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Photodependent Melting of Unmodified DNA Using a Photosensitive Intercalator: A New and Generic Tool for Photoreversible Assembly of DNA Nanostructures at Constant Temperature

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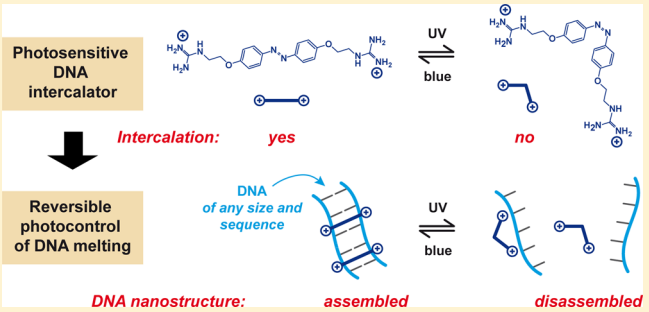
Supporting Information

ABSTRACT: External control of DNA melting and hybridization, a key step in bio- and DNA nanotechnology, is commonly achieved with temperature. The use of light to direct this process is a challenging alternative, which has been only possible with a DNA modification, such as covalent grafting or mismatch introduction, so far. Here we describe the first photocontrol of DNA melting that relies on the addition of a molecule that noncovalently interacts with unmodified DNA and affects its melting properties in a photoreversible and highly robust manner, without any prerequisite in the length or sequence of the target DNA molecule. We synthesize azobenzene-containing guanidinium derivatives and show that a bivalent molecule with a conformation-dependent binding mode, **AzoDiGua**, strongly increases the melting temperature (T_m) of DNA under dark conditions because its *trans* isomer intercalates in the DNA double helix. Upon UV irradiation at 365 nm, the *trans*−*cis* isomerization induced the ejection of **AzoDiGua** from the intercalation binding sites, resulting in a decrease in T_m up to 18 °C. This illumination-dependent T_m shift is observed on many types of DNA, from self-complementary single-stranded or double-stranded oligonucleotides to long genomic duplex DNA molecules. Finally, we show that simply adding **AzoDiGua** allows us to photoreversibly control the assembly/disassembly of a DNA nanostructure at constant temperature, as demonstrated here with a self-hybridized DNA hairpin. We anticipate that this strategy will be the key ingredient in a new and generic way of placing DNA-based bio- and nanotechnologies under dynamic control by light.



KEYWORDS: *DNA nanotechnology, DNA hybridization, azobenzene, photocontrol, intercalator*

The hybridization/melting of DNA is a key step in important biotechnological processes, such as the amplification of DNA in the Polymerase Chain Reaction (PCR).[1−3] It also forms the basis of the developing field of DNA nanotechnology, in which individual nanoscale components are assembled by DNA-base pairing in a highly specific and programmable way.[4−9] Controlling DNA hybridization/melting by external stimuli is therefore a useful and needed strategy that can serve to trigger a broad range of biological and nanotechnological processes relying on DNA assembly/disassembly. Usually, DNA melting is controlled by changing the temperature of the solution, which hinders applications requiring constant temperature or temperature-sensitive systems and renders difficult *in vivo* implementations. A particularly attractive alternative is to use light, a stimulus that offers high tunability (wavelength, intensity), good biocompatibility, and an excellent spatiotemporal resolution.[10−14] To exploit the advantages of light as a trigger for DNA-based systems, photochromic molecules, such as dithienylethene,[15,16] spiropyran,[17−19] or spirooxazine[20] derivatives, were developed and their photoswitchable binding properties toward DNA were studied. However, the photocontrol of DNA melting has only been achieved in a few cases. Asanuma, Komiyama and their collaborators have developed an efficient method based on the covalent incorporation of azobenzene units into synthetic oligonucleotides,[21−24] with applications to *in vitro* photocontrol of DNA replication[21] and transcription.[22] A similar strategy was applied for the photocontrol of the hybridization between peptide nucleic acids and DNA.[25] Nakatani et al. proposed a different approach by adding a photochromic molecule possessing an isomer

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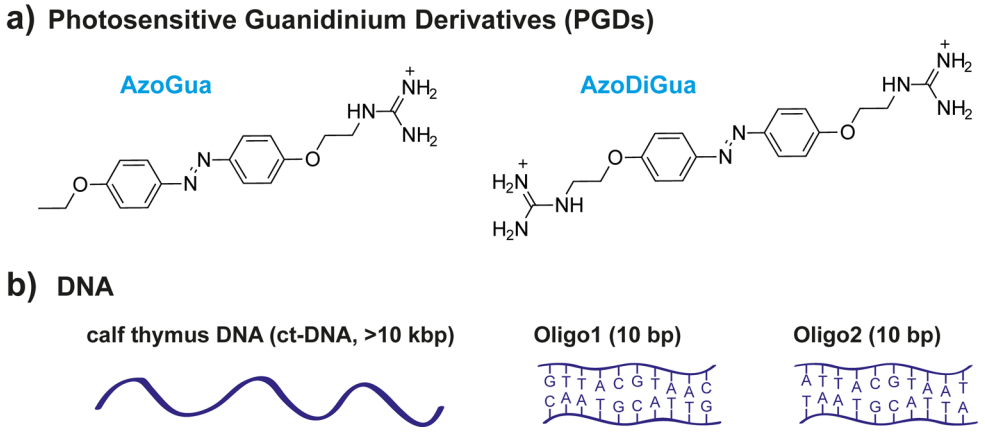

Figure 1. Molecules used in this study. (a) Photosensitive guanidinium derivatives (PGDs, top). (b) DNA.

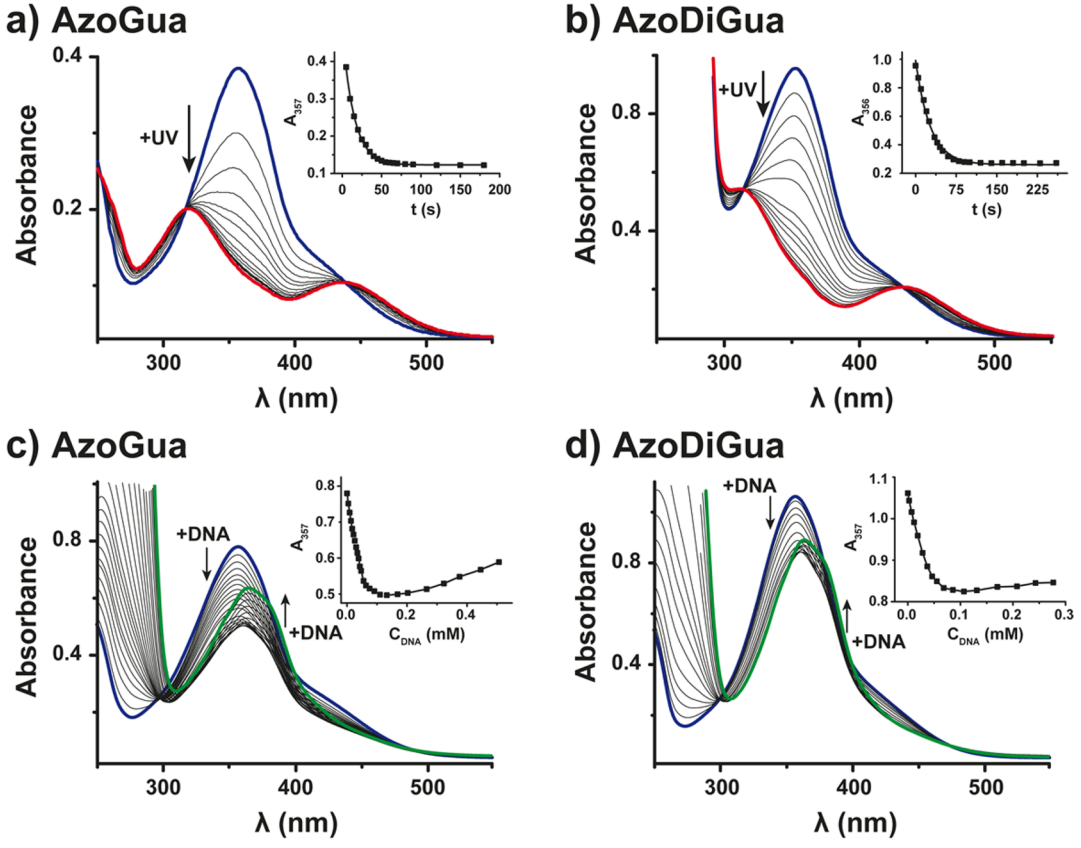

Figure 2. Photochemical and DNA binding properties of PGDs. Absorption of **AzoGua** [31.6 μM in H_2O (a); 50.0 μM in Tris buffer (c)] and **AzoDiGua** [100 μM in H_2O (b); 50.0 μM in Tris buffer (d)] with an increasing time of irradiation at 365 nm (a, b) and with increasing concentrations of ct-DNA (c, d). Insets show the absorption at 356−357 nm as a function of the irradiation time (a, b) and the concentration of ct-DNA (c, d). Solid lines in the insets are exponential fits (a, b) or guides for the eyes (c, d). T = 26 °C.

which specifically binds to a GG mismatch.[26,27] All these methods were shown to be successful but required the ad hoc preparation of specific synthetic oligonucleotides, either covalently modified or with a specific mismatch pattern, therefore limiting the scope of their applicability. Herein, we describe the first generic and reversible photocontrol of DNA melting/hybridization that does not require further DNA modification and can be applied to potentially any kind of DNA molecules, regardless of their length (from short oligonucleotides to long genomic DNA), sequence, and origin (synthetic or natural). It is based on a class of molecules that combine an azobenzene photoisomerizable group and guanidinium units, a functionality that is protonated even under moderate alkaline conditions and known for its high affinity for phosphate groups.[28,29] We describe the synthesis and characterization of these photosensitive guanidinium derivatives (PGDs), investigate their binding with DNA, and analyze their ability to induce photodependent melting/hybridization of DNA molecules of different lengths and sequences, for various PGD:DNA ratios and ionic strength conditions.

We synthesized two photosensitive guanidinium derivatives (PGDs) carrying a photoisomerizable azobenzene moiety and

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one (**AzoGua**) or two (**AzoDiGua**) terminal guanidinium functionalities (Figure 1a, Supporting Information (SI) Scheme S1). We used calf thymus DNA (ct-DNA) as long (>10 kbp) double-stranded DNA, two short (10 bp) self-complementary oligonucleotides with different GC contents (40% and 20% for Oligo1 and Oligo2, respectively, Figure 1b). Both PGDs showed similar absorption and photoisomerization properties (Figure 2a, b). In the dark, they were mainly in the *trans* configuration with a characteristic intense broad absorption band located at 357 nm ($\pi-\pi^*$)[12,13] with a molar extinction coefficient of around 2×10^4 M^{-1} cm^{-1}. Upon UV irradiation at 365 nm (0.35 mW·cm^{-2}), a *trans*–*cis* isomerization was observed with the decrease of the peak at 357 nm accompanied by the appearance of two bands, namely a less intense blue-shifted band at 319 nm ($\pi-\pi^*$) and a broad band at around 440 nm (n–π^*).[12,13] The photostationary *cis*-rich states were reached within similar short illumination times (approximately 50 and 75 s for **AzoGua** and **AzoDiGua**, respectively), whereas thermal *cis*–*trans* relaxation in the dark at room temperature required several hours (SI Figure S1).

Interestingly, when ct-DNA was progressively added to a solution of a fixed PGD concentration in the dark (*trans*-rich state), nonmonotonic behavior of the absorption spectra was observed for both **AzoGua** and **AzoDiGua** with increasing DNA concentration (Figure 2c, d). The intensity of the band at 357 nm first decreased to reach a minimum before increasing again, accompanied by a bathochromic shift of around 10 nm. Both the hypochromic and bathochromic effects at lower DNA concentrations are common features which are usually observed when a ligand binds to DNA.[30−34] In contrast, at higher DNA concentrations, the additional increase of absorption indicates the appearance of a second binding mode, in agreement with reported studies.[34−37] The vanishing isosbestic points at 297 and 304 nm for **AzoGua** and **AzoDiGua**, respectively, with increasing DNA concentrations, support the existence of different binding modes. Note that the second binding mode seems to be more specific to the double-stranded nature of DNA as it was also observed on double-stranded oligonucleotides, while experiments with single-stranded oligonucleotides did not show two-phase binding behavior (SI Figure S2). According to already reported two-phasic binding modes, it may be assumed that at low DNA concentrations nonspecific external association of the PGDs by electrostatic interactions with the DNA phosphate backbone dominates, whereas, in the second phase of the titration experiment, i.e., when an excess of binding sites is available, more specific binding toward the DNA double helix occurs, such as intercalation or groove binding.[34−37]

After it was shown that both PGDs bind to DNA, we studied their effect on the melting properties of DNA. Although **AzoGua** and **AzoDiGua** showed similar spectroscopic properties in the presence of DNA, a marked difference was observed in the melting profiles. While **AzoGua** did not affect the melting curve of neither long (ct-DNA, Figure 3a) nor short DNA (Oligo1, Figure 3b), **AzoDiGua** induced a pronounced shift of the melting curves for both DNAs (Figure 3a, b). For instance, under experimental conditions of Figure 3, the melting temperature of ct-DNA (T_m = 62 °C) did not change after the addition of **AzoGua**, but increased dramatically in the presence of **AzoDiGua** (T_m = 84 °C). This was also observed with the short oligonucleotides Oligo1 (T_m = 19 °C) with **AzoGua** (T_m = 19 °C) and **AzoDiGua** (T_m = 34 °C) (Figure 3b), as well as with Oligo2 (T_m = 11 °C) with **AzoGua** (T_m = 12 °C) and **AzoDiGua** (T_m = 25 °C) (data not shown).

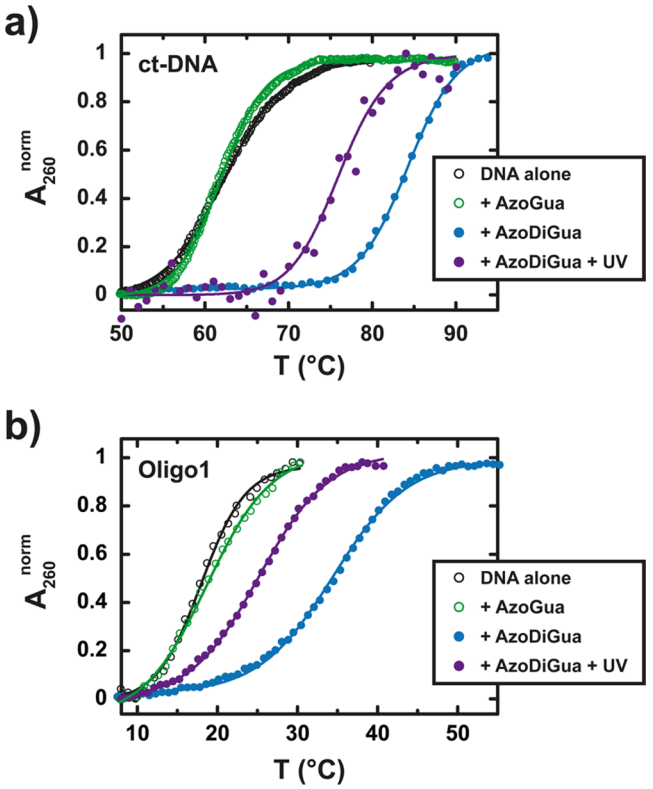

Figure 3. AzoDiGua affects the melting of DNA in a photodependent manner. Thermal denaturation profiles of (a) ct-DNA (40.0 μM in 10 mM Tris buffer, pH = 7.4) and (b) Oligo1 (20.0 μM in 10 mM Tris buffer + 5 mM NaCl, pH = 7.4) in the presence of **AzoGua** and **AzoDiGua** without or with illumination at 365 nm (+UV) during the acquisition of the melting curve (a) or for 1 min before the measurement (b). The PGD concentrations are 20 μM (a) and 40 μM (b). Solid lines represent sigmoidal fits of the experimental data.

The marked difference between **AzoGua** and **AzoDiGua** with respect to their DNA-stabilizing properties might be due to the difference in their hydrophilicity. **AzoGua** is amphiphilic with a short hydrophobic tail allowing it to induce DNA compaction (SI Figure S3) in a way similar to known cationic azobenzene derivatives.[38−41] This property might prevent the binding interaction that is necessary for the helix stabilization. In contrast, **AzoDiGua** is symmetric and hydrophilic and does not induce DNA compaction in the employed range of concentrations (SI Figure S4).

In subsequent studies, we analyzed the effect of light on the melting transition of DNA in the presence of **AzoDiGua**. We first discovered that the conditions of illumination had to be adapted depending on the DNA length. In the case of ct-DNA, the sample had to be permanently illuminated by UV light because of the fast *cis*–*trans* isomerization by thermal relaxation at high temperatures. In contrast, with both oligonucleotides, due to the high photostability of *cis*-**AzoDiGua** at lower temperatures (SI Figure S1b), a short UV irradiation (1 min) before acquiring the melting curve was sufficient. Strikingly, we observed a significant shift of the melting curves toward lower temperatures for both ct-DNA (Figure 3a) and oligonucleotides (Figure 3b and SI Figure S5) upon UV illumination. For instance, for an **AzoDiGua**:ct-DNA ratio r = 0.5, we measured a T_m of 84 °C for −UV and 76 °C for + UV conditions; for an

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Table 1. Melting Temperature (in °C) of ct-DNA (40 μM in 10 mM Tris Buffer, pH = 7.4), Oligo1 (20 μM in 10 mM Tris Buffer, pH = 7.4) and Oligo2 (20 μM in 10 mM Tris Buffer, pH = 7.4) in the Presence of AzoDiGua, for Different AzoDiGua:DNA Ratios (r) and NaCl Concentrations, without (T_m^{-UV}) or with (T_m^{+UV}) illumination at 365 nm[a]

	ct-DNA			Oligo1						Oligo2					
	0 mM NaCl			5 mM NaCl			10 mM NaCl			5 mM NaCl			10 mM NaCl		
r	T_m^{-UV}	T_m^{+UV}	ΔT_m	T_m^{-UV}	T_m^{+UV}	ΔT_m	T_m^{-UV}	T_m^{+UV}	ΔT_m	T_m^{-UV}	T_m^{+UV}	ΔT_m	T_m^{-UV}	T_m^{+UV}	ΔT_m
0	62	–	–	19	18	**−1**	20	20	**0**	11	12	**1**	14	14	**0**
0.1	70	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.25	77	–	–	23	19	**−4**	–	–	–	16	12	**−4**	–	–	–
0.5	84	76	**−8**	27	20	**−7**	27	21	**−6**	19	15	**−4**	24	14	**−10**
1	–	–	–	31	23	**−8**	31	23	**−8**	24	17	**−7**	25	17	**−8**
1.5	–	–	–	32	24	**−8**	–	–	–	25	18	**−7**	–	–	–
2	–	–	–	34	25	**−9**	35	27	**−8**	25	19	**−6**	32	21	**−11**
4	–	–	–	–	–	–	40	36	**−4**	–	–	–	37	24	**−13**

[a]When UV was applied, the sample was illuminated during the whole melting curve acquisition in the case of ct-DNA, and for 1 min before measurements in the case of Oligo1 and Oligo2. $\Delta T_m = T_m^{+UV} - T_m^{-UV}$.

AzoDiGua:Oligo1 ratio r = 2, we found T_m equals 34 °C (−UV) and 25 °C (+UV). This photoinduced decrease of the melting temperature was observed for all studied DNA-**AzoDiGua** complexes and was ranging from 4 to 13 °C depending on buffer composition and the **AzoDiGua**:DNA ratio (Table 1).

To gain further insight into the interaction between **AzoDiGua** and DNA, we acquired circular dichroism (CD) spectra. Hence, the addition of ct-DNA to **AzoDiGua** led to the formation of strong induced CD (ICD) signals in the absorption region of the ligand (300−500 nm), along with the DNA bands (<300 nm), which clearly supports the association of **AzoDiGua** with DNA (Figure 4).[42−44] Such ICD

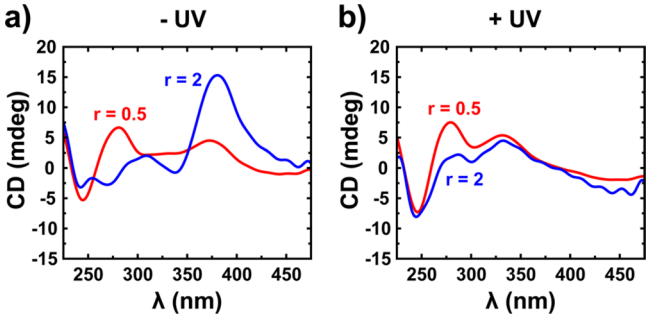

Figure 4. Circular dichroism spectra of **AzoDiGua** in the presence of ct-DNA (50 μM in 10 mM phosphate buffer, pH = 7.0) at **AzoDiGua**:DNA ratio r = 0.5 (red) and r = 2.0 (blue), before (a, −UV) and after (b, +UV) irradiation at 365 nm for 8 min. T = 22 °C.

bands were not observed for the pure components (data not shown). Notably, at low **AzoDiGua**:DNA ratio (r = 0.5) a positive ICD was observed whose maximum matches that of the absorption band, whereas a bisignate ICD signal developed at larger ratios (r = 2.0). This observation is consistent with the nonmonotonic absorption changes upon DNA addition (Figure 2d) and denotes a heterogeneous binding mode depending on the PGD:DNA ratio. In contrast, CD spectra of **AzoGua**:DNA complexes did not exhibit ICD bands (data not shown), showing a probable correlation between the stabilization of the double-helix by **AzoDiGua** (increase in T_m) and the geometric arrangement of the ligand in the DNA environment. After UV irradiation, ICD bands were observed at ca. 320 and 440 nm that match the absorption of the *cis*-isomer and that did not change significantly with increasing **AzoDiGua**:DNA ratio (Figure 4), which indicates the association of *cis*-**AzoDiGua** with DNA in one predominant binding mode.

To obtain additional information about the binding mode of **AzoDiGua** with DNA, linear dichroism (LD) spectroscopic investigations were performed in a rotating Couette flow. In this setup, the DNA molecules are aligned along the flow lines of the resulting hydrodynamic field (flow LD), such that the sign of the LD absorption depends on the orientation of the transition dipole moment of the chromophore relative to the LD reference axis. Hence, the LD signals of the DNA bases and of DNA intercalators are negative, whereas groove binders result in weak positive LD bands.[42,45] Upon complex formation between **AzoDiGua** and ct-DNA, a negative LD signal developed in the absorption region of the ligand which indicates an intercalative binding mode (Figure 5). Notably, upon irradiation of the sample, the LD band of **AzoDiGua** decreased, whereas no maximum appeared in the absorption region of the *cis*-isomer, although photometric analysis of the

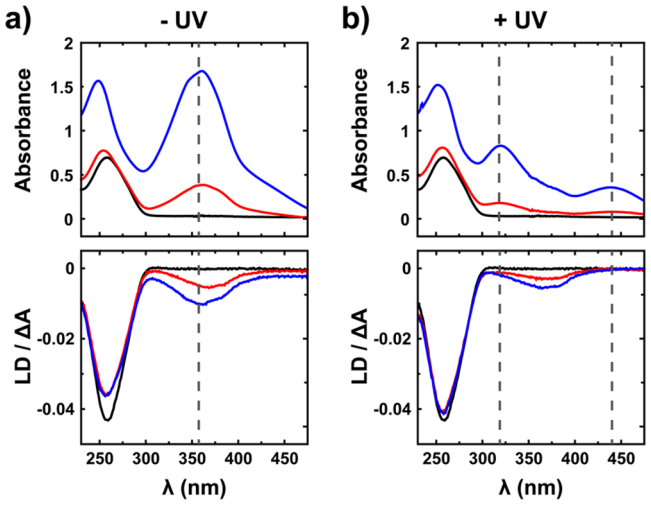

Figure 5. Absorption (top) and linear dichroism (bottom) spectra of **AzoDiGua** in the presence of ct-DNA (50 μM in 10 mM phosphate buffer, pH = 7.0) at **AzoDiGua**:DNA ratio r = 0 (black), r = 0.5 (red), and r = 2.0 (blue), before (a, −UV) and after (b, +UV) irradiation at 365 nm for 8 min. The dashed lines indicate the maxima of absorption of *trans*-**AzoDiGua** (a, 357 nm) and *cis*-**AzoDiGua** (b, 319 and 440 nm).

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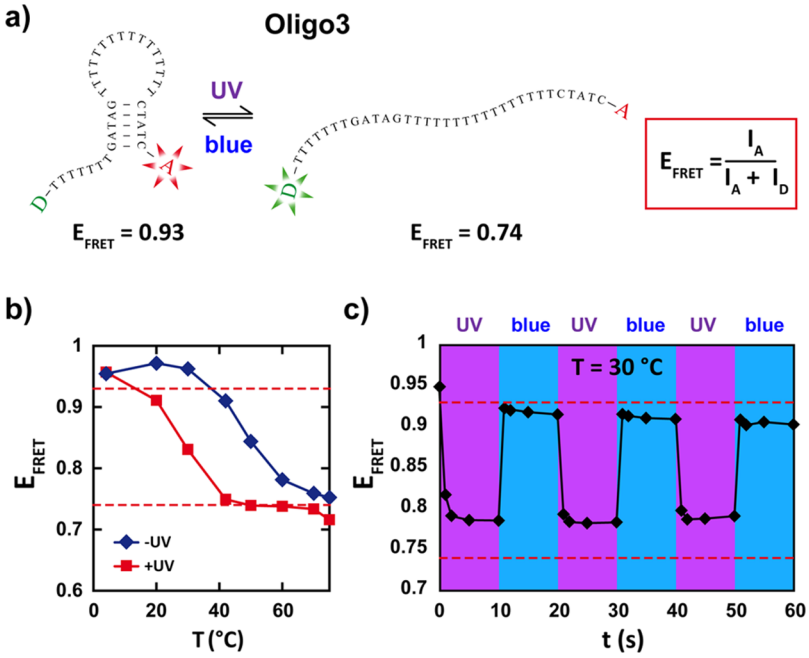

Figure 6. Reversible photocontrol of DNA nanostructure assembly at constant temperature. (a) Oligo3 is a self-complementary hairpin oligonucleotide labeled with a donor D (fluorescein) and an acceptor A (Texas Red) of Förster resonance energy transfer (FRET). The FRET efficiency (E_{FRET}), calculated from the fluorescence intensities of the acceptor (I_A) and of the donor (I_D), decreases/increases upon melting/hybridization of the self-complementary sequences. (b) Oligo3 melting followed by E_{FRET} measurement as a function of temperature, with (+UV) or without (−UV) illumination at 365 nm. The dashed lines indicate the E_{FRET} values at which Oligo3 is hybridized (E_{FRET} = 0.93) and melted (E_{FRET} = 0.74) in the absence of **AzoDiGua**. (c) E_{FRET} of Oligo3 in the presence of **AzoDiGua**, as a function of time, for successive cycles of UV (365 nm) and blue (440 nm) illumination performed at a constant temperature T = 30 °C. [Oligo3] = 33 μM in 10 mM Tris buffer pH 7.4 + 50 mM NaCl; [**AzoDiGua**]:Oligo3 ratio r = 1.

sample confirmed the photoreaction. Thus, the spectrum reveals the intercalating *trans*-**AzoDiGua** that is still present in the photostationary equilibrium, whereas the absence of a LD signal from the *cis* isomer denotes a displacement of the ligand from the intercalation binding site followed by association with the DNA backbone. The maintained association of *cis*-**AzoDiGua** with DNA even after ejection from the intercalation site is supported by the ICD bands (Figure 4). These results are in agreement with the known unfavorable π stacking properties of *cis*-azobenzene units that are covalently attached as base surrogates in double-stranded DNA.[14] Moreover, it has been demonstrated that resembling azobenzene derivatives bind to quadruplex DNA by terminal π stacking and are displaced from this binding site on photoinduced *trans*−*cis* isomerization.[46,47]

Then, we characterized the performance and robustness of the photocontrolled DNA melting in the presence of **AzoDiGua** by measuring T_m with and without UV irradiation, for various DNA sequences, ionic strengths, and **AzoDiGua**:DNA ratios (Table 1 and SI Figure S5). Regardless of irradiation conditions, we found that the T_m was influenced by all of these parameters in a regular manner. T_m systematically increased with an increase in DNA length (T_m of ct-DNA was much higher than both Oligo1 and Oligo2), GC content (T_m of Oligo1 with 40% GC was higher than T_m of Oligo 2 with 20% GC), or ionic strength due to the well-established stabilization of the double helix by these parameters. Interestingly, for all of these conditions, we observed a reproducible effect of UV irradiation: T_m was systematically lowered by the application of UV irradiation, and its variation (ΔT_m) ranged from −4 to −13 °C. Such a robustness can be explained by the fact that ΔT_m originates from a binding mode change, which is primarily controlled by the light-induced *trans*−*cis* isomerization of **AzoDiGua** rather than by the DNA characteristics or ionic strength conditions. To our knowledge, this shows for the first time that light can be used to change the T_m of DNA without any prerequisite on its sequence or length, for various concentration and ionic strength conditions.

All of our results have shown that adding **AzoDiGua** to a DNA solution allowed us to change its T_m by UV irradiation, regardless of its sequence. We then wanted to assess the possibility to control the DNA hybridization and melting processes by light at a constant temperature and apply it for reversible photocontrol of a simple hybridization-based DNA nanostructure. We used a self-complementary oligonucleotide (Oligo3) labeled at its extremities by a pair of fluorophores engaged in Förster resonant energy transfer (FRET) (Figure 6a). The opening/assembly of this nanostructure could thus be followed by the decrease of FRET due to the increase of a mean distance between the fluorophores.[48,49] We computed a normalized FRET efficiency $E_{FRET} = I_A/(I_A + I_D)$ where I_A and I_D were the fluorescence intensity of the FRET acceptor and FRET donor, respectively. Under our experimental conditions, we found E_{FRET} = 0.93 when Oligo3 was self-hybridized (T = 0 °C) and E_{FRET} = 0.74 when it was melted (T = 30 °C). We then established the melting curve of Oligo3 in the presence of **AzoDiGua**, by plotting E_{FRET} as a function of temperature, without (−UV, *trans*-rich solution) or with (+UV, *cis*-rich solution) illumination at 365 nm (Figure 6b). For both isomers, increasing temperature resulted in the melting of Oligo3 as observed by the strong decrease of E_{FRET}. In agreement with previous data using ct-DNA, Oligo1, and Oligo2, the

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temperature to achieve full DNA melting was larger than that in the absence of **AzoDiGua**, which is attributed to the stabilization of the self-hybridized part of Oligo3 by **AzoDiGua**. Interestingly, the melting curve with UV illumination (+UV) was shifted to lower temperatures by about 18 °C compared to that without illumination (−UV), confirming the higher stabilization of the hybridized state of DNA by the *trans*-isomer. This experiment thus confirms the UV-induced decrease of T_m in the presence of **AzoDiGua** in the case of a self-complementary oligonucleotide.

Then we fixed the temperature at 30 °C and we followed the state of the hairpin nanostructure in the presence of **AzoDiGua**, by measuring E_{FRET} as a function of time for successive cycles of UV/blue illumination (Figure 6c). **AzoDiGua** was initially in the *trans* conformation, and at this temperature, Oligo3 was self-hybridized and the nanostructure was properly assembled (high E_{FRET}). Upon UV illumination, E_{FRET} rapidly decreased to reach a plateau of $E_{FRET} \approx 0.78$ within about 2 s. This E_{FRET} value was close to but slightly larger than the value for fully melted Oligo3 (0.74), indicating that most Oligo3 molecules were probably in an open configuration but some interactions remained between the self-complementary sequences. The rapid kinetics can be explained by the use of a more powerful UV light source for this experiment (10 mW·cm^{-2}) compared to the spectroscopic studies (Figure 2a) where low power (0.35 mW·cm^{-2}) was preferred for a detailed analysis of the isomerization properties of **AzoDiGua**. This fast response also shows that the UV-induced isomerization of **AzoDiGua** from *trans*- to *cis*- is rapidly converted into a structural response of the DNA hairpin, probably due to the rapid ejection of the *cis*-isomer from the intercalating binding sites. Interestingly, subsequent illumination of this sample with blue light induced an almost immediate increase of E_{FRET} to a high plateau value ($E_{FRET} \approx 0.92$) indicating almost complete rehybridization of Oligo3 and reassembly of the hairpin nanostructure. Three consecutive UV/blue cycles accompanied by rapid opening/assembly of the hairpin through DNA melting/hybridization were successfully realized. When this experiment was performed in the absence of **AzoDiGua**, Oligo3 remained either in the hybridized state (at T = 0 °C) or in the melted state (at T = 30 °C) without being affected by the UV/blue illumination cycles (SI Figure S6), showing that **AzoDiGua** was necessary for both the stabilization of Oligo3 at 30 °C and for the photoreversible melting/hybridization to occur at this temperature. When **AzoGua** was used instead of **AzoDiGua**, Oligo3 behaved as in the absence of **AzoDiGua** (SI Figure S6), confirming that **AzoDiGua** only had double-helix stabilization properties and was able to induce photodependent DNA melting properties. This control experiment with **AzoGua** also shows that the fluorescence intensity variations observed in our experiments with **AzoDiGua** were not due to the photo-dependent spectroscopic properties of azobenzene but to the unique photodependent binding mode of **AzoDiGua** that was accompanied by marked photosensitive DNA melting behavior. To our knowledge this is the first example of photoreversible DNA nanostructure assembly at constant temperature through photoinduced DNA melting/hybridization that does not require any covalent modification of DNA.

In summary, we have developed a class of DNA binding molecules containing both guanidinium substituents and photoswitchable azobenzene moieties. The two synthesized photosensitive guanidinium derivatives (PGDs) exhibited strong interactions with DNA, but only the hydrophilic, bivalent compound (**AzoDiGua**) had a strong influence on the melting properties of DNA. We showed that, in the dark or after blue illumination, **AzoDiGua** was mainly composed of the *trans* isomer, having an intercalative binding mode strongly stabilizing the DNA double helix and increasing its T_m by several tens of degrees. Upon UV illumination, the *trans*−*cis* isomerization induced an ejection of **AzoDiGua** of the intercalating binding sites, resulting in a significant decrease of T_m of up to 18 °C. The resulting photodependent melting behavior was shown to be highly robust, sequence-independent, and applicable in a broad variety of concentration and ionic strength conditions. As a consequence, we achieved the melting and the hybridization of a self-complementary oligonucleotide at constant temperature upon successive UV/blue illumination, in a highly dynamic and reversible manner. Over existing methods, the marked advantage of our approach lies in the use of a new type of molecule that interacts with DNA in a noncovalent manner and without any prerequisite in terms of DNA length or sequence. The modification and manipulation of DNA nanostructures by noncovalent interactions is becoming increasingly important,[51] but to our knowledge, we describe here the first example of a stimuli-responsive platform for DNA nanotechnology. With our photosensitive intercalator, any DNA molecule can be melted/hybridized by straightforward UV/blue light illumination at constant temperature, bringing revolutionary perspectives for dynamic spatiotemporal photocontrol of DNA hybridization-based biotechnology (e.g., PCR,[1−3] gene regulation[21,22]) and nanotechnology (e.g., DNA origamis,[5,6] nanostructures,[7,8] particle assembly[9]).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.5b04762.

Materials and Methods; Synthesis of PGDs (Scheme S1); Thermal relaxation of PGDs (Figure S1); Binding of **AzoDiGua** to double- and single-stranded oligonucleotides (Figure S2); Fluorescence microscopy of T4 DNA compaction by **AzoGua** (Figure S3); Static light scattering of ct-DNA as a function of PGD concentration (Figure S4); Melting of Oligo1 and Oligo2 as a function of AzoDiGua:DNA ratio and illumination conditions (Figure S5); Control experiments for the photoreversible melting of Oligo3 (Figure S6) (PDF)

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Author Contributions

D.B. supervised the work. A.B. performed PGD synthesis, absorbance measurements, DNA melting curves, and compaction studies. S.R. performed FRET measurements with contributions from T.L.S. and M.M. H.I. performed CD and LD spectroscopic studies and interpretation of the data with contributions from A.B. All authors analyzed the data.

Notes

The authors declare no competing financial interest.

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