Published in final edited form as:

Acc Chem Res. 2014 January 21; 47(1): 45–55. doi:10.1021/ar400036a.

Optochemical Control of Deoxyoligonucleotide Function Via a Nucleobase-Caging Approach

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Conspectus

Synthetic oligonucleotides have been extensively applied to control a wide range of biological processes such as gene expression, gene repair, DNA replication, and protein activity. Based on well-established sequence design rules that typically rely on Watson-Crick base pairing interactions researchers can readily program the function of these oligonucleotides. Therefore oligonucleotides provide a flexible platform for targeting a wide range of biological molecules, including DNA, RNA, and proteins. In addition, oligonucleotides are commonly used research tools in cell biology and developmental biology. However, a lack of conditional control methods has hampered the precise spatial and temporal regulation of oligonucleotide activity, which limits the application of these reagents to investigate complex biological questions. Nature controls biological function with a high level of spatial and temporal resolution and in order to elucidate the molecular mechanism of biological processes, researchers need tools that allow for the perturbation of these processes with Nature's precision.

Light represents an excellent external regulatory element as irradiation can be easily controlled spatially and temporally. Thus, researchers have developed several different methods to conditionally control oligonucleotide activity with light. One of the most versatile strategies is optochemical regulation through the installation and removal of photo-labile caging groups on oligonucleotides. To produce switches that can control nucleic acid function with light, chemists introduce caging groups into the oligomer backbone or on specific nucleobases to block oligonucleotide function until the caging groups are removed by light exposure.

In this Account, we focus on the application of caged nucleobases to the photo-regulation of DNA function. Using this approach we have both optochemically activated and deactivated gene

expression at the transcriptional and translational level with spatial and temporal control. Specifically, we have used caged triplex-forming oligomers and DNA decoys to regulate transcription, and we have regulated translation with light-activated antisense agents. Moreover, we also discuss strategies that can trigger DNA enzymatic activity, DNA amplification, and DNA mutagenesis by light illumination. More recently, we have developed light-activated DNA logic operations, an advance that may lay the foundation for the optochemical control of complex DNA calculations.

1. Introduction

In order to elucidate the underlying mechanisms that maintain and control biological systems, synthetic approaches have been developed for conditional control of cellular processes. Light is a versatile regulatory element, as it is fully orthogonal to most cellular components, is non-invasive, and can be easily controlled in timing and localization to certain tissues, cells, and even sub-cellular compartments.¹ Coupled with commonly employed gene regulatory methods, light has been used to control biological activities with high spatial and temporal resolution. This precise control enables, for example, the study of genes essential for embryonic development by deactivating them at specific time points and locations thus minimizing embryonic lethality.² The light regulation of biological processes is often based on photo-labile protecting groups, so called 'caging groups',^{3, 4} that are installed on small molecules, proteins, or oligonucleotides, thereby serving as light-triggered switches for cellular mechanisms.⁵⁻¹¹

The use of nucleic acids as tools to control biological activity dates back to the 1970s,¹² and synthetic oligonucleotides have been designed to interfere at all three levels of the flow of genetic information.¹³⁻¹⁷ Optochemical control of these regulatory oligonucleotides has been developed and tested for its potential to modulate and investigate gene function in single cells and multicellular organisms. For example, the regulation of translation can be achieved with light-activated antisense agents¹⁸⁻²⁰ small interfering RNAs,²¹⁻²⁴ and antagomirs.²⁵⁻²⁷ The regulation of transcription is possible by using caged triplex-forming oligonucleotides²⁸ and DNA decoys,²⁹ and the regulation of protein activity has been reported with light-controlled aptamers.³⁰

In order to accomplish the photo-regulation of oligonucleotide function, several different approaches have been developed that all take advantage of the high modularity and synthetic accessibility of oligonucleotides (Scheme 1). One of the early and most widely used strategies involves the insertion of a photo-labile moiety within the oligonucleotide backbone.³¹ Upon irradiation, the oligomer is cleaved, thereby inactivating its function (Scheme 1A). To optically activate oligonucleotide function, a complementary inhibitor strand is tethered to a functional strand through a photo-cleavable linker (Scheme 1B). In the absence of light irradiation the caged duplex is inactive; after light irradiation, the linker is cleaved and the active, functional strand is released. Most photo-cleavable linkers are based on *ortho*-nitrobenzyl moieties,^{18, 32, 33} but coumarin groups have been applied in this approach as well.³⁴ Since its introduction in 1995,³¹ light-cleavable linkers have been applied to DNA,³² 2'-*O*-methyl RNA,²⁶ PNA,³⁵ and morpholino oligonucleotides.^{2, 18, 33} However, three synthetic components are needed for this strategy (two oligonucleotide strands and the photo-cleavable linker) and require specific design rules for efficient on/off switching behavior.^{20, 34}

Recently, light-activated circular oligonucleotides, generated by linking both ends of a linear oligonucleotide through photo-cleavable moieties, were introduced as a modification of the above approach. Compared to linear oligonucleotides, circular ones show reduced structural

flexibility in binding to their complementary target sequences due to the induced curvature. Thus, antisense agents can be rendered inactive through circularization and are readily activated through photolysis of a linker, enabling duplex formation with target strands (Scheme 1C).³⁶⁻³⁹

A versatile approach to optically trigger a wide range of oligonucleotide functions involves the installation of caging groups onto oligomers at various positions, including backbone phosphates,^{24, 40, 41} 2'-OH groups,^{22, 42} and nucleobases. Among these approaches, caged nucleobases have demonstrated the broadest applicability in our lab and are the focus of this account. With caging groups blocking Watson-Crick hydrogen bonding, the oligonucleotide is inactive until photolysis of the caging groups restores oligonucleotide function (Scheme 1D). The corresponding light-deactivation of function is accomplished via a caged self-complementary strand (Scheme 1E). The functional arm can still bind to its target sequence before light irradiation, but a hairpin structure is formed and the activity of the oligonucleotide is inhibited after photolysis.

Since the introduction of the caged nucleobase **1** in 2004,⁴³ several other caging groups have been installed on specific nucleobases (Figure 1). For example, the 1-(*ortho*-nitrophenyl)-ethyl (NPE) group has been applied to five nucleobases **2**, **5**, **9**, **13**, and **17**.⁴⁴⁻⁴⁶ In order to improve decaging rates, the 2-(*ortho*-nitrophenyl)propyl (NPP) caged nucleotides **3**, **6**, **10**, and **14** were developed.^{45, 47, 48} To red-shift the absorption maximum and thereby the decaging wavelength and to increase the stability of the caging group during oligonucleotide synthesis, we installed the 6-nitropiperonyl methyl group (NPM) on cytosine (**15**) and its corresponding hydroxymethylene analog (NPOM) on guanine (**7**), thymine (**11**), and uracil (**18**).^{27, 28, 49, 50} The decaging wavelength of all these groups falls within the UV range, typically 360-366 nm, and thus is orthogonal to all commonly used fluorescent proteins. This enables the direct interfacing with many reporter systems,⁴ while maintaining low cellular and organismal toxicity due to light exposure.^{51, 52} Furthermore, those caging groups are stable under ambient light and no additional precautions are required during synthesis and handling. The application of coumarin caging groups allows for multi-wavelength control of different biological processes;⁵³⁻⁵⁵ however, their applicability to nucleobase caging is limited as they require a leaving group with a low pK_a.⁵⁶ Recently, the diethylaminocoumarin (DEACM) caged guanine **8** was reported,⁵⁷ enabling photolysis at >405 nm. In addition, two-photon caging groups have been introduced, which enable activation at 720-800 nm, promising enhanced three-dimensional resolution and deeper tissue penetration.⁵⁸ For example, the nitrodibenzofuran (NDBF) group was employed in the caging of **4**, **12**, and **16**; however, its application in optochemical oligonucleotide control has yet to be demonstrated in biological systems.^{46, 59}

Perturbation effects of nucleobase caging groups vary, based on the oligonucleotide function, the number of caged nucleobases, and their position.⁴⁸ In certain cases, especially the regulation of DNA:protein interactions and catalytic DNase activity, a single caging group located at a crucial site is sufficient to block activity until light exposure.^{49, 50, 60, 61} However, multiple caging groups are usually required to regulate base-pairing interactions between oligonucleotides. Generally, one caging group every 5-6 bases evenly distributed throughout an oligonucleotide fully abrogates hybridization to its complement, until the caging groups are removed through irradiation.^{45, 62, 63}

Different aspects of the photo-regulation of oligonucleotide function have been summarized in the literature and the interested reader is directed to those review articles in order to obtain a comprehensive overview of the field.⁶⁷⁻⁶⁹ In this account, we are focusing on applications of the photocaged nucleobase approach to select examples of the light-regulation of biological processes in our lab.

2. Optochemical control of DNA:DNA interaction

One of the most important functions of DNA strands is their ability to form double-stranded oligonucleotide duplexes via programmed hybridization to their complementary sequences through Watson-Crick base pairing. Thus, the optochemical control of DNA:DNA hybridization through nucleobase-caging lays the foundation for a wide range of developments in the light-regulation of oligonucleotide function.^{30, 45, 47, 50, 70-72} Selected applications of optochemical control of DNA:DNA interactions are discussed below.

2.1 Light-activated DNA logic gate

DNA computation based on the assembly of complex circuits of DNA logic gates has recently attracted substantial attention due to its ability to perform complex algorithms while interacting with biological and chemical environments.⁷³ Compared to biochemical inputs, light inputs provide several advantages, including higher spatial and temporal control over the computational event and the ability to bridge the gap between DNA computation and silicon-based electrical circuitry.

A light-triggered AND gate,⁷⁴ based on the caged thymidine **11**, was designed. It consisted of a fluorophore strand G_F , a quencher strand G , and a toe-hold containing strand G_T , which interacted with the caged strand A and the non-caged strand B (Scheme 2).⁶³ Two light inputs were used: the first light input ($I_1 = 365$ nm) cleaved the NPOM caging groups on strand A and enabled hybridization to the toe-hold of G_T . After a toe-hold displacement reaction,⁷⁵ A and G_T left the gate complex and the toe-hold on G_F was exposed to strand B , leading to displacement of G and separating the quencher from the fluorophore. The second light input ($I_2 = 532$ nm) induced excitation of the fluorophore resulting in a fluorescence light output ($O = 576$ nm). Therefore, only when both inputs are present at the AND gate, an output signal is obtained. The light activation enabled temporal (Scheme 2B) and spatial (Scheme 2C) control of logic gate operation. This light-activated DNA AND gate may be used with other DNA-based logic operations in the construction of complex DNA circuits that can be programmed with light.

2.2 Light-activated and light-deactivated polymerase chain reaction

The polymerase chain reaction (PCR) is widely applied in the amplification of double-stranded DNA.^{76, 77} Primers containing the caged nucleobase **11** have been used for the optochemical control of PCR by inhibiting hybridization of the primer to the DNA template until photolysis of the caging groups initiates DNA polymerization (Scheme 3A).⁷⁰ To inactivate the PCR primer, and thus the reaction, a caged hairpin primer was designed that forms a hairpin structure and inactivates itself upon nucleobase decaging (Scheme 3B). The simultaneous use of both primers enabled the light-switching from one DNA amplification reaction to another. This approach provides a non-invasive activation of PCR at a defined time point and thus has potential for the reduction of non-specific amplification.⁷⁸

2.3 Light-activated DNA decoy

A DNA decoy is a short double-stranded DNA molecule that contains the binding sequence for a transcription factor. The decoy sequesters the transcription factor and prevents it from binding to its genomic promoter region, thereby inhibiting transcription.⁷⁹ Since their introduction in 1990, several DNA decoys have been reported as versatile antigene reagents to deactivate gene function.^{79, 80}

Similar to the Approach E in Scheme 1, the caged thymidine **11** was incorporated into dumbbell DNA decoys that were designed to sequester the NF- κ B transcription factor (Scheme 4A).²⁹ The decoys were inactive until light-induced dumbbell formation through

cleavage of the nucleobase-caging groups led to inhibition of NF- κ B, as demonstrated in the optochemical control of a secreted alkaline phosphatase (SEAP) reporter gene in mammalian tissue culture (Scheme 4B). Light-regulated DNA decoys allow for promoter-specific silencing of genes that may not be achieved through genetic means, since transcription factors can form homo- and heterodimers as active species that control different sets of genes. Targeting transcription instead of translation holds promise as the catalytic formation of mRNA is inhibited, in contrast to targeting mRNA function directly. However, the delivery of DNA decoys to the nucleus remains challenging, potentially limiting their efficiency.⁷⁹

2.4 Light-activated and light-deactivated triplex-forming oligonucleotides

In addition to the optochemical control of DNA duplex formation via nucleobase-caging (as discussed under 2.1-2.3), the generation of triplex DNA structures can be controlled using the same principles. Triplex-forming oligonucleotides (TFOs) can site-specifically bind to double-stranded DNA forming a triplex helix. TFOs have been successfully applied as tools in gene repair,⁸¹ gene modification,⁸² inhibition of gene expression and DNA replication.⁸³ As an approach to regulate gene expression at the transcriptional level, TFOs targeting a promoter sequence can prevent transcription factor binding and thereby induce gene silencing.⁸⁴

Two different approaches (E and F in Scheme 1) were applied to the design of optochemical gene ON and OFF switches based on TFOs: a light-activated TFO containing the caged nucleobase **11** blocking the interaction of the TFO with the duplex DNA target until light exposure (Scheme 5A) and a light-deactivated TFO bearing **13** that binds to the duplex DNA target until irradiation removed the caging groups from an inhibitory sequence generating an inactive dumbbell structure (Scheme 5B). Caged TFOs targeting the cyclin D1 promoter region, which is involved in several neoplastic diseases, were analyzed using a luciferase reporter (Scheme 5C). Excellent optochemical ON $\rightarrow$ OFF switching (H₂) and OFF $\rightarrow$ ON switching (D₂) of gene function was observed in mammalian cells.

3. Optochemical control of DNA:RNA interaction

The generality of the nucleobase-caging approach allows for direct translation of the light-activation of DNA:DNA hybridization (as discussed in 2.1-2.3) to the optochemical control of DNA:RNA interactions. This is important in the light control of gene silencing through mRNA cleavage or suppression of translation.⁸⁵ Towards this goal, various reagents have been introduced, including antisense agents, siRNAs, and DNazymes.

Antisense agents are oligonucleotides that are complementary to their target mRNA and can sequence-selectively silence gene expression at the translational level. A wide range of antisense agents with different backbone and sugar modifications have been introduced.⁸⁶ These antisense agents have been widely used tools in the study of gene function. In order to control the activity of antisense agents, and thus the function of their gene targets, with spatial and temporal resolution, photo-cleavable linkers and photo-caged nucleobases have been introduced into their sequences and have been successfully applied in cell culture and in multi-cellular organisms.^{18, 36, 38, 62}

As previously mentioned, over 15 photo-caged nucleobases bearing 6 different caging groups have been incorporated into oligonucleotides (Figure 1). Among those caging groups, the NPOM thymidine **11** has been applied in the optochemical control of phosphodiester DNA and phosphorothioate DNA (PS DNA) antisense agents (Scheme 6A). The localized activation of an NPOM-caged PS DNA antisense agent was demonstrated by localized irradiating in mammalian tissue culture and thus only silencing reporter gene

expression in that particular location through RNase H-recruitment to the light-induced DNA:RNA duplex (Scheme 6B-C).⁶²

This example shows spatial control over gene expression induced by light-activation of nucleobase-caged antisense agents. Antisense agents that are temporarily deactivated by a photocleavable inhibitor strand (Scheme 1B) have also been applied to control gene expression.^{18, 20, 33, 87} Only one light-cleavable group built into the linker is needed in this approach, but the linked inhibitor needs to be carefully designed.^{20, 34} Recently, light-cleavable circular MOs (cMOs) were developed and used in the optochemical gene silencing in zebrafish embryos, alleviating the need for inhibitor strand design.^{36, 38}

In order to enable the optochemical switching of mRNA translation in both directions (ON → OFF and OFF → ON), approaches to activate gene expression with light have been developed as well. Following the design shown in Scheme 1E, a self-complementary antisense agent with nucleobase-caging groups installed on the inhibitor strand was synthesized in order to achieve light-activation of gene function through antisense agent deactivation (Scheme 7A). A DsRed reporter gene was targeted in mammalian cells and in the absence of UV light, the caged oligomer successfully silenced DsRed expression, while light-induced removal of the caging groups from the nucleobases led to antisense agent deactivation through hairpin formation, thereby activating DsRed expression (Scheme 7B-C).⁸⁸ A simultaneously imaged GFP reporter gene was not affected.

In alternative approaches, the installation of a photo-labile linker within the oligomer backbone (Scheme 1A)³² and the addition of a light-cleavable polyethyleneglycol⁸⁹ can also be used to achieve the light-deactivation of antisense activity. The nucleobase-caging approach has also been successfully applied to the optochemical control of other polymer:RNA interactions, for example morpholino oligomers¹⁹ and antagomirs.²⁷

4. Optochemical control of DNA:protein interactions

The direct light-regulation of DNA:protein interactions was also accomplished via caged nucleobases, as shown in the optochemical control of aptamer binding,³⁰ restriction enzyme activity,⁶⁰ and DNA/RNA polymerase activity.^{45, 90}

Building onto the light-activated PCR (Scheme 3A), a new DNA mutagenesis methodology was developed.⁹¹ A PCR primer bearing a nucleobase-caged thymidine **11** followed by additional oligonucleotide sequences (shown in blue in Scheme 8A) was applied in a PCR, generating a linear plasmid with sticky ends since the polymerase cannot extend through the thymidine caging group. Upon light-induced caging group removal and transformation of the generated nicked plasmid into *E. coli*, the final, mutagenized construct was obtained after an intracellular nicking repair.⁹² The same approach was used to delete a designated sequence from a plasmid (shown in blue in Scheme 8B) or to induce single/multiple site mutations. Compared to previous mutagenesis approaches, this methodology only requires two caged primers and a DNA polymerase for the entire site-directed mutagenesis, sequence addition, or sequence deletion. Moreover, this approach does not rely on restriction enzymes, providing high flexibility in DNA manipulation.

A strikingly similar methodology was developed independently, which further proved its applicability.⁹³

5. Light-activated DNAzymes

Catalytic deoxyribozymes (DNAzymes) were first discovered in 1994 through the evolution of an artificial DNA oligonucleotide that can cleave RNA in a sequence-specific manner.⁹⁴

Since then, various DNAzymes with different functions have been engineered and applied as biological sensors, DNA computation devices, ligases, and potential gene silencing agents.⁹⁵⁻⁹⁹ Following the strategies shown in Scheme 1A and 1B and through the caging of nucleotides directly involved in catalysis, the light-regulation of DNAzyme (and ribozyme) activity has been achieved using the caged deoxyadenosine **1**,⁴³ the caged thymidine **11**,^{50, 88} photo-cleavable backbone linkers,^{100, 101} and caged allosteric modulators.^{102, 103}

Peroxidase DNAzymes have been reported based on hemin intercalation into G-quadruplexes or G-wires,¹⁰⁴ oligonucleotide assemblies formed by guanine rich sequence through hydrogen bonding.¹⁰⁵ It was found that the presence of only one caged deoxyguanosine **7** was sufficient to disrupt G-wire formation and thus inhibited peroxidase DNAzyme activity (Scheme 9A).⁴⁹ Full recovery of G-wire formation and enzymatic activity was obtained upon decaging as shown by an oxidation assay (Scheme 9B-C).

6. Summary and outlook

The examples discussed above demonstrate the potential of nucleobase-caged deoxyoligonucleotides in the optochemical regulation of a wide range of biological processes including DNA transcription, mRNA translation, and enzymatic activity. This nucleobase-caging approach can be readily applied to a wide-range of oligomers with different sequences, structures, and functions by simply incorporating caged monomers into the oligonucleotide through standard solid-phase synthesis methods. Thus nucleobase-caged oligonucleotides provide the programmability and flexibility to target any selected sequence or gene with high specificity, and, depending on the design, either allow for light-induced activation or deactivation of biological processes. Additionally, recent reports show the potential of nucleobase-caging in emerging oligonucleotide technologies, including DNA computation, DNA/RNA sensing, and DNA nanotechnology.^{71, 106, 107} With the success of many “proof of principle” experiments, the development of modified oligomer backbones is expected to expand the use of nucleobase-caging in the study of gene function, effects of non-coding RNA, embryo development, cell motility, and other areas of research that require molecular tools that can be activated and deactivated with both spatial and temporal resolution.^{36, 87, 108, 109}

To further broaden the applicability of caged oligonucleotides to biological problems, technology developments that allow for multi-wavelength and reversible optochemical regulation through use of new chromophores and photoswitchable handles are ongoing.^{57, 64, 65, 110} In addition, other oligomer compositions, such as locked nucleic acids or peptide nucleic acids, are being investigated for nucleobase-caging approaches.¹¹¹

The developments in the maturing field of optochemical oligonucleotide control via nucleobase-caging, or other light-regulation approaches, are expected to enable biological discoveries in the coming years that may not be made without the exquisite external control that light conveys to a biological system.

Acknowledgments

The work summarized in this account has been supported in part by the NIH (R01GM79114), the Beckman Foundation (Beckman Young Investigator Award to AD), and Research Corporation (Cottrell Scholar Award to AD).

Biography

Qingyang Liu received her B.S. from Zhejiang University in China and is currently a graduate student in Dr. Deiters' group working on the development of light-activated oligonucleotides and oligonucleotide analogs.

Alexander Deiters is a Professor of Chemistry at the University of Pittsburgh. His research interests range from synthetic organic chemistry and the discovery of small molecule modifiers of biological pathways to protein and nucleic acid engineering. His lab has developed several general methods for the photochemical control of oligonucleotide function and cellular processes. Alex is a recipient of a Cottrell Scholar Award, a Beckman Young Investigator Award, an NSF CAREER Award, a Teva USA Scholars Grant, an American Cancer Society Research Scholar Grant, a Basil O'Connor Starter Scholar Award, and a Sigma Xi Research Faculty Award.

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Photo-cleavable linkers**A) Deactivation** **B) Activation** **C) Activation** **Photo-caged nucleobases****D) Activation** **E) Deactivation** **Reversible photo-switches****F)** **Scheme 1.**

Different caging approaches to regulate oligonucleotide hybridization with light. A) Photo-deactivation via light-induced strand breakage. B) Photo-activation via light-induced release of an inhibitor strand. C) Photo-activation via linearization of a light-cleavable circular oligonucleotide. D) Photo-activation via photolysis of caged nucleobases. E) Photo-deactivation via removal of nucleobase-caging groups from an inhibitor strand. F) Reversible control over oligomer hybridization via diazobenzene incorporation (outside the scope of this account).^{64, 65} Target strands, typically mRNA, are shown in green; functional oligonucleotides, e.g., an antisense agent, are shown in blue; inhibitor strands are colored dark red; nucleobase-caging groups and light-cleavable linkers are indicated by a red circle.

Figure 1. Chemical structures of caged nucleobases, grouped by letter code, that have been incorporated into oligonucleotides.^{23, 28, 43-50, 57, 59, 66} The light-removable caging groups are shown in red.

Scheme 2.

Photoactivation of a DNA logic gate. A) Design of the light-controlled AND gate. B) Temporal control of logic gate function demonstrating a step response. C) Spatial control of logic gate function through localized irradiation of a low-melt agarose gel containing all gate components. Only areas exposed to UV irradiation ($I_1 = 365\text{ nm}$) performed a logic gate operation and provided a fluorescence output when excited ($I_2 = 532\text{ nm}$). Adapted from *J. Am. Chem. Soc.* **2012**, *134*, 3810.

Scheme 3.

Photo-regulation of PCR through the application of nucleobase-caged primers. Adapted from *Chem. Comm.* **2008**, 462.

Scheme 4.

Activation of NF- κ B decoy function through light-induced dumbbell formation. A) Light-activation of the dumbbell DNA decoy D₂ through triggering of DNA hybridization via removal of nucleobase-caging groups. B) Photo-deactivation of SEAP expression with caged DNA decoys in HEK293 cells. D₀: inactive, negative control DNA decoy. D₁: non-caged, positive control decoy. D₂: caged decoy. Adapted from *J. Am. Chem. Soc.* **2011**, 133, 13176.

Scheme 5.

Light-regulation of TFO activity using a nucleobase-caging approach. A) Light-activation of a caged TFO through decaging of the dsDNA binding site. B) Light-deactivation of a TFO through decaging of an inhibitor strand, inducing dumbbell formation and thus blocking the dsDNA binding site. C) Optochemical regulation of cyclin D1 gene expression based on a luciferase reporter in HEK293T cells. H₀: inactive, scrambled TFO (negative control). H₁: non-caged TFO (positive control). H₂: caged TFO. D₁: inactive dumbbell TFO. D₂: caged dumbbell TFO. Adapted from *ACS Chem. Biol.* **2012**, 7, 1247.

Scheme 6.

A) Light-activation of gene silencing through nucleobase-caged antisense agents. B, C) Spatial activation of a caged PS DNA antisense agent targeting *Renilla* luciferase expression. A layer of NIH 3T3 cells was irradiated with UV light within the dashed circle and luminescence was detected by a Xenogen Lumina system after 24 hrs. B) Negative control in the absence of the antisense agents. C) Transfection of the caged PS DNA antisense agent. Adapted from *ChemBioChem* **2008**, 9, 2937.

Scheme 7.

A) Optochemical deactivation of an antisense agent through light-induced hairpin formation. B) The caged hairpin antisense agent is active and silences DsRed expression. C) Light irradiation removes the nucleobase-caging groups, leads to antisense agent deactivation through hairpin formation, and thus turns on DsRed expression. Adapted from *J. Am. Chem. Soc.* **2010**, *132*, 36183.

Scheme 8.

Mutagenesis approach based on the light regulation of DNA polymerization. In addition to the shown insertion (A) or deletion (B) of DNA sequences of any length, the developed methodology also allows for the site-specific introduction of mutations. Adapted from *Nucleic Acids Res.* **2009**, 37, e58.

Scheme 9.

Optochemical regulation of peroxidase DNzyme activity. A) Light activation of G-wire formation using the nucleobase-caged deoxyguanosine **7**. B) Oxidation of ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) in the presence of hemin before UV irradiation and C) after UV irradiation. D₀ non-caged DNzyme, D₁-D₅ and D_{3,4} caged DNzymes containing 1-2 NPOM groups at defined guanosine sites, preventing G-wire formation until UV exposure. Adapted from *Mol. BioSyst.* **2008**, *4*, 508.