

Photocaged functional nucleic acids for spatiotemporal imaging in biology

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Abstract

Imaging of species in living organisms with high spatiotemporal resolution is essential for understanding biological processes. While functional nucleic acids (FNAs), such as catalytic nucleic acids and aptamers, have emerged as effective sensors for a wide range of molecules, photocaged control of these FNAs has played a key role in translating them into bioimaging agents with high spatiotemporal control. In this review, we summarize methods and results of photocaged FNAs based on photolabile modifications, photoisomerization, and photothermal activation. Future directions, including strategies to improve the performance of these photocaged FNAs, are also described.

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1. Introduction

Biological processes build upon many delicate regulatory events of molecules from nucleic acids and proteins to organic metabolites and metal ions, whose concentrations vary widely with time and location in cells or other biological systems. Consequently, detecting, visualizing, and controlling these molecules in living organisms with high spatiotemporal resolution is very important in our understanding of biological processes. To achieve this goal, photocaged molecules, which are inactive in the absence of light and then activated upon light irradiation, have emerged as perhaps the most effective method because light activation is both kinetically fast and has high spatial resolution, allowing control of both the timing and location of the activation. A primary example is optogenetics in which precise modulation of intracellular signaling in intact cells and multicellular organisms has been achieved at subcellular resolution within seconds of light irradiation [1,2]. While optogenetics has been applied to control the functions of nucleic acids and proteins, it has been challenging to detect and control small organic metabolites and metal ions.

To overcome the limitation in the controlled detection of small molecule metabolites and metal ions, probes based on functional nucleic acids (FNAs) have been developed. FNAs are nucleic acids with either catalytic activities, called catalytic nucleic acids,

including ribozymes [3,4] and deoxyribozymes (DNAzymes) [5], or selective binding ability, called riboswitches [6] and DNA aptamers [7,8]. The FNAs can be obtained using a combinatorial process called in vitro selection or Systematic Evolution of Ligands by EXponential enrichment (SELEX) from a large DNA or RNA library of up to 10^{15} random sequences to bind not only large biomolecules like proteins but also small molecules like metabolites and metal ions [7–9]. After obtaining FNAs that can bind the targets selectively, signal transducers such as fluorophores can be conjugated to these FNAs to transform them into different in vitro sensors or in vivo imaging agents [10–*16,17,**18,**19–*21].

While FNA sensors and imaging agents have significantly expanded the number of targets of biological probes, because it takes time to deliver FNAs into cells and other living organisms, the sensing action may occur during the delivery process. To overcome this limitation, FNAs can be protected using photocaged groups. In this way, the function of FNAs is inhibited during the delivery process. Once the FNAs reach the desired location within the living organism, light irradiation can remove the caging group and reactivate the FNAs. More importantly, through control of the timing and location of the light irradiation, spatiotemporal control of the FNA sensors is achieved.

In this review, we summarize photocaging methods and strategies for spatiotemporal control of catalytic nucleic acids and aptamers, and how these photocages facilitate regulation of the FNA biosensors with spatiotemporal precision. We also provide recent

examples for improvements made to the caging groups and demonstrate their impact in practical applications such as imaging of metal ions. Finally, current limitations and potential opportunities for future photocontrollable FNA sensors are also discussed.

2. Spatiotemporal control of catalytic nucleic acids

2.1 Photocontrol of catalytic nucleic acids based on photolabile modifications

Photolabile modification is a frequently used caging method for controlling the configuration and activity of nucleic acids. These photolabile modifications include photoresponsive small molecule caging of a single nucleotide and photocleavable (PC) linkers between two nucleotides. Detailed properties and development of these photolabile groups in controlling DNA nanomaterials have been reviewed elsewhere [22], and we will focus on the application of these photolabile groups in functional nucleic acid research in this review.

The photoresponsive small molecule caging of a single nucleotide can be used to inhibit the activity of the catalytic core, prevent or enable the formation of a functional structure, or block the cleaving site through various modifications, including on the nucleobase, 2'-OH group, or phosphodiester backbone (Figure 1A-D). Removing these photocaged protection groups with light, ranging from the ultraviolet to near-infrared (NIR) region, restores or disrupts the conformation or configuration for the FNA function (Figure

1E-H). One example of such photocontrolled small molecule cages is the modification of a nitrobenzyl to the active site of ribonucleotide-cleaving catalytic nucleic acids. This method works by modifying the 2'-OH group of the ribonucleotide at the cleavage site in the substrate strand with the caging group, which prevents the cleavage. Uncaging with a 308 nm light regenerated the 2'-OH group and restored the activity of the hammerhead ribozyme (Figure 1A,E) [23]. Later, the Lu group has transformed this caging method into practical applications to image metal ions in vivo by applying the caging to the RNA-cleaving 8-17 and NaA43 DNAzymes, whose activities depend on Zn^{2+} or Na^{+} , respectively (Figure 2A) [24–26]. Before these demonstrations, most DNAzyme-based sensors were used outside living cells, because the DNAzyme can be cleaved during the delivery process before reaching the intended final cellular location. Using this photocaging method can not only address this issue but also allow imaging of metal ions with high spatiotemporal resolution.

Other than modifying the cleavage site of the catalytic nucleic acids, an 8-(2-(4-imidazolyl)ethyl-1-thio)-2'-deoxyribo adenosine (C8 adenosine) has been used to cage the catalytic loop of the 8-17 DNAzyme to inhibit its cleavage activity (Figure 1F). When irradiated with 254-310 nm UV light for 60 min, $\approx 30\%$ of RNA cleavage efficiency was restored [27]. To improve the chemical stability and increase the uncaging rate, a 6-nitropiperonyloxymethyl modification was developed to disrupt the hydrogen bonds between nucleic acid strands, thus controlling the activity of the 10-23 DNAzyme by

perturbing hybridization of the substrate strand to its enzyme strand (Figure 1G) [28,29].
 This system was also demonstrated to achieve the reverse, such that irradiation of the
 caging molecule inactivates an initially active DNAzyme. The same caging molecule was
 employed to prevent the blocking of both the binding arm and the catalytic core by adding
 an antisense tail sequence with multiple caged nucleotides (Figure 1H) [29].

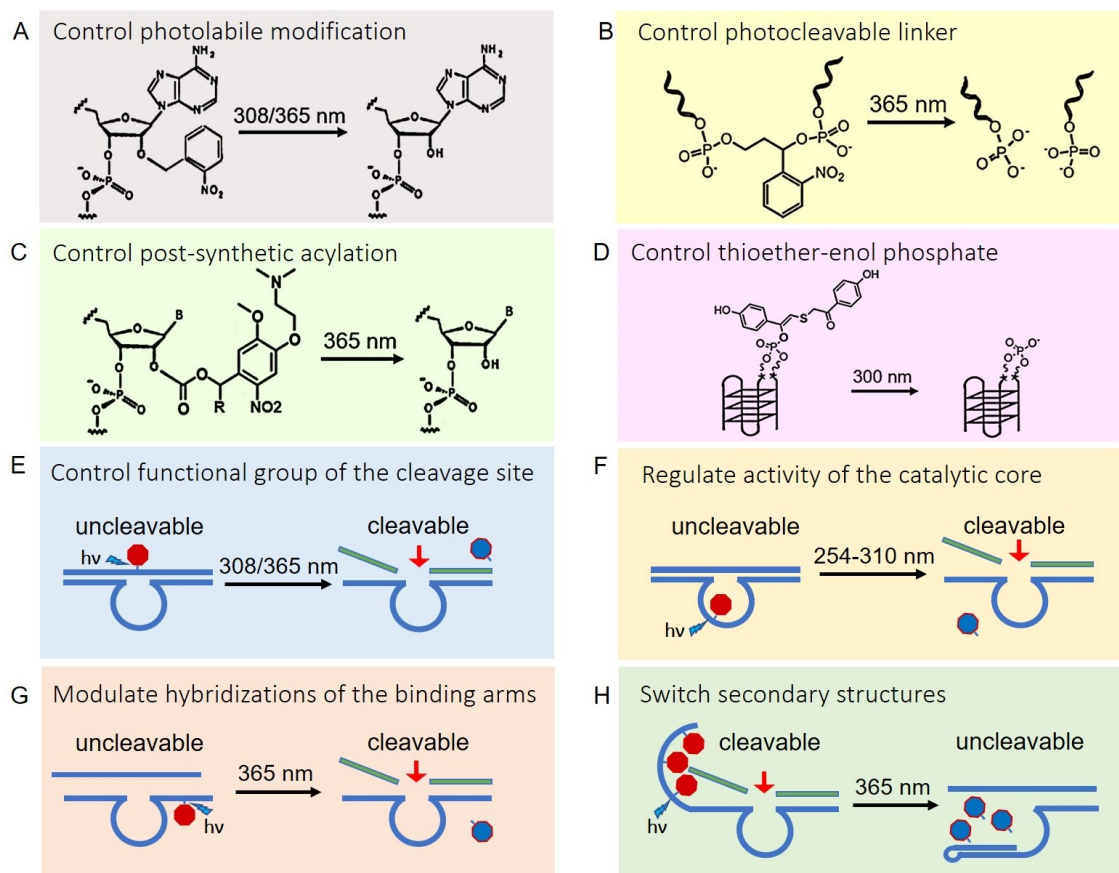


Figure 1 Photocaged control based on the cleavage of photolabile small molecules.
 (A) Photolysis of a nitrobenzyl modification on the 2'-OH group of a ribonucleotide.
 (B) Structure of the PC linker and photocleavage process.
 (C-D) Post-synthetic photocaging modifications of nucleic acids.
 (E-H) Different strategies for photocaged control of DNAzyme sensors based on the cleavage of
 photolabile small molecules.

In addition to photocaging of nucleotides, PC linkers are another class of photolabile

modifications that are readily available with commercial nucleic acid synthesis. Upon light irradiation, the PC linker is broken (Figure 1B), introducing a nick site in the DNA/RNA strand. As a result, the shorter strand can be designed to have a lower melting temperature to its complementary strand than the ambient temperature and thus is released to allow optical control of the sensing and imaging processes [30]. For example, such PC linkers have been used to turn off or turn on DNAzyme functions by light-induced disruption or regeneration of the catalytic components of DNAzymes [31].

While the above studies have demonstrated the potential of using photolabile cleavage to control DNAzyme activities, several improvements have been made for these methods to be applied for biosensing and imaging. First, most of these studies used UV light for optical control. While the UV-light allows efficient and fast uncaging, its exposure to cells often damages the cells. Since NIR light is less phototoxic and has better penetration into cells and living animals, a two-photon photocleavable version of the PC linkers using NIR light has been reported [32]. In addition, by blocking the cleavage site of the 8-17 DNAzyme with a nitrobenzyl group, and conjugating the DNAzyme onto lanthanide-doped upconversion nanoparticles (UCNPs), the Lu group has taken advantage of the ability of UCNPs to upconvert 980 nm NIR light to 365 nm UV light to minimize phototoxicity while maintaining high uncaging efficiency. With this system, imaging of Zn^{2+} in both live HeLa cells and zebrafish has been demonstrated (Figure 2B) [**18].

Second, most photocaging methods are on-and-off controls using a fluorophore and quencher pair, which is challenging to be used to quantify the target, especially in living cells, since the delivery amount of the sensor could vary widely between different cells. To allow quantification in cells, the fluorophore and quencher pair have been replaced by a FRET donor and acceptor pair. The ratiometric signal from the FRET donor and acceptor allowed quantification of Zn^{2+} in living cells (Figure 2C) [30].

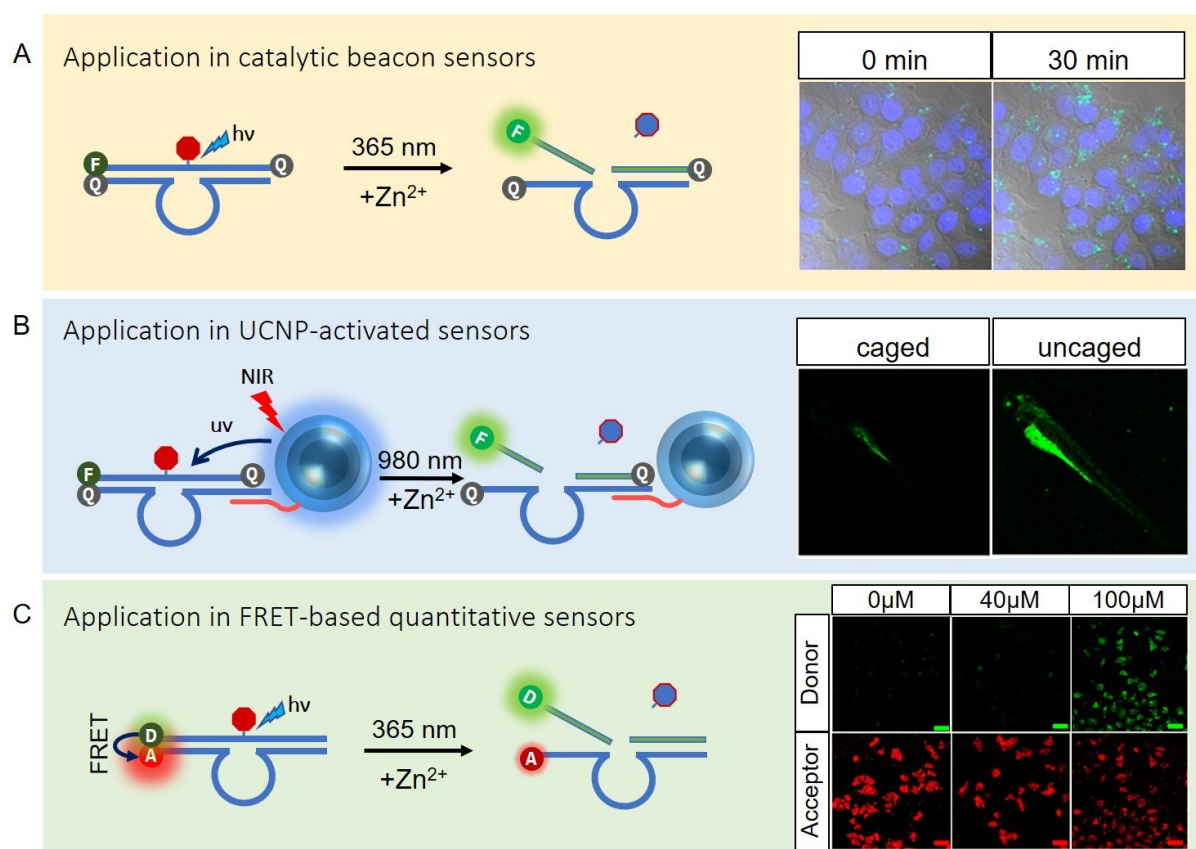


Figure 2 Examples of photolabile molecule based control of DNAzyme sensors.

(A) Photocaged NaA43 DNAzyme detects Na^+ influx in living cells. Nitrobenzyl modification was applied to block the cleavage site. Reprint from [24].

(B) UCNP-controlled Zn^{2+} imaging in Hela cells and zebrafish. Nitrobenzyl modification was applied to block cleavage site. Reprint from [**18].

(C) FRET-based Zn^{2+} quantification and imaging in Hela cells. Reprint from [33].

Finally, progress has been made to simplify the photocaging synthesis process. Since adding the photocaging groups during the solid-phase syntheses of nucleic acids requires special equipment and expertise, caging nucleic acids after the nucleic acids are synthesized has also been developed. For example, post-synthetic acylation of the 2' hydroxyls of RNA resulted in reversible optical control of the activity of the hammerhead ribozyme (Figure 1C) [*34]. Furthermore, a thioether-enol phosphate (TEEP-OH) was incorporated into the DNA phosphodiester backbone to block the activity of the peroxidase-mimicking DNAzyme. Under 300 nm light irradiation, this TEEP-OH modification can transform into a native DNA phosphodiester and regenerate the DNAzyme activity (Figure 1D) [*35].

2.2 Photocontrol of catalytic nucleic acids based on photoisomerization

In contrast to irreversible control based on photolabile cleavage, optical control based on photoisomerization is reversible. Different types of photoregulated switches and their applications in DNA nanotechnology has been reviewed by Lubbe et al. [**36]. Among these photoresponsive molecules, azobenzene and its derivatives are most popular due to their stability, accessibility, and ease of synthesis [**22], and will be discussed in this review (Figure 3A-C). A common usage of the azobenzene-based photocage is taking advantage of its conformational change and base-pair stacking ability to introduce reversible DNA hybridization that can interfere with the conformation and activity of the DNAzyme (Figure 3A). Azobenzene modifications can be incorporated into the catalytic

171 core or binding arms of DNazymes to block the catalytic activity or substrate strand
172 hybridization of DNazymes (Figure 3D-E). With the reversible conformation change of
173 azobenzene, the activity of a DNzyme can be turned on and off multiple times [37,38].
174 To increase the caging efficiency, an extended antisense sequence carrying azobenzene
175 was introduced to block both the catalytic core and the binding arm of DNazymes (Figure
176 3F) [39]. Another strategy of adding extra azobenzene-containing overhang linkers to both
177 ends of a DNzyme has allowed reversible optical control between the inactive DNzyme
178 conformation that sandwiches the substrate within a hairpin structure and its active
179 conformation (Figure 3G) [40].

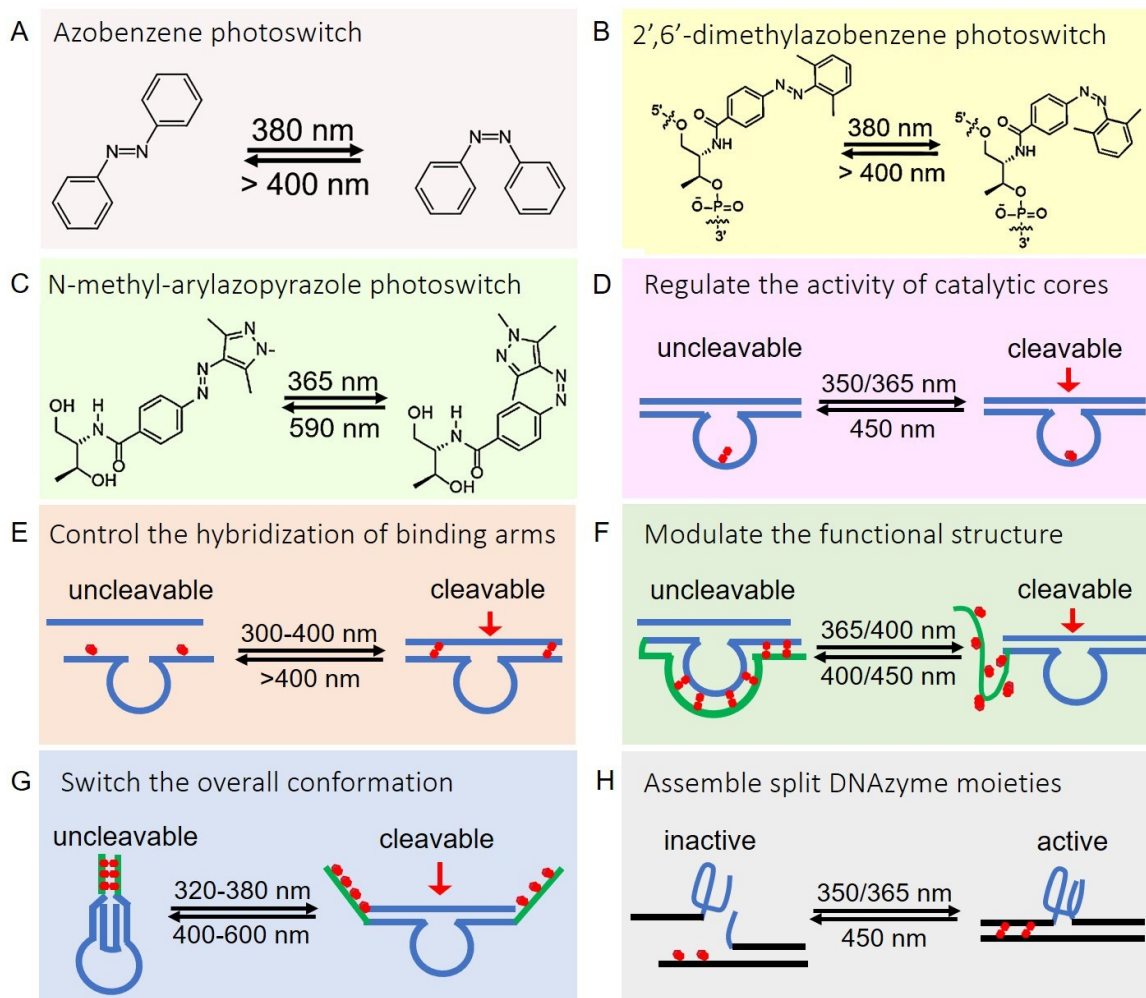


Figure 3. Photocaged control of catalytic FNAs based on the isomerization of photoswitches. (A-C) Structure of the isomerization of azobenzene and its derivatives. (D-H) Photoswitches control the activity of FNAs via catalytic core blocking, substrate hybridization, and split DNAzyme assembly.

In order to increase the photoswitching efficiency, new photoswitches, such as 2',6'-dimethylazobenzene and N-methyl-arylazopyrazole that are derived from azobenzene, have been developed (Figure 3B-C). These photoswitches have been applied to a split horseradish peroxidase-mimicking DNAzyme for reversible control of logic gates, which could be further applied to biosensing applications (Figure 3H) [41]

2.3 Photocontrol of catalytic nucleic acids based on photothermal activation

Photothermal activation is another method for controlling FNA function. It uses light to induce heat from light-absorbing nanomaterials to change the hybridization patterns and the resulting conformation of FNAs that are immobilized on the light-absorbing nanomaterials so that they can transform from an inactive form into an active form. For example, a short DNA strand that is complementary to a part of the enzyme strand of the 8-17 DNAzyme is conjugated to a gold nanoshell. In the absence of NIR light, the short DNA strand hybridizes to the DNAzyme, preventing the formation of the DNAzyme catalytic core. Upon absorbing 808 nm light, the nanoshell generates heat to increase the surrounding temperature enough to dehybridize the short DNA strand from the enzyme strand, restoring the DNAzyme to its active conformation for metal ion detection (Figure 4A). Using this strategy, Wang et al. detected Zn^{2+} in living HeLa cells under NIR light control (Figure 4B) [42].

Expanding from this work, yolk-shell nanorod-satellite structures have been constructed to replace the gold nanoshell for near-infrared circular polarized light activation. Interestingly, their probe showed handedness-dependent performance. Right circular polarized light activated the probe in buffer with the presence of metal ion while left circular polarized light only activated the probe inside of cells. This chiral property enabled their FNA sensors to detect intracellular metal ions with minimal interference from

metals in the culture media. With this system, single and multiple metal ions are detected with multi-channel readouts by fluorescent and UCNP luminescent signals (Figure 4C) [43,*44].

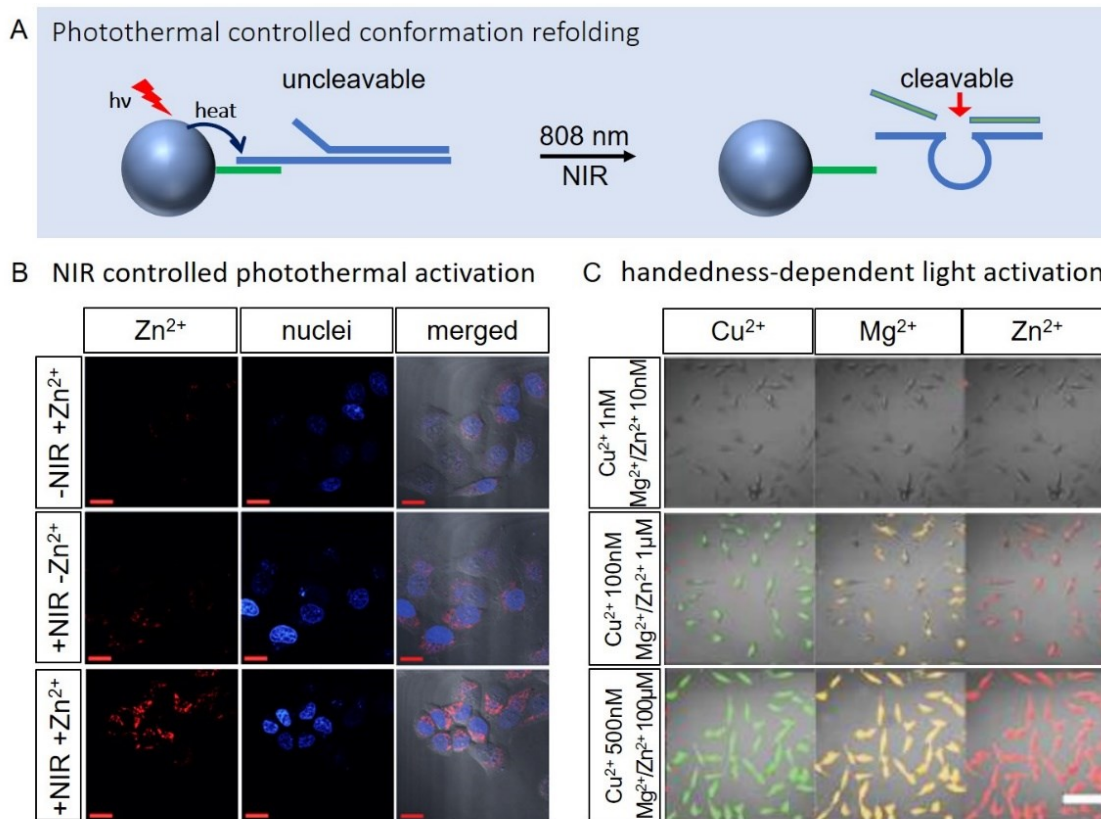


Figure 4 Photothermal-based photocage.

(A) Light-induced temperature increases by nanoparticles dehybridize blocking DNA to restore conformation and function of the DNAs.

(B-C) Cellular imaging of Zn²⁺ and other metal ions in Hela cells. Reprint from [**42,43].

3 Spatiotemporal control of aptamers

3.1 Photocontrol of aptamers based on photolabile modifications

Photolabile groups have also been used in regulating the functions of aptamers by blocking the binding interface for target recognition (Figure 5A). For example,

224 incorporating photocaged thymidine phosphoramidites into the target-binding site of the
225 thrombin aptamer (TBA) prevented thrombin from binding, and the binding can be
226 restored by using 366nm light to remove the photocage group [45]. Instead of blocking
227 the binding domain of the aptamers using a photocage group, other studies prefer to
228 control the conformation of aptamers (Figure 5B). For example, an alkyne-modified 2' -
229 deoxythymidine has been incorporated into a C10 aptamer, which recognizes human
230 Burkitt's lymphoma cells. Click chemistry was then used to lock the aptamer into a
231 bicircularized structure to inhibit the binding of the aptamer. Upon 365 nm light activation,
232 the cleavage of the intramolecular ring enabled efficient aptamer binding and uptake into
233 the cells (Figure 5G) [*46]. Similar to the improvements made for photocontrol of
234 DNazymes, strategies such as RNA acylation and TEEP-OH modification have also been
235 applied to control aptamer function by blocking the conformation and binding site of the
236 aptamers (Figure 5C,H) [*34,*35].

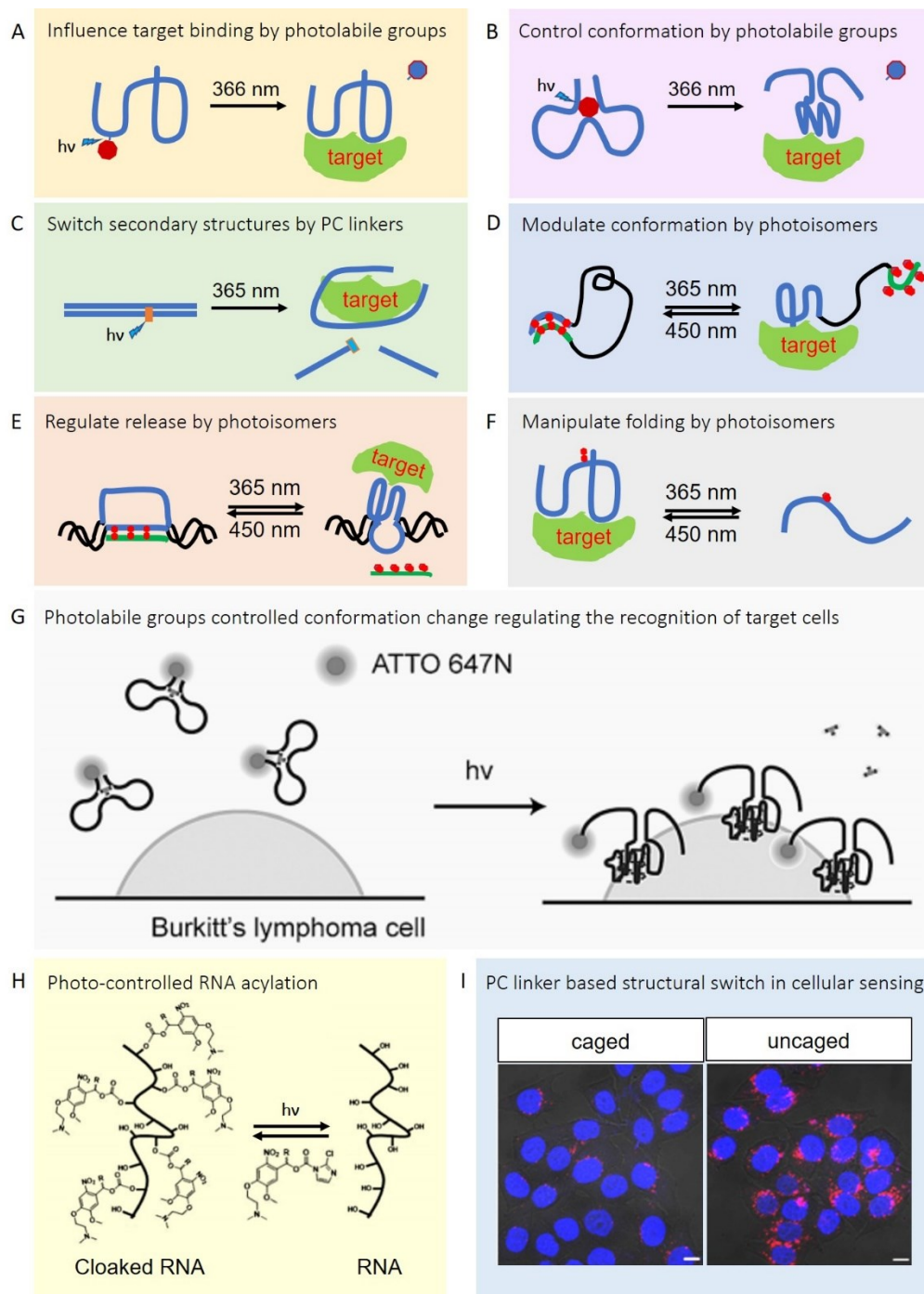


Figure 5 Photocage-controlled aptamers.

(A-F) Photocage control of the function of aptamers by blocking target recognition sites and conformation formation.

(G) Photolabile groups regulate aptamers binding onto and uptake by cells via controlling the conformation. Reprinted from [*46].

(H) Photocontrolled RNA acylation. Reprinted from [*34].

(I) PC linker-based ATP aptamer in cellular sensing and imaging. Reprinted from [**47]

In addition to photoresponsive small molecule caging, PC linkers have also been used for controlling the conformation of aptamers. With light, PC linkers within the DNA strand are cleaved, producing a nick in the DNA, which changes the melting temperature of the shorter strand of the nicked DNA, resulting in release of the shortened strand and activation of the aptamer (Figure 5C). When conjugated to UCNP or specific delivery reagents, ATP has been detected with high spatial resolution using this method (Figure 5I) [47,48].

3.2 Photocaged control of aptamers based on photoisomerization

Photoisomerization has also been applied to regulate the function of aptamers by controlling their hybridization and conformation properties (Figure 5D-E). Using an azobenzene-containing DNA strand that is complementary to the sequence of the thrombin binding aptamer (TBA), the hybridization and release of the TBA were regulated by the isomerization of azobenzene under UV or visible light, affecting the thrombin binding function of the TBA [49]. By adjusting the number of azobenzenes used in the hybridization system, the binding affinity of the TBA to thrombin can be modulated [50]. In addition to regulating DNA hybridization, azobenzene derivatives have also been used to regulate the conformation of aptamers (Figure 5F). For example, by incorporating an azobenzene group into the unstructured loop region of the TBA, the formation of an active thrombin aptamer has been reversibly controlled by light [51].

Recent efforts have been made to improve the chemical properties of azobenzene derivatives for their applications in controlling aptamers. For example, to enhance the control of double-stranded hybridization, a locked azobenzene C-nucleoside has been introduced with an increased hybridization affinity [52]. To minimize phototoxicity, new photoisomerization groups, such as stilbenyl-azopyrroles, have been synthesized to allow two-photon excitation using 800nm NIR light [53]. In addition to azobenzene, anthracene photochromism has also been used to force the conformation change of the functionalized bottom loop of the TBA and thus optically control the activity of thrombin [54].

4 Summary and future directions

In this review, we have summarized the recent progress of spatiotemporal control of photocaged functional nucleic acids for sensing and imaging in biology by discussing methods to control the functions of catalytic nucleic acids and aptamers based on photolabile modifications, photoisomerization, and, in the case of DNAzymes, photothermal activation. While many uncaging methods have been reported, the efficiency is still relatively low. Developing novel photolabile and photoswitching groups that are highly efficient, including a fast rate and high yield, in response to light irradiation will have a significant impact in this field. To apply photoregulation in live biological systems, especially nontransparent animals such as mice, the phototoxicity, tissue heating effect, and tissue penetration depth are still limiting factors for photocaging

methods. To enhance the performance, deep tissue penetrating NIR-II light (in the 1000–1700 nm window) will be an option that may significantly increase the depth of photoactivation. New photocaging groups that directly respond to NIR light via multi-photon events may be used for deep tissue activation. Another frontier lies in using interdisciplinary approaches (e.g., nanomaterials, optogenetic constructs) to generate activating molecules (e.g., ROS, enzymes) by light to trigger existing chemocaging strategies. For instance, nucleotide modifications that could be removed by ROS, restriction enzymes, or other chemicals are reported to block the function of FNAs [55–57], and could potentially be removed indirectly by light if the light could trigger the release or synthesis of these activation chemicals or enzymes. In this way, many published FNA chemocaging and activation methods may be expected to be converted into a light-activatable version for photocaging designs. Finally, since light has a low penetration depth, photocaged control of FNAs are limited so far to cell culture and zebrafish [**18]. To expand this field to even more biological systems, such as living animals or human bodies, the use of photoacoustic imaging that use sound that has a higher penetration depth as a detecting mechanism, as demonstrated recently using a photoacoustic aptamer-based sensor, is promising [58]. Given the significant progress made in the past two years, especially the breakthroughs in sensor activation in vivo, this research field is full of opportunities in developing novel light controlled FNA sensors.

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Conflict of interest statement

Nothing declared.

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