Sign Inversion in Photopharmacology: Incorporation of Cyclic Azobenzenes in Photoswitchable Potassium Channel Blockers and Openers

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Abstract. Photopharmacology relies on ligands that change their pharmacodynamics upon photoisomerization. Many of these ligands are azobenzenes that are thermodynamically more stable in their elongated *trans* configuration, which predominates in the dark. Often, they are biologically active in this form and lose activity upon irradiation and photoisomerization to their *cis*-isomer. Recently, cyclic azobenzenes, so-called diazocines, have emerged. They are thermodynamically more stable in their bent *cis*-form than in their elongated *trans*-form. Incorporation of these switches into a variety of photopharmaceuticals could convert dark-active ligands into dark-inactive ligands, which is preferred in most biological applications. This "pharmacological sign-inversion" is demonstrated for a photochromic blocker of voltage-gated potassium channels, termed **CAL**, and a photochromic opener of G-protein-coupled inwardly rectifying potassium (GIRK) channels, termed **CLOGO**.

Photopharmacology endeavors to bestow light sensitivity to a multitude of biological targets using synthetic photoswitches.[1–5] Its scope has been demonstrated with a vast array of biological targets ranging from enzymes[6–9] to elements of the cytoskeleton,[10] voltage- and ligand-gated ion channels,[11–16] G protein-coupled receptors (GPCRs),[17–20]

and transporters.[21–23] Amongst other photoswitchable molecules, azobenzenes have emerged as the photoswitch of choice due to a variety of reasons, including fast photoswitching, good photostationary states, lack of phototoxicity, compatibility with physiological conditions, fatigue-resistance, tunability, and the relative ease of their chemical synthesis.[1] Several research groups have developed a number of ingenious ways to red-shift the photoswitching of azobenzenes and modulate their thermal bistability and conformational preferences.[24,25]

Standard azobenzenes are thermodynamically more stable in their elongated *trans*-form and can be isomerized to their bent *cis*-form with light (Fig. 1). Depending on their electronic nature and substitution pattern, they can thermally isomerize back to their *trans* form in a tunable fashion, with thermal half-lives ranging from nanoseconds to days under physiological conditions. Although several azobenzene photoswitches have been reported to be biologically active in their thermodynamically less stable *cis*-form,[10,26–28] the majority are more active in the dark, i.e. as their thermodynamically more stable *trans*-isomers. This is, in part, because their parent compounds tend to bind in elongated or stretched conformations, as demonstrated by a recent computational analysis of drug-like molecules that are suitable to azologization.[29]

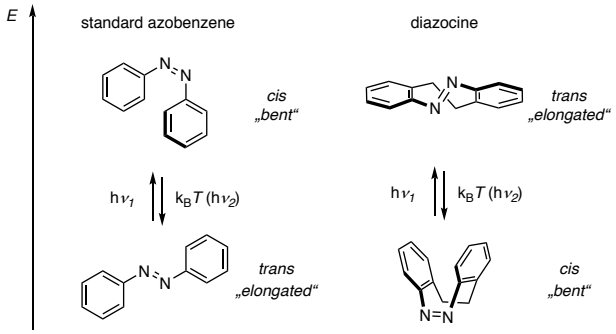

Figure 1. The logic of standard azobenzenes and diazocines. The former are thermodynamically more stable in their elongated *trans*-forms whereas the latter are more stable in their bent *cis*-forms. Both can be switched back and forth with a combination of irradiation and thermal relaxation. The wavelengths needed for photoisomerization and the kinetics of thermal relaxation can be tuned.

Cyclic azobenzene photoswitches, also known as diazocines, wherein the diazene unit is embedded in an eight-membered ring, have been known for many years but were only recently photophysically and conformationally characterized by Temps and Herges.[30–33] In contrast to standard azobenzenes, their bent *cis*-isomer is thermodynamically more stable and predominates in the dark, while exposure to light in the violet or deep blue range of the visible light spectrum promotes the formation of the thermodynamically less stable, elongated *trans*-isomer. We postulated that the stable *cis*-isomer of the diazocine could

effectively mimic the *cis*-isomer of a standard azobenzene, whereas the elongated *trans* diazocine would sterically resemble the *trans*-isomer of a standard azobenzene. This could allow for an inversion of the intrinsic dark-activity of a photoswitch (Fig. 1). Furthermore, diazocines photoisomerize in both directions with visible light, therefore circumventing the need to use potentially harmful UV light.

Herein, we demonstrate that freely diffusible biologically active photoswitches can have the sign of their pharmacological activity, i.e. active (+) vs. inactive (–), reversed by employing a diazocine photoswitch.[34] We envisaged that this concept could be applied to a wide range of dark-active azobenzenes to create their more biologically applicable dark-inactive variants. While this manuscript was in preparation, Ellis-Davies[35] and co-workers reported on diazocine photopharmaceuticals, adapting a photoswitchable glutamate derivative and photoswitchable ion channel blockers developed in our laboratory.[11,12,36] This prompted us to disclose our own results and provide more evidence that the pharmacological sign inversion of azobenzene photoswitches with diazocines is likely to be a generally applicable concept.

In 2008, we introduced photoswitchable azobenzene blockers to control the function of voltage-gated ion channels with light.[11] These compounds, which were derived from lidocaine and its permanently charged congener QX-314, have been continuously refined and have been used in a broad effort to restore vision with photopharmacology[37–40] (Fig. 2). Representative examples include the permanently charged blocker **DENAQ,**[13,38,39] the bis-quaternary ammonium ion **QAQ**,[12,41,42] and the red-shifted lidocaine derivative **DAD**.[40] Ion channel blockers of this type have also been employed in a program that aims to control nociception with light.[42] With one exception, these azobenzenes were more active blockers in their elongated *trans*-form, i.e. in the dark-adapted form.[12] This elongated *trans*-isomer appears to be a better fit for the binding site of use-dependent ion channel blockers in the inner cavity of voltage-gated ion channels than the bent *cis*-form. Incorporation of a diazocine should overcome the issue of dark-activity and provide compounds that are inactive in their dark-adapted form.[43]

Recently, Ellis-Davies introduced a version of **DENAQ** that incorporates a diazocine as a photoswitch. This compound, termed **LAB-QA** (Fig. 2b) was designed to be inactive or less active in the dark, where the bent *cis*-form predominates, and becomes an active blocker upon isomerization to the elongated *trans*-form. This design is similar to that of **CQAQ**, a **C**yclic version of **QAQ**, developed in our laboratories, as well as **CAL**, a **C**yclic **A**zobenzene version of **L**idocaine (Fig. 2c,d). **CQAQ** is permanently charged and presumably requires the presence of additional import channels, such as P2X or TPV1-channels, to reach its binding site, whereas **CAL** can exist as a charged ammonium ion or

in a neutral form that enables it to cross biological barriers and partition effectively into membranes.

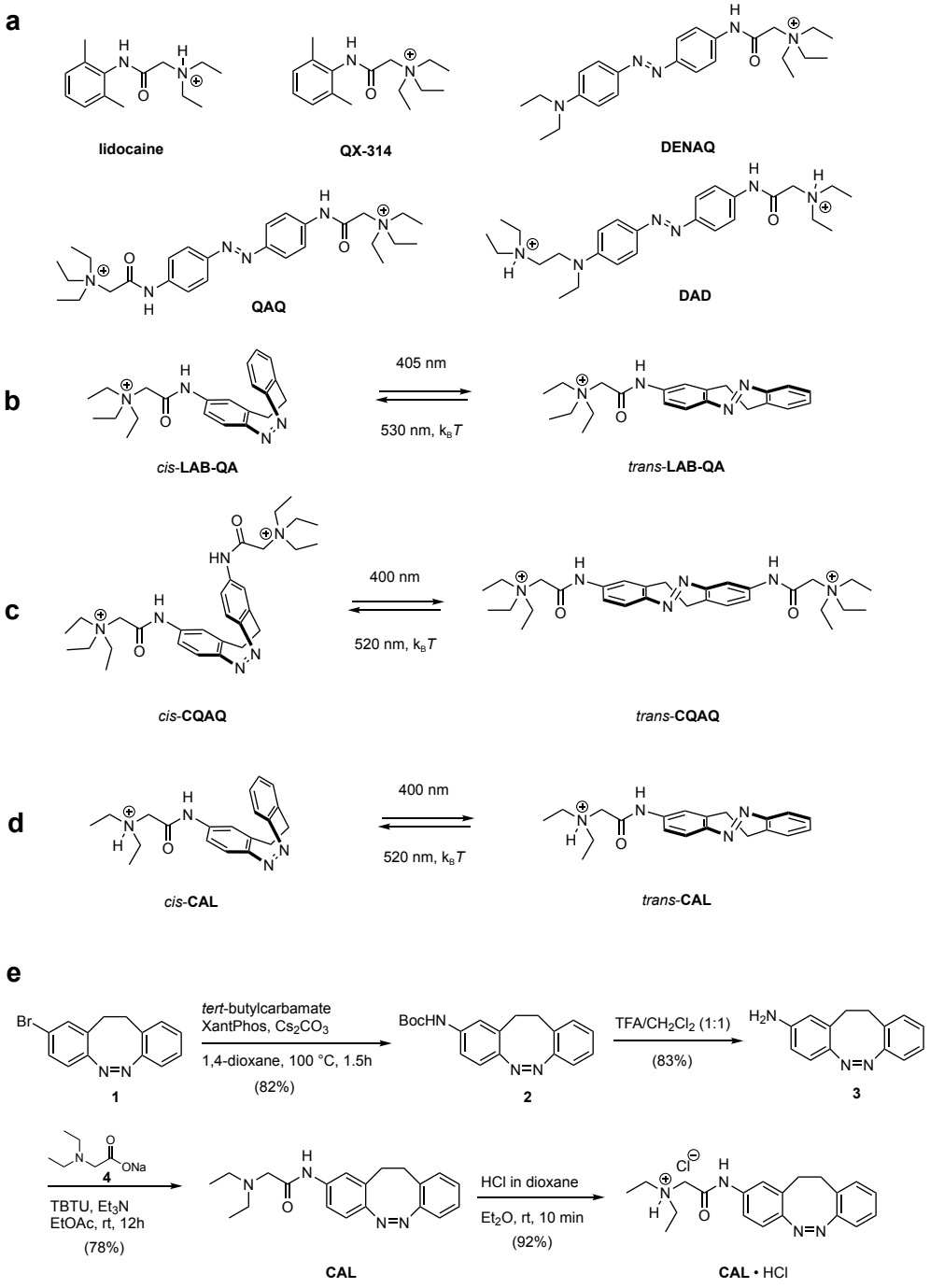

Figure 2. a) Structure of lidocaine, QX-314, photoswitchable K_v blockers **DENAQ**, **QAQ** and **DAD**. b) Structure and photoisomerization of **LAB-QA**. c) Structure and photoisomerization of **CQAQ**. d) Structure and photoisomerization of **CAL**. e) Synthesis of **CAL**·HCl**.**

The synthesis of **CQAQ** was achieved in one step from the known diazocine dianiline[44] and the respective carboxylic acid **12** through a peptide coupling with TBTU in DMF (Fig. S1). HPLC purification yielded the molecule as a formate salt. The synthesis of **CAL** started from the known bromodiazocine **1** and is shown in Fig. 2e. It involved a high-yielding Buchwald-Hartwig cross-coupling of **1** with *tert*-butylcarbamate, followed by deprotection, peptide coupling with diethyl sodium glycinate (**4**) and formation of the quaternary ammonium salt. To enhance solubility, **CAL** was prepared as a hydrochloride salt (**CAL**·HCl).

Given the importance of lidocaine as an antiarrhythmic agent and local analgesic and the pharmacokinetic intricacies of **CQAQ**, we decided to focus our photophysical and biological investigations on **CAL**. In the dark, under ambient light conditions, and under 520 nm light, **CAL** exists almost exclusively in its *cis*-form (> 98%), as determined by UV-Vis spectroscopy and NMR spectroscopy (Fig. 3a,b; Fig. S2). Upon irradiation with 385 – 405 nm light, we were able to enrich the *trans* isomer (Fig. 3a). At 390 nm, using a high-power LED, we observed a PSS of 41:59 = *cis*:*trans* in buffered, aqueous solution as determined by NMR spectroscopy (Fig. S2a). Upon illumination with 470 – 560 nm light **CAL** could be quickly reverted into the thermodynamically more stable *cis*-form (Fig. 3b).

Next, we investigated the effect of **CAL** on inactivation-removed *Shaker* K^+ channel[45] heterologously expressed in HEK293T cells using patch-clamp recordings in the voltage-clamp mode. For technical reasons, we employed 385 nm and 405 nm light to enrich the *trans*-form and 470 nm light to revert to the thermodynamically more stable *cis*-form. Under 385 nm illumination, where there is a substantial concentration of the elongated *trans*-isomer (ca. 50%), we observed robust channel block by **CAL** (500 μM). This block was greatly diminished under 470 nm illumination, where the switch is exclusively in the bent *cis*-form (Fig. 3c). The photoswitch index of **CAL** was determined to be 40.4 ±4.9% (n = 4 cells at 470 nm and 405 nm light; see SI).[37] The block was reversible with light, as demonstrated in Fig. 3d. Optical control of the current-voltage (I-V) relationships is shown in Fig. 3e (n = 3 cells). Thus, **CAL** shows robust photoswitching of *Shaker* K^+ channel currents with the opposite sign of previous use-dependent photoswitchable blockers, such as **DENAQ** and **DAD**.

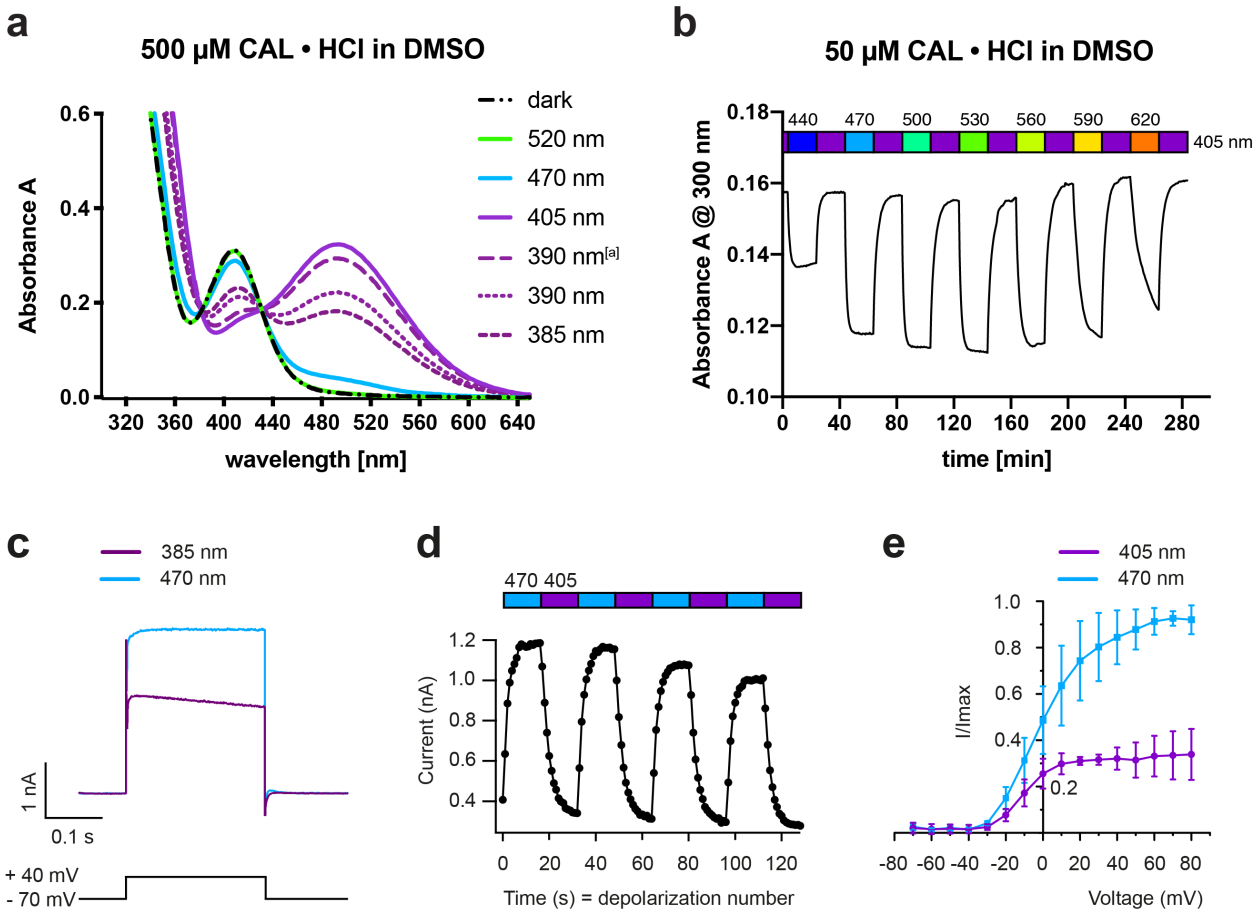

Figure 3. Photophysical characterization and electrophysiological characterization of CAL: a) Photophysical characterization of 500 μM **CAL** in DMSO solution. The absorption spectra after illumination with 385 nm, 390 nm, 405 nm, 470 nm, and 520 nm light. b) **CAL** can be isomerized from *trans* to *cis* with a wide range of wavelengths. c) Reversible optical control of *Shaker* K^+ channel currents with **CAL**. Representative current traces in the presence of **CAL** (500 μM) at 385 nm and 470 nm illumination, respectively, are shown. Outward K^+ currents were elicited by depolarizing cells from –70 mV to +40 mV at 1 Hz for time intervals of 250 ms. The last triggered current responses after 16 depolarizations at respective illuminations are shown. d) K^+ currents under 405 nm and 470 nm illumination demonstrating reversible optical control. e) I-V relationship of *Shaker* K^+ channel under 405 nm and 470 nm illumination. K^+ currents were elicited by stepping from –70 mV to +80 mV, in 10 mV intervals, at 1 Hz for 250 ms.
[a] Illumination with a high-intensity LED (Prizmatix).

Having successfully demonstrated the concept of sign-inversion with one of our photoswitchable ion channel blockers, we turned our attention to photochromic channel openers. In recent years, we have developed a series of photoswitchable openers of G protein-coupled inwardly rectifying potassium channels (GIRK channels). GIRK

channels are attractive biological targets since they play key roles in neuronal silencing and are involved in a wide range of complex biological processes, including nociception, cognition and cardiac output. Moreover, they are associated with numerous neurological and cardiovascular (patho-)physiological conditions.[46,47] We envisioned that our previous photoswitchable GIRK-activator, termed **LOGO**[48] (Fig. 4a), would be a suitable candidate for sign inversion because it behaves as a potent agonist in the dark (*trans*-isomer), whilst the *cis*-isomer is significantly less active. The same was true for a red-shifted tetrafluoro-derivative that operated with visible light and was named **VLOGO**[49] (Fig. 4a). Substitution of the regular azobenzene or tetrafluoro-azobenzene in these compounds with a diazocine afforded the **C**yclic azobenzene **L**ight **O**perated **G**IRK channel **O**pener (**CLOGO**), the structure and basic switching behavior of which is shown in Fig. 4b.

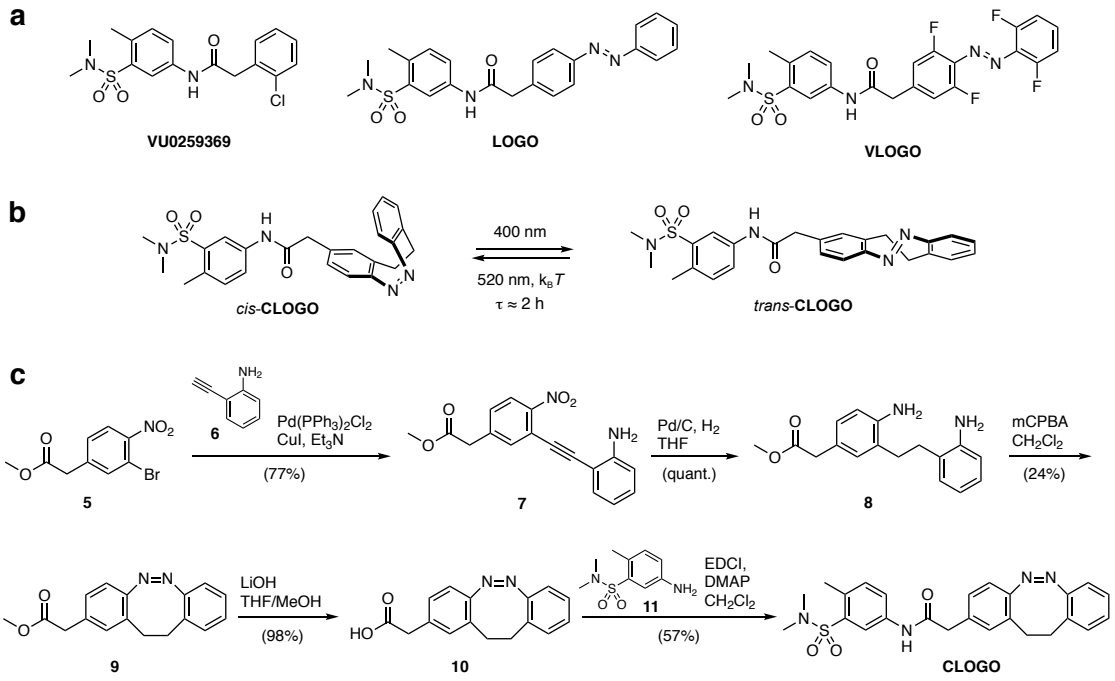

Figure 4. Design and synthesis of the GIRK channel agonist **CLOGO**. a) Chemical structures of the GIRK channel agonists **VU0259369**[50] and the photoswitchable GIRK channel openers **LOGO** and **VLOGO** b) Isomerization of **CLOGO** upon illumination. c) Synthesis of **CLOGO** using a Sonogashira/reduction/cyclization strategy.

The synthesis of **CLOGO** is shown in Fig. 4c. Various syntheses of cyclic azobenzenes have been reported.[51] However, these methods are often hampered by poor yields and many of them only afford cyclic azobenzenes bearing symmetrical substitution patterns. We therefore envisaged that a Sonogashira/reduction/cyclization strategy could be a viable route to afford key diazocine acid **10**, with a subsequent amide coupling with aniline **11** affording our desired product (Fig. 4c). Sonogashira cross-coupling of nitro bromide **5**

with aniline **6** efficiently provided nitro alkyne **7** in 77% yield. Subsequent reduction in the presence of palladium on carbon followed by cyclization using *m*CPBA afforded diazocine **9** in acceptable yield. Hydrolysis of the methyl ester then provided the acid **10** and an amide coupling with aniline **11** yielded **CLOGO**.

With **CLOGO** in hand, we evaluated its potential as a photochromic agonist of GIRK channels. We determined its optimal photoswitching wavelengths to be violet (400 nm, *trans*-isomer) and green light (520 nm, *cis*-isomer) using UV-Vis spectroscopy (Fig. 5a). We then applied this information to our electrophysiological experiments conducted in HEK293T cells heterologously expressing recombinant GIRK1/2 channels. Gratifyingly, we found that at high external potassium concentrations and a membrane potential of –60 mV, **CLOGO** is an excellent photoswitchable agonist of GIRK1/2 channels, whilst exhibiting the desired reversal in intrinsic activity. The *cis*-isomer (dark-state or green light) proved to be a significantly less active GIRK channel agonist than the *trans*-isomer (violet light). By changing the wavelength of illumination between green light (520 nm) and different wavelengths of UV, violet and blue light (340 – 440 nm, Fig. 5b), we were able to confirm that switching between 400 nm and 520 nm provided the largest change in observed current (Fig. S4). We next established that photoactivation of **CLOGO** (10 μM) is highly reversible and robust, with nearly no loss of photocurrent over several switching cycles (Fig. 5c). Similar results were also obtained when operating in current clamp mode (Fig. S5).

We then examined the stability of *trans*-**CLOGO** in the dark in a biological system (Fig. 5d). After the full *trans*-isomer content had been reached at 400 nm light illumination, **CLOGO** was exposed to dark conditions. This resulted in a minimal decrease of inward current on the second timescale. The full content of the trans-isomer was restored under violet light illumination (400 nm) and deactivation of GIRK channels could then be achieved using green light (520 nm). These results demonstrate that constant illumination of **CLOGO** is not required to maintain the maximum *trans*-isomer content.

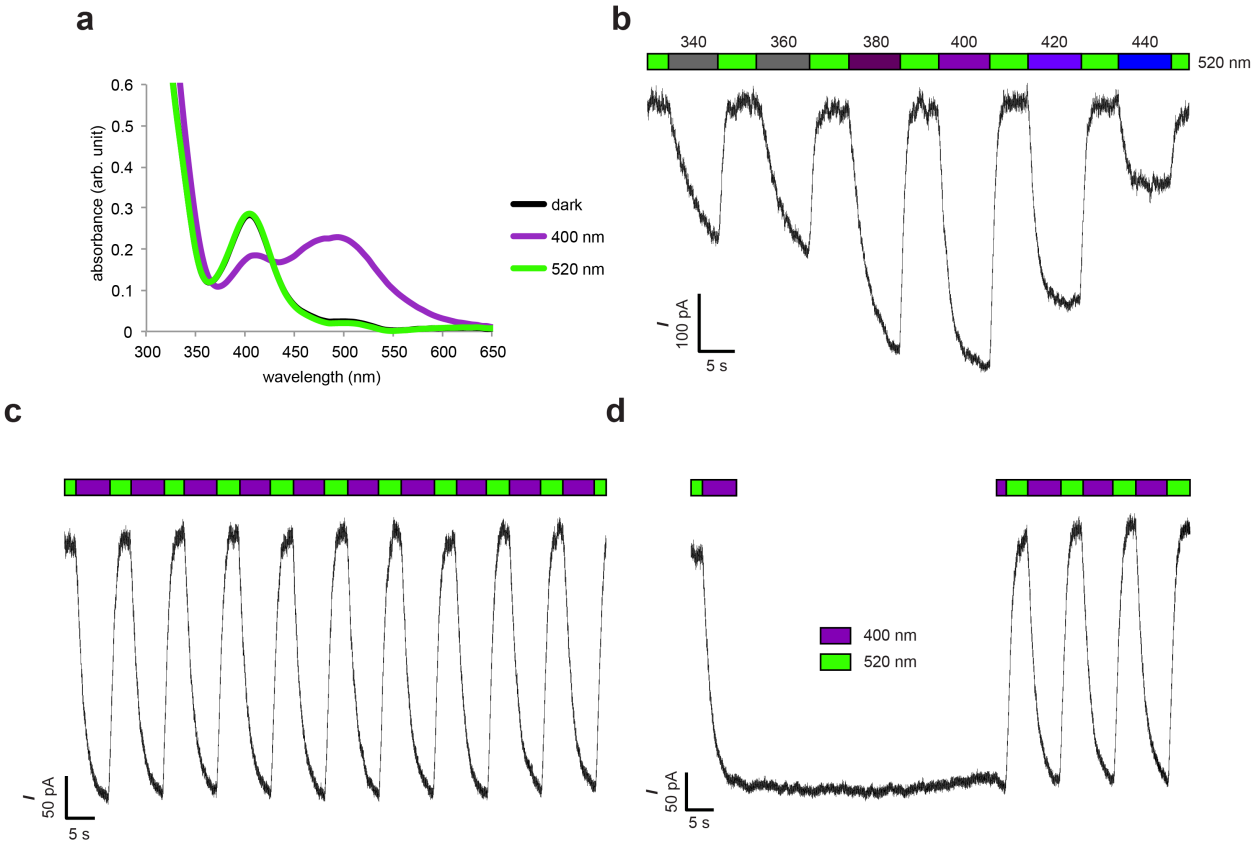

Figure 5. UV-Vis spectroscopy and whole-cell patch clamp electrophysiology characterization of **CLOGO**. a) UV-Vis absorption spectrum of **CLOGO** (500 μM in DMSO) in the dark and after illumination with violet (400 nm) and green light (520 nm). b) Action spectrum of **CLOGO** (10 μM) showing the accurate control of HEK293T cell currents by switching the irradiation wavelength between 520 nm and 340 – 440 nm. c) Highly reproducible photoswitching of **CLOGO** (10 μM) recorded in voltage-clamp mode. d) The *trans*-isomer of **CLOGO** was shown to be relatively stable on the second timescale when there was no light stimulus. Traces representative of n = 5 cells.

Having shown that the cyclic azobenzene photoswitch in the molecule **CLOGO** can effectively alter the intrinsic agonist activity when compared to the photoswitch **LOGO**, we next wanted to quantify the efficacy of the *cis*- and *trans*-isomers of **CLOGO** against a saturating concentration of the non-photoswitchable GIRK channel agonist **VU0259369** (Fig. S6) using patch clamp electrophysiology. To accomplish this, we first washed in a known concentration of **CLOGO** and converted it to its *cis*- and *trans*-isomers. We then washed in **VU0259369** (30 μM) and compared the inward current observed to the inward current obtained for each of the *cis*- and *trans*-isomers of **CLOGO**. The data show that *cis*-**CLOGO** (10 μM) exhibited 23% of **VU0259369** (30 μM) activation on GIRK1/2 channels. This is significantly different from the 65% activation exhibited by *trans*-**CLOGO**

(10 μM). Increasing the concentration of **CLOGO** (30 μM) led to an increase of GIRK channel opening as was the case for *cis*-**CLOGO** (34%). In comparison, the *trans*-isomer exhibited no significant increase in GIRK channel opening (68%), showing that at saturating *trans*-**CLOGO** concentration had almost been attained when using 10 μM.

To evaluate the potency, efficacy and selectivity of **CLOGO** between different GIRK channel subunit combinations, **CLOGO** was evaluated using the fluorescence-based thallium influx assay as previously described.[48,49] When various concentrations of **CLOGO** were tested on the predominantly neuronal GIRK subunit combination, GIRK1 + GIRK2, the predominantly cardiac subunit combination GIRK1 + GIRK4 and homomeric GIRK2 we found the compound to be completely inactive on homomeric GIRK2 channels while **CLOGO** activated GIRK1 + GIRK2 channels with a potency of 6.7 (6.0 - 7.4 95% CI) μM and GIRK1 + GIRK2 channels with a potency of 7.1 μM (5.8 - 8.7 95% CI) (Fig. S7). Further, we observed that **CLOGO** is a more effective activator of GIRK1 + GIRK2 channels compared to GIRK1 + GIRK4 channels. Our findings are in accordance with the GIRK subunit selectivity profile of the parent molecule **VU0259369**; the ability to activate GIRK1-containing GIRK channels but not non-GIRK1-containing channels.

Finally, we wanted to investigate if **CLOGO** could be used to control GIRK channels in excitable cells. As shown in Fig. 6, **CLOGO** silenced spontaneous action potential firing in mouse CA1 hippocampal neurons in its elongated *trans*-state at 400 nm illumination. Firing was, however, reversibly restored by isomerization into bent *cis*-**CLOGO** by illuminating with blue/green light (500 nm).

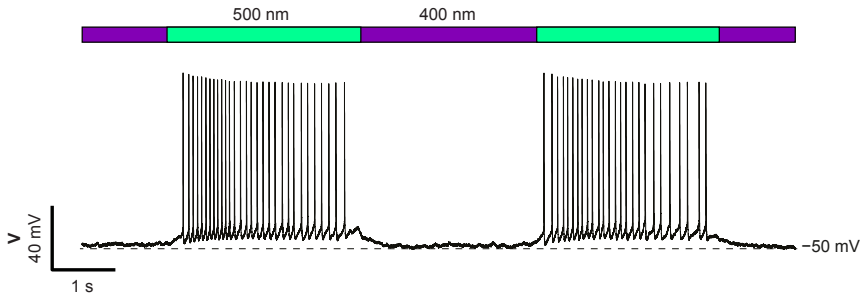

Figure 6. Optical control of action potential firing in mouse hippocampal neurons in the presence of **CLOGO** (100 μM). Switching between the *cis*- and *trans*-isomers of **CLOGO** in current clamp mode enables the optical control of spontaneous action potential firing at depolarizing holding currents. Trace representative of n = 5 neurons.

Discussion

Photopharmacology greatly benefits from the development of new types of photoswitches. Although the improvement of their photophysical properties remains an important aspect,

their structure can also have a profound effect on pharmacology. In some cases, the photopharmacological sign of the biological effect, i.e. active (+) vs. inactive (–), can be "inverted", as we have demonstrated here with a photoswitchable potassium channel blocker and a photoswitchable potassium channel opener.

The substitution of standard azobenzenes with diazocines enabled us to convert a dark-active channel blocker, such as **DENAQ** or **DAD**, into one that shows little if any activity in the dark, like **CAL**. The approach also proved successful for **LOGO** and **VLOGO**, two GIRK-channel openers that could be used to control neuronal activity with light but had a functional feature that may have prevented them from being widely adopted: they were active in the dark. We have overcome this drawback with a diazocine version, *viz*. **CLOGO**, that is inactive in the dark or at 500 nm but can be activated with violet light (400 nm) and used to effectively silence neuronal activity. As such, **CAL** and **CLOGO** are complementary tools to optically control neural networks with minimal effect in the absence of light.

The high concentrations needed for **CAL** and related channel blockers are not a major concern since the dark-adapted form is the pharmacologically inactive one. The active form is generated upon irradiation and its concentration can be fine-tuned with the wavelength of the light used ("color-dosing"). Like all diazocines reported to date, **CAL** thermally reverts slowly to the *cis*-form, but can be quickly and quantitatively switched back with a longer wavelength (Fig. S3). The photoswitch index of **CAL**, i.e. the strength of the photoswitching effect, is higher than that of the permanently charged compound **LAB-QA**. Whereas **CAL** could be simply added to the extracellular solution, **LAB-QA** needed to be applied via a patch pipette to be effective.[35] We anticipate that thermally bistable diazocines, such as **CAL**, **CQAQ**, and **LAB-QA** will be more useful as switchable analgesics and antiarrhythmics than as use-dependent channel blockers in vision restoration. Compared with red-shifted standard azobenzenes, which have been employed in this context, diazocines have slow relaxation kinetics, which limits their application where fast thermal back-isomerization to the default form is important. Whether the relaxation kinetics can be tuned through substitution of the diazocine remains to be determined. The pharmacokinetics of diazocine blockers, in particular with respect to the duration of their effect, is another point of concern that needs to be investigated in the future. It is likely that lipophilic cations of this type interact with membranes and serum differently than their conventional azobenzene counterparts.

Freely diffusible azobenzene photoswitches that are active in their *trans*-form have been developed for a variety of targets. These include GPCRs, such as the μ-opioid receptor,[17] the M1 muscarinic receptor,[52] the sphingosine phosphate receptor $S1PR_1$,[53] and the

metabotropic glutamate receptor mGluR5,[18] and ion channels, such as $GABA_A$,[54,55] α7-nAChR,[14] and ionotropic glutamate receptors.[15,36,56] Dark active photopharmaceuticals have also been used to optically control transporters, such as GAT1,[21] EAAT1-3,[22,23] and F1Fo-ATPase,[57] as well as enzymes.[58] Given the success of **CAL**, **LAB-QA**, **CLOGO**, and the glutamate derivative **LAB-Glu**[35] that targets NMDA receptors, it seems likely that the photopharmacological sign inversion of *trans*-active azobenzenes through substitution with diazocines is a generally applicable concept. The incorporation of diazocines into covalently tethered photoswitches (PTLs) is also likely to expand the reach of photopharmacology. In any case, the optimization of biologically active diazocine switches and their systematic incorporation in photopharmaceuticals will require efficient synthetic access to diazocines with various substitution patterns. Efforts in this direction are currently underway in our laboratories and will be reported in due course.

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Live subject statement

Animal procedures were in accord with EU and national law and were licensed by the Regierung Oberbayern.

Conflict of Interest CDW is an owner of WaveFront Biosciences, maker of the thallium-sensitive fluorescent dye, Thallos, and the kinetic imaging plate reader, Panoptic, used in this manuscript. No other authors have any conflicts of interest to declare.

Notes and References

(1) Hüll, K.; Morstein, J.; Trauner, D. In Vivo Photopharmacology. *Chem. Rev.* **2018**, *118* (21), 10710–10747.

(2) Fehrentz, T.; Schönberger, M.; Trauner, D. Optochemical Genetics. *Angew. Chem. Int. Ed.* **2011**, *50* (51), 12156–12182.

(3) Beharry, A. A.; Woolley, G. A. Azobenzene Photoswitches for Biomolecules. *Chem. Soc. Rev.* **2011**, *40* (8), 4422–4437.

(4) Szymański, W.; Beierle, J. M.; Kistemaker, H. A. V.; Velema, W. A.; Feringa, B. L. Reversible Photocontrol of Biological Systems by the Incorporation of Molecular Photoswitches. *Chem. Rev.* **2013**, *113* (8), 6114–6178.

(5) Lerch, M. M.; Hansen, M. J.; van Dam, G. M.; Szymanski, W.; Feringa, B. L. Emerging Targets in Photopharmacology. *Angew. Chem. Int. Ed.* **2016**, *55* (37), 10978–10999.

(6) Broichhagen, J.; Jurastow, I.; Iwan, K.; Kummer, W.; Trauner, D. Optical Control of Acetylcholinesterase with a Tacrine Switch. *Angew. Chem. Int. Ed.* **2014**, *53* (29), 7657–7660.

(7) Falenczyk, C.; Schiedel, M.; Karaman, B.; Rumpf, T.; Kuzmanovic, N.; Grøtli, M.; Sippl, W.; Jung, M.; König, B. Chromo-Pharmacophores: Photochromic Diarylmaleimide Inhibitors for Sirtuins. *Chem. Sci.* **2014**, *5* (12), 4794–4799.

(8) Reisinger, B.; Kuzmanovic, N.; Löffler, P.; Merkl, R.; König, B.; Sterner, R. Exploiting Protein Symmetry To Design Light-Controllable Enzyme Inhibitors. *Angew. Chem. Int. Ed.* **2014**, *53* (2), 595–598.

(9) Chen, X.; Wehle, S.; Kuzmanovic, N.; Merget, B.; Holzgrabe, U.; König, B.; Sotriffer, C. A.; Decker, M. Acetylcholinesterase Inhibitors with Photoswitchable Inhibition of β-Amyloid Aggregation. *ACS Chem. Neurosci.* **2014**, *5* (5), 377–389.

(10) Borowiak, M.; Nahaboo, W.; Reynders, M.; Nekolla, K.; Jalinot, P.; Hasserodt, J.; Rehberg, M.; Delattre, M.; Zahler, S.; Vollmar, A.; et al. Photoswitchable Inhibitors of Microtubule Dynamics Optically Control Mitosis and Cell Death. *Cell* **2015**, *162* (2), 403–411.

(11) Fortin, D. L.; Banghart, M. R.; Dunn, T. W.; Borges, K.; Wagenaar, D. A.; Gaudry, Q.; Karakossian, M. H.; Otis, T. S.; Kristan, W. B.; Trauner, D.; et al. Photochemical Control of Endogenous Ion Channels and Cellular Excitability. *Nat. Methods* **2008**, *5* (4), 331–338.

(12) Banghart, M. R.; Mourot, A.; Fortin, D. L.; Yao, J. Z.; Kramer, R. H.; Trauner, D. Photochromic Blockers of Voltage-Gated Potassium Channels. *Angew. Chem. Int. Ed.* **2009**, *48* (48), 9097–9101.

(13) Mourot, A.; Kienzler, M. A.; Banghart, M. R.; Fehrentz, T.; Huber, F. M. E.; Stein, M.; Kramer, R. H.; Trauner, D. Tuning Photochromic Ion Channel Blockers. *ACS Chem. Neurosci.* **2011**, *2* (9), 536–543.

(14) Damijonaitis, A.; Broichhagen, J.; Urushima, T.; Hüll, K.; Nagpal, J.; Laprell, L.; Schonberger, M.; Woodmansee, D. H.; Rafiq, A.; Sumser, M. P.; et al. AzoCholine Enables Optical Control of Alpha 7 Nicotinic Acetylcholine Receptors in Neural Networks. *ACS Chem. Neurosci.* **2015**, *6* (5), 701–707.

(15) Barber, D. M.; Liu, S.-A.; Gottschling, K.; Sumser, M.; Hollmann, M.; Trauner, D. Optical Control of AMPA Receptors Using a Photoswitchable Quinoxaline-2,3-Dione Antagonist. *Chem. Sci.* **2017**, *8* (1), 611–615.

(16) Hartrampf, F. W. W.; Barber, D. M.; Gottschling, K.; Leippe, P.; Hollmann, M.; Trauner, D. Development of a Photoswitchable Antagonist of NMDA Receptors. *Tetrahedron* **2017**, *73* (33), 4905–4912.

(17) Schonberger, M.; Trauner, D. A Photochromic Agonist for Mu-Opioid Receptors. *Angew. Chem. Int. Ed.* **2014**, *53* (12), 3264–3267.

(18) Pittolo, S.; Gómez-Santacana, X.; Eckelt, K.; Rovira, X.; Dalton, J.; Goudet, C.; Pin, J.-P.; Llobet, A.; Giraldo, J.; Llebaria, A.; et al. An Allosteric Modulator to Control Endogenous G Protein-Coupled Receptors with Light. *Nat. Chem. Biol.* **2014**, *10* (10), 813–815.

(19) Frank, J. A.; Yushchenko, D. A.; Fine, N. H. F.; Duca, M.; Citir, M.; Broichhagen, J.; Hodson, D. J.; Schultz, C.; Trauner, D. Optical Control of GPR40 Signalling in Pancreatic β-Cells. *Chem. Sci.* **2017**, *8* (11), 7604–7610.

(20) Broichhagen, J.; Johnston, N. R.; von Ohlen, Y.; Meyer-Berg, H.; Jones, B. J.; Bloom, S. R.; Rutter, G. A.; Trauner, D.; Hodson, D. J. Allosteric Optical Control of a Class B G-Protein-Coupled Receptor. *Angew. Chem. Int. Ed.* **2016**, *55* (19), 5865–5868.
(21) Quandt, G.; Höfner, G.; Pabel, J.; Dine, J.; Eder, M.; Wanner, K. T. First Photoswitchable Neurotransmitter Transporter Inhibitor: Light-Induced Control of γ-Aminobutyric Acid Transporter 1 (GAT1) Activity in Mouse Brain. *J. Med. Chem.* **2014**, *57* (15), 6809–6821.
(22) Cheng, B.; Shchepakin, D.; Kavanaugh, M. P.; Trauner, D. Photoswitchable Inhibitor of a Glutamate Transporter. *ACS Chem. Neurosci.* **2017**, *8* (8), 1668–1672.
(23) Hoorens, M. W. H.; Fu, H.; Duurkens, R. H.; Trinco, G.; Arkhipova, V.; Feringa, B. L.; Poelarends, G. J.; Slotboom, D. J.; Szymanski, W. Glutamate Transporter Inhibitors with Photo-Controlled Activity. *Adv. Ther.* **2018**, *1* (2), 1800028.
(24) Bléger, D.; Schwarz, J.; Brouwer, A. M.; Hecht, S. *o*-Fluoroazobenzenes as Readily Synthesized Photoswitches Offering Nearly Quantitative Two-Way Isomerization with Visible Light. *J. Am. Chem. Soc.* **2012**, *134* (51), 20597–20600.
(25) Samanta, S.; Beharry, A. A.; Sadovski, O.; McCormick, T. M.; Babalhavaeji, A.; Tropepe, V.; Woolley, G. A. Photoswitching Azo Compounds in Vivo with Red Light. *J. Am. Chem. Soc.* **2013**, *135* (26), 9777–9784.
(26) Broichhagen, J.; Schönberger, M.; Cork, S. C.; Frank, J. A.; Marchetti, P.; Bugliani, M.; Shapiro, A. M. J.; Trapp, S.; Rutter, G. A.; Hodson, D. J.; et al. Optical Control of Insulin Release Using a Photoswitchable Sulfonylurea. *Nat. Commun.* **2014**, *5*, No. 5116.
(27) Laprell, L.; Repak, E.; Franckevicius, V.; Hartrampf, F.; Terhag, J.; Hollmann, M.; Sumser, M.; Rebola, N.; DiGregorio, D. A.; Trauner, D. Optical Control of NMDA Receptors with a Diffusible Photoswitch. *Nat. Commun.* **2015**, *6*, No. 8076.
(28) 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*, No. 7118.
(29) Morstein, J.; Awale, M.; Reymond, J.-L.; Trauner, D. Mapping the Azolog Space Enables the Optical Control of New Biological Targets. *ACS Cent. Sci.* **2019**.
(30) Paudler, W. W.; Zeiler, A. G. Diazocine Chemistry. VI. Aromaticity of 5,6-Dihydrodibenzo[b,f][1,2]Diazocine. *J. Org. Chem.* **1969**, *34* (11), 3237–3239.
(31) Siewertsen, R.; Neumann, H.; Buchheim-Stehn, B.; Herges, R.; Näther, C.; Renth, F.; Temps, F. Highly Efficient Reversible Z–E Photoisomerization of a Bridged Azobenzene with Visible Light through Resolved S1(Nπ*) Absorption Bands. *J. Am. Chem. Soc.* **2009**, *131* (43), 15594–15595.
(32) Siewertsen, R.; Schönborn, J. B.; Hartke, B.; Renth, F.; Temps, F. Superior Z → E and E → Z Photoswitching Dynamics of Dihydrodibenzodiazocine, a Bridged Azobenzene, by S1(Nπ*) Excitation at λ = 387 and 490 Nm. *Phys. Chem. Chem. Phys.* **2010**, *13* (3), 1054–1063.
(33) Hammerich, M.; Schütt, C.; Stähler, C.; Lentes, P.; Röhricht, F.; Höppner, R.; Herges, R. Heterodiazocines: Synthesis and Photochromic Properties, Trans to Cis Switching within the Bio-Optical Window. *J. Am. Chem. Soc.* **2016**, *138* (40), 13111–13114.
(34) Some of these results have been presented at the Second International Symposium on Photopharmacology, Vic/Barcelona, November 1, 2018.
(35) Thapaliya, E. R.; Zhao, J.; Ellis-Davies, G. C. R. Locked-Azobenzene: Testing the Scope of a Unique Photoswitchable Scaffold for Cell Physiology. *ACS Chem. Neurosci.* **2019**.
(36) Volgraf, M.; Gorostiza, P.; Szobota, S.; Helix, M. R.; Isacoff, E. Y.; Trauner, D. Reversibly Caged Glutamate: A Photochromic Agonist of Ionotropic Glutamate Receptors. *J. Am. Chem. Soc.* **2007**, *129* (2), 260–261.
(37) Polosukhina, A.; Litt, J.; Tochitsky, I.; Nemargut, J.; Sychev, Y.; De Kouchkovsky, I.; Huang, T.; Borges, K.; Trauner, D.; Van Gelder, R. N.; et al. Photochemical Restoration of Visual Responses in Blind Mice. *Neuron* **2012**, *75* (2), 271–282.
(38) Tochitsky, I.; Polosukhina, A.; Degtyar, V. E.; Gallerani, N.; Smith, C. M.; Friedman, A.; Van Gelder, R. N.; Trauner, D.; Kaufer, D.; Kramer, R. H. Restoring Visual Function to Blind Mice with a Photoswitch That Exploits Electrophysiological Remodeling of Retinal Ganglion Cells. *Neuron* **2014**, *81* (4), 800–813.

(39) Tochitsky, I.; Helft, Z.; Meseguer, V.; Fletcher, R. B.; Vessey, K. A.; Telias, M.; Denlinger, B.; Malis, J.; Fletcher, E. L.; Kramer, R. H. How Azobenzene Photoswitches Restore Visual Responses to the Blind Retina. *Neuron* **2016**, *92* (1), 100–113.
(40) Laprell, L.; Tochitsky, I.; Kaur, K.; Manookin, M. B.; Stein, M.; Barber, D. M.; Schön, C.; Michalakis, S.; Biel, M.; Kramer, R. H.; et al. Photopharmacological Control of Bipolar Cells Restores Visual Function in Blind Mice. *J. Clin. Invest.* **2017**, *127* (7), 2598–2611.
(41) Fehrentz, T.; Kuttruff, C. A.; Huber, F. M. E.; Kienzler, M. A.; Mayer, P.; Trauner, D. Exploring the Pharmacology and Action Spectra of Photochromic Open-Channel Blockers. *ChemBioChem* **2012**, *13* (12), 1746–1749.
(42) Mourot, A.; Fehrentz, T.; Le Feuvre, Y.; Smith, C. M.; Herold, C.; Dalkara, D.; Nagy, F.; Trauner, D.; Kramer, R. H. Rapid Optical Control of Nociception with an Ion-Channel Photoswitch. *Nat. Methods* **2012**, *9* (4), 396–402.
(43) Damijonaitis, A. J. Photoswitchable molecules for the optical control of GPCRs and ion channels. PhD Thesis, Ludwig-Maximilians-Universität München, **2015**, deposited 22. Oct 2018, https://edoc.ub.uni-muenchen.de/23011/.
(44) Samanta, S.; Qin, C.; Lough, A. J.; Woolley, G. A. Bidirectional Photocontrol of Peptide Conformation with a Bridged Azobenzene Derivative. *Angew. Chem. Int. Ed.* **2012**, *51* (26), 6452–6455.
(45) Hoshi, T.; Zagotta, W. N.; Aldrich, R. W. Biophysical and Molecular Mechanisms of Shaker Potassium Channel Inactivation. *Science* **1990**, *250* (4980), 533–538.
(46) Hibino, H.; Inanobe, A.; Furutani, K.; Murakami, S.; Findlay, I.; Kurachi, Y. Inwardly Rectifying Potassium Channels: Their Structure, Function, and Physiological Roles. *Physiol. Rev.* **2010**, *90* (1), 291–366.
(47) Luján, R.; Marron Fernandez de Velasco, E.; Aguado, C.; Wickman, K. New Insights into the Therapeutic Potential of GIRK Channels. *Trends Neurosci.* **2014**, *37* (1), 20–29.
(48) Barber, D. M.; Schönberger, M.; Burgstaller, J.; Levitz, J.; Weaver, C. D.; Isacoff, E. Y.; Baier, H.; Trauner, D. Optical Control of Neuronal Activity Using a Light-Operated GIRK Channel Opener (LOGO). *Chem. Sci.* **2016**, *7* (3), 2347–2352.
(49) Trads, J. B.; Burgstaller, J.; Laprell, L.; Konrad, D. B.; de la Osa de la Rosa, L.; Weaver, C. D.; Baier, H.; Trauner, D.; Barber, D. M. Optical Control of GIRK Channels Using Visible Light. *Org. Biomol. Chem.* **2016**, *15* (1), 76–81.
(50) Ramos-Hunter, S. J.; Engers, D. W.; Kaufmann, K.; Du, Y.; Lindsley, C. W.; Weaver, C. D.; Sulikowski, G. A. Discovery and SAR of a Novel Series of GIRK1/2 and GIRK1/4 Activators. *Bioorg. Med. Chem. Lett.* **2013**, *23* (18), 5195–5198.
(51) Joshi, D. K.; Mitchell, M. J.; Bruce, D.; Lough, A. J.; Yan, H. Synthesis of Cyclic Azobenzene Analogues. *Tetrahedron* **2012**, *68* (41), 8670–8676.
(52) Agnetta, L.; Kauk, M.; Canizal, M. C. A.; Messerer, R.; Holzgrabe, U.; Hoffmann, C.; Decker, M. A. A Photoswitchable Dualsteric Ligand Controlling Receptor Efficacy. *Angew. Chem. Int. Ed.* **2017**, *56* (25), 7282–7287.
(53) Morstein, J.; Hill, R. Z.; Novak, A. J. E.; Feng, S.; Norman, D. D.; Donthamsetti, P. C.; Frank, J. A.; Harayama, T.; Williams, B. M.; Parrill, A. L.; et al. Optical Control of Sphingosine-1-Phosphate Formation and Function. *Nat. Chem. Biol.* **2019**, DOI: 10.1038/s41589-019-0269-7.
(54) Yue, L.; Pawlowski, M.; Dellal, S. S.; Xie, A.; Feng, F.; Otis, T. S.; Bruzik, K. S.; Qian, H.; Pepperberg, D. R. Robust Photoregulation of GABAA Receptors by Allosteric Modulation with a Propofol Analogue. *Nat. Commun.* **2012**, *3*, No. 1095.
(55) Stein, M.; Middendorp, S. J.; Carta, V.; Pejo, E.; Raines, D. E.; Forman, S. A.; Sigel, E.; Trauner, D. Azo-Propofols: Photochromic Potentiators of GABAA Receptors. *Angew. Chem. Int. Ed.* **2012**, *51* (42), 10500–10504.
(56) Laprell, L.; Hüll, K.; Stawski, P.; Schön, C.; Michalakis, S.; Biel, M.; Sumser, M. P.; Trauner, D. Restoring Light Sensitivity in Blind Retinae Using a Photochromic AMPA Receptor Agonist. *ACS Chem. Neurosci.* **2016**, *7* (1), 15–20.
(57) Eisel, B.; Hartrampf, F. W. W.; Meier, T.; Trauner, D. Reversible Optical Control of F1Fo-ATP Synthase Using Photoswitchable Inhibitors. *FEBS Lett.* **2018**, *592* (3), 343–355.

(58) Tsai, Y.-H.; Essig, S.; James, J. R.; Lang, K.; Chin, J. W. Selective, Rapid and Optically Switchable Regulation of Protein Function in Live Mammalian Cells. *Nat. Chem.* **2015**, *7* (7), 554–561.