Photoswitching of model ion channels in lipid bilayers

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ABSTRACT

Membrane proteins can be regulated by alterations in material properties intrinsic to the hosting lipid bilayer. Here, we investigated whether the reversible photosomerization of bilayer-embedded diacylglycerols (OptoDArG) with two azobenzene-containing acyl chains may trigger such regulatory events. We observed an augmented open probability of the mechanosensitive model channel gramicidin A (gA) upon photosomerizing OptoDArG’s acyl chains from trans to cis: integral planar bilayer conductance brought forth by hundreds of simultaneously conducting gA dimers increased by typically >50% – in good agreement with the observed increase in single-channel lifetime. Further, (i) increments in the electrical capacitance of planar lipid bilayers and protonation length of aspirated giant unilamellar vesicles into suction pipettes, as well as (ii) changes of small-angle X-ray scattering of multilamellar vesicles indicated that spontaneous curvature, hydrophobic thickness, and bending elasticity decreased upon switching from trans- to cis-OptoDArG. Our bilayer elasticity model for gA supports the causal relationship between changes in gA activity and bilayer material properties upon photo-isomerization. Thus, we conclude that photolipids are deployable for converting bilayers of potentially diverse origins into light-gated actuators for mechanosensitive proteins.

1. Introduction

Key to the mechanical sensitivity of a membrane protein is the hydrophobic coupling between its hydrophobic transmembrane domains and the embedding bilayer’s hydrophobic core [1,2]. Mismatches between the thicknesses of the latter are generally accommodated by elastic deformations of the softer membrane [1,3,4]. Membrane channels may reduce such mismatch-associated energetic costs stored within the elastically deformed bilayer by undergoing conformational changes, which have functional consequences [5,6]. Some channels can be driven over their whole dynamic range with mechanical stress alone; others only change their transport rates in response to mechanical stimuli [7]. To simplify the discussion, we will refer to both types as being mechanically-responsive (MS) channels.

The apparent change in Gibbs free energy for a MS protein structural change, $\Delta G_{\text{rot}}$, comprises both a protein-intrinsic, $\Delta G_{\text{prot}}$, and a lipid-deformation component, $\Delta G_{\text{def}}$ [8]. Earlier approaches at characterizing $\Delta G_{\text{def}}$ were focused on the interaction between depth-dependent changes in protein cross sectional area, $A(z)$, with the lateral pressure profile intrinsic to lipid bilayers [9]; in that framework, an increase in lateral pressure within the acyl chain region mediated by, e.g., a change in lipid composition can be thought of as promoting a reduction in $A(z)$ there, i.e., a structural change that might be of functional consequence. However, the bilayer-inherent distribution of lateral pressures is difficult to access experimentally and its determination thus typically relies on computational approaches [10]. Later, it was demonstrated that the “lateral pressure model” for membrane protein regulation by Cantor [11] can be equivalently expressed using the theory of elasticity of lipid bilayers introduced by Helfrich [12,13]. The latter approach of quantifying $\Delta G_{\text{def}}$ using a set of experimentally-accessible bilayer material properties – e.g. thickness, intrinsic curvatures of the constituent lipids, compressibility and bending modulus, B, membrane tension – has prevailed [14]. Hence, $\Delta G_{\text{def}}$ depends on the material properties of the embedding bilayer as well as protein structure and changes thereof [15]. In other words, alterations in elastic and structural parameters of the embedding medium may contribute to membrane protein regulation in a...
MS membrane channels frequently respond to an increase in membrane tension, $\sigma$, – characterizing the imbalance between bilayer-inherent integral lateral pressure and tension components [16] – by in-plane area expansion. For example: (a) the prototypical prokaryotic mechanosensitive channel of large conductance (MscL) expands by about $20 \text{ nm}^2$ and thereby establishes a large aqueous pore in response to near-lytic membrane tension, thus reflecting its role as an osmotic emergency valve [17]; (b) voltage-gated potassium ion channels such as Shaker and the Kv1.2 paddle chimera are modulated by membrane tension in the range of $0.5–4 \text{ mN/m}$ in so far as their voltage-activation curves are shifted [18]. At the same time, however, the high-threshold tension-gated MscL is exquisitely modulated by alterations in membrane hydrophobic thickness, $d_{hc}$, [19] and spontaneous monolayer formations and increasing fluidity in supported lipid bilayers [21,22].

We have previously used photolipids to reversibly assemble or dissolve ordered lipid domains that are thicker than the surrounding disordered lipid domains [23]. The first reported attempt to trigger a mechanosensitive channel’s opening using photolipids involved MscL. [24]. Unfortunately, it remained unclear whether MscL was functionally reconstituted and served as the origin for the illumination-triggered current spikes. A study wherein a reversible photoswitch was anchored to MscL was more successful [25]. Similarly, it appears possible to gate ion channels by using photoswitchable ligands [26,27]. Yet, the applicability of photolipids as reversible modulators of bilayer physical properties with the prospect of MS protein regulation has not yet been demonstrated. Achieving such control might allow for the creation of biological permeability barriers under tight spatiotemporal regulation using self-assembled photoswitchable membranes with embedded native MS proteins. Such membranes might constitute an alternative to e.g. photo-tunable crystalline metal–organic frameworks towards achieving photoswitchable membrane separation [28].

To that effect, we studied the implications of photosomeration of a membrane-embedded photolipid termed OptoDArG (Fig. 1) on the activity profile of the MS model ion channel gramicidin A (gA). The latter is a sensitive reporter for both mechanical and structural changes in its immediate surroundings [29], as well as for global changes in bilayer material properties [8]. We found that gA channel lifetime and activity – i.e. the time-averaged number of simultaneously conducting gA dimers – increased in planar bilayers upon switching from trans- to cis-OptoDArG. Importantly, unlike earlier work where the covalent attachment of photosensitive moieties to peptides and proteins rendered the products responsive to light [30–33], we did not chemically modify gA. To complement these results on protein function, we characterized changes in bilayer material properties that accompanied photosomerization (i) by the geometric analysis of aspirated giant unilamellar vesicles (GUVs) as well as capacitance measurements on planar bilayers and (ii) via small-angle X-ray scattering (SAXS). Finally, we sought to use the obtained experimental results for calculating spontaneous lipid curvatures including trans- and cis-OptoDArG by adapting a recent model for elastic deformations incurred by different states of gA based on continuum mechanics of bilayer elasticity [34].

2. Materials and Methods

2.1. Solvent-free Planar Bilayers

Dioleoylphosphatidylcholine (DOPC) and diphytanoylphosphatidylcholine (DPhPC) were obtained from Avanti Polar Lipids. We synthesized OptoDArG as previously described [26] and stored it at $-80$ °C. Lipid mixtures for planar bilayer experiments comprising 90 mol% DOPC/DPhPC +10 mol% OptoDArG were prepared via dissolving lipids in chloroform and mixing the solutions at the appropriate molar ratio within amber glass micro reaction vessels. Subsequently, the solvent was evaporated deploying a mild vacuum gradient (Rotavapor, Büchi Labortechnik AG); dried lipid mixtures were flooded with argon and stored at ultralow temperature until deployment.

Solvent-depleted free-standing vertical planar bilayers were folded from monolayers in a $\sim100 \mu\text{m}$ wide aperture of a $25 \mu\text{m}$ thick PTFE septum (Goodfellow Cambridge Ltd) [35,36]. Before usage, the PTFE septum was pretreated with hexadecane and clamped vertically between the halves of an in-house manufactured chamber made from PTFE. Each half comprised an UV-transmitting fused quartz window which allowed for the projection of light onto the aperture within the PTFE septum resting at approximately the center of the assembled chamber. Dried lipid was retrieved from the ultralow temperature freezer and dissolved in hexane to a concentration of $10–20 \text{ mg mL}^{-1}$. Several microliters of this solution were spread on top of the upper air–water interfaces of the aqueous buffer-containing compartments separated by the PTFE septum to form lipid monolayers. The Faraday cage within which the assembled chamber rested possessed an orifice through which light could be shone onto a collimating lens that focused incident light onto the aperture to accelerate the photosomerization process. The appropriate wavelength of light originating from a xenon arc lamp operated at $1000 \text{ W}$ was selected by a double-grating monochromator (model MS257, Oriel Instruments). All experiments on planar bilayers were performed at room temperature.
temperature.

2.2. Ensemble Measurements on Planar Bilayers

The buffer deployed for ensemble measurements was 100 mM KCl, 10 mM N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES) at pH 7.0. Gramicidin A from Bacillus brevis (SKU 50845, Fluka) dissolved in ethanol was stored at 4 °C. gA was symmetrically added to both compartments of the assembled chamber (see above) to a final concentration in the three-digit picomolar range, effectively yielding integral bilayer conductances in the one- to two-digit nanosiemens range [35]. Ag/AgCl electrodes within both compartments were connected to a DSP dual-phase lock-in amplifier (model 7265, PerkinElmer, Inc.). A sinusoidal reference voltage (amplitude: 5 mV) was applied across the faces of the folded planar bilayer. The evoked current was amplified via a current amplifier (model 428, Keithley Instruments) and subsequently fed back to the lock-in amplifier, which determined the in-phase (conductive) and quadrature (capacitive) current contributions at the reference frequency [57]. The latter were digitized (model USB-6210, National Instruments) and recorded with WinWCP (University of Strathclyde, Glasgow). Data analysis was facilitated by Origin (OriginLab), Mathematica (Wolfram Research, Inc.), and Excel (Microsoft).

2.3. Single-channel Measurements

The buffer deployed for single-channel measurements was 500 mM KCl, 10 mM HEPES at pH 7.0. gA was symmetrically added to both compartments of the assembled chamber (see above) to a final concentration in the one-digit picomolar range. Ag/AgCl electrodes within both compartments were connected to (a) a current amplifier (model 428, Keithley Instruments) or (b) the headstage of a patch-clamp amplifier (EPC 9, HEKA Elektronik GmbH). The capacitance of planar bilayers and changes thereof upon photosoisomerization of embedded OptoDarG were (a) monitored using the lock-in amplifier (see above) or (b) determined by a rectangular voltage protocol via the PULSE software (HEKA Elektronik GmbH) controlling the EPC 9. Following successful bilayer formation, a transmembrane potential difference of 100 mV was applied. Upon using the current amplifier, the amplified current signal was analogously low-pass filtered via a low-noise preamplifier (model SR560, Stanford Research Systems) at a cutoff frequency of 30 Hz, digitized at 250 Hz, and recorded with WinWCP. Upon using the EPC 9, the current signal was filtered at 100 Hz, digitized at 250 Hz, and recorded with PULSE. The obtained current traces were analyzed deploying WinWCP, TAC X4.3.3, and TACFit X4.3.3 (Bruxton Corporation) – single-channel events were assigned manually. Data analysis was facilitated by Origin and Excel.

2.4. Giant Unilamellar Vesicles and Microaspiration

The preparation of GUVs and aspiration pipettes has been described in detail earlier [38]. In brief, a modified electroformation protocol was used to prepare GUVs [39,40]. Droplets of lipid dissolved in chloroform were deposited onto two parallel platinum wires (PT005157 Platinum Wire, Goodfellow Cambridge Ltd.). Following partial dehydration under a stream of argon, the lipid reservoirs were rehydrated with GUV formation buffer (5 mM KCl, 5 mM HEPES pH 7.2 and sucrose, 450 mMosm) and GUVs grown under an alternating voltage applied between the platinum wires [38].

Microaspiration pipettes with a long even taper were pulled with a pipette puller (Model P-97 Flaming/Brown Micropipette Puller, Sutter Instrument Company) from borosilicate glass capillaries (GB150-10, Science Products GmbH). Their tips had an inner diameter, Dpip, of 5–9 μm. For later alignment of the tip axis to the focal plane of the objective, the tapered part of the pipette was bent to about 135° using another pipette puller (PP-83, Narishige). Freshly produced microaspiration pipettes were filled with GUV bath buffer (5 mM KCl, 5 mM HEPES pH 7.2 and glucose, 450 mMosm). Filled pipettes were mounted via a holder (OptoPatch, ALA Scientific Instruments) onto a micromanipulator (PatchStar, Scientifica) and gas-tightly connected with a micrometer burette-style dispenser (Gilmont GS-1200-A, Cole-Parmer GmbH) via 1 mm inner diameter tubing (Rotilabo PTFE Tubing, Carl Roth).

The shape of microaspirated GUVs in their equatorial plane was monitored by a confocal laser scanning microscope (LSM510 Meta, Zeiss) – for this purpose, 0.004 wt% Atto633-DPPE (ATTO-TEC) was added to the lipid mixtures prior to electroformation. Image series were recorded during repeated photoswitching of OptoDarG-containing liposomal membranes by monochromatic light from a 150 W Xenon lamp (Polychrome V, TILL Photonics). Membrane area AGUV(t) as a function of time was determined as described in detail elsewhere [38]; the fractional change in surface area, a, was calculated acc. \( a = \frac{A_{GUV}(t)}{A_{GUV,0}} \) with \( A_{GUV,0} \) denoting surface area at \( t = 0 \), i.e., prior to the first trans to cis photosomerization.

2.5. Sample Preparation for SAXS

Stock solutions were prepared by dissolving weighed amounts of DOPC and OptoDarG in chloroform/methanol (2:1 volume/volume) and consecutively mixed at an appropriate ratio to obtain a DOPC/OptoDarG (9:1 mol/mol) mixture. A dry lipid film was obtained by evaporating the organic solvent first under a gentle stream of nitrogen and then by storing the sample overnight in a vacuum. Fully hydrated multilamellar vesicles (MLVs) were formed by hydrating the dry lipid film in 18 MΩcm −1 water to a concentration of 15 wt%. The hydrated sample was vortexed every 15 min for 1 h and incubated overnight at room temperature. This was followed by 5 freeze/thaw cycles by using liquid nitrogen.

2.6. Small-angle X-ray Scattering

SAXS experiments were performed on a SAXSpace compact camera (Anton Paar, Graz, Austria) equipped with an Eiger R 1 M detector system (Dectris, Baden-Daettwil, Switzerland) and a 30 W-Genix 3D microfocus X-ray generator (Xenocs, Sassenage, France) supplying Cu-Kα (\( \lambda = 1.54 \) Å) radiation. Liposomal dispersions were measured using 1 mm quartz glass capillaries (μ-cell, Anton Paar) and equilibrated at 25 °C for 10 min using a Peltier controlled sample stage (TC 150, Anton Paar). The total exposure time was 30 min, and the sample-to-detector distance was set to 308 mm. Data reduction, including sectorial data integration and corrections for sample transmission and background scattering, was performed using the program SAXSanalyis (Anton Paar). A torchlight UV source (\( \lambda = 365 \) nm) was used to switch OptoDarG from trans to cis. The total UV exposure time was 90 min. A 450 nm light source was used to switch the system back to its trans state (exposure time: 2 h). To ensure that all OptoDarG molecules are in trans configuration, samples were illuminated with the same light source for 15 min before the exposure to UV light.

Data were analyzed in terms of a previously established full q-range analysis [41,42], which allows deriving membrane structural parameters, as well as the bending fluctuations. Concerning structural parameters, of specific interest are here the lamellar repeat distance d and the steric bilayer thickness \( d_g = 2(z_{H} + \sigma_g) \), where \( z_{H} \) and \( \sigma_{g} \) are the position and width of the headgroup Gaussian of the used electron density model for the bilayer structure. Specifically, \( \sigma_{g} \) was fixed to 3 Å as reported previously for phosphatidylycholine lipids [43].

3. Results and Discussion

Initially, we investigated the effect of photosomerization of 10 mol % OptoDarG within planar bilayers folded from lipid mixtures with DOPC or DPPC by the activity of embedded gA. Therefore, we recorded integral bilayer conductances, \( \Gamma \), caused by gramicidin dimers, \( D_{g} \),...
Dimer formation from monomers, $M_g$, is described by the equation:

\[ M_g + M_g \rightleftharpoons D_g \]  

(1)

where $k_1$ and $k_{-1}$ are the rate constants of dimerization and dissociation, respectively. $I'$ is proportional to the two-dimensional concentration, $D_{gA}$, of the gramicidin dimer within the bilayer:

\[ I' = D_{gA} \cdot A_{gA} \]  

(2)

where $A_{gA}$ denotes the surface area of the planar bilayer and $g$ the single-channel conductance of $gA$. Hence, an increment in $I'$ upon photoisomerization of OptoDArG, $\Delta I' = I'_c - I'_t$, with the suffixes “cis” and “trans” indicating the presence of cis- and trans-OptoDArG, respectively, reports on a change in the absolute number of conducting dimers (Fig. 2). Synchronously, we recorded planar bilayer capacitance, $C$, for it reports on changes in bilayer hydrophobic thickness, $d_{hc}$, upon photoisomerization:

\[ C = \frac{A_{gA}}{d_{hc}} \]  

(3)

where $\varepsilon_0$ and $\varepsilon_r$ designate vacuum and relative permittivity, respectively. As indicated in Fig. 2, synchronous alterations in $I'$ and $C$ remained reversible for multiple cycles of photoisomerization (effectively until membrane rupture); notably, relative increases in $I'$ were larger by far than those in $C$ upon switching to cis-OptoDArG. It is important to note that in our experiments, $\Delta I'$ was not determined by alterations in (i) $A_{gA}$, which is addressed below, and (ii) $g$, which we excluded by $gA$ single-channel recordings in planar bilayers comprising 10 mol% cis- or trans-OptoDArG.

Initially, with OptoDArG’s azobenzene moieties in their trans configuration, $I'_t$ amounts to $\sim 20$ nS (Fig. 2), indicating $\sim 1700$ $gA$ dimers with a single-channel conductance of 12 pS. Photoisomerization to cis-OptoDArG results in $I'_c \approx 35$ nS, whereby the increment $\Delta I' = 15$ nS corresponds to the presence of $\sim 1200$ additional dimers; this requires an initial excess of monomers. Upon assuming an equilibrium association constant $K = k_1/k_{-1}$ of 1 x $10^{14}$ cm$^2$ mol$^{-1}$ obtained in decane-containing dioleoyllecithin planar bilayers [44], we estimate the number of monomers in the presence of $\sim 1700$ dimers to be $\sim 28,000$ – i.e., a sufficient reservoir for the formation of thousands of further dimers.

For $gA$, changes in $K$ report on alterations in bilayer hydrophobic thickness, $d_{hc}$, upon photoisomerization [8] since:

\[ \Delta G_{m1} = \Delta G_{m} = -k_B T \ln K \]  

(4)

where $k_B$ is Boltzmann’s constant and $T$ is the absolute temperature. Attributing $\Delta I'$, i.e., a change in the number of conducting dimers upon photoisomerization, to a change in $K$ requires that this change is not quantitatively accounted for by a concomitant change in the number of $gA$ monomers available for dimer formation. The following considerations show that this requirement is fulfilled. Firstly, there is only a negligible photoisomerization effect on (i) the aqueous-bilayer partition coefficient of $gA$ monomers, and (ii) $A_{gA}$ which is important because an increment in $A_{gA}$ would allow the partitioning of further monomers even at constant monomer concentration $M_{gA}$. (i) takes into account that hydrophobicity and volume of solute and solvent remain unchanged. (ii) is supported by the observation that changes in $C$ can be largely attributed to changes in $d_{hc}$ – as evidenced and explained by the micropipette aspiration experiments outlined below.

We demonstrated reversible photoisomerization-induced changes of the surface area, $A_{GUV}$, of giant unilamellar vesicles of the same lipid composition (90 mol% DOPC +10 mol% OptoDArG, without $gA$) using the micropipette aspiration technique [38, 45]. Keeping both suction pressure and internal volume constant, we observed an increase of the protrusion length in the suction pipette upon photoisomerization from trans- to cis-OptoDArG (Fig. 3A, B), corresponding to a relative increase in $A_{GUV}$ of $\sim 4\%$ (Fig. 3C). The relative decrease in GUV bilayer thickness, $d$, was similar since lipid bilayers are incompressible fluids [46]. $d_{hc}$ is the bilayer thickness diminished by twice the thickness of the headgroup region, $d_{hp}$, of each leaflet: $d_{hc} = d - 2 \times d_{hp}$. The decrease in thickness of a planar bilayer under equally quantitative photoisomerization has to be the same. We note that the observed relative photoisomerization-induced changes in $d$ (Fig. 3C) and $C$ (Fig. 2) are roughly similar. Accordingly to Eq. (3), we may thus entirely attribute the observed changes in $C$ to changes in $d$. The observation suggests that $A_{gA}$ did not change appreciably upon photoisomerization. Consequently, photoswitching did not push the hexadecane torus to the outside. In conclusion, we attribute $\Delta I'$ observed in the presence of $gA$ (Fig. 2) to photoisomerization-induced changes in $K$.

Importantly, the observed increases in $gA$ activity upon bilayer-thinning induced by photoisomerization from trans- to cis-OptoDArG agree with prior observations; mechanistically, a reduction in $d_{hc}$ decreases the positive hydrophobic mismatch between the $gA$ dimer and the surrounding lipids, thereby reducing the magnitude of imposed bilayer deformations [8].

We conducted further recordings analogous to Fig. 2 with planar bilayers folded from 90 mol% DOPC or DPhPC with 10 mol% OptoDArG each; this allowed plotting the experimentally observed fractional increases in conductance, $I'_c/I'_t$, against the respective hydrophobic thicknesses of bilayers thinned by cis-OptoDArG (Fig. 4). The photolipid-mediated effect on $C$ and $I'$ was present in both lipid mixtures. We attribute variations in (i) $I'_c/I'_t$ to variations in $D_{gA}$, and (ii) $C$ to distinct effective concentrations of the photolipid within the membranes as well as differences in light intensity projected onto the bilayer.

A linear fit to $\ln(I'_c/I'_t)$ over $d_{hc,cis}$ yields a slope with an absolute value of $\sim 4$ nm$^{-1}$ for both lipid mixtures (Fig. 4). Interestingly, a previously reported slope linking $gA$ single-channel lifetime, $r$, and $d_{hc}$ as In ($r$) over $d_{hc}$ was twice as large [8]. The observation suggests that photoisomerization-induced bilayer-thinning makes a major contribution to the increase in $D_{gA}$. However, other factors, like alterations in $J_0$ and $B$ upon photoisomerization may have also contributed. In turn, $k_1$ may have changed. $k_1$ is related to $I'$ and $r$:

\[ \frac{I'_c}{I'_t} \approx \frac{k_{cis}}{k_{trans}} \cdot \frac{r_{cis}}{r_{trans}} \]  

(5)

where the indices “cis” and “trans” indicate parameters in the presence of cis- and trans-OptoDArG. We arrived at Eq. (5) starting from an expression for the fractional change of $K$ upon trans to cis.
photosomerization: $\frac{K_{\text{cis}}}{K_{\text{trans}}} = \frac{(k_1^{\text{cis}} \cdot k_{\text{lr}}^{\text{cis}})}{(k_1^{\text{trans}} \cdot k_{\text{lr}}^{\text{trans}})}$, since $\tau = 1/k_{\text{lr}}^{\text{cis}}$. At the same time: $K_{\text{cis}}/K_{\text{trans}} = (D_{\text{GCA}}^{\text{cis}} \cdot M_{\text{GCA}}^{\text{trans}})/(D_{\text{GCA}}^{\text{trans}} \cdot M_{\text{GCA}}^{\text{cis}})$, because we assumed that $M_{\text{GCA}}^{\text{trans}} = M_{\text{GCA}}^{\text{cis}}$. Finally, we calculated weighted mean $d_{\text{hc,cis}}$ from data on capacitance changes recorded in single-channel experiments; the weights were the respective number of single-channel events contributed to the pooled datasets underlying the survivor curves in Fig. 6. We inserted those values into the linear models displayed in Fig. 4 obtaining $U_{\text{cis}}/U_{\text{trans}} = 1.8$ (DOPC/OptodArG) and 1.9 (DPhPC/OptodArG). The observation that both values are larger than 1 supports the notion that photosomerization of the photolipid has an effect on $k_1$ (Eq. (5)).

**3.1. SAXS Reports on Precipitous Changes in B Upon Photosomerization**

We expected an effect of photosomerization on $B$ of bilayers with embedded OptodArG, as has been reported for another photolipid [49]. Hence, we sought to reassure this prospect via SAXS on multilamellar vesicles composed of 90 mol% DOPC + 10 mol% OptodArG. Changing the conformation of OptodArG from trans to cis and back to trans led to small, yet significant shifts of the Bragg peak positions (Fig. 7A). They correspond to changes of the lamellar repeat distance, $d_{\text{hc}} \approx 0.7 \text{ Å}$. Moreover, the peak position shifted back to almost the identical $q$ value after OptodArG was isomerized back to its cis state. An analysis in terms of a full $q$-range model [42], indicated that these changes are significantly different from each other as the absolute error associated with $r$ in these measurements was relatively large. In pure DOPC and DPhPC bilayers $r$ amounted to $r_{\text{DOPC}} = 3.6 \pm 1.1$ s and $r_{\text{DPhPC}} = 4.5 \pm 1.5$ s (both mean ± standard deviation; $N_{\text{DOPC}} = 6, N_{\text{DPhPC}} = 5$).

We only determined average $\tau$ values as we constructed the survivor plots from pooled datasets containing data from membranes with different $d_{\text{hc,cis}}$ (Fig. 6). In contrast, we have distributions of $\Gamma_{\text{cis}}/\Gamma_{\text{trans}}$ (Fig. 4) – we aim to reduce them to singular values (one for each lipid mixture), allowing us to solve Eq. (5) for $k_1^{\text{cis}}/k_1^{\text{trans}}$. Therefore, we calculated weighted mean $d_{\text{hc,cis}}$ from data on capacitance changes recorded in single-channel experiments; the weights were the respective number of single-channel events contributed to the pooled datasets underlying the survivor curves in Fig. 6. We inserted those values into the linear models displayed in Fig. 4 obtaining $U_{\text{cis}}/U_{\text{trans}} = 1.8$ (DOPC/OptodArG) and 1.9 (DPhPC/OptodArG). The observation that both values are larger than 1 supports the notion that photosomerization of the photolipid has an effect on $k_1$ (Eq. (5)).

Fig. 4. Photosomerization-induced changes in $\Gamma$ as function of photolipid-thinned bilayer hydrophobic thickness, $d_{\text{hc,cis}}$. $\Gamma_{\text{cis}}/\Gamma_{\text{trans}}$ denotes the fractional change in integral bilayer conductance due to $g_A$ upon photoswitching from trans- to cis-OptodArG. The corresponding fractional changes in bilayer capacitance, $C_{\text{cis}}/C_{\text{trans}}$, were used for calculating bilayer hydrophobic thicknesses with cis-OptodArG, $d_{\text{hc,cis}}$. $d_{\text{hc,cis}} = d_{\text{hc,trans}} \cdot C_{\text{cis}}/C_{\text{trans}}$. Thereby, we assumed that the addition of nominally 10 mol% trans-OptodArG did not affect bilayer hydrophobic thickness, i.e. $d_{\text{hc,trans}} = 2.77 \text{ nm}$ [47] for DOPC/trans-OptodArG and $d_{\text{hc,trans}} = 2.78 \text{ nm}$ [48] for DPhPC/trans-OptodArG. Linear least-squares fits yielded a slope of $-3.8 \text{ nm}^{-1}$ and $4.1 \text{ nm}^{-1}$ for DOPC and DPhPC bilayers thinned by 10 mol% OptodArG, respectively.

Fig. 5. Representative single-channel current traces. Depicted are representative single-channel current traces of $g_A$ recorded from voltage-clamped planar bilayers composed of 90 mol% DOPC or DPhPC with 10 mol% OptodArG each, in its trans or cis configuration, respectively. Note the seemingly increased single-channel dwell times in the presence of cis-OptodArG.

Fig. 3. Photosomerization-induced bilayer-thinning increased the surface area of GUVs, $A_{\text{GUV}}$. (A) Fluorescence micrograph of a microaspirated GUV composed of $-90$ mol% DOPC, 10 mol% trans-OptodArG, 0.004 wt% Atto633-DPPE. Gray bars indicate the aspiration pipette. (B) Exposure to light at 365 nm transfers the photolipids into their cis conformation. (C) Kinetics of relative area change, $\alpha$. The black and white bars on top indicate illumination at 365 nm and 450 nm, respectively. The baseline exhibits a drift, conceivably because the number of photolipids able to perform the conformational transition back to the trans-state decreases with time.
mostly due to the thinning of the bilayer (Table 1). We note that the shifts in single-channel lifetime, $\tau$, upon photoisomerization. Survivor curves were constructed from gA single-channel events obtained within four distinct lipid environments (distinguished by the main structural lipid, i.e. 90 mol% DOPC (upper panel) or DPhPC (lower panel), with nominally 10 mol% OptoDArG each, in its trans (red) or cis (blue) state, respectively). $N(t)/N(0)$ represents the fraction of channels that is still open after time $t$. The curves were fitted by an exponential model of the form $N(t)/N(0) = \exp(-t/\tau)$ and yielded the following lifetimes (number of underlying single-channel events, $N(0)$, in brackets): DOPC/cis-OptoDArG: 3.4 s (152); DOPC/trans-OptoDArG: 6.0 s (115); DPhPC/cis-OptoDArG: 4.1 s (131); DPhPC/trans-OptoDArG: 6.2 s (123). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

We find $B_{cis}/B_{trans} \approx 0.5$, suggesting that transferring OptoDArG into its cis configuration halves $B$.

Conceivably, such pronounced reduction in $B$ contributed to the observed increase in gA activity. It is expected to reduce the free energy penalty associated with bending annular lipids to accommodate the short gA dimer within a thicker bilayer. Notably, the bilayer-softening effect of OptoDArG is more pronounced than what would be expected from a mere reduction in acyl chain length [51]: reducing $d_{hc}$ by 4% reduces $B$ by only 8% since $B_2/B_1 \approx (d_{hc,2}/d_{hc,1})^2$.

However, the strongly reduced $B$ in cis-OptoDArG-containing bilayers should have steepened the slope of $\ln(I_{cis}/I_{trans})$ over $d_{hc}$ (Fig. 4). Our observation of a twofold decrease in slope in comparison to literature data [52] suggests an opposing effect of spontaneous monolayer curvature, $J_0$.

### 3.2. Calculating Spontaneous Monolayer Curvature, $J_0$

Considering the small head of cis-OptoDArG and the comparatively large cross-sectional area of its hydrophobic tails (Fig. 1), cis-OptoDArG was expected to impose a negative $J_0$ to monolayers that are otherwise built from cylindrical lipids. We suspected that we could calculate $J_0$, since the theoretical framework of elastic membrane deformations reflects the energetics of gA gating [34]. Thus, in $J_0$ we sought to obtain a bilayer interactions, i.e. van der Waals and hydration forces, are not affected by the conformational changes of OptoDArG. Consequently, the bulk compressibility moduli, $K_s$, must be equal to each other in the cis and trans states, i.e. $K_{s,trans} = K_{s,cis}$. Using Caillé theory [50], we thus may estimate the relative change of $B$ from the corresponding changes of $\eta$ (Table 1):

$$\eta = \frac{xk_b T}{2d_{hc}^2 \sqrt{K_s B}} \quad \text{(6)}$$

Comparatively, we sought to obtain a bilayer thickness, $d_{hc}$, of the water layer ($d_{wl} = d_{hc} - d$), we assume that bare bilayer interactions, i.e. van der Waals and hydration forces, are not affected by the conformational changes of OptoDArG. Consequently, the bulk compressibility moduli, $K_s$, must be equal to each other in the cis and trans states, i.e. $K_{s,trans} = K_{s,cis}$. Using Caillé theory [50], we thus may estimate the relative change of $B$ from the corresponding changes of $\eta$ (Table 1):

$$\eta = \frac{xk_b T}{2d_{hc}^2 \sqrt{K_s B}} \quad \text{(6)}$$

Conceivably, such pronounced reduction in $B$ contributed to the observed increase in gA activity. It is expected to reduce the free energy penalty associated with bending annular lipids to accommodate the short gA dimer within a thicker bilayer. Notably, the bilayer-softening effect of OptoDArG is more pronounced than what would be expected from a mere reduction in acyl chain length [51]: reducing $d_{hc}$ by 4% reduces $B$ by only 8% since $B_2/B_1 \approx (d_{hc,2}/d_{hc,1})^2$.

However, the strongly reduced $B$ in cis-OptoDArG-containing bilayers should have steepened the slope of $\ln(I_{cis}/I_{trans})$ over $d_{hc}$ (Fig. 4). Our observation of a twofold decrease in slope in comparison to literature data [52] suggests an opposing effect of spontaneous monolayer curvature, $J_0$.

### Table 1

| Sequence | Conformation | $d_{hc}$ (Å) | $\delta$ (Å) | $d_{wl}$ (Å) | $\eta$ |
|----------|--------------|---------------|--------------|--------------|--------|
| 1        | trans        | 65.0 ± 0.1    | 47.7 ± 0.6   | 17.3 ± 0.6   | 0.10 ± 0.05 |
| 2        | cis          | 64.1 ± 0.1    | 47.0 ± 0.6   | 17.1 ± 0.6   | 0.14 ± 0.05 |
| 3        | trans        | 64.8 ± 0.1    | 48.3 ± 0.6   | 16.5 ± 0.6   | 0.10 ± 0.05 |
|          |              | 0.5 ± 0.5     | 0.6 ± 0.05   |              |        |

![Fig. 6](image1)

**Fig. 6.** Shift in single-channel lifetime, $\tau$, upon photoisomerization. Survivor curves were constructed from gA single-channel events obtained within four distinct lipid environments (distinguished by the main structural lipid, i.e. 90 mol% DOPC (upper panel) or DPhPC (lower panel), with nominally 10 mol% OptoDArG each, in its trans (red) or cis (blue) state, respectively). $N(t)/N(0)$ represents the fraction of channels that is still open after time $t$. The curves were fitted by an exponential model of the form $N(t)/N(0) = \exp(-t/\tau)$ and yielded the following lifetimes (number of underlying single-channel events, $N(0)$, in brackets): DOPC/cis-OptoDArG: 3.4 s (152); DOPC/trans-OptoDArG: 6.0 s (115); DPhPC/cis-OptoDArG: 4.1 s (131); DPhPC/trans-OptoDArG: 6.2 s (123). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

![Fig. 7](image2)

**Fig. 7.** Effects of photoswitching OptoDArG as observed by SAXS. (A) Global analysis of DOPC MLVs containing 10 mol% OptoDArG ($T = 25 \degree C$). Solid lines correspond to the best fits (green: trans; blue: cis; red: trans after switching back). The insert shows experimental data for the first diffraction order only, demonstrating the shift induced by photoswitching. (B) Shown are the first order reflections normalized to the peak maximum, demonstrating the different intensity decays due to changes in the bilayer fluctuations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
parameter important to membrane mechanics from the response of a reporter peptide in photoswitchable membranes. Typically, \( J_0 \) is obtained by SAXS analysis of fully hydrated stress-free inverse hexagonal (H\(_\text{II} \)) phases established from binary mixtures with H\(_\text{II} \)-forming lipids such as DOPE or DAG [53,54].

Our calculated \( J_0 \) values \((J_{\text{DOPE}} = -0.19 \text{ nm}^{-1}, J_{\text{DPhPC}} = -0.17 \text{ nm}^{-1}, J_{\text{DAG-trans}} = -0.18 \text{ nm}^{-1}, J_{\text{DAG-cis}} = -1.1 \text{ nm}^{-1}, \) see Appendix) should be viewed with a grain of salt, as (i) we obtained changes in \( B \) and \( \Gamma \) under distinct illumination conditions, (ii) photosomerization may, in contrast to our assumption, alter the bending rigidity differently for DOPE and DPhPC bilayers, (iii) the variability of both capacitance and conductance after photosomeration hampered the accuracy of our calculations. Nevertheless, the \( J_0 \) values are reasonably close to literature data. The resulting \( J_{\text{DAG-cis}} \approx 1 \text{ nm}^{-1} \) is conceivably similar to that of dioleoylglycerol, whilst \( J_{\text{DAG-trans}} \approx 0.18 \text{ nm}^{-1} \). The spontaneous curvature of DOPE deviates from the experimentally measured values: \(-0.091 [53] \) and \(-0.114 \text{ nm}^{-1} [55] \). The spontaneous curvature of OptoDAG in trans configuration is close to DPhPCs one, which seems reasonable, as both lipids have tails whose volume is somewhat increased with respect to unbranched saturated lipid tails. The absolute value of the spontaneous curvature of OptoDAG in cis configuration is expectedly high, as its molecular shape becomes much more conical and similar to that of dioleoylglycerol, whose spontaneous curvature was reported to be \( \approx 1 \text{ nm}^{-1} [56] \). The spontaneous curvature of DPhPC is close to the experimentally observed values: \( J_0 \approx 0.21 \text{ nm}^{-1} [57] \).

4. Conclusions

We demonstrated that in situ photosomeration of a photoswitchable lipid reversibly gates the embedded molecular force probe gA by altering bilayer material properties, hence emphasizing the applicability of photolipids such as OptoDAG as \textit{bon a fide} regulators of mechanosensitive membrane proteins. We monitored changes in bilayer capacitance upon photoswitching to infer alterations in bilayer hydrophobic thickness and supported these results by imaging aspirated GUVs. The rise in gA activity upon photolipid-induced bilayer-thinning falls short by a factor of two from previously published data [52] on the dependence of single-channel lifetime on bilayer hydrophobic thickness. The literature data did not analyze to what extend alterations of both \( B \) and \( J_0 \) confounded this dependence. In contrast, we know that (i) the rigidity decreases and (ii) negative curvature increases upon switching from \textit{trans}- to \textit{cis}-OptoDAG. Thus, while (i) would amplify the effect exerted by bilayer thinning, (ii) counteracts it.

Jointly, we subjected our experimental findings to photoinduced changes in (i) bilayer material parameters, i.e. \( \delta_{\text{Bil}} \) and \( B \), and (ii) gA activity, i.e. \( \Gamma \) and \( \tau \), to a theoretical physical model. \( J_0 \) calculations of the constituent lipids based on the continuum theory of bilayer elasticity demonstrate the framework’s applicability for the understanding of individual forces relevant for the gating of proteinaceous MS channels.

Photoinduced alterations of the mechanical parameters \( \delta_{\text{Bil}}, J_0 \) and \( B \) may well elicit responses from various MS channels. For example, \( J_0 \) is known to alter the gating characteristics of the bacterial mechanosensitive channels large (MsCl) [58] and small (MsCl5) [59]. \( B \) influences the response of the mammalian MS channel Piezo1 to membrane tension [60] because Piezo1 shapes the membrane outside its channel perimeter into a curved ‘membrane footprint’. Our work paves the way for deploying photolipids for rapidly generating photoswitchable membranes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphotobiol.2021.112320.

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