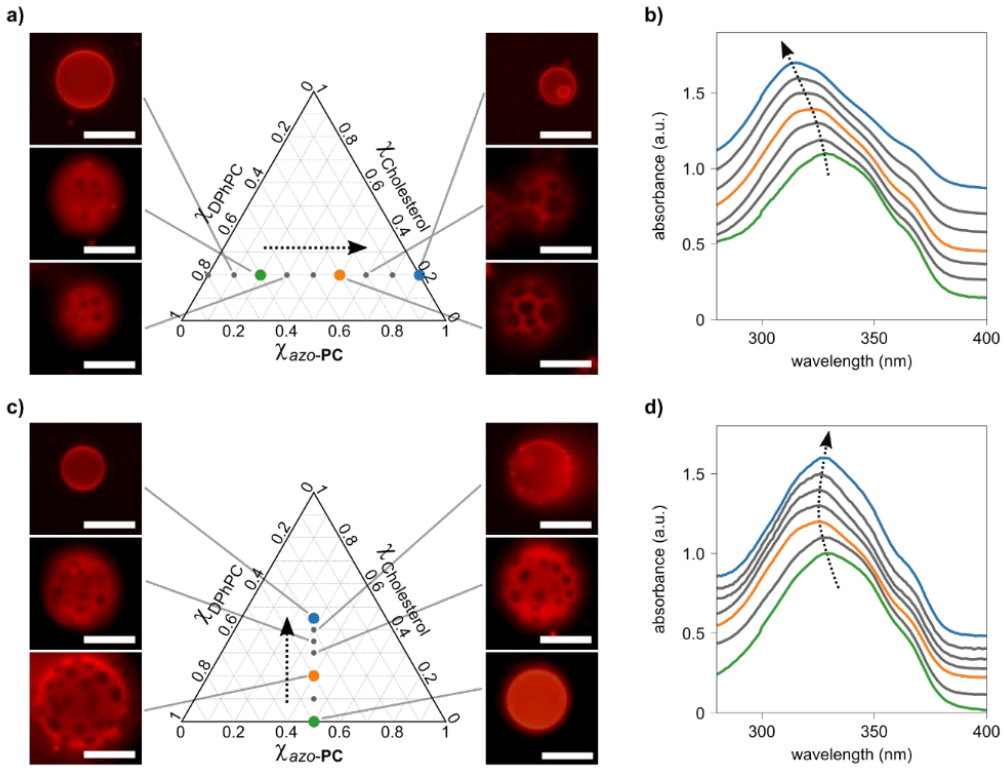

Figure 3. Domains in ternary mixtures of DPhPC and cholesterol with *azo*-**PC**. (a) Fluorescence images of GUVs with a constant cholesterol concentration. The lipid compositions correspond to the points in the phase diagram. (b) Absorption spectra of GUVs for the lipid compositions displayed in (a). The colored lines correspond to the respective points in the phase diagram; the arrow indicates the blue-shift due to a higher concentration of *azo*-**PC**. Spectra are normalized and offset for clarity. (c) Fluorescence images of GUVs with a 1:1 ratio of DPhPC and *azo*-**PC**. Lipid domains are observed for a cholesterol concentration between 10 and 35 mol%. The arrow displayed in the phase diagram indicates the increasing concentration of cholesterol. (d) Absorption spectra of GUVs corresponding to the lipid compositions in (c). The absorption spectra of lipid compositions with domain formation display a stronger hypsochromic shift compared to lipid mixtures where phase separation was not observed. (Scale bar = 20µm)

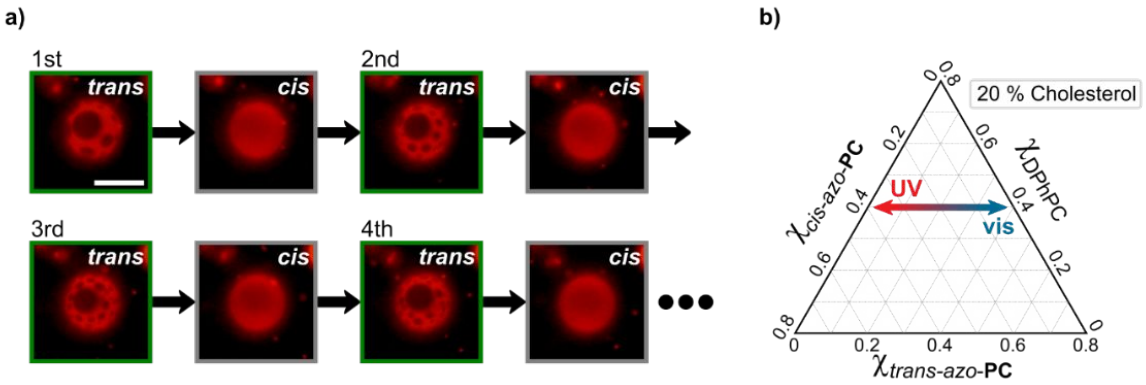

Figure 4. Photoswitching of membrane domains in ternary mixtures with *azo*-**PC**. (a) Domains in GUVs containing DPhPC, *azo*-**PC** and cholesterol (lipid ratio 4:4:2) can be switched reversibly with UV-A and visible light. (Scale bar = 20 µm) (b) Controlling the illumination intensity and duration allows continuous tuning of the concentration of *trans*- and *cis*-*azo*-**PC** in the bilayer membrane.

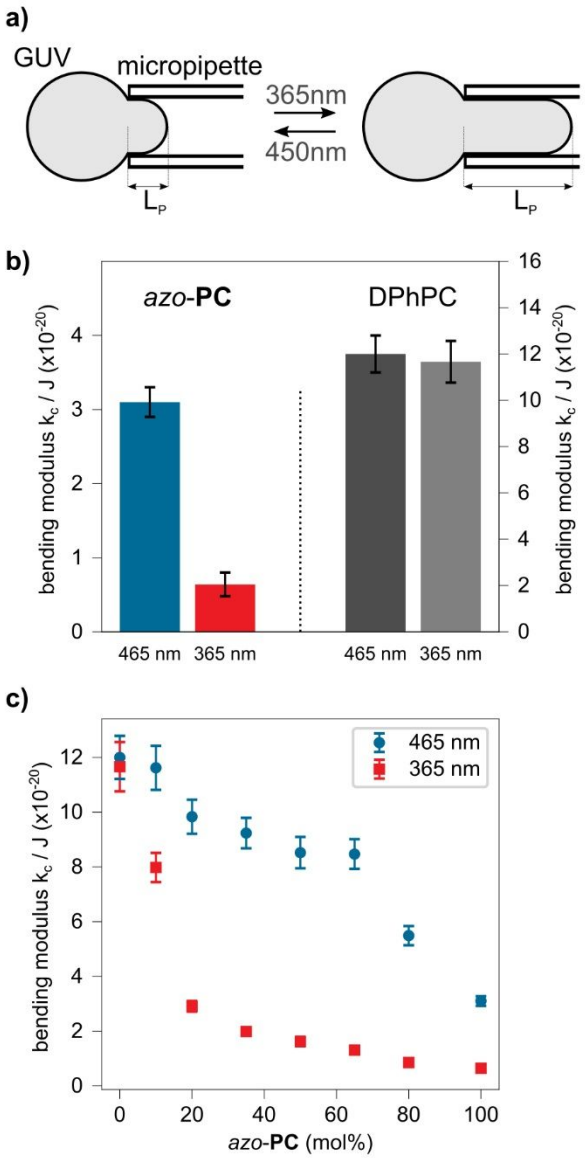

Figure 5. Mechanical properties of *azo*-**PC**/DPhPC bilayer membranes. (a) Schematic representation of the micropipette aspiration setup. GUVs are sucked into a micropipette. The bending modulus of the membrane is calculated from the elongation L_P of the vesicle tube inside the pipette. (b) Bending modulus, k_c, for 100 % *azo*-**PC** GUVs and 100 % DPhPC GUVs, each illuminated with blue or UV-A light. c) Bending moduli, k_c, for membranes composed of mixtures of *azo*-**PC** and DPhPC. Each data point represents the measurements of three different vesicles that were illuminated with visible and UV-A light to switch the photolipids from *trans* to *cis*.

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