6. Flipping the photoswitch:

Ion channels under light control

Catherine K. McKenzie, Inmaculada Sanchez-Romero and Harald Janovjak

C.K. McKenzie • I. Sanchez-Romero • Harald Janovjak (✉)

IST Austria (Institute of Science and Technology Austria)

Am Campus 1

A-3400 Klosterneuburg

Austria

e-mail: harald@ist.ac.at

Phone: +43 2243 9000 4201

Abstract

Nature has incorporated small photochromic molecules, colloquially termed ‘photoswitches’, in photoreceptor proteins to sense optical cues in phototaxis and vision. While Nature’s ability to employ light-responsive functionalities has long been recognized, it was not until recently that scientists designed, synthesized and applied synthetic photochromes to manipulate biological processes with the temporal and spatial resolution of light. Ion channels in particular have come to the forefront of proteins that can be put under the designer control of synthetic photochromes. Photochromic ion channel controllers are comprised of three classes, photochromic soluble ligands (PCLs), photochromic tethered ligands (PTLs) and photochromic crosslinkers (PXs), and in each class ion channel functionality is controlled through reversible changes in photochrome structure. By acting as light-dependent ion channel agonists, antagonist or modulators, photochromic controllers effectively converted a wide range of ion channels, including voltage-gated ion channels, ‘leak channels’, tri-, tetra- and pentameric ligand-gated ion channels, and temperature-sensitive ion channels, into man-made photoreceptors. Control by photochromes is reversible, unlike in the case of ‘caged’ compounds, and non-invasive with high spatial precision, unlike pharmacology and electrical manipulation. Here, we introduce design principles of emerging photochromic molecules that act on ion channels and discuss the impact that these molecules are beginning to have on ion channel biophysics and neuronal physiology.

Keywords

optochemical genetics, optogenetics, photopharmacology, optical control, photochrome, azobenzene, tethered ligand

6.1 Introduction

In all domains of life optical cues control essential physiological and behavioural processes that range from simple forms of phototaxis in unicellular organisms to vision in animals. Multiple molecular mechanisms to sense light evolved independently in the form of distinct classes of photoreceptor proteins. However, with few exceptions (Rizzini et al. 2011), most natural photoreceptors rely on low molecular weight (M_r~200-700Da) photochromes that undergo reversible conformational changes between two isomers upon photon absorption (Ridge and Palczewski 2007; Rockwell and Lagarias 2006). A classic example are rhodopsin photoreceptors and their prokaryotic relatives, where the 11-*cis*-retinal undergoes light-induced isomerization ('switching') into all-*trans*-retinal (or all-*trans*-retinal to 13-*cis*-retinal). In most members of the opsin protein family, photoisomerization of retinal triggers changes in the structure of the transmembrane protein and activates ion flow or activation of downstream signalling cascades.

New fields of laboratory research have been inspired by Nature's highly efficient concept of relaying light-induced structural changes of small photochromes to larger biological molecules. In the past 40 years, photochromes have been combined with small peptides, proteins, lipids and nucleic acids (Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules 2005). One central motivation for this work was found in the recognition that light can be precisely controlled in space and time and offers non-invasive 'remote' control in transparent matrices. Inspired by classic work dating back as far as to the 1970s (Bartels et al. 1971; Lester et al. 1980; Bieth et al. 1969; Deal et al. 1969), photochromes were recently introduced in the ion channel field to contribute these experimental advantages to our current research. Researchers began to exploit photochromes with the help of molecular, chemical and genetic engineering in the fields of photopharmacology and optochemical genetics. New photochromic tools were

developed and meanwhile many classes of ion channels have been 'fitted' for photochromic controllers.

This chapter will focus on the design and impact of photoswitches in ion channel research. In Sections 6.2 and 6.3, we build up photochromic ion channel controllers by explaining the structure and function of synthetic photochromes (**Section 6.2**) and the design approach towards PCLs, PTLs and PXs (**Section 6.3**). In Section 6.4 we discuss specific application of photochromes to the different ion channel families. Our contribution focuses on photochromes, which are in themselves reversible and control ion channels reversibly, and therefore we do not discuss caged compounds, photoaffinity labelling or the use of light-sensitive unnatural amino acids.

6.2 Synthetic photochromes for biological research

By definition, photochromes undergo light-induced and reversible transitions between two isomers that exhibit distinct spectral properties. Light-induced changes of colour were first reported by Fritzsche for tetracene (Fritzsche 1867) and Hirshberg coined the term photochromism (Hirshberg 1950). In common photochromes, photoisomerization either relies on *cis-trans*-isomerization (e.g. in azobenzene (AB) and hemithioindigo (HTI)) or on cyclisation/bond opening (e.g. in spiropyran (SP) or diarylethenes (DAE)). For the control of biological molecules the two isomers of the photochrome ideally have very different geometries and polarities as it is generally assumed that the bigger the transition the more likely it will induce a significant effect. In the following, we describe important classes of synthetic photochromes that are used to manipulate biological processes.

AB likely is the best studied and most commonly applied photochrome. AB undergoes a *cis-trans*-isomerization around the central nitrogen-nitrogen double bond (**Figure 1a**). Thousands of photocycles can be performed with high quantum yield, on remarkably short time scales and without signs of fatigue or toxic side products. These properties collectively make AB a well-suited photochrome for

biology. *Trans*-AB is the dominant isomer at equilibrium in the dark. Photoisomerization to *cis*-AB is typically initiated using UV light (λ~360nm) and the relaxation can either occur thermally or be catalysed by blue or green light (λ~500nm) (Rau 1973). Complete photoisomerization cannot be achieved as the absorption spectra of the two isomers overlap and photostationary states always contain mixtures of *cis*- and *trans*-isomers. While unmodified AB shows thermal *cis*-*trans*-relaxation on the timescale of days at room temperature, AB with red-shifted absorption maxima relax within seconds to minutes (also see **Sections 6.4.1 and 6.4.2**). The photoisomerization of AB is accompanied by changes in structure: The two benzyl rings of the *cis*-isomer are roughly tilted by 55°, while in the *trans*-isomer both rings lie in a plane. The end-to-end distance (measured between the two *para*-positions of the benzyl rings) decreases by about 3.5Å, and modified ABs were synthetized to maximize this distance without generating additional degrees of conformational freedom (Beharry and Woolley 2011; Samanta and Woolley 2011; Standaert and Park 2006).

While not yet combined to ion channels, we briefly introduce other types of photochromes that are applied in biology and likely to transition to ion channel research in the future. SPs consist of two ring systems joined at a quaternary spiro-carbon atom (**Figure 1b**). The twisted and colourless SPs can be converted into the planar and purple coloured merocyanines (MCs) using UV light that breaks the spiro-carbon-oxygen bond (Aldoshin 1990; Berkovic et al. 2000). Relaxation to the thermodynamically favoured SP can be accelerated by green light or occurs thermally. Large changes in geometry and polarity, compatibility with two-photon excitation and fluorescence of the MC isomer make SPs attractive photochromes for biological applications (Mao et al. 2008; Marriott et al. 2008; Petchprayoon et al. 2011). HTIs were synthesized and studied in detail recently (**Figure 1c**) (Cordes et al. 2007; Eggers et al. 2001; Herre 2005; Mostoslavskii 1970; Regner et al. 2012).

HTIs undergo light-induced isomerization from the thermodynamically favoured *Z*-isomer to the corresponding *E*-isomer and both isomers exhibit a planar structure. In contrast to AB and SP, both the photoisomerization and thermal relaxation can be accelerated by visible light. Finally, DAE and fulgides/fulgimides show potential for applications in biological research (**Figure 1d**) (Chen et al. 2011; Fujimoto et al. 2012). These hexatriene compounds undergo light-induced and reversible electrocyclic ring closure and opening (Irie 2000; Yokoyama 2000). While the ring closing requires UV light the opening can be triggered by visible light. It is interesting to note that both classes show bi-stability as thermal relaxation is negligible.

6.3 Design principles of photochromic ion channel controllers

6.3.1 PCLs: one-component photopharmacology

Several complementary designs that build on photochromes have been developed for the control of ion channels. PCLs refer to soluble agonists, antagonists and modulators with photochromic substituents. Biological activity is retained in one isomer but not the other and thereby photoisomerization allows rapid and reversible control of ion channel function. In the first design represented by 4-GluAzo, a PCL of ionotropic glutamate receptors (iGluRs), the natural ligand is coupled to AB (see **Section 6.4** for a detailed discussion of 4-GluAzo). Coupling sites are typically chosen with the help of model molecules with lipophilic tails that test how coupling affects affinity and solubility. In the design represented by AP2, a PCL of $GABA_A$ receptors ($GABA_ARs$), the aromatic group of the ligand propofol is incorporated in AB. Also for this design model compounds are useful, and often model compounds have already been described in literature (e.g. LY339434 in the case of 4-GluAzo or *p*-4-AziC5-propofol in the case of AP2) (**Figure 2a**) (also see **Section 6.4.1**). PCLs are ‘drug-like’ in that they diffuse in tissue and offer similar specificities for ion channels as natural ligands. However, they are advantageous in specific

applications, as spatial and temporal precision can be obtained that surpasses that of normal pharmacology.

6.3.2 PTLs: ligands-on-a-leash

In contrast to PCLs, PTLs target genetically modified ion channels through covalent attachment. The majority of published PTLs consist of AB, maleimide (Mal) as the reactive group for site-specific attachment and a specific ligand group (agonists, antagonists or modulators) (**Figure 2b**). Cysteine (Cys) residues are commonly used as attachment sites of PTLs for two major reasons. First, Cys can be easily introduced into a protein by specific mutation and second, the thiol group is a well-known and highly reactive nucleophile (also see **Chapters 2 and 5 of this book**). PTLs have many of the attributes of PCLs but due to genetic modification they enable the construction of orthogonal ligand-protein-pairs and offer specific control over selected ion channel subtypes. In contrast to PCLs, PTLs are a two-component system that requires introduction of the Cys substitution and expression of the modified ion channel followed by application of the PTL.

6.3.3 PXs: molecular tweezers

An exciting new development for the optical control of ion channels are PXs, which bind amino acids located in two distant parts of the protein through functional groups located at the ends of the photochrome (**Figure 2c**) (Browne et al. 2014). Non-isomerizable bi-functional crosslinkers have for decades been applied in molecular and structural biology as ‘molecular rulers’ during protein function and protein assembly (Fasold et al. 1971; Ji 1983). In 2000, Woolley and colleagues introduced crosslinkers that contain a central AB moiety for the optical control of the secondary structure of peptides. This principle was recently applied to P2X ion channels, where photoisomerization of AB resulted in a change of crosslinker length and

conformational changes leading to channel opening (Browne et al. 2014; Kumita et al. 2000).

6.4 Applications of PCLs and PTLs

6.4.1 PCLs of ligand-gated ion channels

The first PCLs were designed and synthesized in the 1960s and 1970s in the form of AB-substituted acetylcholines and carbachols. These molecules were mono- or bi-functional with a quaternary ammonium (QA) and were applied to control nicotinic acetylcholine receptors (nAChRs) and acetylcholine esterase to study kinetics of ion channel activation and membrane potential shifts in excitable tissue (Bartels et al. 1971; Bieth et al. 1969; Deal et al. 1969; Chabala et al. 1985; Lester et al. 1979; Nargeot et al. 1982). Despite these early successful examples for the optical control of ion channel function, further design and application of PCLs was not revisited until nearly three decades later. The first molecule to follow was 4-GluAzo, a PCL designed for the Kainate receptor-subfamily (KAR) of tetrameric iGluRs. With its lipophilic tail, 4-GluAzo resembles LY339434 and discriminates between GluK1 and GluK2 subtypes in a heterologous expression system (Volgraf et al. 2007). 4-GluAzo is more active in its *trans*-isomer but exhibits markedly reduced potency and efficacy compared to glutamate (Glu). A recent crystal structure of the GluK2 ligand-binding domain (LBD) in complex with 4-GluAzo confirmed several hypothesis made during the design of 4-GluAzo (Reiter et al. 2013). The structure confirmed that the Glu moiety of 4-GluAzo indeed forms those contacts to the ligand-binding pocket that were previously observed for Glu. The structure also revealed that the lipophilic tail protrudes to the protein surface with the formation of additional PCL-protein contacts. Finally, the structure allowed attributing the reduced efficacy to a more limited conformational change of the GluK2 LBD that is characteristic for partial agonists. The clamshell-like LBD of iGluRs is of prokaryotic origin and shared amongst the three major iGluR subfamilies (AMPA receptors (AMPAR), KARs and NMDA

receptors (NMDARs)) (Mayer 2011; Janovjak et al. 2011). However, sufficient structural differences exist between the subfamilies and these differences prevented the direct application or even modification of 4-GluAzo as a PCL of AMPARs and NMDARs. To functionally discriminate between AMPAR and other iGluRs, BnTetAMPA, a highly specific AMPAR agonist with a lipophilic tail was derivatized into AB-tetrazolyl-AMPA-3 (ATA-3) (Stawski et al. 2012). Like 4-GluAzo, ATA-3 is functionally active in its dark-adapted *trans*-isomer. Both 4-GluAzo and ATA-3 are capable of triggering trains of action potentials (APs) in primary neurons by activating endogenous iGluRs.

UV light, which is required for *trans*-*cis*-isomerization of unmodified AB, may harm biological samples and exhibits limited depth of tissue penetration. An important advance in the design of PCLs is thus the development of molecules that react to visible light, which can be achieved by increasing the electron density at the AB using substituents. For instance, *trans*-*cis*-isomerization of ATA-3 occurs readily in blue light because of an electron-donating *para*-dimethylamino substituent (Stawski et al. 2012). Notably, unlike unmodified AB that thermally relaxes to its *trans*-isomer on the time scale of many minutes to hours, such modified ABs relax within seconds. Similar improvements in wavelength sensitivity and thermal relaxation were also achieved in PCLs of voltage gated ion channels and exploited for restoration of retinal function (see **Section 6.4.2**).

A recent variation of PCLs encompasses molecules with the ability to tune the conductance of ligand-gated ion channels rather than to directly control channel opening or closing. Two potentiating PCLs have been developed for pentameric $GABA_AR$s and applied in heterologous expression systems, primary cells and a tadpole model system (Stein et al. 2012; Yue et al. 2012). AP2 and MPC088 are both AB derivatives of propofol, a common amnestic agent and powerful positive allosteric $GABA_AR$ modulator. However, in contrast to MPC0888, in which AB is conventionally coupled to propofol, a new approach was chosen in AP2 by incorporating the phenol

of propofol into the photochrome (**Figures 2** and **3**). Both PCLs potentiate $GABA_AR$s in the *trans-isomer* and remarkably with higher potency but reduced efficacy compared to the parent molecule (Stein et al. 2012; Yue et al. 2012). The development of AP2 also highlighted that synthesis and functional evaluation of many PCL candidates with varying substituents can be required to discover molecules with desired combined photophysical and functional properties.

6.4.2 PCLs of voltage-gated ion channels

Mono-functional and bi-functional PCLs have been developed to act on voltage-gated ion channels. These molecules contain QA ions and resemble both the QA PCLs developed in the 1960s and 1970s (see above) as well as lidocaine or QX-314 (Binshtok et al. 2007). A family of mono-functional AB-QA molecules binds to the tetraethylammonium (TEA) binding site on the inner cavity of K_v channels and functions as open channel blockers (Banghart et al. 2009; Mourot et al. 2011). Experimental evaluation of several AB-QA compounds, including acrylamide-AB-QA (AAQ) and benzoyl-AB-QA (BzAQ), revealed that the length and composition of the lipophilic tail influences potency. Similarly to the PCLs described above, AB-QA compounds were developed that contain modified ABs and are isomerized by visible light with more rapid thermal relaxation (Fehrentz et al. 2012; Mourot et al. 2011). Notably, the green light-sensitive diethylamino-AB-QA (DENAQ) is capable of restoring functional light sensitivity in rat retinas with degenerated photoreceptors (Polosukhina et al. 2012; Tochitsky et al. 2014).

A second family of PCLs of voltage-gated ion channels is bi-functional and represented by the prototypical QA–AB–QA (QAQ). QAQ also resembles the early compound bis-Q (Bartels et al. 1971) and effectively blocks voltage-gated Na^+, K^+ and Ca^{2+} channels in its *trans*-isomer (**Figure 4**) (Mourot et al. 2012). The two permanent charges of QAQ prohibit passive entry into the cells and make QAQ reliant on TRPV1 and P2X7 receptors as an entry route. This entry route allows

selecting for neurons that are being activated by pain stimuli, and thus QAQ can function like an optical local anaesthetic while sparing other sensory modalities (Mourot et al. 2012).

6.4.3 PTLs of ligand-gated ion channels

With the help of a lipophilic Glu-derivative that served as a model molecule, a family of Mal-AB-Glu (MAG) PTLs was developed for the specific control of KARs (Volgraf et al. 2006) (**Figure 2**). GluK2 was the first ion channel to be gated by MAGs and both the length of MAGs as well as the attachment site were shown to determine whether the channels are opened by UV light (*cis*-MAG) or visible light (*trans*-MAG) (Volgraf et al. 2006; Numano et al. 2009). Application of two ion channels with this 'sign inversion' allows experimenters to target two separate neuronal populations, and such experiments are further supported by modified MAGs with *trans*-*cis*-isomerization in response to visible light and rapid thermal relaxation (Kienzler et al. 2013). The light-gated iGluR (LiGluR; GluK2-MAG) was converted from an excitatory channel to an inhibitory channel (HyLighter) by incorporating the transmembrane domain of a prokaryotic K^+-selective amino acid receptor (Janovjak et al. 2010). While LiGluR is capable of activating neurons in culture and *in vivo* with millisecond time resolution, HyLighter is capable of hyperpolarization and neuronal silencing (Szobota et al. 2007; Janovjak et al. 2010). LiGluR has been used to evoke transmitter release in glial cells and chromaffin cells, to conduct neural circuit analysis and restore a retinal light response and visual behaviour to mice with degenerated photoreceptor cells (Caporale et al. 2011; Izquierdo-Serra et al. 2013; Li et al. 2012; Wyart et al. 2009).

Many years before the development of MAGs, a set of classic studies revealed a first PTL of pentameric ligand gated ion channels. Bromomethyl-AB-QA (QBr) attached to a native Cys of endogenous nAChRs and enabled selective channel opening in its *trans*-isomer (Bartels et al. 1971). QBr was subsequently applied to study ion

channel activation kinetics in *Electrophorus* electroplaques and rat myoballs (Chabala and Lester 1986; Lester et al. 1980). Unlike QBr, which was not targeted by genetic manipulation, recent PTLs were designed to act as an agonist (Mal–AB–acylcholine (MAACh)) or antagonist (Mal–AB–homocholine (MAHoCh)) on genetically engineered nAChRs (**Figure 5**) (Tochitsky et al. 2012). The basis for the development of light-activated nAChRs was, similarly to the development of LiGluR and recent light-activated metabotropic Glu receptors (Levitz et al. 2013), a combination of Cys-scanning mutagenesis and molecular modelling.

6.4.4 PTLs of voltage-gated ion channels

The design of the Mal-AB-QA (MAQ) PTL enabled the development of the hyperpolarizing synthetic photoisomerizable AB-regulated K^+ channel (H-SPARK), the first optical tool that could effectively silence a neuronal population (Banghart et al. 2004). In H-SPARK, the Shaker K^+ channel was optimized for the PTL by reducing inactivation and by shifting its voltage dependence to resting potentials. Expression of the channel results in a high conductance that is blocked by MAQ in its *trans*-isomer (Banghart et al. 2004). The excitatory counterpart to H-SPARK, depolarizing SPARK (D-SPARK), was developed shortly after, and in the meantime MAQ has proven to be a potent PTL of a number of K^+-selective channels, including leak channels and channels that are opened by intracellular Ca^{2+} (Chambers et al. 2006; Fortin et al. 2011; Sandoz et al. 2012).

Genetic ‘knock-in’ or ‘knock-out’ or subtype specific pharmacology are the classic approaches to dissect the functional role of selected ion channels *in vivo*. MAQ enabled the creation of a complementary approach called ‘subunit replacement strategy’ using photoswitchable conditional subunits (PCS) (Sandoz et al. 2012). The PCS is an engineered ion channel subunit that has been modified for gating by a PTL but cannot be trafficked to the membrane without the presence of a native subunit. Through this trick, only cells that express native subunits are

photosensitized and the currents under light control are not subject to overexpression. Collectively, subunit replacement strategy represents a functional knock-in (**Figure 6**) (Sandoz et al. 2012), and a PCS of the K2P channel TREK-1 revealed that this channel is a target of modulation by G-protein coupled receptor signaling (Sandoz et al. 2012).

6.5 Outlook

By combining chemical synthesis, photophysics and biophysics, the fields of photopharmacology and optochemical genetics have produced photochromic ion channel controllers that function with striking potency and specificity. The versatility of these photoswitches includes (i) sign inversion, which may enable dissecting effects of one ion channel type versus another (**Section 6.4.3**), (ii) modulation, which may decipher how current magnitudes effect signal integration (**Section 6.4.1**), and (iii) photochromes with modified absorption maxima, which may overcome experimental limitations associated with UV light and slow thermal relaxation (**Sections 6.4.1** to **6.4.3**). Exciting applications of PCLs and PTLs are emerging and include the control of pain sensation (Mourot et al. 2012), the control of visual responses (Polosukhina et al. 2012; Tochitsky et al. 2014; Caporale et al. 2011), and the identification of the targets of non-ionotropic signaling pathways (Sandoz et al. 2012).

We are now able to tailor molecular properties of the photochromic controllers (e.g. choice of agonistic or antagonistic ligand groups, choice of photoisomerization wavelengths) and of the ion channel (e.g. removal of inactivation) to the extent that we are able to rationally design the combined system as a whole. In our contribution to a *Springer* book published three years ago, we envisioned systems that exhibit ‘gated’ photoresponses. We defined gated systems as those that “only respond to light if an additional, external stimulus is present or modulate an external stimulus by the conformational effect of light”. While the second of these two types of gated

system is beautifully represented by AP2 (**Section 6.4.1**), the first type still awaits realization.

An ongoing challenge will be to apply photochromic systems in a broader range of animal models, in particular to connect channel function to the behaviour of freely moving animals. Since PCL and PTLs can behave like small molecule drugs, they are able to photosensitize tissue within seconds or minutes in comparison to days in the case of genetically targeted optogenetic regulators. Delivery of molecules to cortical and deep brain structures in mammalian systems could be achieved by intra-cranial or cannula injection, which has been used for many years in *in vivo* analysis of connectivity and excitability as well as disease-related drug treatment.

The PCLs, PTLs and PXs introduced here have been recently complemented by two approaches that already address some of the challenges mentioned above. Both approaches have in common that they are built on light-sensitive molecules that do not incorporate pharmacologically-active ligands. Optovin is a rhodanine-containing small molecule that modulates TRPA1 channels in response to violet light. Optovin not only responds to visible light but also has been shown to control neurons that express TRPA1 channels *in vivo* (Kokel et al. 2013). PTL-like molecules that were designed to directly gate channels by light can control even those channels that lost their ability to respond to ligands and in addition promise to be of general applicability (Lemoine et al. 2013).

In line with voltage-gated and ligand-gated ion channels being essential for all information flow within and between neurons, photochromic ion channel controllers have been most commonly used to enhance or inhibit neuronal signalling. However, PCLs, PTL and PXs are also of potential relevance for a number of fields other than neuroscience. Voltage-gated and ligand-gated ion channels are involved in a plethora of physiological processes, ranging from metabolism (e.g. insulin secretion in pancreatic β-cells driven by membrane currents) to cancer (e.g. cell proliferation linked ion flow). It should be possible to adapt what has been built and tested on

neurons to open new avenues in these research areas. Specifically the research presented here can irradiate to these disciplines by (i) either providing highly efficient molecular tools or (ii) by providing guidance for the development of new molecular tools for non-ion channel targets. For instance, a PTL designed for metabotropic Glu receptor is similar to MAG and produced rapid and reproducible induction of G-protein coupled signals (Levitz et al. 2013).

It is often noted that the fascinating developments leading to the optical control of ion channels started with the collaboration of specialists from different disciplines. Most commonly chemistry and biophysics are emphasized. Thus this research provides an exciting basis for the continued education of future generations of graduate students and postdocs that will be familiar with chemical synthesis, electrophysiology and molecular modelling. This chapter has highlighted that photochromic ion channel controllers have high potential and harvesting this potential may require scientists from other disciplines, in particular pharmacologists and physiologists, to also play role.

Figure Legends

Figure 1. Prominent photochromes applied to manipulate biological molecules

See main text for a detailed description of each photochrome.

Figure 2. Main classes of photochromic ion channel controllers

(a) In PCLs (here: AP2), AB is either partly incorporated into a ligand or tethered to a ligand (here: incorporated into propofol). AP2 was developed with the help of *p*-4-aziC2-propofol, which served as a 'tether model' to test for potency and solubility of the ligand derivative. AP2 potentiates GABA-induced currents of $GABA_A$Rs in its *trans*-isomer (also see **Section 6.4.1**).

(b) In PTLs (here: MAG), AB is tethered to a native ligand (here: Glu) and a Cys-reactive group (here: Mal). MAG was developed with the help of the lipophilic Glu-derivative 'tether model 3'. MAG activates KARs in its *cis*-isomer but not *trans*-isomer or *vice versa* depending on attachment site of MAG (grey star denotes Cys substitution) (also see **Section 6.4.3**).

(c) PXs are molecular tweezers that function without the need of pharmacologically-active ligand moieties. In BMA, the aliphatic chain of the non-isomerizable model crosslinker bismaleimidohexane is replaced by AB. BMA enforced open channel conformations in trimeric ion channels.

Figure 3. MPC088, a modulating PCL of $GABA_A$Rs

(a) MPC088, here shown in its active *trans*-isomer, acts as a photochromic potentiator of $GABA_A$Rs.

(b) At high concentrations, *trans*-MPC088 gates $GABA_A$Rs, while at low concentrations, GABA currents are potentiated by *trans*-MPC088.

(c) Representative current traces recorded from *X. laevis* oocytes expressing $\alpha_1\beta_2\gamma_2$ $GABA_A$Rs at increasing concentrations of *trans*-MPC088.

(d) Representative current traces recorded from *X. laevis* oocytes expressing $\alpha_1\beta_2\gamma_2$ $GABA_A$Rs at increasing concentrations of *trans*-MPC088 co-applied with 3μM GABA. Part c and d is reprinted by permission from Macmillan Publishers Ltd: Nature Communications (Yue et al. 2012), copyright 2012.

Figure 4. QAQ, a PCL of voltage-gated cation channels

(a) QAQ, here shown in its active *trans*-isomer, acts as a blocker of voltage-gated cation channels.

(b) QAQ enters cells through TRPV1 or P2X ion channels upon activation by noxious stimuli and is active at various ion channel targets (c).

(d) Representative current traces recorded during AP firing in a mammalian neuron with QAQ applied on the intracellular side. Firing rate normally increases with increasing current injection (magenta trace) and this increase is blocked in response to 500nm light (green trace).

Part c and d is reprinted by permission from Macmillan Publishers Ltd: Nature Methods (Mourot et al. 2012), copyright 2012.

Figure 5. MAACh and MAHoCh, agonistic and antagonistic PTLs of nAChRs

(a) MAACh and MAHoCh, here shown in their active *cis*-isomer, act as an agonist and antagonist of nAChRs.

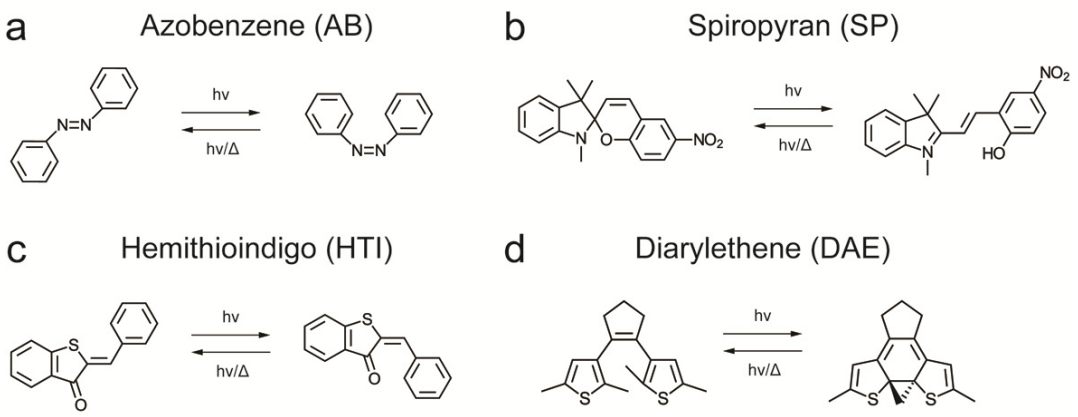
(b) Genetically engineered nAChRs (grey star denotes Cys substitution) are able to bind either MAACh or MAHoCh resulting in activation or competitive inhibition.

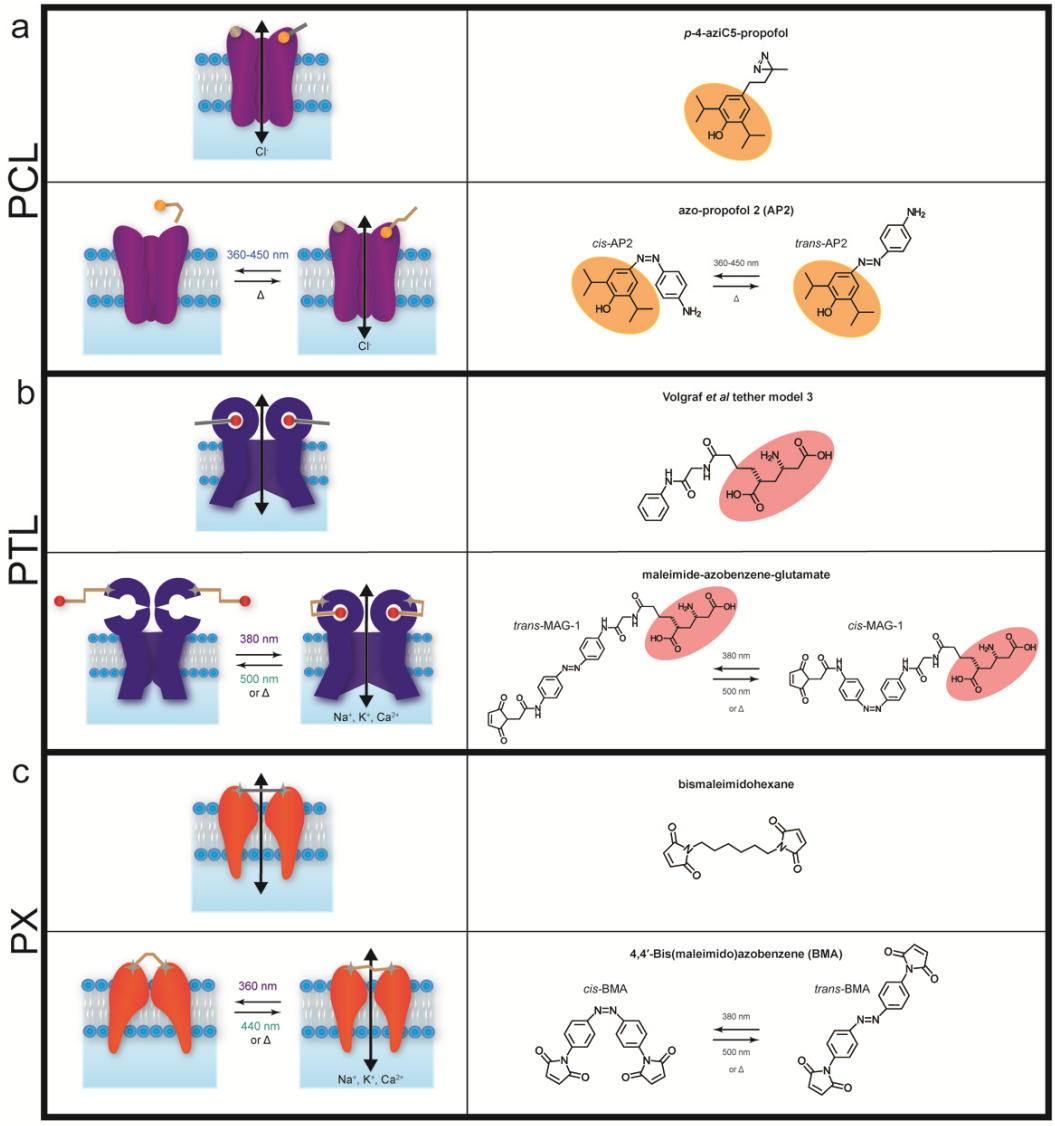
(c) Representative current trace recorded from *X. laevis* oocytes expressing $\alpha_3\beta_4$ E61C nAChRs functionalized with MAACh. Currents are elicited by 380nm light (magenta lines) and turned off by 500nm light (green lines).

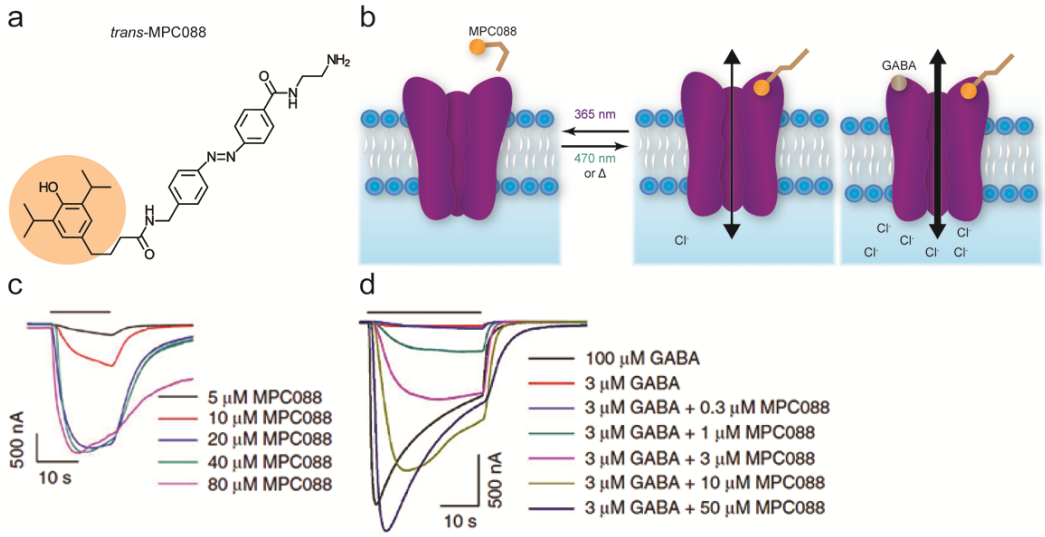
(d) Representative current trace recorded from *X. laevis* oocytes expressing $\alpha_3\beta_4$ E61C AChRs functionalized with MAHoCh. Currents elicited by ACh are reduced in response to 380nm light (magenta lines) but not 500nm light (green lines).
Part c and d is reprinted by permission from Macmillan Publishers Ltd: Nature Chemistry (Tochitsky et al. 2012), copyright 2012.

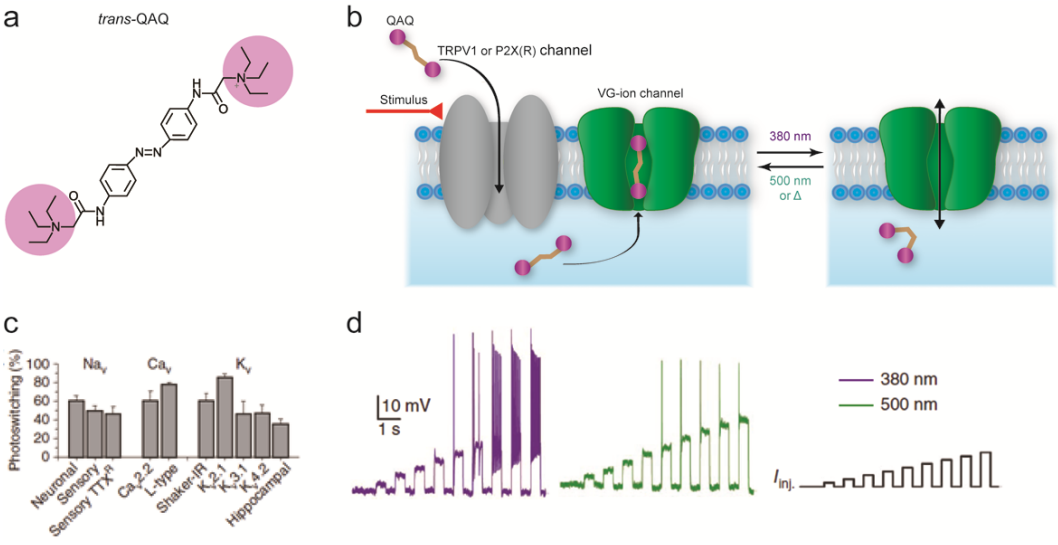
Figure 6. 'Subunit replacement strategy' based on MAQ, a PTL of K+ channels

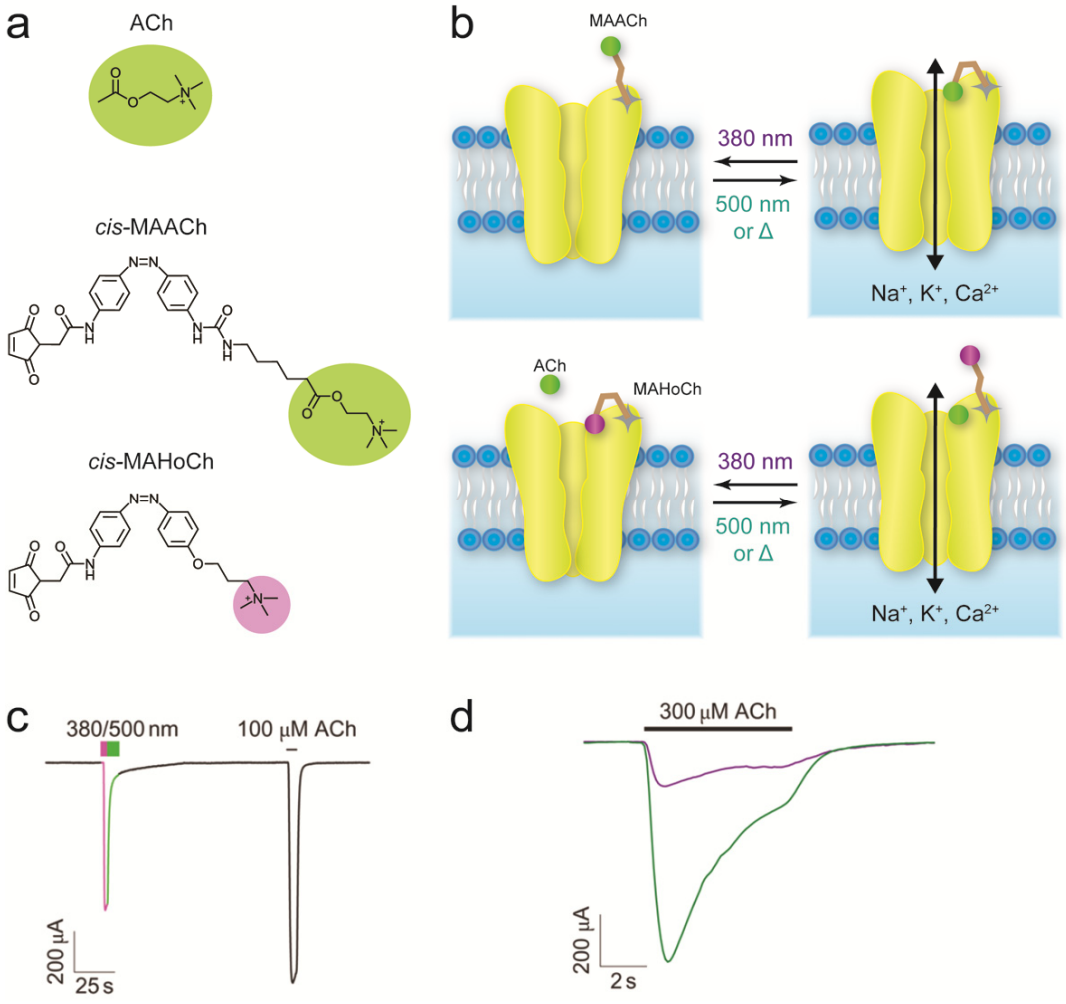
(a) MAQ, here shown in its active *cis*-isomer, acts as a blocker of K+ channels.
(b) PCS (orange) has been altered for PTL attachment and for impaired trafficking to the plasma membrane. In TREK1, retention was achieved by deletion of the C-terminus. As PCS and native subunits (green) assemble, the complex is transported to the membrane and, in this way, currents that resemble endogenous currents are under optical control.
(c) Representative current trace recorded from HEK293 cells expressing TREK1-PCS. Illumination with 380nm light (magenta lines) and 500nm light (green lines) reversibly blocks and releases block of outward current preceding (top) and following GABA application (bottom).
(d) Representative current trace for photomodulation of spontaneous firing in hippocampal neurons expressing TREK1-PCS (left). Firing rate averaged over time (min) during phasic illumination with 500nm and 380nm light (right).
Part c and d is reprinted from Neuron (Sandoz et al. 2012), Copyright 2012, with permission from Elsevier Ltd.

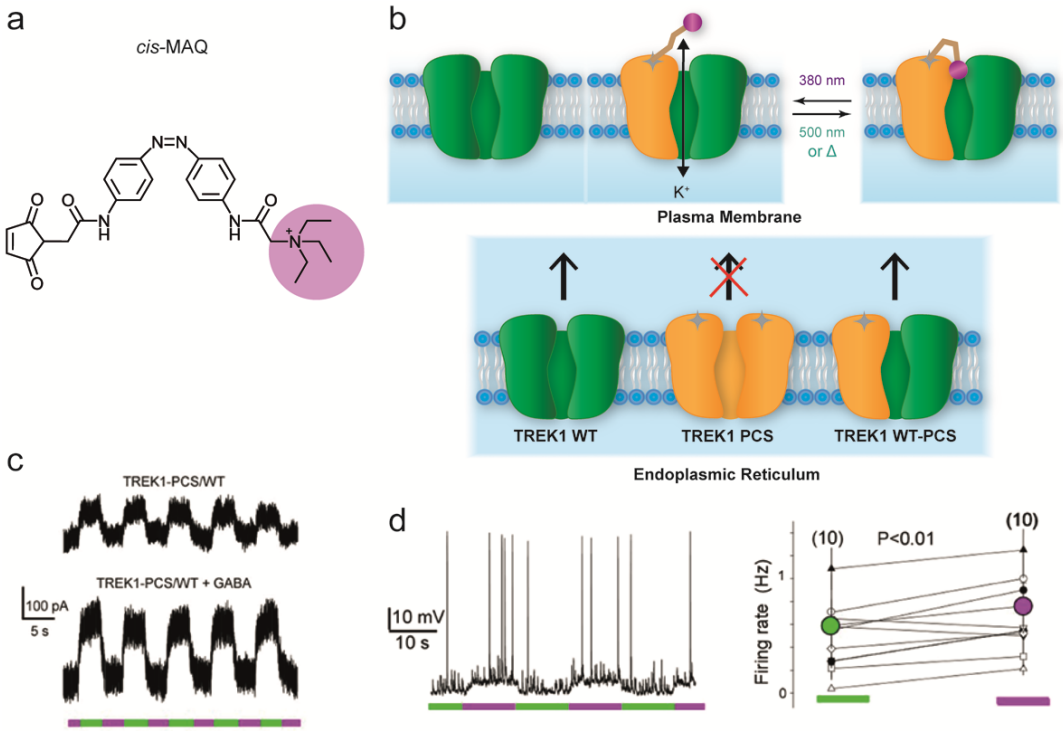

a
Azobenzene (AB)
hv
hv/Δ
b
Spiropyran (SP)
hv
hv/Δ
NO2
NO2
HO
c
Hemithioindigo (HTI)
hv
hv/Δ
d
Diarylethene (DAE)
hv
hv/Δ


a
PCL
Cl⁻
360-450 nm
Δ
Cl⁻
p-4-aziC5-propofol
azo-propofol 2 (AP2)
cis-AP2
360-450 nm
Δ
trans-AP2
b
PTL
Volgraf et al tether model 3
380 nm
500 nm
or Δ
Na⁺, K⁺, Ca²⁺
maleimide-azobenzene-glutamate
trans-MAG-1
380 nm
500 nm
or Δ
cis-MAG-1
c
PX
bismaleimidohexane
360 nm
440 nm
or Δ
Na⁺, K⁺, Ca²⁺
4,4′-Bis(maleimido)azobenzene (BMA)
cis-BMA
380 nm
500 nm
or Δ
trans-BMA


a
trans-MPC088
HO
N=N
NH2
O
N
H
O
b
MPC088
365 nm
470 nm
or Δ
Cl-
GABA
Cl-
Cl-
Cl-
Cl-
Cl-
Cl-
c
500 nA
10 s
5 μM MPC088
10 μM MPC088
20 μM MPC088
40 μM MPC088
80 μM MPC088
d
500 nA
10 s
100 μM GABA
3 μM GABA
3 μM GABA + 0.3 μM MPC088
3 μM GABA + 1 μM MPC088
3 μM GABA + 3 μM MPC088
3 μM GABA + 10 μM MPC088
3 μM GABA + 50 μM MPC088


a
trans-QAQ
b
QAQ
TRPV1 or P2X(R) channel
Stimulus
VG-ion channel
380 nm
500 nm
or Δ
c
Photoswitching (%)
100
80
60
40
20
0
Na$_v$
Ca$_v$
K$_v$
Neuronal
Sensory
Sensory TTX^R
Ca$_v$2.2
L-type
Shaker-IR
K$_v$2.1
K$_v$3.1
K$_v$4.2
Hippocampal
d
10 mV
1 s
380 nm
500 nm
$I_{inj.}$


a
ACh
cis-MAACh
cis-MAHoCh
b
MAACh
380 nm
500 nm
or Δ
Na+, K+, Ca2+
ACh
MAHoCh
380 nm
500 nm
or Δ
Na+, K+, Ca2+
c
380/500 nm
100 μM ACh
200 μA
25 s
d
300 μM ACh
200 μA
2 s


a
cis-MAQ
N=N
O
O
N
H
N
H
O
O
+
N
b
380 nm
500 nm
or Δ
K+
Plasma Membrane
TREK1 WT
TREK1 PCS
TREK1 WT-PCS
Endoplasmic Reticulum
c
TREK1-PCS/WT
TREK1-PCS/WT + GABA
100 pA
5 s
d
10 mV
10 s
(10)
P<0.01
(10)
Firing rate (Hz)
1
0

References

Aldoshin SM (1990) Spiropyrans: structural features and photochemical properties. Russ Chem Rev 59:663-684

Banghart M, Borges K, Isacoff E, Trauner D, Kramer RH (2004) Light-activated ion channels for remote control of neuronal firing. Nat Neurosci 7 (12):1381-1386

Banghart MR, Mourot A, Fortin DL, Yao JZ, Kramer RH, Trauner D (2009) Photochromic blockers of voltage-gated potassium channels. Angew Chem Int Ed Engl 48 (48):9097-9101. doi:10.1002/anie.200904504

Bartels E, Wassermann NH, Erlanger BF (1971) Photochromic activators of the acetylcholine receptor. Proc Natl Acad Sci U S A 68 (8):1820-1823

Beharry AA, Woolley GA (2011) Azobenzene photoswitches for biomolecules. Chem Soc Rev 40 (8):4422-4437. doi:10.1039/c1cs15023e

Berkovic G, Krongauz V, Weiss V (2000) Spiropyrans and spirooxazines for memories and switches. Chem Rev 100 (5):1741-1754

Bieth J, Vratsanos SM, Wassermann N, Erlanger BF (1969) Photoregulation of biological activity by photocromic reagents. II. Inhibitors of acetylcholinesterase. Proc Natl Acad Sci U S A 64 (3):1103-1106

Binshtok AM, Bean BP, Woolf CJ (2007) Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers. Nature 449 (7162):607-610. doi:10.1038/nature06191

Browne LE, Nunes JP, Sim JA, Chudasama V, Bragg L, Caddick S, Alan North R (2014) Optical control of trimeric P2X receptors and acid-sensing ion channels. Proc Natl Acad Sci U S A 111 (1):521-526. doi:10.1073/pnas.1318582111

Caporale N, Kolstad KD, Lee T, Tochitsky I, Dalkara D, Trauner D, Kramer R, Dan Y, Isacoff EY, Flannery JG (2011) LiGluR restores visual responses in rodent models of inherited blindness. Mol Ther 19 (7):1212-1219. doi:10.1038/mt.2011.103

Chabala LD, Gurney AM, Lester HA (1985) Photoactivation and dissociation of agonist molecules at the nicotinic acetylcholine receptor in voltage-clamped rat myoballs. Biophys J 48 (2):241-246. doi:10.1016/S0006-3495(85)83777-2

Chabala LD, Lester HA (1986) Activation of acetylcholine receptor channels by covalently bound agonists in cultured rat myoballs. J Physiol 379:83-108

Chambers JJ, Banghart MR, Trauner D, Kramer RH (2006) Light-induced depolarization of neurons using a modified Shaker K(+) channel and a molecular photoswitch. J Neurophysiol 96 (5):2792-2796

Chen X, Islamova NI, Robles RV, Lees WJ (2011) Photochromic properties of a water-soluble methyl carboxylic acid indolylfulgimide. Photochem Photobiol Sci 10 (6):1023-1029. doi:10.1039/c1pp05016h

Cordes T, Heinz B, Regner N, Hoppmann C, Schrader TE, Summerer W, Ruck-Braun K, Zinth W (2007) Photochemical Z-->E isomerization of a hemithioindigo/hemistilbene omega-amino acid. Chemphyschem 8 (11):1713-1721

Deal WJ, Erlanger BF, Nachmansohn D (1969) Photoregulation of biological activity by photochromic reagents. 3. Photoregulation of bioelectricity by acetylcholine receptor inhibitors. Proc Natl Acad Sci U S A 64 (4):1230-1234

Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules (2005). Wiley-VCH,

Eggers K, Fyles TM, Montoya-Pelaez PJ (2001) Synthesis and characterization of photoswitchable lipids containing hemithioindigo chromophores. J Org Chem 66 (9):2966-2977

Fasold H, Klappenberger J, Meyer C, Remold H (1971) Bifunctional reagents for the crosslinking of proteins. Angew Chem Int Ed Engl 10 (11):795-801

Fehrentz T, Kuttruff CA, Huber FM, Kienzler MA, Mayer P, Trauner D (2012) Exploring the pharmacology and action spectra of photochromic open-

channel blockers. Chembiochem 13 (12):1746-1749. doi:10.1002/cbic.201200216

Fortin DL, Dunn TW, Fedorchak A, Allen D, Montpetit R, Banghart MR, Trauner D, Adelman JP, Kramer RH (2011) Optogenetic photochemical control of designer K+ channels in mammalian neurons. J Neurophysiol 106 (1):488-496. doi:jn.00251.2011 [pii]

10.1152/jn.00251.2011

Fritzsche J (1867). C R Acad Sci 69:1035-1037

Fujimoto K, Kajino M, Sakaguchi I, Inouye M (2012) Photoswitchable, DNA-binding helical peptides assembled with two independently designed sequences for photoregulation and DNA recognition. Chemistry 18 (32):9834-9840. doi:10.1002/chem.201201431

Herre S, Steinle, W., Rück-Braun, K. (2005) Synthesis of photoswitchable hemithioindigo-based ω-amino acids and application in Boc-based peptide assembly. Synthesis 19:3297-3300

Hirshberg Y (1950). C R Acad Sci 231

Irie M (2000) Diarylethenes for memories and switches. Chem Rev 100 (5):1685-1716

Izquierdo-Serra M, Trauner D, Llobet A, Gorostiza P (2013) Optical control of calcium-regulated exocytosis. Biochim Biophys Acta 1830 (3):2853-2860. doi:10.1016/j.bbagen.2012.11.003

Janovjak H, Sandoz G, Isacoff EY (2011) A modern ionotropic glutamate receptor with a K(+) selectivity signature sequence. Nat Commun 2:232. doi:10.1038/ncomms1231

Janovjak H, Szobota S, Wyart C, Trauner D, Isacoff EY (2010) A light-gated, potassium-selective glutamate receptor for the optical inhibition of neuronal firing. Nat Neurosci 13 (8):1027-1032

Ji TH (1983) Bifunctional reagents. Methods Enzymol 91:580-609

Kienzler MA, Reiner A, Trautman E, Yoo S, Trauner D, Isacoff EY (2013) A red-shifted, fast-relaxing azobenzene photoswitch for visible light control of an ionotropic glutamate receptor. J Am Chem Soc 135 (47):17683-17686. doi:10.1021/ja408104w

Kumita JR, Smart OS, Woolley GA (2000) Photo-control of helix content in a short peptide. Proc Natl Acad Sci U S A 97 (8):3803-3808

Lemoine D, Habermacher C, Martz A, Mery PF, Bouquier N, Diverchy F, Taly A, Rassendren F, Specht A, Grutter T (2013) Optical control of an ion channel gate. Proc Natl Acad Sci U S A 110 (51):20813-20818. doi:10.1073/pnas.1318715110

Lester HA, Krouse ME, Nass MM, Wassermann NH, Erlanger BF (1979) Light-activated drug confirms a mechanism of ion channel blockade. Nature 280 (5722):509-510

Lester HA, Krouse ME, Nass MM, Wassermann NH, Erlanger BF (1980) A covalently bound photoisomerizable agonist: comparison with reversibly bound agonists at Electrophorus electroplaques. J Gen Physiol 75 (2):207-232

Levitz J, Pantoja C, Gaub B, Janovjak H, Reiner A, Hoagland A, Schoppik D, Kane B, Stawski P, Schier AF, Trauner D, Isacoff EY (2013) Optical control of metabotropic glutamate receptors. Nat Neurosci 16 (4):507-516. doi:10.1038/nn.3346

Li D, Herault K, Isacoff EY, Oheim M, Ropert N (2012) Optogenetic activation of LiGluR-expressing astrocytes evokes anion channel-mediated glutamate release. J Physiol 590 (Pt 4):855-873. doi:10.1113/jphysiol.2011.219345

Mao S, Benninger RK, Yan Y, Petchprayoon C, Jackson D, Easley CJ, Piston DW, Marriott G (2008) Optical lock-in detection of FRET using synthetic and genetically encoded optical switches. Biophys J 94 (11):4515-4524. doi:10.1529/biophysj.107.124859

Marriott G, Mao S, Sakata T, Ran J, Jackson DK, Petchprayoon C, Gomez TJ, Warp E, Tulyathan O, Aaron HL, Isacoff EY, Yan Y (2008) Optical lock-in detection imaging microscopy for contrast-enhanced imaging in living cells. Proc Natl Acad Sci U S A 105 (46):17789-17794

Mayer ML (2011) Structure and mechanism of glutamate receptor ion channel assembly, activation and modulation. Curr Opin Neurobiol 21 (2):283-290. doi:10.1016/j.conb.2011.02.001

Mostoslavskii MA, Kravchenko, M. D. (1970) Absorption spectra of 3-oxo-2,3-dihydrothionaphthene and its derivatives. Chem Heterocycl Comp 4:45-47

Mourot A, Fehrentz T, Le Feuvre Y, Smith CM, Herold C, Dalkara D, Nagy F, Trauner D, Kramer RH (2012) Rapid optical control of nociception with an ion-channel photoswitch. Nat Methods 9 (4):396-402. doi:10.1038/nmeth.1897

Mourot A, Kienzler MA, Banghart MR, Fehrentz T, Huber FM, Stein M, Kramer RH, Trauner D (2011) Tuning photochromic ion channel blockers. ACS Chem Neurosci 2 (9):536-543. doi:10.1021/cn200037p

Nargeot J, Lester HA, Birdsall NJ, Stockton J, Wassermann NH, Erlanger BF (1982) A photoisomerizable muscarinic antagonist. Studies of binding and of conductance relaxations in frog heart. J Gen Physiol 79 (4):657-678

Numano R, Szobota S, Lau AY, Gorostiza P, Volgraf M, Roux B, Trauner D, Isacoff EY (2009) Nanosculpting reversed wavelength sensitivity into a photoswitchable iGluR. Proc Natl Acad Sci USA 106 (16):6814-6819

Petchprayoon C, Yan Y, Mao S, Marriott G (2011) Rational design, synthesis, and characterization of highly fluorescent optical switches for high-contrast optical lock-in detection (OLID) imaging microscopy in living cells. Bioorg Med Chem 19 (3):1030-1040. doi:10.1016/j.bmc.2010.07.015

Polosukhina A, Litt J, Tochitsky I, Nemargut J, Sychev Y, De Kouchkovsky I, Huang T, Borges K, Trauner D, Van Gelder RN, Kramer RH (2012) Photochemical

restoration of visual responses in blind mice. Neuron 75 (2):271-282. doi:10.1016/j.neuron.2012.05.022

Rau H (1973) Spectroscopic Properties of Organic Azo Compounds. Angew Chem Int Ed Engl 12:224-235

Regner N, Herzog TT, Haiser K, Hoppmann C, Beyermann M, Sauermann J, Engelhard M, Cordes T, Ruck-Braun K, Zinth W (2012) Light-switchable hemithioindigo-hemistilbene-containing peptides: ultrafast spectroscopy of the Z --> E isomerization of the chromophore and the structural dynamics of the peptide moiety. J Phys Chem B 116 (14):4181-4191. doi:10.1021/jp300982a

Reiter A, Skerra A, Trauner D, Schiefner A (2013) A photoswitchable neurotransmitter analogue bound to its receptor. Biochemistry 52 (50):8972-8974. doi:10.1021/bi4014402

Ridge KD, Palczewski K (2007) Visual rhodopsin sees the light: structure and mechanism of G protein signaling. J Biol Chem 282 (13):9297-9301

Rizzini L, Favory JJ, Cloix C, Faggionato D, O'Hara A, Kaiserli E, Baumeister R, Schafer E, Nagy F, Jenkins GI, Ulm R (2011) Perception of UV-B by the Arabidopsis UVR8 protein. Science 332 (6025):103-106. doi:10.1126/science.1200660

Rockwell NC, Lagarias JC (2006) The structure of phytochrome: a picture is worth a thousand spectra. Plant Cell 18 (1):4-14

Samanta S, Woolley GA (2011) Bis-azobenzene crosslinkers for photocontrol of peptide structure. Chembiochem 12 (11):1712-1723. doi:10.1002/cbic.201100204

Sandoz G, Levitz J, Kramer RH, Isacoff EY (2012) Optical control of endogenous proteins with a photoswitchable conditional subunit reveals a role for TREK1 in GABA(B) signaling. Neuron 74 (6):1005-1014. doi:10.1016/j.neuron.2012.04.026

Standaert RF, Park SB (2006) Abc amino acids: design, synthesis, and properties of new photoelastic amino acids. J Org Chem 71 (21):7952-7966

Stawski P, Sumser M, Trauner D (2012) A photochromic agonist of AMPA receptors. Angew Chem Int Ed Engl 51 (23):5748-5751. doi:10.1002/anie.201109265

Stein M, Middendorp SJ, Carta V, Pejo E, Raines DE, Forman SA, Sigel E, Trauner D (2012) Azo-propofols: photochromic potentiators of GABA(A) receptors. Angew Chem Int Ed Engl 51 (42):10500-10504. doi:10.1002/anie.201205475

Szobota S, Gorostiza P, Del Bene F, Wyart C, Fortin DL, Kolstad KD, Tulyathan O, Volgraf M, Numano R, Aaron HL, Scott EK, Kramer RH, Flannery J, Baier H, Trauner D, Isacoff EY (2007) Remote control of neuronal activity with a light-gated glutamate receptor. Neuron 54 (4):535-545

Tochitsky I, Banghart MR, Mourot A, Yao JZ, Gaub B, Kramer RH, Trauner D (2012) Optochemical control of genetically engineered neuronal nicotinic acetylcholine receptors. Nat Chem 4 (2):105-111. doi:10.1038/nchem.1234

Tochitsky I, Polosukhina A, Degtyar VE, Gallerani N, Smith CM, Friedman A, Van Gelder RN, Trauner D, Kaufer D, Kramer RH (2014) Restoring Visual Function to Blind Mice with a Photoswitch that Exploits Electrophysiological Remodeling of Retinal Ganglion Cells. Neuron 81 (4):800-813. doi:10.1016/j.neuron.2014.01.003

Volgraf M, Gorostiza P, Numano R, Kramer RH, Isacoff EY, Trauner D (2006) Allosteric control of an ionotropic glutamate receptor with an optical switch. Nat Chem Biol 2 (1):47-52

Volgraf M, Gorostiza P, Szobota S, Helix MR, Isacoff EY, Trauner D (2007) Reversibly caged glutamate: a photochromic agonist of ionotropic glutamate receptors. J Am Chem Soc 129 (2):260-261. doi:10.1021/ja067269o

Wyart C, Bene FD, Warp E, Scott EK, Trauner D, Baier H, Isacoff EY (2009) Optogenetic dissection of a behavioural module in the vertebrate spinal cord. Nature 461 (7262):407-410

Yokoyama Y (2000) Fulgides for Memories and Switches. Chem Rev 100 (5):1717-1740

Yue L, Pawlowski M, Dellal SS, Xie A, Feng F, Otis TS, Bruzik KS, Qian H, Pepperberg DR (2012) Robust photoregulation of GABA(A) receptors by allosteric modulation with a propofol analogue. Nat Commun 3:1095. doi:10.1038/ncomms2094