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Seeing in the future – a perspective on combining light with chemical biology approaches to treat retinal pathologies

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Abstract: New concepts to treat eye diseases have emerged that elegantly combine unnatural light exposure with chemical biology approaches to achieve superior cellular specificity and, as a result, improvement of visual function. Historically, light exposure without further molecular eye treatment has offered limited success including photocoagulation to halt pathological blood vessel growth or low light exposure to stimulate retinal cell viability. To add cellular specificity to such treatments, researchers have introduced various biological or chemical light-sensing molecules and combined those with light exposure. (Pre-) clinical trials describe the use of optogenetics and channelrhodopsins, i.e. light-sensitive ion channels, in patient vision restoration. In the chemical arena, pharmacological agents, rendered light-sensitive by reversible modification with photosensitive protecting compounds (“caging”), have been applied to eyes of living mice to photo-release specific cellular activities. Among these were successful proof-of-principle experiments that were conducted to establish photo-sensitive gene therapies in the eye. For light-mediated treatment in combination with chemical biology, we wish to describe here the current frontiers of research in vision restoration with an eye on differences between biological and chemical light-sensing molecules, patient requirements, and future outlooks.

1. Introduction

In biomedical research, and especially in neuroscience, novel optical methods provide deeper insights into physiological processes than ever before. Optogenetics, the use of light-sensitive ion channels derived from algae, has revolutionized neurophysiological research.¹ Expression of exogenous, microbial opsins generated functional light-sensitive channels in living animals because the required co-factor *all-trans* retinal is naturally also produced in vertebrates. However, for optogenetics experiments in *Xenopus* oocytes or acute brain slices, the underlying chemical biology would require adding exogenous *all-trans* retinal. Introduction of the so-called channelrhodopsins into excitable cells has equipped researchers with unprecedented possibilities to manipulate neurons with exquisite spatial and temporal resolution based on irradiation with light. The resulting light-induced firing patterns of neurons come close to those induced by natural stimuli so that even complex activity patterns can be recapitulated. Other prominent light-sensing, biologically derived molecules often contain the LOV (light-oxygen-voltage sensing) domain which has been used to molecularly engineer novel LOV fusion proteins.² Fusion proteins include small GTPases, DNA binding proteins, and enzymes among others. Attachment of the LOV domain thus can confer light-sensitivity to an otherwise light-insensitive protein. In the chemical arena, photosensitive protecting compounds allow a similar concept in that small, pharmacologically active molecules can be rendered light-sensitive upon modification with such ‘caging’ moieties.³ ‘Caging’ produces a reversible, yet light-sensitive conjugation with the target molecule whose activity can then be released upon photoactivation between wavelengths of usually around 300-500 nm, depending on the caging chromophore. Previously caged compounds include a wide variety of different molecules such ATP,⁴ calcium chelators,⁵ fluorescent molecules,⁶ neurotransmitters,⁷ lipids,⁸ peptides,⁹ DNA & RNA,¹⁰ and proteins, e.g. transcription factors¹¹ and antibodies.¹² The advantage of caging chemistry is that photo-release occurs on a microsecond timescale while spatially confined irradiation can provide exquisite precision, for example limiting photo-stimulation to individual synapses. The combination of caged pharmacological agents with photoactivation in the living eye thus has the advantage that treatment could be limited to pathological regions of the retina by restricting irradiation to designated areas. In fact, photoactivation could previously be limited to top layers of ~ 150 µm thick *ex vivo* hippocampal brain slices using two-photon microscopy,¹³ offering unprecedented resolution in Z and the exciting possibility to target specific layers of the retina. Being able to spatially restrict irradiation and the photo-release of pharmacological agents also has the added advantage that side-effects should be mostly limited to the target area and because of that, one could potentially afford to employ more potent or aggressive agents as they are not photo-released in healthy, non-targeted tissue areas. The second key advantage of photoactivation of caged pharmacological agents in the eye is the flexibility of being able to fine tune the exact concentration of the agent. High or low concentrations can be titrated as deemed necessary during at each treatment session. The subsequent pharmacological clearance of the agents also renders the eye insensitive to ambient light. The possibility to titrate the agent to target concentrations, its clearance, and the wide breadth of pharmacological molecules available for caging and possible medical intervention highlight photoactivation as a viable treatment option for the future.

The chromophores used in the caging moieties dictate the wavelengths used for photoactivation; there are several excellent reviews discussing the photochemistry.^{3,14} Uncaging wavelengths used to be between 300-400 nm several decades ago, but could be shifted to 400-500 nm and higher in recent years.¹⁴ Because the light-sensitivity of most recently developed caged compounds is considerably high, photo-toxicity from uncaging experiments in biological tissues is rarely reported. For applications in the eye however, additional considerations are warranted as

the photoreceptors are evolutionary optimized for high light-sensitivity. Consequently, a photoactivation procedure that is harmless to skin may cause damage to the retina. Phototoxicity in the eye has been extensively researched especially with the introduction of retinal prostheses and optogenetics as a possible means for patient treatment. The parameters with which retinas can safely be irradiated without causing damage are now well defined.¹⁵ These multitude of parameters include among others wavelength, repetition frequency, and pupil diameter. The latter indicates that these parameters are also patient-specific and clearly emphasize the need for personalized approaches. Another critical parameter is the wavelength used for light exposure. A few publications showed that the required levels of blue light (~450-500 nm) for efficient retinal manipulation were close to or above the safety limits.¹⁶ More recent studies successfully shifted to using lower energy photons at higher wavelengths of 500 nm and above, mostly in the red spectrum (> 590 nm).¹⁷ Newly optimized channelrhodopsins such as ChrimsonR¹⁸ have their peak absorbance in the red. Of note, retinal two-photon imaging has been demonstrated and thus blue light (one photon) absorbing caged compounds could potentially be photoactivated with far red light two-photon irradiation to further decrease photo-toxicity.

While the focus of this perspective is on the *combination* of unnatural light exposure with chemical biology approaches to improve visual function, there are also hundreds of publications in which eye treatments only with light are described. They generally fall into two categories – using light for irreversible tissue photocoagulation to prevent spreading of pathologies to other portions of the retina or using non-toxic levels of light to stimulate retinal cell viability through photochemical mechanisms that are not fully understood. Using only light for treatment offers no cellular specificity and is therefore severely limited in targeting any pathology directly. Nevertheless, both approaches showed some benefits in pathologies such as diabetic retinopathy,¹⁹ glaucoma²⁰ (both photocoagulation), and chorioretinopathy²¹ (subthreshold laser stimulation) but appear to be slowly replaced by more advanced treatments including combinations of light and chemical biology approaches as described here.

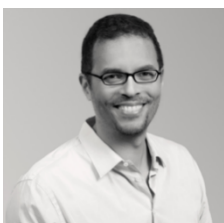
Alexandre SPECHT PhD in chemical biology from Strasbourg University. After post-doctoral research at the Howard Hughes Medical Institute at UCLA (USA), he joined the Centre National de la Recherche Scientifique at Strasbourg in 2004 as researcher (CR CNRS) and was promoted research director (DR CNRS) in 2015. Since 2011, he is group leader at the Faculty of Pharmacy of Strasbourg (UMR 7199, University of Strasbourg / CNRS). His group develops photochemical tools for the regulation of various biological activities and nanoparticles for light induced drug release.



Maxime Klimezak is a 4th year PhD student under the supervision of Dr. Alexandre Specht at the Laboratoire de Chemo-Biologie Synthétique et Thérapeutique (UMR 7199, University of Strasbourg / CNRS). His thesis is based on the synthesis of various photocleavable protecting groups, photosensitizers and self-immolative linkers to trigger the controlled release of drugs through assisted photolysis on nanoparticles.



Sidney Cambridge is currently an independent group leader at the Department of Physiological Chemistry, Johannes Gutenberg University, in Mainz (Germany). He received a Ph.D. in Biological Sciences at Carnegie Mellon University, Pittsburgh (USA) where he pioneered the concept of photoactivated gene expression in *Drosophila* embryos. He continued this line of research first as an EMBO postdoctoral fellow at the Max-Planck-Institute of Neurobiology in Munich (Germany) and then as lecturer and group leader at Heidelberg University (Germany). His current scientific focus is on interdisciplinary, translational research with an emphasis on neuronal plasticity.



2. Optogenetics and applications on retinal pathologies

Optogenetics and the use of light-sensitive channelrhodopsins burst onto the neuroscience scene in 2005.²² In 2006, the first attempt showing some vision restoration in a rodent using a microbial-type rhodopsin was published.²³ About 15 years later, the first clinical trials in patients with retinitis pigmentosa have begun.²⁴ These patients have lost most of their photoreceptors and show advanced retinal degeneration. The key advantage of therapeutic optogenetics is that such an approach could be employed independent of the underlying heterogenous genetic defect, as there are more than 100 known genes that can cause retinal degeneration. Loss of retinal photoreceptors can be compensated for by rendering the remaining healthy retinal cells light-sensitive with channelrhodopsins. Notably, channelrhodopsins typically exhibit an absorption coefficient of approximately $50,000 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at their peak absorption wavelength, accompanied by a photoisomerization quantum yield ranging from 0.2 to 0.6. It was found that surviving ganglion cells existed despite near complete degeneration of photoreceptor cells and that these neurons properly projected to the lateral geniculate nucleus of the visual pathway. Viral transduction of retinal ganglion cells thus appears promising as several groups could show partial vision restoration and even visually guided behavior in mice.²⁵ Notably, in these animal experiments, light-stimulation was not performed with ambient daylight but by use of unnatural light tailored to the peak absorbance of the channelrhodopsin. The key advancement from early studies with 'wild-type' blue light absorbing channelrhodopsins was the introduction of engineered (codon optimization, channel trafficking, etc.) channelrhodopsins (ChrimsonR and ReaChR)²⁶ with peak absorbances around 590 nm in the orange-red spectrum. These red-shifted opsins were originally developed to allow manipulation of separate neuronal populations within the same tissue with two distinct wavelengths (blue and red), but potentially made the biggest difference in therapeutic optogenetics for the eye. With red light stimulation of the eye, the safety limitations of blue light irradiation could be overcome, making such an approach suitable for patient treatment. In the first successful demonstration of partial vision recovery in a patient, the visual world was captured through a camera and projected as monochromatic light (590 nm) onto the treated eye with goggles in real time.²⁴ Four months prior, the eye had received a single intravitreal injection of an optimized adeno-associated virus (AAV) with ChrimsonR to allow for full expression of the channelrhodopsin transgene. The patient, who previously had visual acuity limited to light perception, could now identify objects and reach for them. This indicates functioning visuomotor performances which was also confirmed by EEG recordings in the occipital cortex during the task. Similar experiments were also conducted in non-human primates, again showing improvement of visual perception with patterned light illumination.¹⁷ Retinal ganglion cells were targeted in macaques with ChrimsonR AAVs and transduced retinas could be post-experimentally assessed after the animals were sacrificed.

Fig 1. shows a retinal explant and the ChrimsonR-positive retinal ganglion cells in red with a few axons visible that will project to the optical nerve of the visual path. Because of these powerful animal models and the possibility to analyze cells, tissues, and the anatomy of the visual path in much detail, researchers have the great opportunity to leverage these findings for patient treatment.

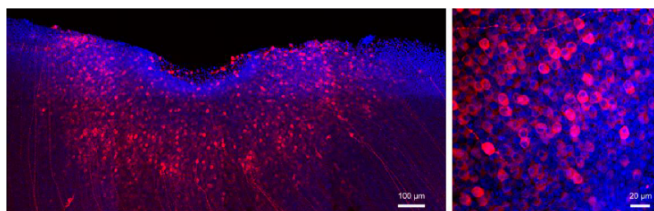


Figure 1. Left: Confocal stack projection of a macaque retina with the fovea in the center. Retinal ganglion cells with virally transduced ChrimsonR in red, DAPI stain in blue. Right: Higher magnification of transduced cells. Reproduced with permission¹⁷.

3. Photoactivatable molecules for the treatment of retinal pathologies: from photodynamic therapies to caged compounds:

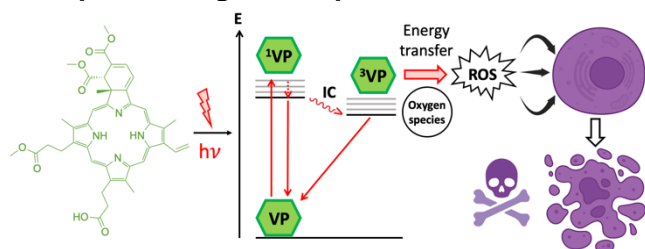


Figure 2: Chemical structure of verteporfin and PDT mechanism for abnormal cell destruction.

Although the concept of treating pathologies with light activated molecules is not new²⁷, recent years have seen a boom on the use of light activatable compounds in particular for the treatment of retinal pathologies. Indeed, light can be readily focused, allowing precise temporal and spatial definition of the stimulus. In addition, light exposure time and intensity can also be precisely controlled and, as a consequence, the strength of the stimulus can be modulated.²⁸ Compared to other tissues, the unique transparency of the vitreous makes it particularly beneficial for light-triggered therapies in eye.

The first example of a well-defined photochemical reaction using exogenous small molecules to treat retinal pathologies is based on Photodynamic Therapy (PDT). PDT relies on three essential elements: a photosensitizer (PS), light, and oxygen. Indeed, once the photosensitizer molecule has absorbed light, the PS excited triplet state can be generated. This state leads to energy or electron transfer to neighboring oxygen or substrate molecules.²⁹ Reactive Oxygen Species (ROS) and $^1\text{O}_2$ which are commonly accepted to be the main cytotoxic species being formed cause cellular damage and result in therapeutic outcomes like the destruction of abnormal cells or tissues. The first documented use of PDT for treating retinal pathologies dates to the nineties.³⁰ PDT was specifically introduced for the treatment of neovascular age-related macular degeneration (AMD). As a result, verteporfin (Visudyne), a hydrophobic photosensitizer capable of generating reactive oxygen species (Figure 2), was approved following clinical trials that demonstrated its efficacy in a liposomal formulation.³¹ The Table 1 summarize the photophysical and the photochemical properties of verteporfin. This therapy causes minimal damage to the neurosensory retina, preventing loss of visual acuity. The benefit of verteporfin therapy is using this therapy for patients who are not eligible for treatment by laser photocoagulation leading to a significant advancement in treating AMD. Noteworthy, photocoagulation is a thermal process in which high-intensity light energy is applied to naturally present light-absorbing chromophores, such as melanin or hemoglobin. Light energy is converted into heat, leading to nonselective coagulative necrosis of the targeted tissue. After PDT was approved for the treatment of neovascular AMD, it was gradually introduced to treat other chorioretinal diseases, such as central serous chorioretinopathy (CSC), polypoidal choroidal vasculopathy (PCV) and choroidal hemangioma.³²

Table 1. Photophysical and Photochemical properties of verteporfin in MeOH

Photosensitizer	Abs. λ_{max} (nm) / ϵ ($\text{M}^{-1} \text{cm}^{-1}$)	Singlet oxygen quantum yield Φ_{Δ}	$\epsilon \Phi_{\Delta}$ ($\text{M}^{-1} \text{cm}^{-1}$) at λ_{max}	Ref.
Verteporfin	686 (red band)/34000	0.76	25840	33

However, this photochemical approach relies on the destruction of abnormal cells or tissues. Conversely, light therapies should be able to precisely control a drug activity, particularly for the release of drugs able to treat retinal pathologies, to minimize side effects and enhance the therapeutic efficacy. On the other hand, many current therapies for the treatment of retina pathologies are based on strategies able to block the progress of neovascularization whether by inhibiting VEGF (vascular endothelial growth factor) either pharmacologically or through anti-VEGF antibodies or by using anti-angiogenic gene therapies. Light activatable versions of blocking VEGF potentially is an exciting development for personalized medicine.³⁴

The emerging field of photopharmacology is based on chemical biological approaches enabling an optical control of drug activities.³ It relies on integrating light-responsive moieties into drug structures, leading to reversible³⁵ or irreversible modulation of their activities. Ultimately this could solve the off-target toxicity of PDT or photocoagulation therapies.

Irreversible modulation involves the concept of ligand directed photocatalysts for the release of a biological effector³⁶ (a recently developed strategy outside the scope of this review) and the incorporation of photocleavable protecting groups (PPGs, also known as 'photo-cages') to mask the pharmacophoric features of a drug.¹⁴ The 'uncaging' process irreversibly removes a photolabile group, resulting in a local concentration increase of the active parent drug. In the following paragraphs we will discuss recent developments of so called 'caged compounds' able to control cellular mechanisms involved in retinal pathologies with a highlight on caged gene expression inducers for photoactivated transgene expression. Our focus is the use of caged compounds for *therapeutical* applications in the retina. However, we want to point out recent reviews in the field of light-responsive biomaterials for ocular drug delivery, such as light sensitive biomaterials based on the uses of photolytic reactions.³⁷

The first use of a caged compounds in living eyes was reported by Mascareñas and Vazquez in 2012.³⁸ This seminal work reported the use of o-nitrobenzyl caged DNA-binding dyes (such as caged DAPI and caged ethidium bromide) to demonstrate that these caged dyes could be activated by external irradiation in the eyes of living mice. However, the PPG used in this work necessitated UV irradiation for the uncaging process, which is characterized by low penetration depth in living tissues due to light absorption of native biological chromophores and high diffusion by scattering tissues, leading to cellular phototoxicity. The chemical challenge during the last three decade was therefore to find ways for 'uncaging' with lower energy light to induce photochemical reactions.¹⁴ Of note, the efficiency of a PPG to undergo a specific chemical bond cleavage using classical one-photon excitation (also called the 'uncaging cross-section') is defined as the product of the molar extinction coefficient (ϵ) and the quantum yield of photolytic reaction (ϕ). In the last decade various PPGs have been described for bond cleavage using visible light. One approach to solve this problem was to modify caging groups by extending π -conjugation of the light-induced excited state during photolysis. This was achieved by introducing heteroatoms and functional groups into the ring system, enabling a larger dipole change upon excitation. As a result, blue to green light-sensitive PPGs have been developed with coumarin,³⁹ o-nitrobenzyl,⁴⁰ cinnamate,⁴¹ and o-nitrophenethyl series.⁴²

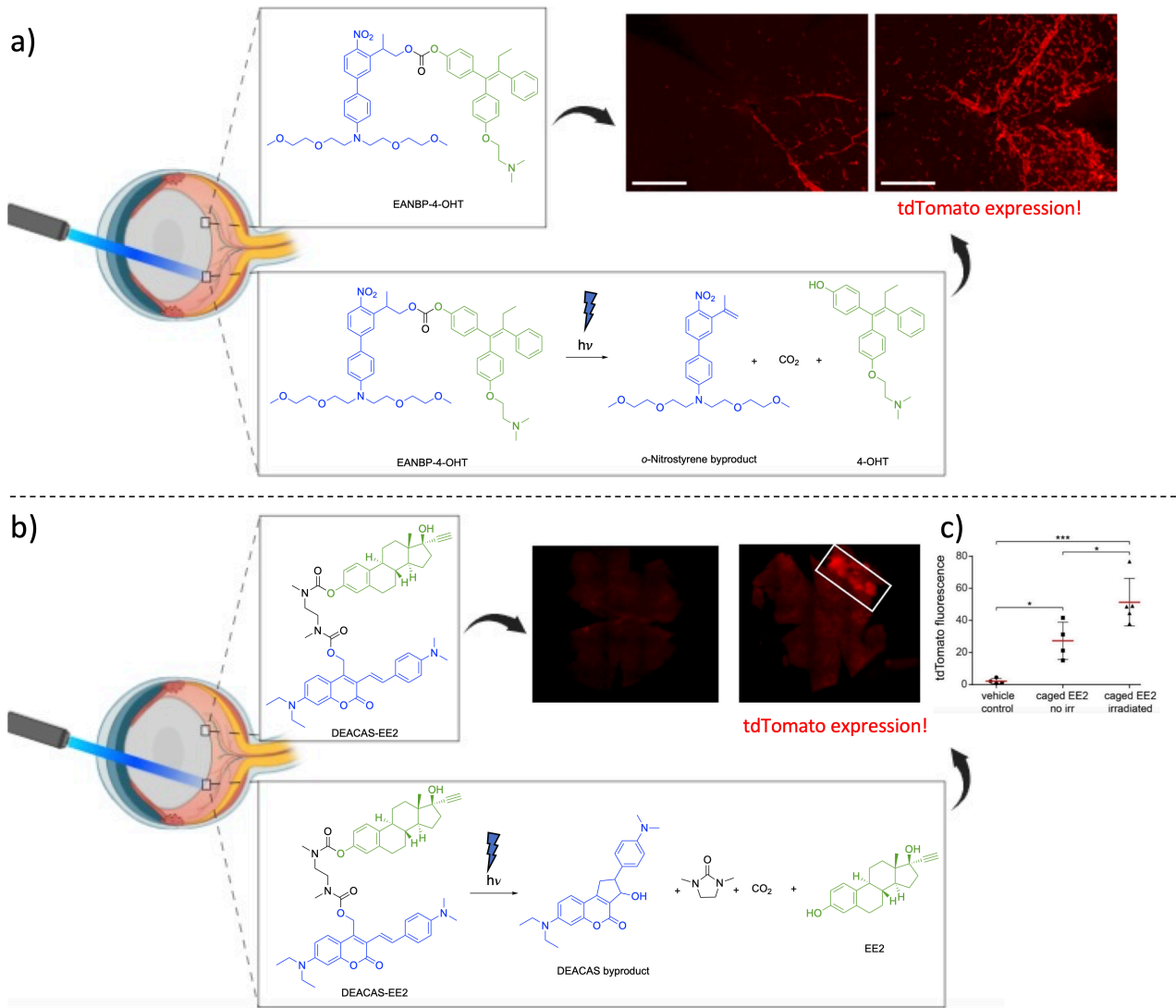


Figure 3: Chemical structure of EANBP-4OHT (a) and DEACAS-EE2 (b), proposed photolytic reactions and application to light induced gene expression using the Cre/lox system, c) Photoactivation of DEACAS-EE2 in mouse eyes in vivo induced retinal tdTomato expression. Each data point represents one retina. Control injections without DEACAS-EE2 did not produce any fluorescence. Irradiation of eyes injected with DEACAS-EE2 increased tdTomato fluorescence (number of tdTomato fluorescence positive cells over threshold per mm² retinal area) compared to unirradiated eyes. Reproduced with permission from ref^{44,46}. EANBP-4OHT = (2-(4'-((di(tris-ethoxy(methoxy))amino)-4-nitro-[1,1'-biphenyl]-3-yl) propan-1-ol)-4-Hydroxy-Tamoxifen, DEACAS-EE2=(DiEthyl-AminoCoumarin-Amino- Stilbene)-ethylestradiol.

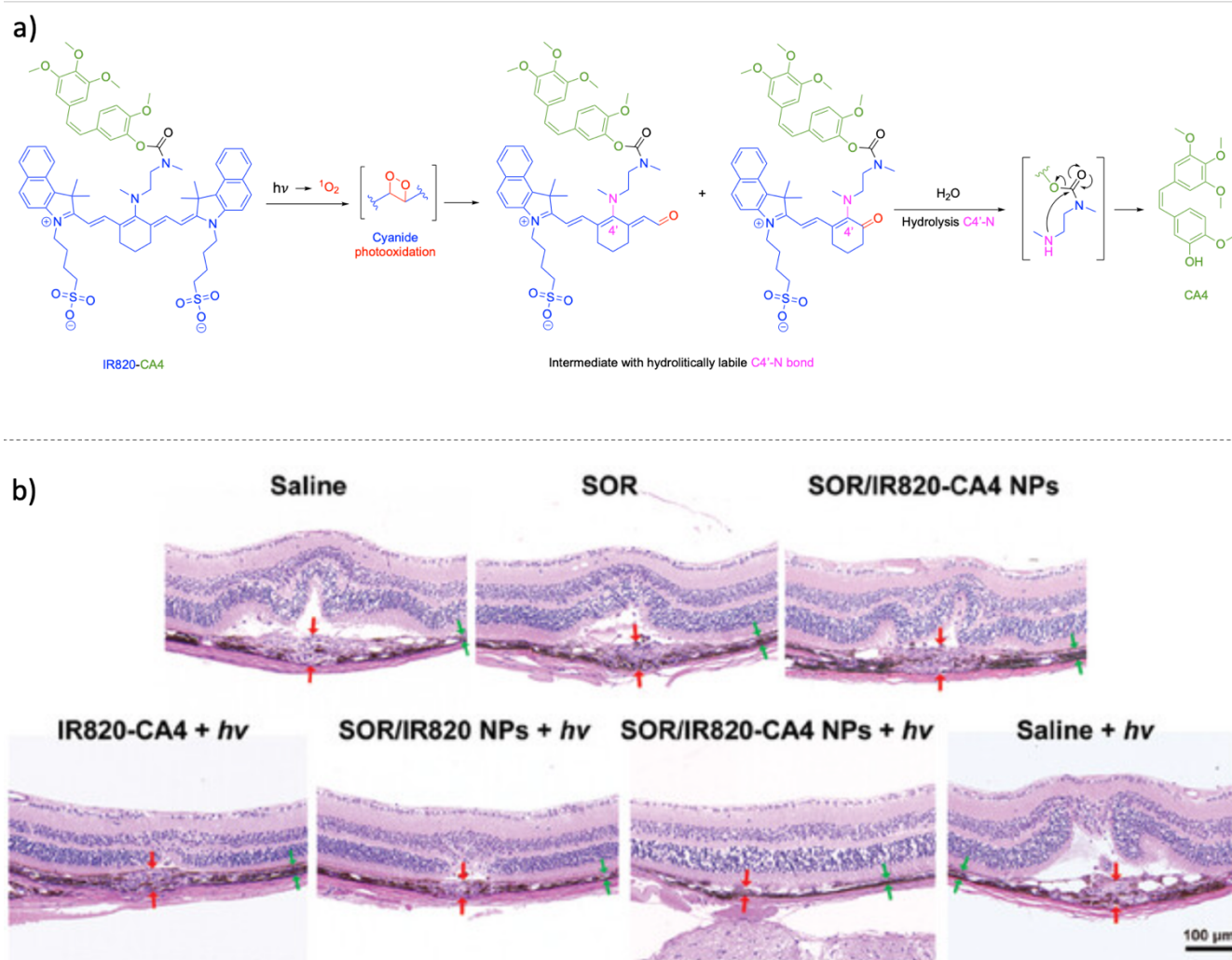


Figure 4: a) Chemical structure and proposed mechanism of the light-assisted chemical bond cleavage via a regioselective photooxidative polyene reaction, b) Representative hematoxylin and eosin (H&E)-stained eye cross-sections from the CNV mice after different treatments. Red arrows denote CNV lesions and green arrows denote normal choroid demonstrating CNV suppression by SOR/IR820-CA4 NPs in a laser-induced CNV mouse model. Reproduced with permission from ref⁴⁸, CA4 = Combretastatin A-4, SOR = Sorafenib, IR820 = Indocyanine PPG.

In a interdisciplinary collaborative research effort between our two laboratories we decided to develop photoactivated transgene expression paradigm using caged molecules for applications in retina pathologies. The idea was to regulate transgene expression with high spatiotemporal control in the eye using light as a noninvasive inducing agent. We focused on photoactivated gene expression paradigm for retinal tissue based on the inducible Cre/lox system and caged Tamoxifen analogs. The inducible Cre/lox system is the most prominent conditional transgene expression system available, as witnessed by almost one thousand different Cre-related transgenic mouse lines (JAX mice) for tissue- and cell-type specific genetic manipulation including vascular pathologies.⁴³ Upon binding of Tamoxifen (analog) to a fusion protein (designated 'CreERT2')⁴⁴ of the mutated human estrogen receptor (hER) and Cre recombinase, excision of DNA is used for permanent induction or deletion of transgenes. Two caged compounds were synthesized, a caged version of Tamoxifen⁴⁵ and a caged version of ethinylestradiol (EE2)⁴⁶ (Figure 3). The caged tamoxifen analogue is based on a EANBP (2-(4'-((di(tris-ethoxy(methoxy))amino)-4-nitro-[1,1'-biphenyl]-3yl) propan-1-ol) PPG.^{42d} Noteworthy, this PPG was coupled to the phenol function of 4-hydroxytamoxifen (4-OHT, an active metabolite of tamoxifen) using a carbonate linkage (see figure 3a for the structure of the EANBP-4-OHT caged compound). Because tamoxifen itself is light sensitive and tends to photodegrade,⁴⁷ we screened a few compounds for their ability to induce transgene expression with the inducible Cre/lox system and discovered that EE2 exhibited similar induction potency as Tamoxifen.⁴⁶ Thus, as an alternative to 4-OHT, EE2 was coupled to a DEACAS (DiEthyl-AminoCoumarin-Amino- Stilbene) PPG^{39d} using a self-immolative carbamate linker (see figure 3b for the structure of the DEACAS-EE2 caged compound). After injecting caged Tamoxifen or caged EE2 into the eyes of Cre/lox transgenic mice, irradiation successfully triggered *in vivo* photoactivation of tdTomato transgene expression in the retinas.⁴⁶ For these experiments, we used double transgenic mice that expressed CreERT2 either under the control of the astrocytic GFAP promoter or the blood vessel-specific Tie2 promoter. In addition, mice had a Cre-dependent tdTomato transgene ('Ai14' line) so that transcriptionally active Tamoxifen or EE2 induced red tdTomato fluorescence.^{45,46} For *in vivo* photoactivated transgene expression in the eyes, intravitreal injections were chosen. Comparison of eyes that were injected with caged EE2 but not irradiated with eyes that were injected with caged EE2 and irradiated, showed that photoactivation roughly

doubled retinal transgene expression (Figure 3 c).⁴⁶ These experiments clearly demonstrated that it is now possible to photoactivate caged compounds in the eye for manipulation of cellular activity.

Recently the group of Wang described the use of a red light sensitive caged compound for the treatment of Choroidal neovascularization (CNV).⁴⁸ The primary treatment for CNV is an anti-vascular endothelial growth factor (VEGF) therapy. However, regular invasive intravitreal administration can lead to serious complications, such as traumatic cataracts and endophthalmitis.⁴⁹ A promising treatment option for CNV is the combination of anti-VEGF medications with vascular disrupting agents that block blood vessels. Combretastatin A-4 (CA4) is a leading candidate for vascular disruption, targeting vessel ablation. As a tubulin-binding agent, CA4 inhibits microtubule polymerization in endothelial cells, resulting in cytoskeletal disruption and regression of CNV. In order to be able to release CA-4 locally in the eye, the group of Wang used a red-light sensitive PPG (IR820), a type of PPG initially developed by the Schnermann group.⁵⁰ In particular, the C4'-dialkylamine-substituted variants of a heptamethine cyanine is able to generate singlet oxygen and can therefore induce a light-assisted chemical bond cleavage via a regioselective photooxidative polyene reaction using 690 nm excitation (Figure 4a). This scaffold was used in combination with a self-immolative linker to liberate CA-4. Noteworthy, this interesting system still suffers from a low uncaging efficiency presumably due to the low efficiency of the photolytic reaction based on a photooxidation reaction followed by scission reaction.⁵⁰ More importantly, the caged CA-4 is able to form a nanoparticle (NP) with the VEGF receptor inhibitor sorafenib (SOR) enclosed. Near infrared irradiation (690 nm) triggered the photolysis of caged CA4 which also caused nanoparticle disassembly, leading to the release SOR (an anti-angiogenic drug) and CA4 (a vasculature disrupting agent). Controlled release of SOR and CA4 in the ocular tissue was achieved after intravenous administration of caged CA4/SOR NPs and light illumination to the eyes. More importantly, this type of caged compound significantly downregulated angiogenic signaling, reduced neovascular leakage, and achieved approximately 87% suppression of CNV growth in a laser-induced CNV mouse model (Figure 4b). Furthermore, caged CA4/SOR NP treatment caused no significant systemic or localized side effects, largely due to the inactivity of the caged-CA4 in the dark and minimal photo-damage upon light irradiation. This study paves the way to new promising approaches which overcome the limitations of traditional intravitreal administration of anti-VEGF treatment by using light-activation of standard treatment for retinal pathologies.

The table 2 summarize the photophysical and the photochemical properties of the caged compounds applied to the treatment of vascular pathologies.

Table 2. Photophysical and Photochemical properties of caged compounds used for the treatment of vascular pathologies in mice models.

Caged compound	Abs. λ_{max} (nm) / ϵ ($M^{-1} cm^{-1}$)	Φ_L	$\epsilon \Phi_L$ ($M^{-1} cm^{-1}$) at λ_{max}	Ref.
EANBP-4OHT	397/7500	0.16	1200	45
DEACAS-EE2	446/41000	0.12	4920	46
IR820-C4A	710/not reported	Not reported	Not reported	48

5. Compliance, clinical complications, and caged compounds

Current treatment options for pathological neovascularization of the retina involve introduction of anti-angiogenic substances such as VEGF blockers. These pharmacological blockers require regular, typically monthly, eye injections. It is not surprising that patient compliance to continuously adhere to such agonizing treatment is waning over time. Optogenetic therapies require only a single viral injection, so this will not constitute a problem. However, the success of caged compounds in retinal treatments will probably increase if caged compounds can be delivered non-invasively through eye drops. Alternatively, as demonstrated by the Wang group, intravenous injections followed by photoactivation in the eye appears also effective. This would probably circumvent the need of regular eye injections and will consequently increase patient compliance. Optimization of pharmacological properties so that the compounds indeed reach the retina following topical administration through eye drops will be key. Immunological responses to eye drops should also be limited compared to injections. This is an advantage as the single viral eye injections for optogenetic therapies in patients and non-human primates required immunosuppression.

6. Summary and Outlook

Optogenetic therapy is a remarkable translational success story of basic research, animal models, and pioneering clinical work to overcome devastating photoreceptor degeneration. Further efforts will continue to improve vision restoration and patient well-being. PDT using photosensitizers, has been used to treat retinal diseases like AMD. Another emerging field, photopharmacology, aims to develop light-activated drugs (caged compounds) that can be precisely controlled for drug release, minimizing side effects and enhancing therapeutic outcomes. For other eye pathologies, innovative approaches combining chemical biology and light illumination for treatment with high cellular specificity and high spatiotemporal control are at early stages of development. It will be exciting to see how further progress of such experimental efforts could impact patient lives.

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Keywords: Ocular pathologies • Light triggers• Optogenetics • PDT • Caged compounds

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