Photoresponsive Control of G-Quadruplex DNA Systems

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ABSTRACT: G-quadruplex (G4) oligonucleotide secondary structures have recently attracted significant attention as therapeutic targets owing to their occurrence in human oncogene promoter sequences and the genome of pathogenic organisms. G4s also demonstrate interesting catalytic activities in their own right, as well as the ability to act as scaffolds for the development of DNA-based materials and nanodevices. Owing to this diverse range of opportunities to exploit G4 in a variety of applications, several strategies to control G4 structure and function have emerged. Interrogating the role of G4s in biology requires the delivery of small-molecule ligands that promote its formation under physiological conditions, while exploiting G4 in the development of responsive nanodevices is normally achieved by the addition and sequestration of the metal ions required for the stabilization of the folded structure. Although these strategies prove successful, neither allows the system in question to be controlled externally. Meanwhile, light has proven to be an attractive means for the control of DNA-based systems as it is noninvasive, can be delivered with high spatiotemporal precision, and is orthogonal to many chemical and biological processes. A plethora of photoresponsive DNA systems have been reported to date; however, the vast majority deploy photoreactive moieties to control the stability and assembly of duplex DNA hybrids. Despite the unique opportunities afforded by the regulation of G-quadruplex formation in biology, catalysis, and nanotechnology, comparatively little attention has been devoted to the design of photoresponsive G4-based systems. In this Perspective, we consider the potential of photoresponsive G4 assemblies and examine the strategies that may be used to engineer these systems toward a variety of applications. Through an overview of the main developments in the field to date, we highlight recent progress made toward this exciting goal and the emerging opportunities that remain ripe for further exploration in the coming years.

KEYWORDS: DNA, G-quadruplexes, Nucleic acids, Supramolecular chemistry, DNA regulation, Photopharmacology, Photoresponsive ligands, Photoswitching

1. INTRODUCTION

G-quadruplexes (G4s) are a class of nucleic acid secondary structures formed from guanine-rich nucleic acid sequences that have generated much interest in recent years from across many scientific disciplines as therapeutic targets, catalysts, and as the basis of functional nanodevices. These structurally distinctive oligonucleotide sequences are formed from self-association of four guanine residues in a square planar arrangement, which are referred to as G-tetrads. G4s are stabilized by Hoogsteen hydrogen bond interactions and coordination to a central metal cation. The stacking of these tetrads upon folding of the oligonucleotide strand gives rise to the G4 architecture. G4-forming sequences are found in the human genome and have been linked to essential genomic functions, such as transcription, replication, repair, and telomere maintenance. This has been further validated by the development of novel chemical biology tools and methods developed to map and visualize these nucleic acid secondary structures in human cells. Moreover, it is now widely acknowledged that G4s are over-represented in the promoter regions of oncogenes (e.g., c-myc, BCL2, and c-kit) and has been proposed that targeting of the folded G4 with small molecules can inhibit the binding of transcription factors leading to downstream silencing of oncogene expression. More recently, putative G4-forming sequences in the genomes of the protozoan parasites Trypanosoma brucei and Leishmania major have also been reported and in viral genomes such as the human papilloma virus (HPV), herpes simplex virus-1 (HSV-1), Epstein–Barr virus (EBV), and the human immunodeficiency virus-1 (HIV-1).

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Due to the polymorphic nature of G-quadruplex DNA structures and their potential involvement on many biological roles, it has been long proposed that targeting of these oligonucleotide sequences with small molecules can help control their biological function. In this context, a wide range of small-molecule ligands have been shown to interact with G4s leading to striking changes in the folding topology of the DNA or even its unfolding. Most systems, however, entail a one-way topological switch whereby the ligand induces a permanent morphological change upon binding to the oligonucleotide sequence. A much more versatile system is such that can toggle between topologies on-demand using an external stimuli.

In this context, stimuli-responsive ligands have emerged involving chemical/supramolecular triggers, although those will not be discussed in the context of this perspective, and also light. Light offers several advantages over chemical stimuli for the regulation of G4 formation/topology in vivo as a potential therapeutic strategy or in the regulation of the G4 structure in order to generate a mechanical or spectroscopic output that can form the basis of a functional system. Unlike chemical stimuli, light can be delivered with much higher spatiotemporal precision than chemical “fuels” by controlling the wavelength, intensity, or irradiation time. Importantly, irradiation in most cases does not contaminate the system, unlike the irreversible addition of chemical stimuli which tends to accumulate, becoming a traceless tool once irradiation has ceased. It has been demonstrated that irradiation with UV (e.g., wavelength of 365 nm) or visible light does not appear to be harmful to DNA, cells, or tissues.

A variety of photoresponsive chemotypes (photocages and photoswitches) have found application in the photoregulation of nucleic acid structures. Thus, light has proven to be an attractive means for the control of DNA-based systems as it can be considered noninvasive and orthogonal to many chemical and biological processes. In particular, photoswitches where irradiation with light causes a change in the molecular structure are particularly attractive, since no waste products are generated. In many examples, two photoisomers of a single molecule can present different activities in the system of interest, allowing the reversible back-and-forth switch between structures and function, which is controlled by the choice of wavelength or thermally. This approach does not require the sequential addition of chemical fuels and offers additional levels of control over the system of interest.

While being an emerging area, studies concerning the photoregulation of G4 structures are less common than examples of photoregulation of duplex DNA hairpins. In this Perspective, we focus our attention on the most recent examples of photoreponsive G4-based systems and the strategies used to engineer these systems toward a variety of applications. For a more comprehensive overview regarding photoresponsive control of oligonucleotide structures, the reader is referred to another recent review.

2. PHOTOCONTROL THROUGH INCORPORATION OF PHOTOSWITCHES ON THE DNA SEQUENCE

One of the possible strategies to afford control of G4 structure is the preincorporation of responsive functionality in the DNA sequence itself. This strategy need not rely on natural DNA structures and can be easily modified to include the necessary functionality. Moreover, this approach allows stimuli-driven changes in folding without the need for external fuel, being particularly useful in the development of functional materials.

In 2009, Ogasawara and Maeda reported the first example of reversible photoregulation of the G4 structure using different UV wavelengths. The team incorporated a modified guanine nucleobase functionalized with the photoisomerisable 8-fluorenylethyl group into the oligonucleotide sequence (Figure 1a). This system allowed the reversible photoregulation of G4 formation and unfolding, which was triggered by alternating irradiation with monochromatic light at 410 and 310 nm. The G4 structure could assemble in the E state of the vinyl functional group, whereas isomerization using 410 nm light to the Z state provoked the unfolding of the system. In this scenario, no waste products are generated.
manner, the folding and refolding of the DNA could be cycled several times. Moreover, they were able to control the affinity of the DNA structure to thrombin by hybridization-controlled catch-and-release of thrombin by simple photoirradiation.

Later, Heckel and co-workers demonstrated the photoresponsive formation of an intermolecular G4 with K+ ions at room temperature by incorporating an azobenzene functionality into the backbone of guanosine tetramers (Figure 1b). In this work, the authors revealed that the E form of the p,p'-substituted azobenzene derivative was capable of forming nonpolymorphic, stacked tetramer G4, whereas after E→Z isomerization with UV light (λ = 365 nm), an unstructured state is formed. These results were in agreement with computational studies using quantum mechanics/molecular mechanics (QM/MM) and molecular dynamics (MD) simulations, where the Faraji group determined that the p,p'-substitution pattern provides more flexibility to the whole G4 structure compared to other substitution patterns, facilitating the reversible folding and unfolding of the G4 by irradiation with UV light. In a similar way to Ogasawara’s system, the system was reversible and could be cycled several times. More recently, the Tucker group engineered a bisanthracene-functionalized G4 thrombin-binding aptamer, whereby (4π + 4π) photocycloaddition reaction of two anthracene groups can modulate the binding, and therefore activity, of thrombin (Figure 1c). The group hypothesize the observed control of bioactivity can be attributed to the distortion of the structure, which inhibits the binding to thrombin. Unlike, the previous examples, the reversible photodimerization of the anthracene unit occurs using both light and thermal triggers, which limits somewhat their potential applications in a biological setting.

Though many exciting applications of these elegant and pioneering approaches to regulate the G4 structure can be envisaged, the synthesis of modified oligonucleotides poses outstanding challenges for researchers due to their large size and complexity. Moreover, this switching strategy has only been exploited for the thrombin-binding aptamer as a model system, which is not a very stable G4 structure. However, it is possible that with further investigation using other more canonical and stable G4s such as pro-oncogene SRC and c-myc DNA sequences among others, further advances using these type of approaches will be disclosed in the coming years.

### 3. PHOTOCONTROL USING PHOTORESPONSIVE LIGANDS

Supramolecular approaches which utilize photoresponsive small molecules could overcome situations where such a modification of the underlying DNA sequence is undesirable and the drawbacks associated with photocontrol of G4 systems through the incorporation of photoswitchable motifs in the oligonucleotide structure. Also, supramolecular strategies offer additional advantages in terms of lower cost and easier access than those based on modified oligonucleotide sequences. Moreover, since such photochromic ligands can be designed to be stimulus-responsive, their effects can be controlled by external factors (e.g., light), affording additional levels of spatiotemporal control, which is desirable for both the development of nanodevices or as the basis of G4-targeted photopharmacology strategies. For more information regarding other supramolecular triggers other than light for G-quadruplex nucleic acid regulation, the reader is referred to several previous works.

In particular, the design and synthesis of photoresponsive small ligands whereby binding and activity of the DNA can be controlled using either a photocaging or photoswitching strategy have attracted much attention in recent years. This type of approach has been mostly used in the general area of photopharmacology, and many illustrative examples can be found for biological or therapeutic applications. For these type of applications to be successful, ideally there should be a significant difference in bioactivity between the two isomers. Though quadruplex DNA has long been studied as a therapeutic target, not many attempts have been made to date to explore the potential of these noncanonical structures as targets for photopharmacology until now.

#### 3.1. Photocaged G4 Ligands

Regarding the development of photocaged G4 ligands, two examples of G4-targeting prodrugs have been reported to date: one based on telomestatin (2, Figure 2) and the other based on a pyridostatin mimic (3, Figure 2), two well-known excellent G4 binders. Both the Nagasawa and Balasubramian groups employed similar strategies to cage the activity of their chosen G4 ligands (telomestatin or pyridostatin, respectively) by incorporating a nitroveratryl group on the ligand that could be cleaved upon UV irradiation. In both cases, the G4-binding ability of the ligand is masked by the caging group, which can be removed in a quantitative yield in 30 min simply by photolysis with 365 nm light, resulting in the unmasking of the compounds’ toxicity. Among different techniques, Förster resonance energy transfer (FRET) melting assays confirmed that the caged ligands do not affect the stability of G4, whereas ligand binding to the G4 (human telomeric G4 for 2 and SCR G4 for 3) is restored after irradiation to a level comparable to that of the unprotected form of the ligand, suggesting the mechanism of action to be the result of light-activated G4 binding. Although, the photocaging approach permits only one-way control of the ligand activity, the overall strategy hints at possible applications in real systems.

#### 3.2. Photoswitching G4 Ligands

Another type of photoresponsive strategy comes from the use of photoswitching ligands whose topology can be modulated by the application of a particular light source. Based on this idea,
azobenzenes are the photoswitches most investigated to date for binding to G4 DNA, despite the availability of several other potential photoresponsive scaffolds which may furnish ligands that provide complementary or improved effects.

### 3.2.1. Azobenzenes

In 2010, Zhou and colleagues reported the first example of a reversible stimuli-responsive ligand designed to influence G4 folding. In this example, the authors found that photoswitchable azobenzene-derived ligand could be used to induce folding of human telomeric G4 DNA (d(TTAGGG)_4), in the absence of cations in water in the E isomer (E-4), while the Z azobenzene moiety (Z-4) did not exhibit such an effect, leading to dissociation of ligand on this conformation (Figure 3). The structural transitions of the nucleic acid were monitored by CD spectra, in which the formation of the parallel G-quadruplex structure was confirmed by a negative band at λ = 240 nm and a positive band at λ = 265 nm. Moreover, the latter band disappeared upon transition to the random-coil structure after irradiation with UV light at 350 nm for 1 min. Both G4 unfolding and refolding processes could be cycled at least 10 times by illumination with UV/vis light without any appreciable photofatigue, as the Z isomer can be generated from the E form by illumination with UV light (λ = 350 nm, 1 min) and can be reversibly isomerized by the subsequent exposure of the system to visible light (λ > 400 nm, 1 min). The authors suggested that the planar E isomer participates in π−π stacking with guanine quartets, while the positively charged side chains displayed a high degree of freedom to interact with the phosphate backbone, facilitating the formation of the G4 structure. On the other hand, the V-shaped Z isomer reduced π−π stacking, and the rotary flexibility of the side chain could not interact sufficiently to effect a conformational change. This very elegant example represented a step forward toward the development of new DNA nanodevices, which could be controlled with light by exploiting the photoisomerisation properties of the ligand. However, this system operated in the absence of metal cations as those...
naturally present and thus commonly employed in biophysical studies of G4s in order to mimic physiological conditions, which limits its applicability.

To overcome this limitation, in a subsequent study, the authors attempted to exploit photoswitchable ligand 4 under physiological conditions but found it to be ineffective. The authors did not provide an explanation for why this system was negatively affected by the presence of metal salts. However, we could hypothesize that perhaps the stabilization on G4 folding topology induced by these metal cations is more difficult to overcome by simple ligand-driven effects, and that improving the ligand design and the switching kinetics of the system might be essential to overcome these issues and develop systems that work under the required high cationic content. More recently, in an elegant proof-of-concept study, the same authors used photoswitchable ligand 4 to regulate the activity of thrombin, demonstrating that a supramolecular approach can be used as a means to control G4-mediated processes (Figure 4).

In order to expand the scope of azobenzene derivatives as G4 ligands, Zhou and colleagues developed further analogues of 4 decorated with morpholino (5) or pyridinium (6) side chains, which appeared to allow the photoregulation of G4 topology in potassium-rich conditions to some extent (Figure 5a). The isomerization processes of these ligands was studied by UV−visible techniques. Upon photoirradiation at 350 nm for 90−120 s, the E isomers were converted into the Z forms, whereas the irradiation of the latter with visible light for 120−230 s allowed the formation of the corresponding photostationary state and not the E forms, which is in agreement with previous studies (Figure 5b). Based on the apparent response observed in the circular dichroism (CD) spectra, the authors proposed the E isomers in the presence of d(TTAGGG)4 DNA induced a topological switch from a mixed-hybrid-type G4 structure (characterized by a strong positive peak at 290 nm with a shoulder at 270 nm) to antiparallel (a positive peak at 295 nm and a negative peak at 265 nm) in the presence of potassium ions, which was also in a reversible manner by photoradiation with UV/vis light (Figure 5c). However, full details on the type of the ligand-induced structures formed or specific changes in binding mode could not be elucidated in the absence of further structural information. In these cases, after irradiation of the E isomers with visible light, the formed Z isomers induced a topological switch from antiparallel to the initial conformation of the telomeric G4 DNA (mixed-hybrid-type) and did not induce the unfolding of the oligonucleotide like the previous compound 4. However, unlike the original system using 4 in the absence of metal cations, the reversibility of these isomers 5 and 6 did not prove to be so robust, allowing only three switches in topology. Nevertheless, these initial reports suggest that reversible regulation of G4 topology is possible with azobenzene ligands and that further improvements and careful ligand design might lead to better molecules for the robust and reversible regulation of such systems that drive G4-folding in physiological conditions, such as in the presence of metal ions, which are required for real-world applications to be realized.

Figure 6. Structure of azobenzene derivatives 7 and the corresponding photoisomerization process using UV/vis light. (b) Schematic representation showing the complexation process between the photochrome and the telomeric G4 DNA via stacking on the top/bottom of the quartets. Reproduced with permission from ref 77. Copyright 2018 Royal Society of Chemistry. (c) Structure of azobenzene 8 as photoresponsive G4 ligands.
the photoisomerization process, but in these cases, the compounds exerted little effect on the topology of the human telomeric G4 and, unfortunately, interacted significantly with duplex DNA. Fluorescence titrations provided evidence that the binding modes of the E ligands to telomeric G4 in both Na+- and K+-rich conditions gave rise to end-stacking binding modes via stacking on the top/bottom of the quartets, which is the most common observed ligand/G4 DNA-binding mode (Figure 6b). The resulting complex is stabilized by a combination of π–π stacking, hydrogen bonding, Coulombic attractions, etc. On the other hand, as it is expected, the molecular switch-bound duplex DNA occurred via intercalation.

In a short study, Titterington and co-workers studied the ability of a series of closely related 4,4′-diaminoazobenzene-derived ligands, such as the most potent compound 8 (Figure 6c), to stabilize telomeric G4 DNA. They conferred only very modest thermal stabilization to G4 structures, indicating that this chemotype does not display high affinity for G4 under physiologically relevant conditions of high ionic strength. In fact, even at high ligand concentrations (40μM), only 4°C thermal stabilization was induced in the G4 structure for the most potent analogue 8 reported in potassium-rich conditions. Although disappointing, these results could be expected since it has been reported that azobenzene derivatives intercalate and stabilize duplex DNA at comparable concentrations.

The poor G4 affinity of azobenzenes could be attributed to comparatively weak stacking interactions between the G-tetrads and the ligand π system, which has a relatively small hydrophobic surface area, which is the binding mode suggested by computational investigations in the original study. Furthermore, rotation about the N-aryl bonds permits conformational freedom in solution, resulting in an entropic penalty to adopt the active conformation for binding to the macromolecule. Unlike azobenzene ligands, stacking interactions between fused ring ligands, and the G-tetrads could be expected to be higher owing to a greater hydrophobic stacking surface and increased rigidity.

These results suggest that this chromophore is more likely suited to intercalating DNA base pairs rather than targeting the specific features of G4. Thus, the applications for these types of ligands may be limited to situations where a high level of quadruplex/duplex selectivity is not required.

3.2.2. Arylstilbazolium. Another type of chromophore employed as a G4 ligand is based on the use of arylstilbazolium moieties. The Juskowiak group developed a series of photo-switchable arylstilbazolium G4 ligands toward regulation of different G4 topologies in metal-rich conditions. For instance, the E,E-9 and E,Z-9 isomers of 1,4-bis(vinylquinolinium)-benzene could interact with parallel and antiparallel tetraplexes but exhibit different binding selectivity, and the E,Z ligand showed higher binding preference for c-myc DNA (a propeller-type quadruplex), whereas the E,E isomer favorably interacted with telomeric DNA (a basket-type quadruplex), demonstrating that these ligands were capable of binding with some selectively to different G4 topologies and also isomerize between E and Z forms in sodium buffer (Figure 7). However, the photo-isomerization was inhibited in the presence of DNA, which limited its applicability somewhat.

Despite similar apparent binding constants (see Table 1), the authors suggested that the variation in binding selectivity observed for both isomers toward a particular G4 structure could be attributed to the planarity of the ligand structure and steric factors since E,E-9 exist in the planar conformation, whereas the E,Z isomer is nonplanar.

3.2.3. Stiff-Stilbenes. In light of the above results, Galan et al. decided to focus efforts on the use of conformationally restricted stilbenes, colloquially termed stiff-stilbenes, that would feature both increased hydrophobic surface area and additional core rigidity that could be beneficial for more effective G4 recognition. In this context, the team reported the discovery of a series of still-stilbene ligands that displayed high affinity for G4 DNA with significant discrimination against duplex DNA and exhibited selective anticancer and antiparasitic activity. In particular, the photoresponsive pyridinium stiff-stilbene ligand 10 (Figure 8) is capable of unfolding telomeric G4 DNA in sodium-rich conditions. This was a particularly surprising finding given that this ligand stabilized the potassium form of the same sequence, namely, telo23. In fact, the FRET melting assay results showed that, at lower concentrations, the E isomer induced a high level of thermal stabilization in the human telomeric G4 in K+-rich conditions, whereas in Na+ conditions, this stabilization was slightly decreased and the stability of duplex DNA was largely unaffected, showing the specificity of the ligand for G4 over duplex DNA. This specificity was also confirmed under competitive conditions by FRET assay. CD titrations showed that E-10 caused dramatic changes in the CD signature of the oligonucleotide in Na+ conditions, while no conformational change was observed in the presence of K+.

These results were in agreement with data from NMR titrations, where a marked decrease in intensity of the imino signals from the DNA (10.4–11.8 ppm) was observed in Na+ conditions, resulting in the disappearance of signals associated with key residues in the oligonucleotide sequence. In addition to spectrometric techniques (CD, UV–vis, and NMR studies),...
molecular dynamics and metadynamics simulations were employed to identify the likely binding site and mechanism of unfolding. These results suggested that the stiff-stilbene 10 targeted the G4 grooves before intercalating into the G4 structure, leading to eventual disruption of the hydrogen bond network of the G-tetrad.

Figure 8. Reversible unfolding of G4 DNA using the photoresponsive stiff-stilbene 10. Reproduced with permission from ref 80. Copyright 2019 Wiley.

In a similar way to stiff-stilbene derivatives, the pyridinium-decorated DTE 12 displayed good affinity for G4 oligonucleotides with significant discrimination against duplex DNA. Using a variety of spectroscopic studies supported by computational methods, the group demonstrated that the closed form of the ligand 12c binds to the G4 structure without perturbing the hybrid G4 fold via an end-stacking mode, while the binding of the open form 12o causes partial disruption to the G-tetrad network, pointing to an intercalative binding mechanism. Moreover, the photoreversible control in a bidirectional manner of ligand-binding mode and oligonucleotide folding occurred in physiologically relevant biologically compatible visible light sources (Figure 9). This is significant, since it was the first time the reversible regulation of G-quadruplex ligand activity exclusively with visible light. The observed control of ligand-binding mode and oligonucleotide folding occurred in physiologically relevant buffered solutions. In particular, the native human telomeric G4 formed in 100 mM potassium phosphate buffer was reversibly disrupted and reformed up to seven times by alternate irradiation of the G4/ligand system with red (635 nm) and blue (450 nm) visible light, which are biologically compatible visible light sources (Figure 9). This is significant, since it was the first example to our knowledge, of reversible regulation of G-quadruplex ligand activity exclusively with visible light. The observed control of ligand-binding mode (telo22) and G-tetrad formation (telo23) hints to a variety of possible applications of DTE derivatives in the development of responsive G4/ligand systems. Toward one such application, we demonstrated that the toxicity of the ligand toward cervical cancer cells appears to be modulated to an extent by the photoisomeric state of the ligand, indicating for the first time the

### Table 1. Summary of Main Properties of Photoresponsive G4 Binders Reported to Date

| core | azobenzene | azobenzene (X = 5 or 6) | azobenzene | arylstilbazolium | stiff-stilbene | DTE |
|------|------------|--------------------------|------------|-----------------|----------------|-----|
|      | E-4        | E-X                      | E-Z        | E-7            | E-E-9         | E-Z-9|
| isomerization from... to... (nm, min, %) | 350 Vis                 | 350 Vis                  | 360      | 515             | 450 heat       | 400 | 450 | 635 |
| morphology regulation | P reversible (10 cycles) | A H reversible (only 3 cycles) | H reversible (12 cycles) | | irreversible | A-D irreversible | A D |
| specificity* | N/A | N/A | N/A | | yes | yes |
| binding constants | 3.9 × 10^6 (M^-1) | N/A | N/A | | 5.8 × 10^6 (M^-1) | N/A | 1.3 × 10^6 (M^-1) | 0.6 × 10^6 (M^-1) |
| binding mechanism | e-s | N/A | N/A | | I e-s | N/A | g-b and I |
| physicochemical conditions | no | yes | yes | | yes | yes | yes |

*Refers to the selectivity for G4 DNA over duplex DNA. Morphology: P = parallel; A = antiparallel; U = unfolded; H = hybrid; D = disruption. Regulation: e-s = end-stacking; I = intercalation; g-b = groove-binding. N/A = Data not available. Incom. = Incomplete. Full data for compound 8 was not available in the literature.

Figure 9. Suggested binding modes of the two photochromic forms 12c/12o to telo23 G4 DNA and cartoon representation of the responsive G4/ligand system. Adapted with permission from ref 81. Copyright 2020 Royal Society of Chemistry.
potential of G4 to serve as a target for photopharmacological strategies.

3.3.4. What Are the Best Ligands? Advantages and Disadvantages. In order to examine the advantages and shortcomings of the different ligands previously described, we summarize the main properties of the photoresponsive G4 binders in Table 1.

While promising results have been obtained using azobenzene scaffolds as photoresponsive ligands to facilitate control of the G4 structure, these type of ligands suffer from several limitations that currently prevent their use for in vivo G4 targeting applications (e.g., photopharmacology). For example, although the process of isomerization of these kinds of ligands is reversible, often it occurs in an incomplete way and requires UV light, which limits their use in live systems. Importantly, the reported photoresponsive ligands were not completely selective for G4 DNA over duplex DNA, e.g., arylstilbazolium ligand

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addition, for G4 DNA over duplex DNA, e.g., arylstilbazolium ligand reported photoresponsive ligands were not completely selective light, which limits their use in live systems. Importantly, the reversible, often it occurs in an incomplete way and requires UV light, which limits their use in live systems. Importantly, the reported photoresponsive ligands were not completely selective for G4 DNA over duplex DNA, e.g., arylstilbazolium ligand

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In addition, 9 also presents other important shortcomings, such as the need for thermal conditions for the isomerization from E,Z isomer to E,E form, the irreversible regulation of both isomers, and exhibiting similar binding constants for telomeric G4 DNA.

On the other hand, stiff-stilbenes can be considered complementary to the azobenzene systems previously reported. These type of ligands have shown to exert dramatic effects on G4 fold even in the presence of metal salts. In particular, stiff-stilbene 10 could induce unfolding against the K-stabilized structure, rather than templating G4 folding in the absence of cations. Unfortunately, the stiff-stilbene was not suitable for reversible photopharmacological applications, as the inactive form of the ligand cannot be converted to the active form in buffered solutions, instead undergoing irreversible oxidative cleavage to give a nonbinding fragment. For photopharmacological applications, it would be much more desirable for the system to function without the need for repeated additions of chemical fuels and the generation of waste products. Nonetheless, the ligand permitted the photoregulation of G4 folding in the presence of ions without the requirement to preincorporate photoresponsive functionality into the biomolecule, suggesting potential applications in the control of natural G4 systems.

To address the shortcomings of previous systems, efforts have also been made toward the identification of alternative chromophores as the basis for a fully photoreversible G4 ligand that functions in physiologically relevant conditions. In this context, the combination of the pyridinium moieties of stiff-stilbene ligand 10, which conferred high G4 affinity and selectivity, as well as induced conformational perturbations in G4 structures, with the superior photochemical qualities of the DTE core, led to a new generation of G4 ligands with more favorable photoswitching properties. In fact, this system allowed truly photoreversible control of G4 folding under much more satisfactory and readily applicable conditions than previously reported systems. The more rigid planar structure of the closed DTE isomers could stack more efficiently with G-tetrads than the conformationally flexible open isomer, which was better at intercalating within the oligonucleotide structure as was confirmed by CD, NMR and modeling experiments. Moreover, the binding mode of DTE ligands can be reversibly manipulated in situ by controlling the structure of the ligand by photoisomerization using exclusively visible light. This represents a clearly advantage over previous reports, such as the azobenzenes or stiff-stilbenes, where the ligand photochemistry is irreversible. While the change in binding mode observed for the antiparallel G4 appears to take place without perturbing the underlying G4 structure, both CD and NMR suggested that the structure of the G-tetrads is affected by the photoisomeric state of the 12o/12c couple. It is also true that the difference in binding modes did not appear to correlate with a significant difference in G4-binding affinities based on the results of the UV/visible titration experiments; however, the different photoisomers of 12o/12c did exert differing toxicity to cancer cells, providing proof-of-principle that photoresponsive G4 ligands may find application in photopharmacological anticancer strategies. To date, this is the only example of a fully reversible system that works using visible light and that targets G4 sequences. However, there is ample room for improvement. Ideally, better photoresponsive chromophores with reversible and improved photoswitching kinetics, which can toggle between states and with differing binding affinities toward a specific target, will lead to better therapeutic ligands for photopharmacology applications.

3.3.5. Predicting Ligand Binding to G4 Is Still a Challenge. As we have shown in the examples described in this Perspective, most known ligands bind to a preformed G4 topology; however, G4 structures are highly dynamic, and the discovery of ligands that can influence the DNA folding equilibria and be used as probes help us understand the dynamic nature of G4s in vivo or to exploit the polymorphism of G4s in the development of molecular devices, which are otherwise difficult to predict with standard techniques. In most instances, such small molecules have been discovered serendipitously, which entails the synthesis and screening of large libraries of compounds, and it can be a very time-consuming process. To address this and encouraged by their previous results, Galan and Mulholland et al.32 recently developed a powerful tool based on molecular dynamics and metadynamics simulations to help predict the diverse binding modes of stiff-stilbene ligands that perturb G4 topology, which will allow the design and development of new chemotypes to influence G4 structure. The team characterized the binding of the stiff-stilbene ligands to the telomeric G4 target experimentally using NMR and CD spectroscopy and in parallel run simulations to predict a variety of binding mechanisms on G4 structure for the different ligands. Good agreement between the simulated and experimentally observed binding modes, binding affinities and ligand-induced perturbation of the G4 structure was observed, demonstrating that the metadynamics tools developed within their work could correctly predict ligands that perturb G4 topology and could be used as a powerful tool to aid the discovery of molecules to influence G4 structure.

4. SUMMARY AND OUTLOOK

There has been tremendous progress over the past few decades on the development of small molecules to target G4 oligonucleotides starting from the pioneering work of Hurley and Neidle in the late 1990s on the development of ligands that could inhibit the telomerase enzyme by targeting G4 structures.33 A lot of efforts have since been devoted to the design and synthesis of ligands that can selectively target G4 sequences over duplex DNA. From these research programs, several lead scaffolds have been identified with promising binding affinities and modes; however, despite the many impressive efforts, no therapeutics based on G4 targeting have made it to the clinic to date, mostly due to issues with selectivity, bioavailability, and off-target side effects.

In this Perspective, we have aimed to provide an overview on the most recent developments in the emerging area of photoresponsive G4-targeting ligands that could potentially be
applied to the study and modulation of the role and function of G4s in biological systems. Light activation as part of a pro-drug strategy has garnered a lot of interest in recent years due to its many benefits when compared to other chemical stimuli; however, its widespread use in the clinical settings does come without associated challenges such effective deliverability in vivo and the need to develop molecules that can be activated under physiological conditions.

We have shown in the examples described here that ligands with the capability to bind G4s specifically and to control G4 structure and behavior offer great potential in the development of novel therapies, technologies, and even novel functional materials. Different strategies including ligand photocaging and photoisomerization have been used thus far to switch on and off the binding to the G4 target and in some cases to toggle between two different binding modes, which results in different bioactivity. It is clear from the examples described in the literature that the choice of wavelength of light required to activate the ligand is important and must be compatible with in vivo applications to avoid poor tissue penetration if the end goal is to reach the clinic. Thus, it is exciting to see the emergence of new strategies for the in situ activation of G4 ligands using visible light that could be exploited toward therapeutic ends. It has also become apparent that ligand design is equally important and that due to the highly dynamic nature of G4 topology, there is a need for the development of computational methods to help predict ligand-binding modes to drive ligand design and development and for molecular-level understanding of ligand-binding mechanisms and associated topological perturbation of G4 structures. Moreover, thousands of ligands have already been reported to date to be able to target G4 sequences, and thus data mining and artificial intelligence (AI) strategies are needed to compile and analyze data already available, which will help predict the next generation of G4-binding molecules.

All of the very elegant systems described to date represent proof-of-principle that can be optimized to better control the in situ release of G4-targeting pro-drugs. It is thus hoped that within the next few years we will expect to see new developments in the design of improved ligands that can operate in physiological conditions, preferably in the presence of K+ ions within the next few years. It is hoped that due to the highly dynamic nature of G4 topology, there is a clear need for the development of computational methods to help predict ligand-binding modes to drive ligand design and development and for molecular-level understanding of ligand-binding mechanisms and associated topological perturbation of G4 structures. Moreover, thousands of ligands have already been reported to date to be able to target G4 sequences, and thus data mining and artificial intelligence (AI) strategies are needed to compile and analyze data already available, which will help predict the next generation of G4-binding molecules.

All of the very elegant systems described to date represent proof-of-principle that can be optimized to better control the in situ release of G4-targeting pro-drugs. It is thus hoped that within the next few years we will expect to see new developments in the design of improved ligands that can operate in physiological conditions, preferably in the presence of K+ ions and with high selectivity toward telomeric G4 DNA, as the prevalence and importance of these types of DNA sequences in cancer, viruses, parasites, and bacteria has now been validated. As we have already discussed in the previous sections, there are still clear limitations and challenges remaining in this field, particularly for systems to work in biological applications. However, as we learn more about the molecular features required for both improved binding to G4 DNA and reversible photoswitching kinetics for bespoke applications such as in photopharmacology (e.g., using visible light, working under physiological conditions, fully reversible systems, ligands where one isomer is active, while the other is not), new chromophores with unique binding modes and bioactivities are likely to emerge.

Looking forward, lessons learned from these pioneering studies will help pave the way for the design of improved photoresponsive ligands for in vivo applications in the clinic, which is one of the ultimately goals. This is an exciting time in the field and we look forward to following the progress in the coming years.

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Notes

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■ REFERENCES

(1) Sen, D.; Gilbert, W. Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis. Nature 1988, 339 (6218), 364-6.
(2) Neidle, S. Quadruplex Nucleic Acids as Novel Therapeutic Targets. J. Med. Chem. 2016, 59 (13), 5987–6011.
(3) Wang, C.; Jia, G.; Li, Y.; Zhang, S.; Li, C. Na+/K+ switch of enantioselectivity in G-quadruplex DNA-based catalysis. Chem. Commun. 2013, 49 (95), 11161–3.
(4) Canale, T. D.; Sen, D. Hemin-utilizing G-quadruplex DNAzymes are strongly active in organic co-solvents. Biochem. Biophys. Acta, Gen. Subj. 2017, 1861 (5), 1455–1462.
(5) Alberti, P.; Mergny, J. L. DNA duplex-quadruplex exchange as the basis for a nanomolecular machine. Proc. Natl. Acad. Sci. U. S. A. 2003, 100 (4), 1569–73.
(6) Alberti, P.; Bourdoncle, A.; Sacca, B.; Lacroix, L.; Mergny, J. L. DNA nanomachines and nanostructures involving quadruplexes. Org. Biomol. Chem. 2006, 4 (18), 3383–91.
(7) Hanna, R.; Flamier, A.; Barabino, A.; Bernier, G. G-quadruplexes originating from evolutionary conserved L1 elements interfere with neuronal gene expression in Alzheimer’s disease. Nat. Commun. 2021, 12 (1), 1828.
(8) Hansel-Hertsch, R.; Beraldi, D.; Lensing, S. V.; Marsico, G.; Zyner, K.; Parry, A.; Di Antonio, M.; Pike, J.; Kimura, H.; Narita, M.; Tannahill, D.; Balasubramanian, S. G-quadruplex structures mark human regulatory chromatin. Nat. Genet. 2016, 48 (10), 1267–72.
(9) Rhodes, D.; Lippis, H. J. G-quadruplexes and their regulatory roles in biology. Nucleic Acids Res. 2015, 43 (18), 8627–8637.
(10) Bedrat, A.; Lacroix, L.; Mergny, J. L. Re-evaluation of G-quadruplex propensity with G4Hunter. Nucleic Acids Res. 2016, 44 (4), 1746–1759.
(11) Fleming, A. M.; Ding, Y.; Burrows, C. J. Oxidative DNA damage is epigenetic by regulating gene transcription via base excision repair. Proc. Natl. Acad. Sci. U. S. A. 2017, 114 (10), 2604–2609.
(12) Raguseo, F.; Chowdhury, S.; Minard, A.; Di Antonio, M. Chemical-biology approaches to probe DNA and RNA G-quadruplex structures in the genome. Chem. Commun. 2020, 56 (9), 1317–1324.
(13) Shivalingam, A.; Izquierdo, M. A.; Marois, A. L.; Vysniauskas, A.; Suhling, K.; Kuimova, M. K.; Vilar, R. The interactions between a small molecule and G-quadruplexes are visualized by fluorescence lifetime imaging microscopy. Nat. Commun. 2015, 6, 8178.
(14) Sperti, F. R.; Charbonnier, T.; Lejault, P.; Zell, J.; Bernhard, C.; Valverde, I. E.; Monchaud, D. Biomimetic Smart, and Multivalent Ligands for G-Quadruplex Isolation and Biorthogonal Imaging. ACS Chem. Biol. 2021, 16 (5), 905–914.

(15) Di Antonio, M.; Ponjavic, A.; Radzevicius, A.; Ranasinghe, R. T.; Catalano, M.; Zhang, X.; Shen, J.; Needham, L. M.; Lee, S. F.; Kleinerman, D.; Balasubramanian, S. Single-molecule visualization of DNA G-quadruplex formation in live cells. Nat. Chem. 2020, 12 (9), 832–837.

(16) Siddiqui-Jain, A.; Grant, C. L.; Beare, D. J.; Hurley, L. H. Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2002, 99 (18), 11593–8.

(17) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(18) Fernández, I. E.; Monchaud, D. Biomimetic, Smart, and Multivalent Ligands for G-Quadruplex Identification in the Genome of Protozoan Parasites. J. Am. Chem. Soc. 2021, 1525 (1), 1319–22.

(19) Cimino-Reale, G.; Zaffaroni, N.; Folini, S.; Pochard, P.; Daskalogianni, C.; Beauvineau, C.; Guetta, C.; Jamin, D.; Ruggiero, E.; Richter, S. N. Viral G-quadruplexes: New frontiers at Room Temperature. ChemPhotoChem. 2020, 4 (11), 2720–2731.

(20) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(21) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(22) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(23) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(24) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(25) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(26) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(27) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(28) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(29) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(30) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(31) Agrawal, P.; Lin, C.; Mathad, R. I.; Carver, M.; Yang, D. The major G-quadruplex formed in the human BCL-2 proximal promoter: evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (20), 10785–90.

(32) Lejault, P.; Mitteaux, F.; Sperti, F. R.; Monchaud, D. How to untie G-quadruplex knots and why? Cell Chem. Biol. 2021, 28 (4), 436–455.

(33) Mergny, J. L.; Sen, D. DNA Quadruplex Helices in Nano-technology. Chem. Rev. 2019, 119 (10), 6290–6325.

(34) Wang, F.; Liu, X.; Willner, I. DNA switches: from principles to applications. Angew. Chem. Int. Ed. 2015, 54 (4), 1098–129.

(35) Vojnovich, A.; Pandoh, D.; Bassi, L.; Schwable, H.; Blum-Charron, K.; Dash, J. Small molecule regulated dynamic structural changes of human G-quadruplexes. Chem. Sci. 2016, 7 (5), 3279–3285.

(36) Vojnovich, A.; Pandoh, D.; Bassi, L.; Schwable, H.; Blum-Charron, K.; Dash, J. Small molecule regulated dynamic structural changes of human G-quadruplexes. Chem. Sci. 2016, 7 (5), 3279–3285.

(37) Vojnovich, A.; Pandoh, D.; Bassi, L.; Schwable, H.; Blum-Charron, K.; Dash, J. Small molecule regulated dynamic structural changes of human G-quadruplexes. Chem. Sci. 2016, 7 (5), 3279–3285.

(38) Vojnovich, A.; Pandoh, D.; Bassi, L.; Schwable, H.; Blum-Charron, K.; Dash, J. Small molecule regulated dynamic structural changes of human G-quadruplexes. Chem. Sci. 2016, 7 (5), 3279–3285.

(39) Vojnovich, A.; Pandoh, D.; Bassi, L.; Schwable, H.; Blum-Charron, K.; Dash, J. Small molecule regulated dynamic structural changes of human G-quadruplexes. Chem. Sci. 2016, 7 (5), 3279–3285.

(40) Vojnovich, A.; Pandoh, D.; Bassi, L.; Schwable, H.; Blum-Charron, K.; Dash, J. Small molecule regulated dynamic structural changes of human G-quadruplexes. Chem. Sci. 2016, 7 (5), 3279–3285.
(55) Kamiya, Y.; Asanuma, H. Light-driven DNA nanomachine with a photosresponsive molecular engine. Acc. Chem. Res. 2014, 47 (6), 1663–72.

(56) Kou, B.; Zhang, J.; Huai, X.; Liang, X.; Xiao, S.-J. Light-driven reversible strand displacement using glycerol azobenzene inserted DNA. RSC Adv. 2015, 5 (7), 5055–5058.

(57) Wang, S.; Yue, L.; Li, Z. Y.; Zhang, J.; Tian, H.; Willner, I. Light-Induced Reversible Reconfiguration of DNA-Based Constitutional Dynamic Networks: Application to Switchable Catalysis. Angew. Chem., Int. Ed. 2018, 57 (27), 8105–8109.

(58) Avagliano, D.; Sanchez-Murcia, P. A.; Gonzalez, L. Directional and regioselective hole injection of spiropyran photo-switches intercalated into A/T-duplex DNA. Phys. Chem. Chem. Phys. 2019, 21 (32), 17971–17977.

(59) Heinrich, B.; Bouzoune, K.; Wojcik, M.; Bakowsky, U.; Vázquez, O. Ortho-Fluoroazobenzene derivatives as DNA intercalators for photocatalysis of DNA and nucleosome binding by visible light. Org. Biomol. Chem. 2019, 17 (7), 1827–1833.

(60) Chen, H.; Li, R.; Li, S.; Andreasson, J.; Choi, J. H. Conformational Effects of UV Light on DNA Origami. J. Am. Chem. Soc. 2017, 139 (4), 1380–1383.

(61) Andersson, J.; Li, S.; Lincoln, P.; Andreasson, J. Photoswitched DNA-binding of a photochromic spiropyran. J. Am. Chem. Soc. 2008, 130 (36), 11836–7.

(62) Sanchez, M. I.; Vazquez, O.; Vazquez, M. E.; Mascaréns, J. L. Light-controlled DNA binding of bisbenzamidines. Chem. Commun. 2011, 47 (39), 11107–9.

(63) Kolsch, S.; Ihmels, H.; Pattaradom, J.; Mattay, J.; Sewald, N.; Patrick, B. O. Reversible photoswitching of the DNA-binding properties of styrylquinolizinium derivatives through photochromic [2 + 2] cycloaddition and cycloreversion. Beilstein J. Org. Chem. 2020, 16, 111–124.

(64) Ogasawara, S.; Maeda, M. Reversible photoswitching of a G-quadruplex. Angew. Chem., Int. Ed. 2009, 48 (36), 6671–4.

(65) Thevarpadam, J.; Bessi, I.; Binus, O.; Gonçalves, D. P. N.; Slavov, C.; Jonker, H. R. A.; Richter, C.; Wachtveitl, J.; Schwalbe, H.; Heckel, A. Photosensitive Formation of an Intermolecular Minimal G-Quadruplex Motif. Angew. Chem., Int. Ed. 2016, 55 (8), 2738–2742.

(66) Gholamjani Moghaddam, K.; Giudetti, G.; Sipma, W.; Faraji, S.; Vázquez, M.; Mascarenas, J. L. Theoretical insights into the effect of size and substitution patterns of azobenzene derivatives on the DNA G-quadruplex. Phys. Chem. Chem. Phys. 2020, 22 (46), 26944–26954.

(67) Ali, A.; Bullen, G. A.; Cross, B.; Dafforn, T. R.; Little, H. A.; Manchester, J.; Peacock, A. F. A.; Tucker, J. H. R. Light-controlled thrombin catalysis and clot formation using a photoswitchable G-quadruplex. Angew. Chem., Int. Ed. 2016, 55 (39), 5627–5630.

(68) Hansel-Hertsch, R.; Di Antonio, M.; Balasubramanian, S. DNA G-quadruplexes in the human genome: detection, functions and therapeutic potential. Nat. Rev. Mol. Cell Biol. 2017, 18 (5), 279–284.

(69) Neidle, S. Quadruplex Nucleic Acids as Novel Therapeutic Targets. J. Med. Chem. 2016, 59 (13), 5987–6011.

(70) Amrane, S.; Kerrou, A.; Bedat, A.; Viallet, B.; Andreola, M. L.; Murph, J. L. Topology of a DNA G-quadruplex structure formed in the HIV-1 promoter: a potential target for anti-HIV drug development. J. Am. Chem. Soc. 2014, 136 (14), 5249–52.

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

(72) Nakamura, T.; Iida, K.; Tera, M.; Shin-ya, K.; Seimiya, H.; Nagasawa, K. A caged ligand for a telomeric G-quadruplex. ChemBioChem 2012, 13 (6), 774–7.

(73) Murat, P.; Gormaly, M. V.; Sanders, D.; Antonio, M. D.; Balasubramanian, S. Light-mediated in cell downregulation of G-quadruplex-containing genes using a photo-caged ligand. Chem. Commun. 2013, 49 (76), 8453–8455.

(74) Wang, X.; Huang, J.; Zhou, Y.; Yan, S.; Wang, X.; Wu, X.; Deng, M.; Zhou, X. Conformational switching of G-quadruplex DNA by photoregulation. Angew. Chem., Int. Ed. 2010, 49 (31), 5305–9.

(75) Xing, X.; Wang, X.; Xu, L.; Tai, Y.; Dai, L.; Zheng, X.; Mao, W.; Xu, X.; Zhou, X. Light-driven conformational regulation of human telomeric G-quadruplex DNA in physiological conditions. Org. Biomol. Chem. 2011, 9 (19), 6639–45.

(76) Tian, T.; Song, Y.; Wang, J.; Fu, B.; He, Z.; Xu, X.; Li, A.; Zhou, X.; Wang, S.; Zhou, X. Small-Molecule-Triggered and Light-Controlled Reversible Regulation of Enzymatic Activity. J. Am. Chem. Soc. 2016, 138 (3), 955–61.

(77) Dudek, M.; Deiana, M.; Pokladek, Z.; Mlynarz, P.; Samoc, M.; Matczyszyn, K. Light-driven chiral photoswitchable DNA assemblies mediated by bioinspired photoresponsive molecules. Nanoscale 2018, 10 (24), 11302–11306.

(78) McCallum, J. E. B.; Coyle, C. W.; Elson, R. R.; Titterington, B. A. Interactions of 4,4′-diaminoazobenzene derivatives with telomeric G-quadruplex DNA. Nucleosides, Nucleotides Nucleic Acids 2018, 37 (3), 169–178.

(79) Czerwińska, I.; Juskowiak, B. Photosomerizable arylistilbazolium ligands recognize parallel and antiparallel structures of G-quadruplexes. Int. J. Biol. Macromol. 2012, 51 (4), 576–82.

(80) O’Hagan, M. P.; Haldar, S.; Duchi, M.; Oliver, T. A. A.; Mulholland, A. J.; Morales, J. C.; Galan, M. C. A Photoresponsive Stiff-Stable Ligand Fuels the Reversible Unfolding of G-Quadruplex DNA. Chem. Sci. 2019, 58 (13), 4334–4338.

(81) O’Hagan, M. P.; Ramos-Soriano, J.; Haldar, S.; Sheikh, S.; Morales, J. C.; Mulholland, A. J.; Galan, M. C. Visible-light photoswitching of ligand binding mode suggests G-quadruplex DNA as a target for photopharmacology. Chem. Commun. 2020, 56 (38), 5186–5189.

(82) O’Hagan, M. P.; Haldar, S.; Morales, J. C.; Mulholland, A. J.; Galan, M. C. Enhanced sampling molecular dynamics simulations correctly predict the diverse activities of a series of stiff-stable G-quadruplex DNA ligands. Chem. Sci. 2021, 12 (4), 1415–1426.

(83) Sun, D.; Thompson, B.; Cathers, B. E.; Salazar, M.; Kerwin, S. M.; Trent, J. O.; Jenkins, T. C.; Neidle, S.; Hurley, L. H. Inhibition of human telomerase by a G-quadruplex-interactive compound. J. Med. Chem. 1997, 40 (14), 2113–6.