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How to cite: *Angew. Chem. Int. Ed.* **2025**, *64*, e202420232
doi.org/10.1002/anie.202420232

Photochemistry **Hot Paper**

Control of Bilayer Transport through a Photoswitchable Membrane-Stiffening Agent

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Abstract: The mobility of proteins in the bilayer membrane is affected by (local) changes in lipid environment, which is important to their biological functioning. Artificial molecular systems that—to some extent—imitate tasks of membrane-embedded proteins are increasingly developed, however, they are usually controlled through responsive units in their core structure. Here we present an alternative approach based on an amphiphilic stiff-stilbene derivative that enables control of membrane fluidity by light. The fluidity increase upon *E*-to-*Z* isomerization is shown to enhance the activity of known synthetic anion transporters as a result of a higher mobility. The photoisomerization process is studied by UV/Vis and ^{1}H NMR spectroscopy in solution and in POPC vesicles, where the light-induced changes in fluidity and hence, activity of anion transporters, are monitored by fluorescence spectroscopy. Dynamic light-scattering (DLS) and cryo-EM studies show that vesicle integrity is not impaired by photoswitching. Our work introduces a versatile approach to control solute transport by carrier molecules. Moreover, the photocontrol over membrane fluidity and, with that, mobility could eventually be used for directed motion, which we expect to be key in achieving active transport in the future.

Membrane-embedded proteins are involved in many important cellular processes, such as ion transport, cell signaling and recognition.[1] These proteins vary in mobile states, ranging from being immobile to undergoing random (Brownian) motion, which is dependent on their size/shape and aggregation behavior. Importantly, protein mobility is known to be influenced by changes in (microdomain) lipid composition, and relates to biological function.[2]

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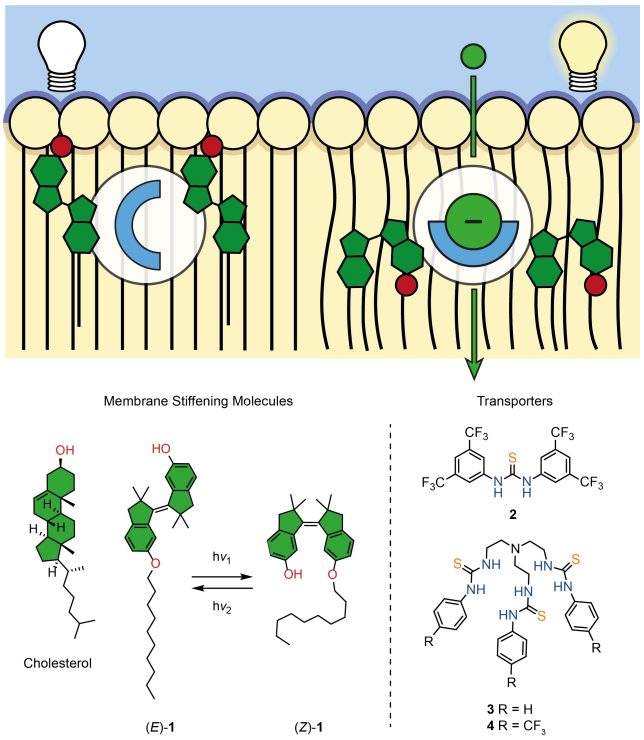
An area of significant interest is the development of photoswitchable amphiphiles.[3] Some of these are capable of self-assembling into bilayer nanostructures solely,[4] where others have been blended with naturally occurring phospholipids to form liposomes.[5] Photoisomerization of these amphiphiles was shown to cause morphological changes, nanostructure disassembly, or increased bilayer permeability (to release cargo). Particularly worth noting here are the azobenzene-appended phospholipids reported by the groups of Trauner and Lohmüller, which were used to modulate various bilayer membrane properties by light,[6] which was additionally shown to affect the diffusion of membrane-embedded proteins.[7]

Since the malfunctioning of transport proteins has been associated to serious illnesses, many efforts have been devoted to creating fully artificial bilayer transport systems, in particular for anions.[8] In recent years, the focus has shifted towards making these systems responsive to environmental stimuli,[9] for example, to allow local (de)activation when used as therapeutics or as physiological tools to study faulty transport-related diseases. Current designs that allow switching of transport activity are mostly based on carriers of which the binding affinity for chloride can be modulated by stimuli such as pH change, light, or redox agents.[10] Hence, these carriers always contained the responsive unit in their core structure. Yet, we envisioned that influencing the performance of a synthetic transporter via subtle, stimuli-induced changes in membrane properties would provide a more versatile method to control the activity.

It is well known that cholesterol can stiffen lipid membranes, which has been shown to affect the activity of synthetic anion transporters.[11] Cholesterol—composed of a rigid core, a polar head-group, and flexible alkyl tail (Scheme 1)—inserts directionally into the bilayer, as the alcohol group favorably interacts with the polar head-groups of the phospholipids, and the rigid core and alkyl tail are preferentially situated within the phospholipid fatty-acid chain region.[1] We aimed to develop a stimuli-responsive stiffening agent and considered the use of a stiff-stilbene photoswitch as its core. This photoswitch is advantageous for this purpose as it has a high structural rigidity, excellent thermal stability, and undergoes a large geometrical change upon isomerization,[12,13] More specifically, we selected a stiff-stilbene derivative having dimethyl substituents in the five-membered rings, which has been shown to improve photostationary state (PSS) ratios and fatigue resistance.[10j,14] Its functionalization with alcohol and alkyl groups was expected to afford a photoswitchable mem-



Scheme 1. Schematic representation of photocontrol over membrane fluidity and transport using a photoswitchable membrane-stiffening agent and chemical structures of cholesterol and compounds **1**–**4** used in this study.

brane-stiffening agent, of which isomerization would impact membrane fluidity and with that, the mobility of membrane-embedded transmembrane transporters. This would enable a new approach towards activity control and furthermore, such control of membrane mobility could be used in the future to steer the motion of transporters in membranes, ultimately facilitating active transport.

Here we describe photoswitchable membrane stiffening agent **1** (Scheme 1), whose isomers can be interconverted with UV light. We demonstrate that its insertion into POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) vesicles enables control of membrane fluidity, without impairing vesicle bilayer integrity. Further, the influence of this change in fluidity on the activity of known non-responsive anion transporters **2**–**4** is investigated. Our results show that—in case the transport activity is diffusion limited—it is higher in the more fluid membrane.

Membrane-stiffening agent (*E*)-**1** was synthesized starting by McMurry homocoupling of dimethylated and TBDMS-protected 6-hydroxy-1-indanone (Scheme S1 in the Supporting Information). This reaction afforded the (*E*)-isomer as the major product alongside trace amounts of (*Z*)-isomer, which could be easily separated by column chromatography (isolated yield 67 % and 3 %, respectively). Subsequent silyl deprotection of the fraction of (*E*)-isomer using TBAF, followed by mono-alkylation with bromodecane, gave the desired product (See the Supporting Information for synthetic details and characterization).

During the course of the synthesis, single crystals suitable for X-ray structure determination were obtained of the TBDMS-protected (*Z*)-stiff-stilbene and the bis-hydroxy (*E*)-stiff-stilbene intermediates by cooling concentrated solutions in $CHCl_3$. The crystal structures illustrate the large difference in dihedral angle and oxygen-oxygen interatomic distance between isomers (Figure 1).[15] Where in the (*E*)-isomer the central dihedral angle (C_{Ar}–C=C–C_{Ar}) is −168.29(13)° and resultantly, the oxygen atoms are relatively far apart from each other at a distance of 10.0589-(14) Å,[16] in the (*Z*)-isomer this dihedral angle is −17.92(19) and the oxygen atoms are positioned closer to each other at a distance of 5.4921(13) Å. These structures highlight that photoswitching between isomers of **1** would drastically change the relative position of the polar hydroxyl head group and the apolar alkyl tail, as well as the volume occupancy of the molecule. Therefore, a large change in (membrane stiffening) properties is expected upon isomerization.

First, the photoswitching behavior was investigated by UV/Vis spectroscopy in DMSO. Compound (*E*)-**1** showed an absorption maximum at $\lambda = 350$ nm (Figure 2A). Upon irradiation with 340 nm light, this maximum decreased, whereas the longer wavelength absorption increased, which is indicative of *E*-to-*Z* isomerization.[12–14] Subsequent irradiation with 395 nm light gave the reverse spectral changes, revealing that the (*E*)-isomer was (partially) regained. Importantly, a clear isosbestic point was maintained at $\lambda =$ 368 nm (Figures S13 and S14 in the Supporting Information), and alternating use of 340 and 395 nm light showed excellent fatigue resistance over multiple cycles (Figure 2B).

The photostationary state (PSS) ratios were determined by ^{1}H NMR spectroscopy in DMSO-d_6. Here, irradiation of (*E*)-**1** with 340 nm light led to the appearance of a new set of signals corresponding to the (*Z*)-isomer, and subsequent irradiation with 395 nm light resulted in an increased integral ratio of the original signals of the (*E*)-isomer, confirming back isomerization (Figures S11 and S12 in the Supporting Information). By ^{1}H NMR signal integration, PSS_{340} and PSS_{395} (*E*/*Z*) ratios of 20:80 and 75:25 were determined, similar to the ratios reported for other stiff-stilbene derivatives containing methyl substituents in the five-membered rings.[10j,14]

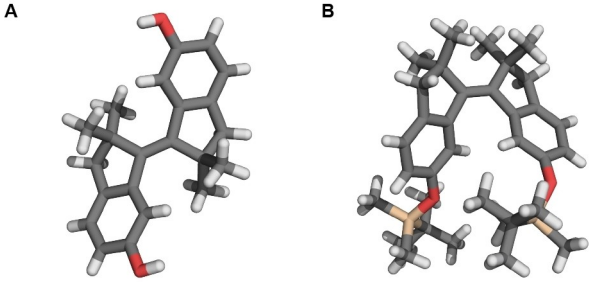


Figure 1. Single crystal X-ray structures of the (*E*)-isomer of bis-hydroxy stiff-stilbene **S5** (A) and the (*Z*)-isomer of TBDMS-protected stiff-stilbene **S4** (B), shown in stick representation.

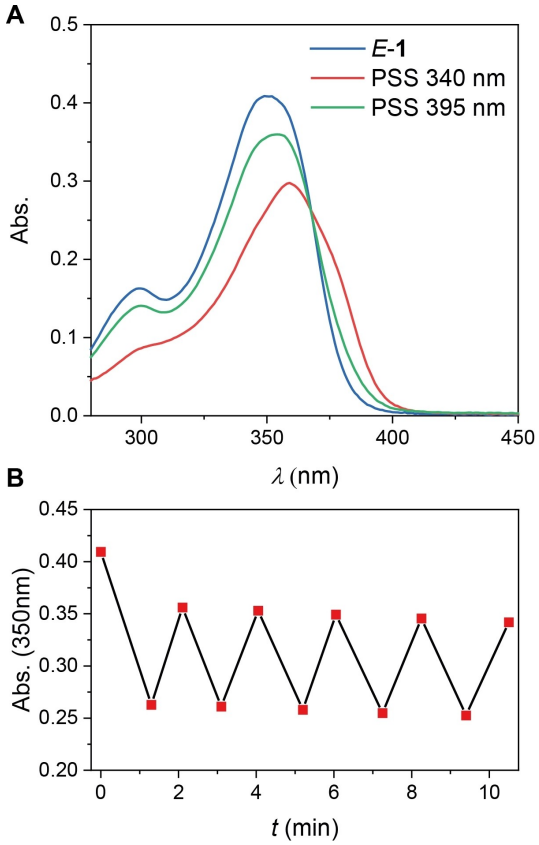


Figure 2. UV/Vis spectral changes of (*E*)-**1** (2.0×10^{-5} M) in DMSO upon irradiation with 340 nm and 395 nm light (A), and the change in absorption (at $\lambda = 350$ nm) upon multiple 340/395 nm irradiation cycles (B).

Subsequently, the quantum yields for 340 nm light induced isomerization were determined by using potassium ferrioxalate as actinometer. From the photon flux and the rate of formation of (*Z*)-**1** from (*E*)-**1** (Figures S20 and S21 in the Supporting Information), the quantum yield for *E*-to-*Z* isomerization was calculated as $\Phi_{E\rightarrow Z} = 40.5 \pm 0.72\,\%$. Using the PSS_{340} ratio and molar absorptivities, it was determined that the reverse *Z*-to-*E* isomerization process has a quantum yield of $\Phi_{Z\rightarrow E} = 26.9 \pm 0.48\,\%$.

Next, we investigated whether photoswitching is still feasible when (*E*)-**1** is embedded in the lipid bilayer membrane. Therefore, it was incorporated in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) vesicles at a desired molar ratio of 2:8 (**1**/POPC), and an incorporation efficiency of 94 % was calculated (Figures S15 and S16 in the Supporting Information). The isomerization process in the vesicles was followed with UV/Vis spectroscopy. Again, irradiation with 340 nm light led to a decrease in the absorption maximum and an increase in the longer wavelength absorption, while subsequent irradiation with 395 nm light gave the reverse spectral changes (Figures S17–S19 in the Supporting Information). Also, when (*E*)-**1** was embedded in the vesicles, multiple switching cycles could be performed with limited signs of fatigue. Importantly, photoswitching did not lead to any significant change in size of the vesicles as measured by dynamic light scattering (DLS) (Figure S22 in the Supporting Information). Moreover, no significant differences were observed between vesicles containing either (*E*)-**1** or (Z_{PSS})-**1** by cryo-transmission electron microscopy (cryo-EM) (Figure S23 and S24 in the Supporting Information). These results demonstrate that photoswitching of **1** does not affect the integrity of the vesicles.

We then assessed the effect of photoswitching on the bilayer membrane properties using the polarity-sensitive fluorescent probe 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan).[17] In tightly packed membranes, where hydration is low, this probe exhibits an emission maximum around 440 nm. Yet, in more fluid, and thus more hydrated, membranes this maximum is red-shifted to around 490 nm. Even though it constitutes an indirect method as the emission shift relates to variations in water content, taking the ratio between the intensity of the short and long wavelength emissions allows quantification of the change in membrane fluidity. This ratio is defined as the general polarizability (GP) value. When Laurdan was pre-incorporated in vesicles containing (*E*)-**1** a GP value of 0.03 was found (Figure S25 in the Supporting Information). After irradiation of these vesicles with 340 nm light to generate (Z_{PSS})-**1**, the GP value dropped to −0.06, indicating that the membrane had become more fluid.[18]

Since for artificial chloride transporters, it has often been shown that membrane-stiffening by doping with cholesterol affects their transport activity,[11] we set out to determine whether the fluidity change induced by photoisomerization of **1** enables activity control. This would require the use of a transporter of which the activity is dependent on its rate of diffusion across the bilayer membrane, which most likely relates to the transport mechanism by which it operates. We selected widely used chloride transporters **2**–**4** (see Scheme 1) for our transport studies, as their mechanisms were extensively studied by Gale and co-workers.[19] They found that bis(CF_3)-substituted thiourea transporter **2** is not selective for either Cl^- uniport H^+/Cl^- symport (or Cl^-/OH^- antiport), whereas tren-based tris-thiourea **3** is selective for Cl^- uniport, and CF_3-substituted tren tris-thiourea **4** is selective for H^+/Cl^- symport (or OH^-/Cl^- antiport).

The transport studies were performed using POPC vesicles that had (*E*)-**1** incorporated in the bilayer and that were loaded with the pH-sensitive dye 8-hydroxypyrene-1,3,6-trisulfonate (HPTS).[20] These vesicles were suspended in an aqueous NaCl solution buffered to pH 7.0 with HEPES, and the transporters were added to this solution from DMSO, after which a pH gradient was applied across the membrane through addition of a base pulse (NaOH). The ability of the transporters to dissipate this pH gradient through H^+/Cl^- symport (or OH^-/Cl^- antiport) was then monitored by the change in HPTS emission. The activity of the transporters was quantified by Hill analysis to determine the half-maximal effective concentrations (EC_{50}, expressed in mol % with respect to lipids, see Figures S26–S34 in the Supporting Information).[21] Compound **2** showed good transport capability, and interestingly, after 340 nm irradi-

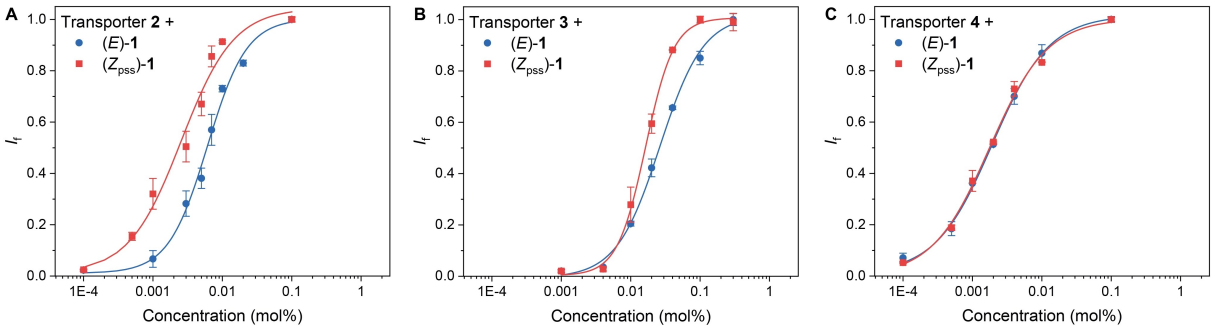


Figure 3. Normalized Hill plots of transporter **2** (A), transporter **3** (B) and transporter **4** (C) in vesicles containing (*E*)-**1** or (Z_{PSS})-**1**.

ation of the vesicles to generate (Z_{PSS})-**1** in situ, the activity increased by 2.4 fold (Figure 3A and Table 1). To rule out that this difference is (partially) due to altered membrane partitioning—instead of membrane mobility—as has been suggested in studies using cholesterol-doped POPC vesicles,[11b,e] we performed additional studies where **2** was first allowed to incorporate in vesicles with (*E*)-**1**, followed by irradiation to (Z_{PSS})-**1**. Gratifyingly, this gave no observable difference in activity, indicating that membrane incorporation is unchanged upon *E*-to-*Z* isomerization of the membrane-stiffening agent (Figure S35 in the Supporting Information).

For **3**, the activity increased by 1.6 fold after irradiation of the vesicles containing (*E*)-**1**. In stark contrast, the activity of **4** was virtually the same before and after irradiation of the vesicle solution. These results indicate that the activities of **2** and **3** are limited by diffusion, while the rate-limiting step in the transport mediated by **4** appears to be another process than its shuttling between the inner and outer membrane interfaces. Given that **4** is a potent HCl transporter, a tentative explanation is that its (de)protonation kinetics are rate-limiting instead. It should be noted that similar observations were made in other studies where POPC vesicles where doped with cholesterol. That is, not in all cases membrane-stiffening by cholesterol reduced transport activity.[11b,d,e] Whether the activity of a transporter shows dependency on membrane fluidity is thus determined by the specifics of the mechanism under which it operates. In other words, activity is only influenced when it is limited by diffusion and not by the rate of (de)protonation or anion complexation. To our best knowledge, this aspect still needs to be explored in greater depth in the field of transmembrane (anion) transport. Furthermore, detailed investigation will be needed to determine if there is a more general role for Cl^- uniport vs H^+/Cl^- symport in fluidity dependency, as we observed in our case.

In short, we have demonstrated that insertion of a photoswitchable membrane-stiffening agent into the lipid bilayer offers control of fluidity, without impairing vesicle integrity or changing its morphology. That is, POPC vesicles containing the (*E*)-isomer of the membrane-stiffening agent showed an increased membrane fluidity upon irradiation to generate the (Z_{PSS})-isomer. In addition, we used this fluidity change to control the activity of (diffusion limited) known anion transporters. Our work represents the first example of photocontrol of membrane fluidity as a means to modulate the activity of non-responsive transporters, which is expected to be of great importance in the future design of stimuli-controlled and -driven transport systems. We expect that control of the mobility of molecular receptors in the bilayer will ultimately facilitate the route towards directed motion and active transport.

Table 1: Transport properties of **2**–**4** in vesicles containing the different isomers of **1**.

Transporter	EC_{50} in (*E*)-1/POPC vesicles[a] (mol%)	EC_{50} in (Z_{PSS})-1/POPC vesicles[a] (mol%)	$F_{(E/Z)}$[b]
2	0.0061	0.0026	2.4
3	0.026	0.016	1.6
4	0.0019	0.0018	1.1

[a]EC_{50} defined as the effective concentration needed to reach 50% of the maximum activity at $t = 360$ s; Values are reported in transporter-to-lipid molar ratio. [b]Factor of enhancement in chloride transport activity between vesicles containing the (*E*)- and (Z_{PSS})-isomer of **1** [$F_{(E/Z)} = EC_{50(E)}/EC_{50(Z_{PSS})}$].

Supporting Information

The authors have cited additional references within the Supporting Information.[22–26]

Acknowledgements

We gratefully acknowledge financial support from the European Research Council (Starting Grant no. 802830 to S.J.W.) and the Leiden Institute of Chemistry (LIC). This work benefited from access to the Netherlands Centre for Electron Nanoscopy (NeCEN) at Leiden University, an Instruct-ERIC center with assistance from dr. Willem Noteborn.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Amphiphiles · Lipid Bilayers · Molecular Switches · Photochromism · Anion Transport

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Manuscript received: October 18, 2024
Accepted manuscript online: December 11, 2024
Version of record online: December 19, 2024