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Artificial Base zT as Functional "Element" for Constructing Photoresponsive DNA Nanomolecules

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Abstract

In contrast to small molecules, DNA and RNA macromolecules can be accurately formulated with base "elements" abbreviated as A, T, U, C, and G. However, the development of functionally artificial bases can result in the generation of new biomaterials with unique properties and applications. Therefore, we herein report the design and synthesis of a photoresponsive base as a new functional or molecular "element" for constructing DNA nanomolecules. The new base is made by fusion of an azobenzene with a natural T base (zT). zT, a new molecular element, is not only the most size-expanded T analogue but also a photoresponsive base capable of specific self-assembly through hydrogen bonding. Our results showed that stable and selective self-assembly of double-stranded DNAs occurred through zT-A base pairing, but it could still be efficiently dissociated by light irradiation. The photoresponsive DNA bases will provide the versatility required for constructing desired DNA nanomolecules and nanodevices.

Small molecules are angstrom-scaled, and their chemical formulas contain structural information. In contrast, nanoscaled molecules DNA and RNA are generally formulated with A, T, U, C, and G, and this type of formula (i.e., the base sequence) of DNA and RNA can also accurately represent their structural information. Hence, we propose an alias for A, T, U, C, G and numerous artificial bases as "base elements", molecular "elements" for

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Notes

The authors declare no competing financial interest.

Supporting Information

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Experimental details, melting curves, and NMR and ESI-MS spectra (PDF)

constructing DNA and RNA nanomolecules. Apart from storing biological information, DNA has been widely exploited as an intelligent and advanced material in biomedical sciences, materials science, and nanotechnology. [1–7] Artificial DNA bases are highly desirable because they expand A, T, U, C, and G to construct various novel DNA nanomolecules as biomaterials with new functions and thus unique applications.[8]

Separation of nucleic acid double helices into two single strands is the reverse process of DNA self-assembly. In living systems, this is accomplished by polymerase, while thermal denaturing separates two strands of nucleic acids in PCR technology.[9] Synthetic DNAs provide promising solutions for controllable dissociation of duplexes. However, while the use of single-stranded oligonucleotides is easily achieved in the self-assembly of DNA structures, controllable dissociation of hybridized duplexes remains challenging. Photoregulation of DNA-duplex structures by incorporation of azobenzene was pioneered by Asanuma and Komiyama,[10–12] and diverse backbones[13,14] were developed to tether functionality. Studies have also shown that the incorporation of different photo-responsive functionalities onto DNA bases may result in the dissociation of a DNA duplex by photon energy.[15–18] Using a method whereby a phosphoramidite monomer bearing an azobenzene is synthesized from D-threoninol, we previously constructed DNA-based nanodevices for bioengineering studies.[19,20]

Based on the structural homogeneity and simplicity intrinsic to the design of photoresponsive DNAs, in 2010 we proposed the concept of zT as a new and functional “element” with preliminary results.[21] The width of a natural DNA duplex is uniformly between 2.2 and 2.6 nm. However, Kool and coworkers reported that the width could be expanded without sacrificing self-assembly ability by insertion of a benzene ring into the heterocycle of natural bases.[22,23] To explore the limitation of the width, they gradually increased the size by exploring naphtha-homologated analogues.[24] These expanded DNAs could be unique tools in bioanalysis and biotechnology. [25] Since *trans*-azobenzene is wider than naphthalene in dimension, the addition of azobenzene with thymine leads to zT (Scheme 1a), the most expanded analogue under these conditions. Moreover, thermally stable *trans*-azobenzene is coplanar and can be efficiently isomerized to the *cis* isomer by irradiation with UV light.[26] As a result, *trans*-zT specifically hybridizes with A. However, because *cis*-zT can neither stabilize the duplex by π-stacking nor form hydrogen bonds with the corresponding A for its unmatched orientation, such hybridization can also be dissociated by UV irradiation (Scheme 1b, 1c). Based on this design, we attempted to embed a “photoswitch” to control the self-assembly of the DNA duplex through zT-A base pairing.

In this study, we tested the concept using the zT analogue with an expanded thymine base as our model. The synthesis of the corresponding deoxyriboside (dzT) was developed, and the stacking and pairing properties of azoDNA were studied by thermodynamics experiments. Subsequently, the photoresponsive properties were characterized for DNAs incorporated with element zT.

As shown in Scheme 2, dzT was synthesized using a fragment-condensation approach. Nitroso compound **1** was prepared as one of the fragments from 2-amino-4-nitrobenzoic acid. Pd-mediated Heck reaction of glycal **2** with *N*-(3-iodophenyl) acetamide gave the

coupled product **3** in good yield in optimized conditions. Protecting group TBDPS was removed, and the resulting ketone intermediate was stereo-selectively reduced to give compound **4**. Aniline **5** was prepared as the other fragment by removal of the acetyl group of compound **4**. The condensation of fragment **5** with fragment **1** efficiently gave zT deoxynucleoside **6** as a red powder. The preparation of compound **7** was achieved from dzT **6** in two steps. Phosphoramidite **7** was synthesized from starting compound **2** in seven steps with a 21% yield.

From phosphoramidite **7**, zT was incorporated into oligonucleotides (ODNs) by a standard DNA synthesizer. The sequences of ODNs used in this study are listed in Table 1.

To investigate the properties of zT, we prepared azoDNAs (ODN 11–14, Table 1) for different studies. **ODN 11** is an oligo with a self-complementary sequence, two strands of which may form a duplex structure through zT-A base pairing (Scheme 3a). Similarly, **ODN 12** may form a hairpin structure with intramolecular duplexes (Scheme 3b). Both double-stranded duplexes and intramolecular duplexes can be dissociated at a specific temperature, namely the melting temperature (T_m), by heating the solution.

Thermodynamic studies of **ODN 11** and **ODN 12** confirmed the formation of a duplex structure through zT-A base pairing. The melting temperature of the double-stranded azoDNA duplex is 58.6 °C (entry 1, Table 2), which is higher than that of the control duplexes (**ODN 15**, entry 5, Table 2). The melting temperature of the intramolecular azoDNA duplex is 86.5 °C (entry 2, Table 2), which is also higher than that of its control duplexes (entries 6 and 7, Table 2). The results indicate that zT-A base pairing is more stable than natural T-A and C-G base pairing. The thermodynamic stability of the azoDNA duplex may be produced by extending the π-system[24] of zT. The binding of dangling zT to self-complementary oligo 5′-CGCGCG dramatically stabilizes the core duplex by affording stronger π-stacking (entry 4, Table 2) compared with its control oligo containing dangling natural T at 5′-prime (ODN-18, entry 8, Table 2).

We have proved the formation of an intramolecular duplex in **ODN-12** as a hairpin structure through zT-A base pairing. To further investigate the selectivity of the zT-A base pair, we prepared **ODN-13** (Table 1) by replacing the stem sequence AAAAAA of **ODN-12** with TTTTTT. Depending on the strength of this nonspecific zT-T, a hairpin structure could also form in **ODN-13** through zT-T base pairing. This self-assembly places fluorescein (FAM) at the 5′-end and Dabcyl quencher on the 3′-end in close proximity, thereby yielding a weak fluorescence signal (molecular beacon in the OFF state). In contrast, either heating the solution of molecular beacons in buffer or addition of complementary DNA (cDNA, for sequence information see Table S1 as ODN-23) of the loop to the buffer solution should dissociate the stem duplex structure, resulting in an open ON state. Accordingly, with such a dramatic increase of fluorescence intensity, the self-assembly property of azoDNAs with hairpin structure would be easy to characterize by variations in fluorescence.

Figure 1 shows the signal enhancement after hybridization of **ODN-12** with cDNA (ODN-12+cDNA) in 20 mM PIPES buffer at pH 7.0. The fluorescence intensity of **ODN-12** is increased by approximately 6-fold, confirming the formation of a hairpin structure

through zT-A base pairing. In contrast, no obvious fluorescence changes were observed when cDNA was added to the solution of **ODN-13** in 20 mM PIPES buffer at pH 7.0. In addition, fluorescence-monitored thermal melting experiments of **ODN-13** showed only negligible changes in fluorescence intensity with temperature, suggesting that no duplex formed in **ODN-13** through either mismatched zT-T base pairing or base stacking. These results indicate that base pairing of zT-A is both stable and selective.

Since azo bases are expanded bases merged with azobenzene, the resulting azoDNAs may be conferred with photoresponsive properties. Thus, self-assembly through *trans*-zT-A base pairing can be switched on and off by photoirradiation. As such, azoDNAs are afforded a unique property by which both self-assembly and dissociation of DNA duplexes composed of azo bases can be controlled. To examine the photoswitchable properties, **ODN-12**, previously verified with hairpin structure, was subjected to UV irradiation at 365 nm for 5 min. Fluorescence variation of **ODN-12** in PIPES buffer, both before (Figure 1, **ODN-12**) and after irradiation (Figure 1, **ODN-12**-UV), was recorded, and the fluorescence intensity was increased by approximately 3-fold after photoisomerization, suggesting the efficient dissociation of the duplex by UV irradiation.

Based on this photoresponsive phenomenon, it is essential to demonstrate the reversible hybridization of zT-A base pairing by repeated on/off cycles of photoswitching. Therefore, a kinetic fluorescent experiment was designed to characterize the reversibility of zT as a photoswitch. As shown in Figure 2, the kinetic fluorescence intensity of 1 μM **ODN-12** solution in PIPES buffer was recorded for 25 s as a start, after which the solution was subjected to UV irradiation (Figure 2, Process A) for 10 min, and the kinetic fluorescence of the UV-irradiated solution was recorded immediately. After 25 s, the solution was subjected to visible light for 10 min (Figure 2, Process B, the reverse of A), and the kinetic fluorescence was recorded thereafter. Three cycles of variations in fluorescence were recorded, and the on/off ratio of the third cycle was approximately 2, confirming the reversibility of zT as a photoswitch.

In conclusion, we have designed and synthesized oligonucleotides incorporated with zT for characterization of their thermodynamic and photoresponsive properties. The fusion of azobenzene with thymine leads to zT. This provides extra space between the pyrimidine ring and phosphate backbone to embed a “switch” with which to exogenously control the self-assembly of the DNA duplex. The synthetic methods of dzT were developed, and a series of azoDNAs was prepared to study their properties. Our experimental results showed that zT specifically forms stable base pairs with natural A and that the duplex composed of zT-A base pairing is more stable than the duplex composed of natural C-G base pairing. Subsequently, the photoresponsive properties were characterized, and our results showed that the self-assembly of azoDNA could be switched on and off by photon energy. With more artificial DNA bases prepared as molecular elements with different properties, we will be able to construct functional molecules with artificial DNA bases using the same base connection chemistry by a commercial DNA synthesizer. It is expected that these molecular elements with different properties will lead to facile synthesis of a variety of designer and functional molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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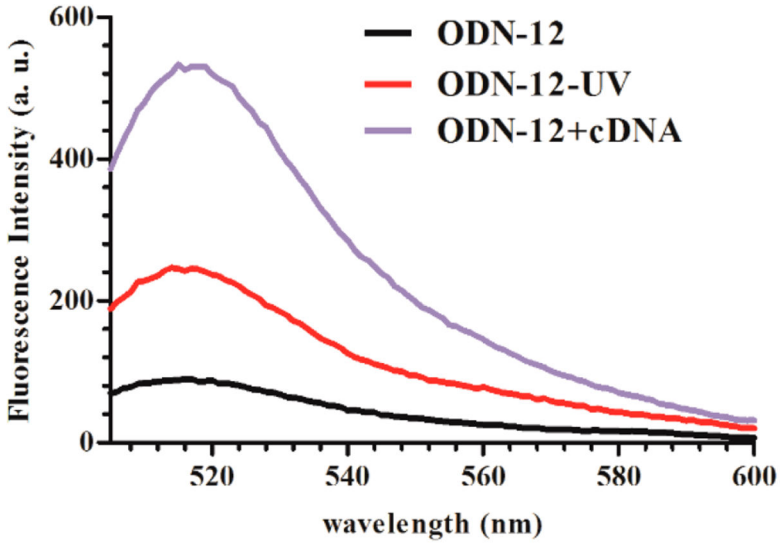


Figure 1.

Fluorescence changes of **ODN-12** in PIPES buffer when subjected to the addition of cDNA or UV irradiation.

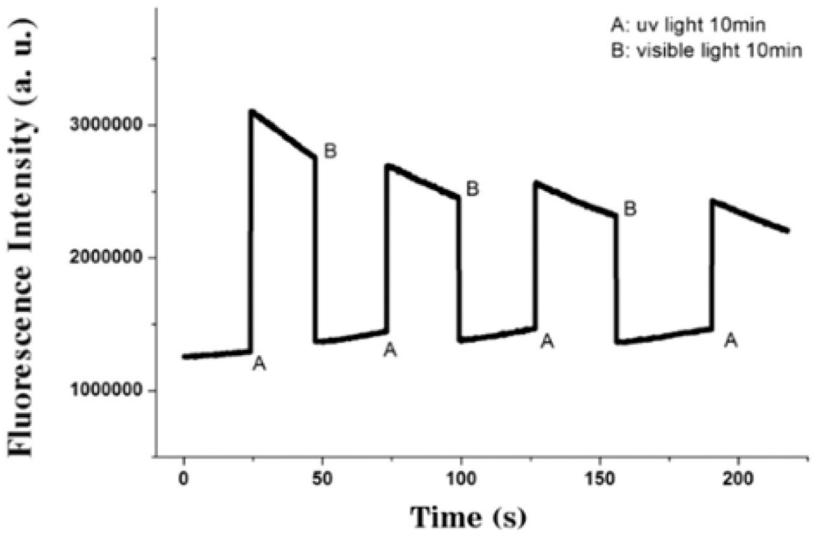
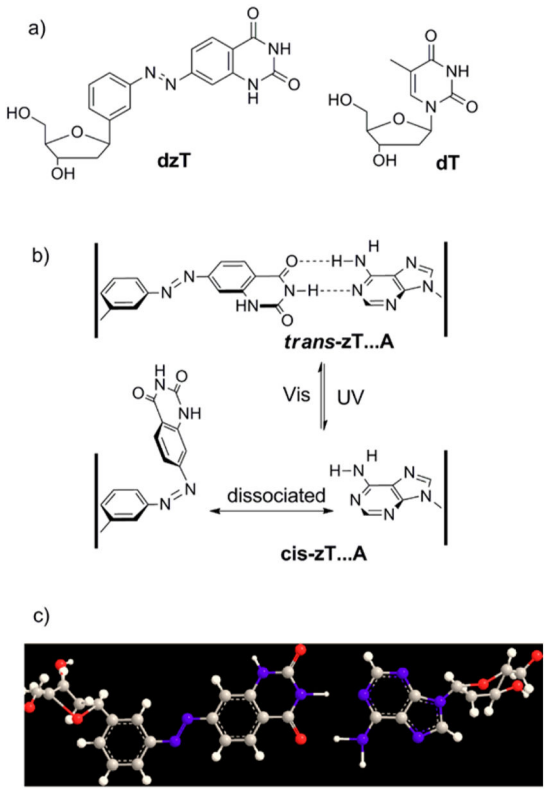
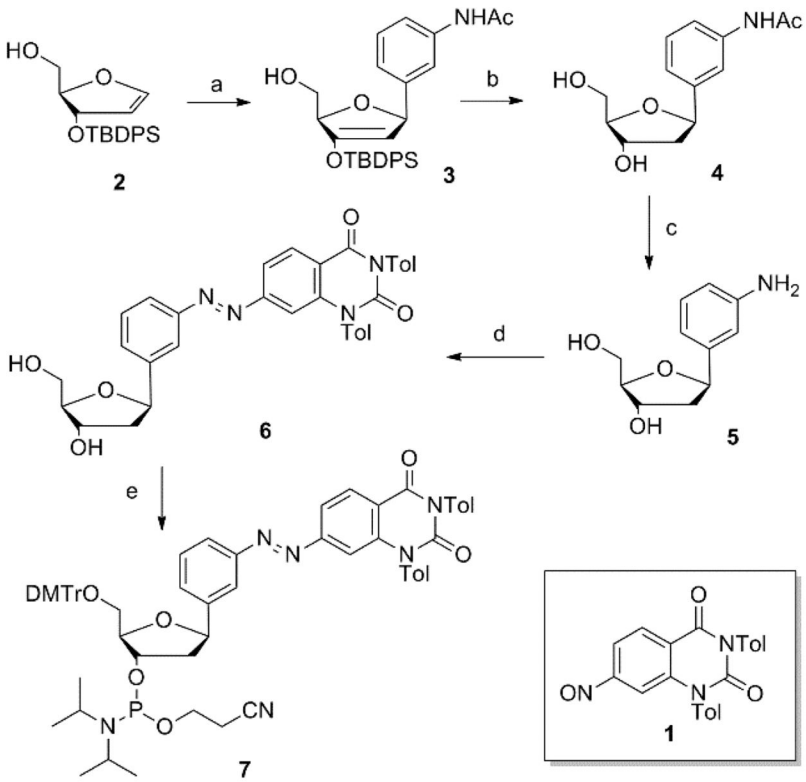


Figure 2.
Kinetic variations in fluorescence of **ODN-12** for repeated on/off photoswitching cycles.



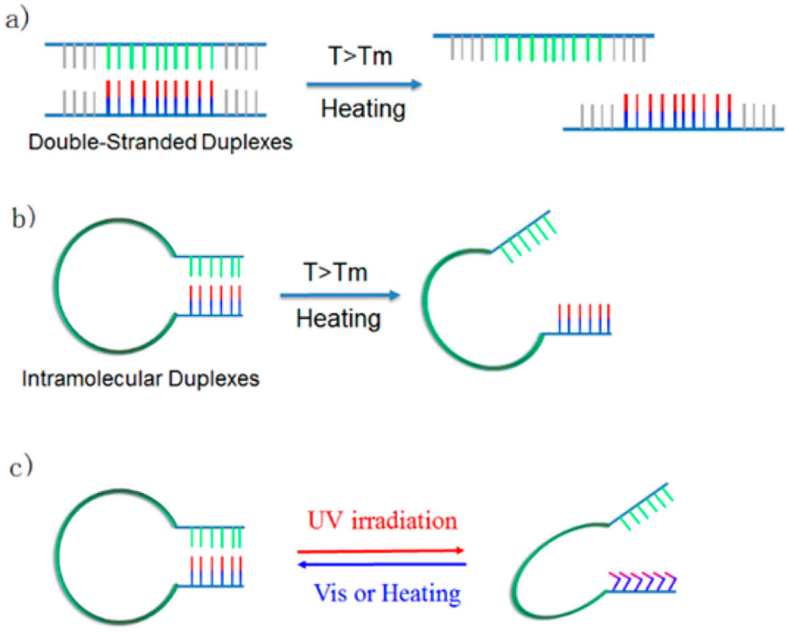
Scheme 1.

(a) Structures of Deoxyribonucleosides dzT and Natural dT; (b) Proposed Base Pair Pattern for zT-A; (c) Proposed Base Pairing Model for zT-A



Scheme 2. Synthesis of dzT 6 and Corresponding Phosphoramidite 7[a]

[a]Reagents and conditions: (a) *N*-(3-iodophenyl)acetamide, $Pd(OAc)_2$, $AsPh_3$, Et_3 N, $CHCl_3$, reflux, 55%; (b) (I) TBAF, THF, 0 °C; (II) $Na(OAC)_3BH$, THF, −10 °C, 82%; (c) 3 M HCl methanolic solution, reflux, 93%; (d) AcOH, 25 °C, 75%; (e) (I) DMTrCl, Pyridine, 25 °C, 79%; (II) chlorodiisopropylcyanoethyl phosphoramidite, DIEA, CH_2Cl_2, 0–25 °C, 85%.



Scheme 3.

(a) Thermal Dissociation of Double-Stranded Duplexes; (b) Thermal Dissociation of Intramolecular Duplexes; (c) Photo-controlled Dissociation and Reversible Process

Table 1

Sequences of Oligonucleotides

ODNs	Sequence[a]
11	5′-TTTTT**zTAzTzTAzTAAzTA**TTTTT
12	5′-Dabcyl-**zTzTzTzTzTzT**TCTAAATCACTATGGTCGCAAAAAA-Fam
13	5′-Dabcyl-**zTzTzTzTzTazT**TCTAAATCACTATGGTCGCTTTTTT-Fam
14	5′-**zT**CGCGCG
15	5′-TTTTTTATTATAATATTTTT
16	5′-Dabcyl-TTTTTTTCTAAATCACTATGGTCGCAAAAAA-Fam
17	5′-Fam-GCGCGCTCTAAATCACTATGGTCGCGCGCGC-Fam
18	5′-TCGCGCG

[a] **zT** indicates the unit synthesized from phosphoramidite **7**.

Table 2

Melting Temperature (T_m) of DNA Duplexes[a]

Entry	ODN	T_m (°C)	Entry	ODN	T_m (°C)
1	11	58.6	5	15	41.7
2	12	86.5	6	16	33.7
3	13	–	7	17	79.2
4	14	62.4	8	18	51.5

[a]Conditions: 1 μM DNA, 100 mM NaCl, 10 mM $MgCl_2$, 10 mM PIPES, pH 7.0. The heating rates were 0.5 °C/min.